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Ibogaine

Scientific Literature Overview

composed by
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Tabel of Content

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——CHAPTER 1——

IBOGAINE: A REVIEW

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I. Introduction, Chemical Properties, and Historical Time Line

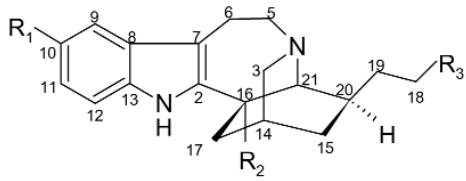
A. Introduction

Ibogaine, a naturally occurring plant alkaloid with a history of use as a medicinal and ceremonial agent in West Central Africa, has been alleged to be effective in the treatment of drug abuse. The National Institute on Drug Abuse (NIDA) has given significant support to animal research, and the U.S. Food and Drug Administration (FDA) has approved Phase I studies in humans. Evidence for ibogaine's effectiveness includes a substantial preclinical literature on reduced drug self-administration and withdrawal in animals, and case reports in humans. There is relatively little financial incentive for its development by the pharmaceutical industry because ibogaine is isolated from a botanical source in which it naturally occurs, and its chemical structure cannot be patented. This has left the academic community and the public sector with a crucial role in research on ibogaine, which was a major reason for organizing the First International Conference on Ibogaine.

A major focus of the Conference was the possible mechanism(s) of action of ibogaine. Ibogaine is of interest because it appears to have a novel mechanism of action distinct from other existing pharmacotherapeutic approaches to addiction, and it potentially could provide a paradigm for understanding the neurobiology of addiction and the development of new treatments. Another important focus of the Conference was to review human experience with ibogaine and preclinical and clinical evidence of efficacy and safety. The Conference also featured presentations related to the sociological and anthropological aspects of the sacramental context of the use of iboga in Africa and the distinctive ibogaine subculture of the United States and Europe.

B. Chemical Structure and Properties

Ibogaine (10-methoxyibogamine) (Figure 1) is an indole alkaloid with molecular formula $C_{20}H_{26}N_2O$ and molecular weight 310.44. Ibogaine is the most abundant alkaloid in the root bark of the Apocynaceous shrub *Tabernanthe iboga*, which grows in West Central Africa. In the dried root bark, the part of the plant



Alkaloid	R_1	R_2	R_3
Ibogaine	OCH_3	H	H
Noribogaine	OH	H	H
(±)-18-Methoxycoronaridine	H	CO_2CH_3	OCH_3

FIGURE 1. CHEMICAL STRUCTURES OF IBOGAINE, NORIBOGAINE, AND 18-METHOXYCORONARIDINE. The ibogamine skeleton above is numbered using the LeMen and Taylor system in which ibogaine is designated as 10-methoxyibogamine and noribogaine as 10-hydroxyibogamine. Alternatively, according to the Chemical Abstracts numbering system for the ibogamine skeleton which is frequently encountered in the biological and medical literature, ibogaine and noribogaine have respectively been referred to as 12-methoxyibogamine and 12-hydroxyibogamine.

in which alkaloid content is highest, total alkaloid content is reportedly 5 to 6% (*1*).

Ibogaine has a melting point of 153°, a pK_a of 8.1 in 80% methylcellosolve, and it crystallizes as prismatic needles from ethanol. Ibogaine is levorotatory $[\alpha]_D$ –53° (in 95% ethanol), soluble in ethanol, ether, chloroform, acetone and benzene, but it is practically insoluble in water. Ibogaine is decomposed by the action of heat and light. Ibogaine hydrochloride decomposes at 299°, is also levorotatory $[\alpha]_D$ –63° (ethanol), $[\alpha]_D$ –49° (H_2O), and is soluble in water, methanol, and ethanol, slightly soluble in acetone and chloroform, and practically insoluble in ether (*2*). The X-ray crystal analysis that confirmed the structure of ibogaine has been described (*3*). The literature provides references to the mass spectrum of ibogaine (*4*), and the proton (*5,6*) and the ^{13}C (*7-9*) NMR spectra of ibogaine and other *iboga* alkaloids. Analytic chemical methods for extraction, derivatization, and detection of ibogaine utilizing combined gas chromatography-mass spectometry have been described (*10-13*).

Ibogaine undergoes demethylation to form its principal metabolite, noribogaine, also known as *O*-desmethylibogaine or 10-hydroxyibogamine. 18-methoxycoronaridine (18-MC, see Glick *et al.* in this volume) is an ibogaine congener that appears to have efficacy similar to ibogaine in animal models of drug dependence with evidence of less potential toxicity.

C. Historical Time Line

The following time line outlines the historical events relating to the development of ibogaine as a treatment for drug dependence. Elsewhere in this volume, Alper *et al*. provide a more detailed contemporary history of ibogaine in the United States and Europe.

1864: The first description of *T. iboga* is published. A specimen is brought to France from Gabon. A published description of the ceremonial use of *T. iboga* in Gabon appears in 1885 (*14*).

1901: Ibogaine is isolated and crystallized from *T. iboga* root bark (*15-17*).

1901-1905: The first pharmacodynamic studies of ibogaine are performed. During this period ibogaine is recommended as a treatment for "asthenia" at a dosage range of 10 to 30 mg per day (*14*).

1939-1970: Ibogaine is sold in France as Lambarène, a "neuromuscular stimulant," in 8 mg tablets, recommended for indications that include fatigue, depression, and recovery from infectious disease (*14*).

1955: Harris Isbell administers doses of ibogaine of up to 300 mg to eight already detoxified morphine addicts at the U.S. Addiction Research Center in Lexington, Kentucky (*18*).

1957: The description of the definitive chemical structure of ibogaine is published. The total synthesis of ibogaine is reported in 1965 (*19-21*).

1962-1963: In the United States, Howard Lotsof administers ibogaine to 19 individuals at dosages of 6 to 19 mg/kg, including 7 with opioid dependence who note an apparent effect on acute withdrawal symptomatology (*22*,*23*).

1967-1970: The World Health Assembly classifies ibogaine with hallucinogens and stimulants as a "substance likely to cause dependency or endanger human health." The U.S. Food and Drug Administration (FDA) assigns ibogaine Schedule I classification. The International Olympic Committee bans ibogaine as a potential doping agent. Sales of Lambarène cease in France (*14*).

1969: Dr. Claudio Naranjo, a psychiatrist, receives a French patent for the psychotherapeutic use of ibogaine at a dosage of 4 to 5 mg/kg (*24*).

1985: Howard Lotsof receives a U.S. patent for the use of ibogaine in opioid

withdrawal (*22*). Additional patents follow for indications of dependence on cocaine and other stimulants (*23*), alcohol (*25*), nicotine (*26*), and polysubstance abuse (*27*).

1988-1994: U.S. and Dutch researchers publish initial findings suggestive of the efficacy of ibogaine in animal models of addiction, including diminished opioid self-administration and withdrawal (*28-30*), as well as diminished cocaine self-administration (*31*).

1989-1993: Treatments are conducted outside of conventional medical settings in the Netherlands involving the International Coalition of Addict Self-Help (ICASH), Dutch Addict Self Help (DASH), and NDA International (*22,32-35*).

1991: Based on case reports and preclinical evidence suggesting possible efficacy, NIDA Medication Development Division (MDD) begins its ibogaine project. The major objectives of the ibogaine project are preclinical toxicological evaluation and development of a human protocol.

August 1993: FDA Advisory Panel meeting, chaired by Medical Review Officer Curtis Wright, is held to formally consider Investigational New Drug Application filed by Dr. Deborah Mash, Professor of Neurology at the University of Miami School of Medicine. Approval is given for human trials. The approved ibogaine dosage levels are 1, 2, and 5 mg/kg. The Phase I dose escalation study begins December 1993, but activity is eventually suspended (*36*).

October 1993-December 1994: The National Institute on Drug Abuse (NIDA) holds a total of four Phase I/II protocol development meetings, which include outside consultants. The resulting draft protocol calls for the single administration of fixed dosages of ibogaine of 150 and 300 mg versus placebo for the indication of cocaine dependence (*37*).

March 1995: The NIDA Ibogaine Review Meeting is held in Rockville, Maryland, chaired by the MDD Deputy Director, Dr. Frank Vocci. The possibility of NIDA funding a human trial of the efficacy of ibogaine is considered. Opinions of representatives of the pharmaceutical industry are mostly critical, and are a significant influence in the decision not to fund the trial. NIDA ends its ibogaine project, but it does continue to support some preclinical research on *iboga* alkaloids.

Mid 1990s-2001: Ibogaine becomes increasingly available in alternative settings, in view of the lack of approval in the Europe and the United States. Treatments in settings based on a conventional medical model are conducted in

Panama in 1994 and 1995 and in St. Kitts from 1996 to the present. Informal scenes begin in the United States, Slovenia, Britain, the Netherlands, and the Czech Republic. The Ibogaine Mailing List (*38*) begins in 1997 and heralds an increasing utilization of the Internet within the ibogaine medical subculture.

II. Mechanisms of Action

A. Neurotransmitter Activities

1. General Comments

Elsewhere in this volume, Glick *et al.*, Sershen *et al.*, and Skolnick review the mechanism of action of ibogaine. Popik and Skolnick (*39*) provide a recent, detailed review of ibogaine's receptor activities. Ibogaine appears to have a novel mechanism of action that differs from other existing pharmacotherapies of addiction, and its mechanism of action does not appear to be readily explained on the basis of existing pharmacologic approaches to addiction. Ibogaine's effects may result from complex interactions between multiple neurotransmitter systems rather than predominant activity within a single neurotransmitter system (*39-42*).

Several laboratories have reported on the results of pharmacological screens of the receptor binding profile of ibogaine (*40,43-45*). Ibogaine has low micromolar affinities for multiple binding sites within the central nervous system, including *N*-methyl-D-aspartate (NMDA), kappa- and mu-opioid and $sigma_2$ receptors, sodium channels, and the serotonin transporter. Although not apparent in binding studies, functional studies indicate significant activity of ibogaine as a noncompetitive antagonist at the nicotinic acetylcholine receptor (*46-50*).

Although *in vitro* activities in the micromolar range are often described as ancillary in attempting to characterize a drug's *in vivo* mechanism of action, micromolar activity may be pharmacologically important with regard to ibogaine or noribogaine due to the relatively high concentrations reached in the brain (*40,44,51*). Hough *et al.* (*51*) noted a brain level of ibogaine of 10 μM in female rats at 1 hour after the administration of 40 mg/kg ibogaine intraperitoneally (i.p.), which is the usual dosage, animal, gender and route of administration used in that laboratory to investigate ibogaine's effects on drug self-administration and withdrawal. Brain levels of ibogaine, and its major metabolite noribogaine, ranged from 1 to 17 μM between 15 minutes and 2 hours in male rats following the oral administration ibogaine at a dose of 50 mg/kg (*44*).

2. Glutamate

Elsewhere in this volume, Skolnick reviews the possible relevance of

ibogaine's activity as a glutamate antagonist to its putative effects in drug dependence. There is evidence that suggests that antagonists of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor are a potentially promising class of agents for the development of medications for addiction (*52-54*). Ibogaine's apparent activity as a noncompetitive NMDA antagonist has been suggested to be a possible mechanism mediating its putative effects on drug dependence (*39,41,55-58*).

Ibogaine competitively inhibits the binding of the NMDA antagonist MK801 to the NMDA receptor complex, with reported affinities in the range of 0.02 to 9.8 μM (*40,45,55-57,59,60*). Functional evidence supporting an antagonist action of ibogaine at the NMDA receptor includes observations of reduced glutamate-induced cell death in neuronal cultures, reduction of NMDA-activated currents in hippocampal cultures (*55,58*), prevention of NMDA-mediated depolarization in frog motoneurons (*59*), and protection against NMDA-induced convulsions (*61*). Glycine, which acts as an NMDA co-agonist by binding at the NMDA receptor, attenuates ibogaine's effect of blocking naloxone-precipitated jumping (*58*). MK801 and ibogaine do not produce identical effects, as evidenced by the observation that in the rat brain ibogaine lowered the concentration of dopamine while increasing the level of its metabolites, whereas MK801 did not have these effects (*62,63*).

3. Opioid

It has been suggested that ibogaine's or noribogaine's activity as a putative agonist at mu-opioid receptors might explain ibogaine's apparent efficacy in opioid withdrawal (*36,64,65*). Ibogaine binds to mu-opioid receptors with reported binding affinities in the range of 0.13 to 26 μM (*40,45,64,66*), with one study reporting a result in excess of 100 μM (*43*). Ibogaine behaves as an agonist in a functional assay for mu-opioid receptors, the binding of [35^S]-GTPγS (*65*). However, some observations are difficult to reconcile with a mu-agonist action of ibogaine. Ibogaine did not behave as a mu-opioid agonist in assays with isolated smooth muscle preparations (*67*). Unlike mu-opioid agonists, ibogaine (*68-70*) and noribogaine (*71*) do not appear by themselves to have antinociceptive effects.

Some findings suggest the intriguing possibility that ibogaine may act at the level of second messenger signal transduction to enhance the functional activity of mu-opioid receptors independently of any direct agonist interaction at opioid receptors. Both ibogaine and noribogaine reportedly potentiated morphine-induced inhibition of adenylyl cyclase *in vitro* with opioid receptors already occupied by the maximally effective concentration of morphine, but did not affect adenylyl cyclase in the absence of morphine (*72*). A similar interpretation might also explain the finding that ibogaine inhibited the development of tolerance to the antinociceptive effect of morphine in mice, without by itself affecting nociception (*73*).

Ibogaine binds to kappa-opioid receptors with reported binding affinities in the range of 2.2 to 30 μM (*43,45,56,66*). Evidence consistent with a kappa-opioid action of ibogaine includes the observation that the kappa-opioid antagonist, norbinaltorphimine antagonized some of the effects of ibogaine in morphine-treated rats (*74,75*). Kappa-opioid agonists reportedly can imitate certain effects of ibogaine, such as reduced cocaine and morphine self-administration (*76*), and reduction in locomotor activation to morphine accentuated by prior morphine exposure (*77*). Sershen, on the other hand, attributes a kappa-opioid antagonist action to ibogaine, based on the observation that stimulated dopamine efflux from mouse brain slices was decreased by a kappa-opioid agonist, and the decrease was offset by the addition of ibogaine (*78*). However, ibogaine's interactions with multiple neurotransmitter systems raises the possibility that the finding could be accounted for by mechanisms that do not involve the kappa-opioid receptor, as dopamine efflux is modulated by multiple neurotransmitters.

4. Serotonin

Ibogaine and serotonin both contain an indole ring in their structure, and ibogaine has been shown to bind to the serotonin transporter and to increase serotonin levels in the nucleus accumbens (NAc) (*41,79,80*). The demonstration that ibogaine blocks serotonin uptake (*81*) suggests that the effect of ibogaine on extracellular serotonin levels may be mediated by uptake inhibition, in addition to release (*80*). The reported affinity of ibogaine for the serotonin transporter ranges from 0.55 to 10 μM (*39,44,45,79,81*), and the affinity of noribogaine for the serotonin transporter is approximately 10-fold stronger (*45,79*). The magnitude of the effect of ibogaine on serotonin release is reportedly large and is comparable to that of the serotonin releasing agent fenfluramine, with noribogaine having a lesser effect, and 18-MC no effect (*80*). Some authors suggest a role for modulatory influence of serotonin in ibogaine's effects on dampening dopamine efflux in the NAc (*41,80*).

Ibogaine's hallucinogenic effect has been suggested to involve altered serotonergic neurotransmission (*42,80*). Ibogaine is reported in some studies to bind the 5-HT_{2A} receptor, which is thought to mediate the effects of "classical" indolealkylamine and phenethylamine hallucinogens (*82*), with three studies reporting affinities in the range of 4.1 to 12 μM (*40,45,83*), one reporting a value of 92.5 μM (*84*), and with two other studies reporting no significant affinity (*43,44*). Drug discrimination studies provide some functional evidence for the action of ibogaine as an agonist at the 5-HT_{2A} receptor, which is apparently a significant, although nonessential, determinant of the ibogaine stimulus (*84*) (see Section II.B, "Discrimination Studies"). Ibogaine binds to the 5-HT_3 receptor with reported affinities of 2.6 and 3.9 μM (*40,45*), and it was without significant affinity in two other studies (*43,83*). The 5-HT_3 receptor is apparently not involved in the ibogaine discriminative stimulus (*85*).

5. Dopamine

Ibogaine does not appear to significantly affect radioligand binding to D_1, D_2, D_3, or D_4 receptors (*40,43,44*) and is a competitive blocker of dopamine uptake at the dopamine transporter with affinities in the range of 1.5 to 20 μM (*81*). Where affinities for the serotonin and dopamine transporter have been estimated within the same study, the reported affinity of ibogaine for the serotonin transporter has generally been 10 to 50 times stronger than its affinity for the dopamine transporter (*44,79,81*). Ibogaine does not apparently affect norepinephrine reuptake (*44,45*).

French *et al.* (*86*) studied the electrophysiological activity of dopamine neurons in the ventral tegmental area (VTA) of rats given up to 7.5 mg/kg ibogaine intravenously and found a significant increase in firing rate. Ibogaine given i.p. at a dose of 40 mg/kg did not affect the spontaneous firing of VTA dopamine neurons or the response of VTA dopamine neurons to cocaine or morphine. Ibogaine reportedly lowers the concentration of dopamine, while increasing the level of its metabolites, indicating diminished release of dopamine in the brain of the rat (*62,63*) and the mouse (*87*). Decreased release of dopamine could possibly explain the observation of increased prolactin release following ibogaine administration (*62,63,88*). Staley *et al.* (*44*) have suggested that ibogaine might act at the dopamine transporter to inhibit the translocation of dopamine into synaptic vesicles, thereby redistributing dopamine from vesicular to cytoplasmic pools. As a result, the metabolism of dopamine by monoamine oxidase could explain the observation of decreased tissue dopamine content with increased levels of its metabolites.

The effects of ibogaine on dopamine efflux in response to the administration of drugs of abuse are described in Section III.E, “Dopamine Efflux”.

6. Acetylcholine

Ibogaine is a nonselective and weak inhibitor of binding to muscarinic receptor subtypes. Reported affinities are 7.6 and 16 μM and 5.9 and 31 μM, respectively, for the M_1 and M_2 muscarinic receptor subtypes (*40,45*), with another study reporting no significant affinity of ibogaine for muscarinic receptors (*43*). Functional evidence consistent with a muscarinic cholinergic agonist effect of ibogaine includes the observations of the elimination of ibogaine-induced EEG dyssynchrony by atropine in cats (*89*), decreased heart rate following ibogaine administration in rats (*90*), and the attribution of the effect of cholinesterase inhibition to ibogaine in the older literature (*1,91*). The affinity of noribogaine for muscarinic receptors is apparently similar to that of ibogaine (*44,45*).

Several laboratories have reported that ibogaine produces noncompetitive functional inhibition of the nicotinic acetylcholine receptor, apparently involving open channel blockade (*46,48-50*). As with a number of other channel blockers, binding studies involving channels associated with nicotinic receptors have been

limited by the lack of appropriate ligands, and investigations of the affinity of ibogaine for the nicotinic acetylcholine receptor have mainly involved functional assays. Utilizing $^{86}Rb^{+}$ efflux assays, Fryer and Lukas (*50*) found that ibogaine inhibited human ganglionic and muscle-type nicotinic acetylcholine receptors with IC_{50} values of 1.06 and 22.3 μM, respectively. Badio *et al.* (*48*) found that ibogaine inhibited $^{22}Na^{+}$ influx through rat ganglionic and human muscle-type nicotinic acetylcholine receptors with IC_{50} values of 0.020 μM and 2.0 μM, respectively. Noribogaine was 75-fold less active than ibogaine in the rat ganglionic cell assay. In mice, ibogaine at a dose of 10 mg/kg completely blocked the central antinociceptive nicotinic receptor-mediated response to epibatidine. Ibogaine has been associated with decreased acetylcholine-stimulated nicotinic receptor mediated catecholamine release in cultured cells (*49*) and decreased dopamine release evoked by nicotine in the NAc of the rat (*46,92*).

7. Sigma Receptors

Elsewhere in this volume, Bowen discusses ibogaine's action at the sigma receptor. The affinity of ibogaine for the $sigma_2$ receptor is strong relative to other known CNS receptors, and the reported range is 0.09 to 1.8 μM (*45,60,93,94*). The affinity of ibogaine for the $sigma_1$ receptor is reportedly on the order of 2 to 100 times weaker than its affinity for the $sigma_2$ receptor (*45,60,93,94*). The neurotoxic effects of ibogaine may involve activity at the $sigma_2$ receptor, which reportedly potentiates the neuronal response to NMDA (*95*).

8. Sodium Channels

The reported affinity of ibogaine for sodium channels ranges from 3.6 to 9 μM (*40,43*). There is apparently no experimental evidence regarding the functional significance of ibogaine's action at sodium channels.

B. Discrimination Studies

Elsewhere in this volume, Helsley *et al.* discuss the topic of ibogaine and drug discrimination. Drug discrimination studies offer a possible approach to the issue of ibogaine's mechanism of action and may help resolve the distinction between ibogaine's therapeutic and hallucinogenic effects. The $5\text{-}HT_{2A}$ receptor appears to be a significant, but nonessential, determinant of the ibogaine stimulus (*84,96*). The ibogaine stimulus is reportedly generalized to the indolealkylamine hallucinogen D-lysergic acid diethylamide (LSD) and the phenethylamine hallucinogen 2,5-dimethoxy-4-methylamphetamine (DOM), and this generalization is abolished by the addition of a $5\text{-}HT_{2A}$ receptor antagonist (*96*). The addition of a $5\text{-}HT_{2A}$ receptor antagonist did not attenuate stimulus control of ibogaine itself in the ibogaine-trained animals, indicating that $5\text{-}HT_{2A}$ is not an essential component of the ibogaine discriminative stimulus. The $5\text{-}HT_{2C}$

receptor, which plays a modulatory role in hallucinogenesis, is also involved, but is not essential to the ibogaine stimulus, and the 5-HT_{1A} and 5-HT_3 receptors are apparently not involved in the ibogaine stimulus (*85*). The ibogaine discriminative stimulus reportedly is potentiated by the serotonin reuptake inhibitor fluoxetine (*85*), and has an insignificant degree of generalization to the serotonin releaser D-fenfluramine (*97*).

Ibogaine showed a lack of substitution for phencyclidine (*98,99*), and substituted for MK 801 only at high (100 mg/kg) doses in mice (*58,61*), but not at lower (10 mg/kg) doses in rats (*99,100*), suggesting that the NMDA receptor is not a significant determinant of the ibogaine stimulus. $Sigma_2$, and mu- and kappa-opioid activity may be involved in the ibogaine discriminative stimulus (*99*). A high degree of stimulus generalization is reported between ibogaine and some of the Harmala alkaloids, a group of hallucinogenic beta-carbolines that are structurally related to ibogaine (*101,102*). While the discriminative stimulus for both the Harmala alkaloids and ibogaine apparently involves the 5-HT_2 receptor (*84,85,103*), it does not appear essential to generalization between ibogaine and harmaline, as generalization to the harmaline stimulus was unaffected by the addition of a 5-HT_2 antagonist in ibogaine-trained animals (*84*). Ibogaine-trained rats generalize to noribogaine (*100,104*), which in one study was more potent than ibogaine itself in eliciting ibogaine-appropriate responses (*100*).

C. Effects on Neuropeptides

Both ibogaine and cocaine given in multiple administrations over 4 days to rats reportedly increase neurotensin-like immunoreactivity (NTLI) in the striatum, substantia nigra, and NAc (*105*). However, unlike cocaine, which increased NTLI in the frontal cortex, ibogaine had no effect on frontal cortical NTLI. Ibogaine pretreatment prevented the increase of NTLI in striatum and substantia nigra induced by a single dose of cocaine. Substance P, like NTLI, was increased in the striatum and substantia nigra after either cocaine or ibogaine, with an increase in frontal cortex with cocaine and no effect with ibogaine (*106*). Ibogaine–induced increases in NTLI or substance P were blocked by administration of a D_1 antagonist.

Unlike the NTLI or substance P responses, ibogaine alone had no effect on dynorphin. However, ibogaine pretreatment dramatically enhanced cocaine-induced increases in dynorphin, a kappa-opioid agonist (*107*). The authors suggested that the increase in dynorphin related to cocaine's interaction with ibogaine could result in enhanced kappa-opioid activity. Kappa-opioid agonists reportedly decrease cocaine intake in animal models (*108,109*).

D. Possible Effects on Neuroadaptations Related to Drug Sensitization or Tolerance

There is some evidence to suggest that ibogaine treatment might result in the "resetting" or "normalization" of neuroadaptations related to drug sensitization or tolerance (*110*). Ibogaine pretreatment blocked the expression of sensitization-induced increases in the release of dopamine in the NAc shell in response to cocaine in cocaine-sensitized rats (*111*). The effect of ibogaine on diminished locomotor activity and dopamine efflux in the NAc in response to morphine is more evident in animals with prior exposure to morphine (*112,113*), which is consistent with a relatively selective effect of ibogaine on neuroadaptations acquired from drug exposure. Similarly, the observation that ibogaine inhibited the development of tolerance in morphine-tolerant mice, but had no effect on morphine nociception in morphine-naïve mice (*114*), suggests a selective effect on acquired neuroadaptations related to repeated morphine exposure.

Ibogaine appears to have persistent effects not accounted for by a metabolite with a long biological half-life (*29,115*). Ibogaine's action could possibly involve the opposition or reversal of persistent neuroadaptive changes thought to be associated with drug tolerance or sensitization. Such an action could be related to persistent effects on second messengers (*72,116*). For example, sensitization to both opiates and cocaine is thought to involve enhanced stimulation of cyclic AMP (*117*). Ibogaine has been reported to potentiate the inhibition of adenylyl cyclase by serotonin (*72*), an effect that would be expected to oppose the enhanced transduction of cyclic AMP that is reportedly associated with stimulant sensitization (*117*).

III. Evidence of Efficacy in Animal Models

A. Drug Self-Administration

Evidence for ibogaine's effectiveness in animal models of addiction includes observations of reductions in self-administration of morphine or heroin (*29,31,118-120*), cocaine (*29,31,119,121*), and alcohol (*122*), and reduced nicotine preference (*75*). According to some reports, effects of ibogaine on drug self-administration are apparently persistent. Sershen *et al.* (*121*) administered ibogaine i.p. to mice as two 40 mg/kg dosages 6 hours apart, and found a diminution of cocaine preference that was still evident after 5 days. Glick *et al.* (*29,119*) noted reductions in cocaine and morphine self-administration that persisted for at least 2 days and were dose dependent in the range of 2.5 to 80 mg/kg. ibogaine given i.p. The persistence of an effect beyond the first day

suggests a specific action of ibogaine on drug intake, as water intake was also suppressed initially by ibogaine on the first, but not the second day. Cappendijk and Dzoljic (*31*) found reductions in cocaine self-administration that persisted for more than 48 hours in rats treated with ibogaine at a dose of 40 mg/kg i.p., given as a single administration, or repeatedly on 3 consecutive days or three consecutive weeks.

In the studies by Glick *et al.* there was variation between results in individual rats with some showing persistent decreases in morphine or cocaine intake for several days or weeks after a single injection and others only after two or three weekly injections. The authors noted evidence of a continuous range of individual sensitivity to ibogaine among the experimental animals and that it appeared as if adjustments of the dosage regimen could produce long-term reductions in drug intake in most animals (*29*). Similarly, Cappendijk and Dzoljic (*31*) found the largest effects on cocaine self-administration occurred when ibogaine was given weekly for three consecutive weeks. This result suggests the possibility that the optimal schedule of ibogaine administration to limit cocaine intake may involve modification of the single dose regimen which has been used for opioid detoxification (*32,123*).

Dworkin *et al.* (*118*) found that pretreatment with ibogaine at a dose of 80 mg/kg i.p. diminished the response for heroin and cocaine, and also for food, suggesting a nonspecific confound. A 40 mg/kg i.p. dose of ibogaine sharply reduced heroin self-administration in the absence of a significant effect on food response, although the effect did not persist beyond 24 hours (*118*). Dworkin *et al.* cited methodologic factors relating to differences in gender, strain, and reinforcement schedule to explain the apparent discrepancy between their results and other studies that reported persistent effects (*29,31,119,121*).

Noribogaine has also been reported to reduce cocaine and morphine self-administration (*124*). The effect of noribogaine on drug self-administration persisted for 2 days, after the response for water, which was initially suppressed on the first day, had returned to baseline. Other *iboga* alkaloids have also been reported to reduce morphine and cocaine self-administration in rats for a period of a day or longer following a single i.p dose (*119*). Some of the *iboga* alkaloids tested in this study produced tremors, which typically occurred for a period of 2 to 3 hours, and were independent of persistent effects of drug self-administration. An ibogaine congener, 18-methoxycoronaridine (18-MC) (*45*), reportedly reduces in rats the self-administration of cocaine (*120*), morphine and alcohol (*125*), and nicotine preference (*75*) without any apparent reduction in the response for water.

B. Acute Opioid Withdrawal

Dzoljic *et al.* (*28*) administered ibogaine in a dose range of 4 to 16 µg intra-

cerebroventricularly to rats and observed a dose-dependent attenuation of naloxone-precipitated withdrawal signs. This same group also found an attenuation of morphine withdrawal signs in rats with 40 mg/kg ibogaine administered i.p., and also norharman, an endogenously occurring hallucinogenic beta-carboline and a structural relative of ibogaine (*126*). Glick *et al.* have reported dose-dependent reduction of the signs of naltrexone-precipitated morphine withdrawal in rats administered ibogaine at doses of 20, 40, or 80 mg/kg i.p (*127*) or 18-MC (*128*) at doses of 20 and 40 mg/kg i.p. Attenuation of withdrawal signs was reported in morphine-dependent monkeys given 2 or 8 mg/kg ibogaine subcutaneously (*129*). In their chapter in this volume, Parker and Siegel report that 40 mg/kg ibogaine administered i.p attenuated naloxone-precipitated morphine withdrawal in rats, as well as withdrawal-induced place aversion.

Sharpe and Jaffe (*130*) reported that ibogaine in dosages ranging between 5 and 40 mg/kg administered subcutaneously failed to attenuate naloxone-precipitated withdrawal in rats, although they did find that one sign (grooming) was reduced, and noted the possible effect of methodological issues such as morphine exposure and withdrawal procedures, or the route of administration of ibogaine. Popik *et al.* (*58*) and Layer *et al.* (*56*) found that ibogaine at doses ranging from 40 to 80 mg/kg i.p. reduced naloxone-precipitated jumping in morphine dependent mice, although Francés *et al.* (*69*) found the opposite effect with 30 mg/kg ibogaine administered i.p. in mice. As pointed out by Popik and Skolnik (*39*), the divergent results in morphine dependent mice might relate to ibogaine having been given prior to the administration of naloxone in the studies by Popik *et al.* (*58*) and Layer *et al.* (*56*), whereas ibogaine was administered after naloxone in the study by Francés *et al.*

C. Conditioned Place Preference

Parker and Siegel review ibogaine and place preference in this volume. Ibogaine is reported to prevent the acquisition of place preference when given 24 hours before amphetamine (*131*) or morphine (*132*). The effect of ibogaine on blocking the acquisition of place preference was diminished across multiple conditioning trials. Ibogaine given after morphine did not apparently attenuate the expression of previously established morphine place preference (*133*).

D. Locomotor Activity

Pretreatment with ibogaine and its principal metabolite, noribogaine reportedly diminishes locomotor activation in response to morphine (*74,112,113,124,134-136*). The effect of ibogaine in reducing locomotor activity in response to morphine is reportedly greater in female than in male rats, probably reflecting the

relatively greater bioavailability of ibogaine in females (*135*). The literature on cocaine appears to be less consistent, with some reports of decreased locomotor activation (*87,137-139*), and others reporting increases (*127,137,140,141*). This apparent disparity may be related in part to the species of experimental animal that was used, as Sershen *et al.* (*137*) report increased locomotor activity in response to cocaine in the rat, with the opposite result in the mouse.

Stereotypy is a methodologic issue that might explain some of the disparate results regarding ibogaine's interaction with the locomotor response to cocaine. Higher doses of stimulants can produce strereotypy, which could decrease the amount of measured locomotion relative to an animal that is experiencing less locomotor stimulation at a lower stimulant dose. Thus, the potentiation by ibogaine of locomotor activity related to cocaine administration can result in less measured movement in animals experiencing locomotor stimulation to the point of stereotypy (*110*). Ibogaine pretreatment reportedly potentiates stereotypy in rats receiving cocaine or methamphetamine (*111,142*).

E. Dopamine Efflux

Reductions in dopamine efflux in the NAc in response to morphine have been reported in animals pretreated with ibogaine (*113,115,134*), noribogaine (*124*), or 18-MC (*120,143*). Similarly, reductions in dopamine efflux in the NAc in response to nicotine have been reported in animals pretreated with ibogaine (*46,92*) and 18-MC (*42*).

As with locomotor stimulation, methodological issues may have played a part in apparently divergent results regarding ibogaine's effect on dopamine efflux in the NAc in response to cocaine or amphetamine, which is reportedly increased as measured by microdialysis (*134*), although the opposite result was observed in a study on cocaine using microvoltammetry (*139*). Dosage is an additional consideration that might influence ibogaine's effect on dopamine efflux in the NAc in response to cocaine, with a larger ibogaine dose reportedly producing an increase and a smaller dose producing a decrease (*144*).

Dopamine efflux in response to cocaine may also depend on whether dopamine measurements are made in the NAc core versus shell. Szumlinski *et al.* (*111*) found that ibogaine pretreatment (given 19 hours earlier) abolished the sensitized dopamine efflux in response to cocaine in the NAc shell in rats that had been sensitized by repeated prior exposure to cocaine. The same ibogaine pretreatment had no apparent effect on dopamine efflux in the NAc shell in response to "acute" (administered without prior cocaine exposure) cocaine. The authors noted a prior study in their laboratory that found a potentiation by ibogaine pretreatment of dopamine efflux in response to acute cocaine in which the position of the recording probe spanned both the core and shell regions of the NAc (*134*). These results indicate the possibility of a differential effect of ibogaine on dopamine

efflux in response to cocaine between the NAc shell, which is thought to play a relatively greater role in the motivational aspects of drugs of abuse, and the NAc core, which, in turn, is thought to play a relatively greater role in motor behavior (*145*). The authors suggested that the effect of ibogaine on reduced cocaine self-administration may be mediated by the observed reduction in dopamine efflux in response to cocaine in the NAc shell in cocaine-sensitized animals (*111*). On the other hand, the enhancement by ibogaine preatreatment of locomotor activity seen in response to acute or chronic cocaine administration may be mediated by increased dopamine efflux in the NAc core. The observed increase in dopamine efflux with ibogaine pretreatment in the NAc core in response to acute cocaine (*134*) is consistent with such a formulation, although this group has yet to report on the result of the same experiment in cocaine-sensitized animals.

Ibogaine and 18-MC reportedly decrease dopamine release evoked by nicotine in the NAc of the rat (*46,92*). In the study by Benwell *et al.* (*46*), the decreased NAc dopamine release following ibogaine was independent of any change in locomotor activity, which was viewed as notable given the usual association between NAc dopamine efflux and locomotor activity in response to nicotine. The authors cited previous work in which a similar dissociation between NAc dopamine efflux and locomotor activity in response to nicotine was produced by treatment with NMDA antagonists, and they suggested that their findings might be related to ibogaine's NMDA antagonist activity.

IV. Evidence of Efficacy and Subjective Effects in Humans

A. Evidence Of Efficacy

1. Acute Opioid Withdrawal

One line of clinical evidence suggesting ibogaine's possible efficacy are the accounts of the addicts themselves, whose demand has led to the existence of an "informal" treatment network in Europe and the United States. Opioid dependence is the most common indication for which addicts have sought ibogaine treatment, which has been typically administered as a single dose. Common reported features of case reports describing ibogaine treatment (*35,36,146-149*) are reductions in drug craving and opiate withdrawal signs and symptoms within 1 to 2 hours, and sustained, complete resolution of the opioid withdrawal syndrome after the ingestion of ibogaine. These case studies appear consistent with general descriptions of ibogaine treatment (*33,34,150*).

Alper *et al.* (*32*) summarized 33 cases treated for the indication of opioid detoxification in nonmedical settings under open label conditions. These cases

are a subset of those presented at the NIDA Ibogaine Review Meeting held in March, 1995 (*151*). A focus on acute opioid withdrawal may offset some of the methodological limitations of the informal treatment context because the acute opioid withdrawal syndrome is a clinically robust phenomenon that occurs within a relatively limited time frame and yields reasonably clear outcome measures. Despite the unconventional setting and the lack of structured clinical rating instruments, the lay "treatment guides" who reported on the case series might reasonably be expected to be able to assess the presence or absence of the relatively clinically obvious and unambiguous features of opioid withdrawal.

The subjects in this series of cases reported an average daily use of heroin of 0.64 ± 0.50 g, primarily by the intravenous route, and received an average dose of ibogaine of 19.3 ± 6.9 mg/kg p.o. (range of 6 to 29 mg/kg). Resolution of the signs of opioid withdrawal without further drug seeking behavior was observed in 25 patients. Other outcomes included drug seeking behavior without withdrawal signs (four patients), drug abstinence with attenuated withdrawal signs (two patients), drug seeking behavior with continued withdrawal signs (one patient), and one fatality, possibly involving surreptitious heroin use (see Section VI, "Safety"). The reported effectiveness of ibogaine in this series suggests the need for a systematic investigation in a conventional clinical research setting.

In their chapter in this volume, Mash *et al.* report having treated more than 150 subjects for substance dependence in a clinic located in St. Kitts, West Indies. A subset of 32 of these subjects was treated with a fixed dose of ibogaine of 800 mg for the indication of opioid withdrawal. Physician ratings utilizing structured instruments for signs and symptoms of opioid withdrawal indicated resolution of withdrawal signs and symptoms at time points corresponding to 12 hours following ibogaine administration and 24 hours after the last use of opiates, and at 24 hours following ibogaine administration and 36 hours after the last use of opiates. The resolution of withdrawal signs and symptoms was sustained during subsequent observations over an interval of approximately one week following ibogaine administration. Reductions of measures of depression and craving remained significantly reduced one month after treatment (*123*). The authors noted that ibogaine appeared to be equally efficacious in achieving detoxification from either methadone or heroin. The reported efficacy of ibogaine for the opioid withdrawal syndrome observed in the St. Kitts facility appears to confirm the earlier impressions of the case study literature (*32-36,146-150*).

2. Long-Term Outcomes

There is very little data regarding the long-term outcomes in patients treated with ibogaine. Lotsof (*151*) presented a summary of 41 individuals treated between 1962 and 1993 at the NIDA Ibogaine Review Meeting held in March 1995. The data consisted of self-reports obtained retrospectively, which are essentially anecdotal, but apparently represent the only formal presentation of a

systematic attempt to determine long-term outcomes in patients treated with ibogaine. Thirty-eight of the 41 individuals presented in the summary reported some opioid use, with approximately 10 of these apparently additionally dependent on other drugs, mainly cocaine, alcohol, or sedative-hypnotics. The use of tobacco or cannabis was not apparently assessed. Across the sample of 41 individuals, nine individuals were treated twice and one was treated three times for a total of 52 treatments. The interval of time following treatment was recorded for which patients reported cessation of use of the drug or drugs on which they were dependent. Fifteen (29%) of the treatments were reportedly followed by cessation drug use for less than 2 months, 15 (29%) for at least 2 months and less than 6 months, 7 (13%) for at least 6 months and less than one year, 10 (19%) for a period of greater than one year, and in 5 (10%) outcomes could not be determined.

B. Subjective Effects

There appear to be common elements to experiences generally described by patients treated with ibogaine. The "stages" of the subjective ibogaine experience presented below are a composite derived by the author from interviews with patients and treatment guides, and general descriptions and case studies provided by the literature (*33-35,146,150*). Ibogaine has generally been administered in non-hospital settings, as a single p.o. dose, usually given in the morning. Vomiting is reportedly common and usually occurs relatively suddenly as a single episode in the first several hours of treatment. Patients generally lie still in a quiet darkened room throughout their treatment, a practice that is possibly related to the cerebellar effects of ibogaine, and because vomiting tends to be more frequent with movement. Patients later in treatment often experience muscle soreness, possibly due to reduced motor activity earlier in treatment, that resolves with motion, stretching, or massage.

1. Acute

The onset of this phase is within 1 to 3 hours of ingestion, with a duration on the order of 4 to 8 hours. The predominant reported experiences appear to involve a panoramic readout of long-term memory (*152*), particularly in the visual modality, and "visions" or "waking dream" states featuring archetypal experiences such as contact with transcendent beings, passage along a lengthy path, or floating. Descriptions of this state appear more consistent with the experience of dreams than of hallucinations. Informants appear to emphasize the experience of being placed in, entering, and exiting entire visual landscapes, rather than the intrusion of visual or auditory hallucinations on an otherwise continuous waking experience of reality. Ibogaine-related visual experiences are reported to be strongly associated with eye closure and suppressed by eye

opening. The term "oneiric" (Greek, *oneiros*, dream) has been preferred to the term "hallucinogenic" in describing the subjective experience of the acute state. Not all subjects experience visual phenomena from ibogaine, which may be related to dose, bioavailability, and interindividual variation.

2. Evaluative

The onset of this phase is approximately 4 to 8 hours after ingestion, with a duration on the order of 8 to 20 hours. The volume of material recalled slows. The emotional tone of this phase is generally described as neutral and reflective. Attention is still focused on inner subjective experience rather than the external environment, and it is directed at evaluating the experiences of the acute phase. Patients in this and the acute phase above are apparently easily distracted and annoyed by ambient environmental stimuli and prefer as little environmental sensory stimulation as possible in order to maintain an attentional focus on inner experience.

3. Residual Stimulation

The onset of this phase is approximately 12 to 24 hours after ingestion, with a duration in the range of 24 to 72 hours or longer. There is a reported return of normal allocation of attention to the external environment. The intensity of the subjective psychoactive experience lessens, with mild residual subjective arousal or vigilance. Some patients report reduced need for sleep for several days to weeks following treatment. It is not clear to what extent such reports might reflect a persistent effect of ibogaine on sleep or a dyssomnia due to another cause.

V. Pharmacokinetics

A. Absorption

Jeffcoat *et al.* (*153*) administered single oral doses of ibogaine of 5 mg/kg and 50 mg/kg to rats, and estimated oral bioavailabilities of 16 and 71% at the two dosages, respectively, in females, and 7 and 43% in males. The dose-dependent bioavailability was interpreted as suggesting that ibogaine absorption, and/or first pass elimination, is nonlinear, and the greater bioavailability in females was viewed as consistent with gender-related differences in absorption kinetics. Pearl *et al.* (*135*) administered ibogaine at a dose of 40 mg/kg i.p. and found whole brain levels at 1, 5, and 19 hours post-administration of 10, 1, and 0.7 μM in female rats, and 6, 0.9, and 0.2 μM in male rats, respectively. In the same study, brain levels of noribogaine at 1, 5, and 19 hours post-administration were 20, 10,

and 0.8 μM in female rats, and 13, 7, and 0.1 μM and male rats respectively. In addition to gender differences in bioavailability, the data also provide evidence for the pharmacologic relevance of micromolar activities of ibogaine and noribogaine measured *in vitro* (*40,44*).

Upton (*154*) reported on observations in rats given ibogaine in the form of oral suspension, oral solution, or via IV or intraperitoneal routes, and also reviewed data obtained in beagle dogs, cynomologous monkeys, and human subjects. Absorption of the oral suspension in rats was noted to be variable and incomplete. As in the study cited above by Jeffcoat (*153*), peak levels and bioavailability were greater in female than in male rats.

B. Distribution

Hough *et al.* (*51*) administered 40 mg/kg ibogaine by the intraperitoneal and subcutaneous routes and evaluated its distribution in plasma, brain, kidney, liver, and fat at 1 and 12 hours post-administration. Ibogaine levels were higher following subcutaneous versus intraperitoneal administration, suggesting a substantial "first pass" effect involving hepatic extraction. The results were consistent with the highly lipophilic nature of ibogaine; ibogaine concentrations at 1 hour postadministration were 100 times greater in fat, and 30 times greater in brain, than in plasma. These authors suggested that the prolonged actions of ibogaine could relate to adipose tissue serving as a reservoir with release and metabolism to noribogaine over an extended period of time (*51*). The apparently greater levels of ibogaine in whole blood versus plasma suggests the possibility that platelets might constitute a depot in which ibogaine is sequestered (*42*). If there is conversion of ibogaine to noribogaine in the brain, then the significantly greater polarity of noribogaine relative to ibogaine could prolong the presence of the more polar metabolite in the CNS after conversion from ibogaine (*42*).

C. Metabolism

The major metabolite of ibogaine, noribogaine, is formed through demethylation, apparently via the cytochrome P-450 2D6 (CYP2D6) isoform (*155*). Consistent with first pass metabolism of the parent drug, noribogaine is reportedly detectable in brain tissue within 15 minutes after oral administration of 50 mg/kg ibogaine (*44*). Noribogaine is itself pharmacologically active and is discussed in this volume by Baumann *et al.*

In pooled human liver microsomes, Pablo *et al.* identified two kinetically distinguishable ibogaine *O*-demethylase activities which corresponded, respectively, to high and low values of the apparent Michaelis constant (K_{mapp}) (*155*). The low K_{mapp} ibogaine *O*-demethylase activity was attributable to CYP2D6 and accounted for greater than 95% of the total intrinsic clearance in pooled human

liver microsomes. The authors noted that the apparent involvement of the CYP2D6 suggests possible human pharmacogenetic differences in the metabolism of ibogaine. "Poor metabolizers" who lack a copy of the CYP2D6 gene (*156*) would be expected to have relatively less CYP2D6-catalyzed activity to metabolize ibogaine to noribogaine. Consistent with such an expectation, a subject identified as a phenotypic CYP2D6 poor metabolizer possessed only the high K_{mapp} ibogaine *O*-demethylase activity, which had accounted for only a small fraction of the intrinsic clearance. In another study, analysis of ibogaine and noribogaine levels in human subjects yielded a distribution interpreted as indicating three groups of rapid, intermediate, and poor metabolizers (*157*), a pattern consistent with the observed pharmacogenetic polymorphism of CYP2D6 in human populations (*156*).

D. Excretion

Ibogaine has an estimated half-life on the order of 1 hour in rodents (*158*), and 7.5 hours in man (Mash *et al.*, this volume). Ibogaine and its principal metabolite, noribogaine, are excreted via the renal and gastrointestinal tracts. In rats, Jeffcoat *et al.* (*153*) noted 60 to 70% elimination in urine and feces within 24 hours, and Hough *et al.* (*51*) found plasma and tissue levels to be 10 to 20-fold lower at 12 hours versus 1 hour post dose.

Upton and colleagues (*154*) cited several pharmacokinetic issues of potential concern based on their analysis of data obtained from rats. These include evidence for presystemic clearance potentially resulting in low bioavailability and interpatient variability, and saturable first pass clearance, which could also generate intrapatient variability. The possibility of saturable systemic clearance was also noted. Mash *et al.* (*36*) suggested the possibility of species or strain differences in ibogaine metabolism and clearance rates and cited the rapid elimination of ibogaine from the blood of primates, as opposed to rats or humans, as an example.

In human subjects, 90% of a 20 mg/kg p.o. dose of ibogaine was reportedly eliminated within 24 hours (*36*). Noribogaine is apparently eliminated significantly more slowly than ibogaine, and observations in human subjects indicate persistently high levels of noribogaine at 24 hours (*36,79,123,* Mash *et al.* in this volume). The sequestration and slow release from tissues of ibogaine or noribogaine and the slow elimination of noribogaine have been suggested to account for the apparently persistent effects of ibogaine.

VI. Safety

A. Neurotoxicity

1. Neuropathology

Multiple laboratories have reported on the degeneration of cerebellar Purkinje cells in rats given ibogaine at a dose of 100 mg/kg i.p. (*159,160*). However, the available evidence suggests that the neurotoxic effects of ibogaine may occur at levels higher than those observed to have effects on opioid withdrawal and self-administration. Molinari *et al.* (*161*) found no evidence of cerebellar Purkinje cell degeneration with 40 mg/kg i.p. administered as a single dose, which is reported to reduce morphine or cocaine self-administration or morphine withdrawal in rats (*29,119,126,161*). Xu *et al.* (*162*) evaluated biomarkers of cerebellar neurotoxicity in rats treated with single doses of ibogaine of 25, 50, 75, and 100 mg/kg i.p. The biomarkers used in this study included the specific labeling of degenerating neurons with silver, and Purkinje neurons with antisera to calbindin. Astrocytes were identified with antisera to glial fibrillary acidic protein (GFAP), a marker of reactive gliosis, a general response of astrocytes to CNS injury. The 25 mg/kg dosage was found to correspond to a no-observable-adverse-effect-level (NOAEL). Helsley *et al.* (*102*) treated rats with 10 mg/kg ibogaine every other day for 60 days and observed no evidence of neurotoxicity.

Regarding the question of neurotoxicity in brain areas outside the cerebellum, O'Hearn and Molliver (*163*) have stated, "Evidence of neuronal injury following ibogaine administration in rats appears to be almost entirely limited to the cerebellum." While the cerebellum appears to be the brain region most vulnerable to neurotoxic effects of ibogaine, some research has addressed the issue of neurotoxicity in other brain regions. O'Callaghan *et al.* (*164*) examined GFAP in male and female rats exposed to either an "acute" regimen of ibogaine administered at doses of 50, 100, or 150 mg/kg i.p. daily for 3 days or a "chronic" regimen of daily oral administration of 25, 75, or 150 mg/kg for 14 days. The acute i.p. regimen produced elevations of GFAP in animals of either gender that were not restricted to the cerebellum, and were observed in the cerebellum and hippocampus at the 50 mg/kg dosage level, and in the cortex, hippocampus, olfactory bulb, brain stem, and striatum at the 100 mg/kg level. The effect of the acute ibogaine regimen on GFAP was no longer evident at 14 days with either dosage in male rats, and was restricted to the cerebellum with the 100 mg/kg dose in female rats. GFAP levels were examined at 17 days after the completion of the chronic dosing regimen. No elevations of GFAP were found in any of the brain regions examined at any of the dosages administered utilizing the chronic regimen in males, and elevations of GFAP were found only in females, which were restricted to the hippocampus with the 25 mg/kg dosage regimen and were

present in the hippocampus, olfactory bulb, striatum, and brain stem with the 150 mg/kg dosage regimen.

O'Hearn *et al.* (*159*) found GFAP elevations in the cerebellum only, and not the forebrain of male rats administered 100 mg/kg doses i.p on up to 3 consecutive days. Elevations of GFAP are relatively sensitive, but not specific to, neuronal degeneration (*162*). Using a silver degeneration-selective stain as a histologic marker of neurodegeneration, Scallet *et al.* (*165*) examined diverse brain regions in rats and mice treated with single 100 mg/kg doses of ibogaine administered i.p. and found evidence of neurodegeneration only in the cerebellum in rats, whereas mice showed no evidence of neurodegeneration. In rats that received a dose of ibogaine of 100 mg/kg i.p., neuronal degeneration was confined to the cerebellum as revealed by staining with Fluoro-Jade, a recently developed sensitive and definitive marker of neuronal degeneration (*166,167*).

Sensitivity to ibogaine neurotoxicity appears to vary significantly between species. The monkey appears to be less sensitive to potential ibogaine neurotoxicity than the rat (*36*). Mash *et al.* observed no evidence of neurotoxicity in monkeys treated for 5 days with repeated oral doses of ibogaine of 5 to 25 mg/kg, or subcutaneously administered doses of 100 mg/kg (36). Another species difference in sensitivity is the mouse, which unlike the rat shows no evidence of cerebellar degeneration at a 100 mg/kg i.p. dose of ibogaine (*165*).

2. Mechanisms of Neurotoxicity

Ibogaine's cerebellar toxicity could be related to excitatory effects mediated by $sigma_2$ receptors in the olivocerebellar projection, which sends glutaminergic excitatory input to cerebellar Purkinje cells, whose synaptic redundancy makes them particularly vulnerable to excitotoxic injury (*160*). $Sigma_2$ agonists are reported to potentiate the neuronal response to NMDA (*95*), and potentiation of glutamatergic responses at Purkinje cells might lead to the observed neurotoxicity. $Sigma_2$ agonists have also been shown to induce apoptosis, and activation of $sigma_2$ receptors by ibogaine results in direct neurotoxicity via induction of apoptosis in *in vitro* cell culture systems (*168,169*). Elsewhere in this volume, Bowen discusses the effects of *iboga*a alkaloids at $sigma_2$ receptors. It is possible therefore that ibogaine's neurotoxic effect on the highly sensitive Purkinje neurons is the result of combined direct neurotoxicity and excitotoxicity due to the enhancement of glutamatergic activity, both effects being mediated by $sigma_2$ receptors. The agonist activity of ibogaine at the $sigma_2$ receptor might explain the apparent paradox of ibogaine-induced excitotoxicity, despite its properties as an NMDA antagonist (*42*). The neurotoxic effects of *iboga* alkaloids can apparently be dissociated from their putative effects on addiction, since $sigma_2$ receptors appear not to be involved in the suppression of drug self-administration. 18-MC, an ibogaine congener with relatively much less $sigma_2$ affinity, reportedly produces effects similar to ibogaine on morphine and cocaine

administration in rats, but has shown no evidence of neurotoxicity, even at high dosages (*42,75,120*).

Ibogaine's NMDA antagonist activity has been cited as a rationale for a patent for its use as a neuroprotective agent to minimize excitotoxic damage in stroke and anoxic brain injury (*170*). In methamphetamine-treated mice, ibogaine is reported to protect against hyperthermia and the induction of heat shock protein, which are possible mediators of methamphetamine neurotoxicity (*171*). Binienda *et al.* in this volume report an accentuation of delta amplitude in ibogaine pretreated animals given cocaine, and they suggest a "paradoxical" proconvulsant effect resulting from the interaction of cocaine and ibogaine, similar to interactions reported between cocaine and other noncompetitive NMDA antagonists. However, ibogaine is reported to protect against convulsions produced by electroshock (*61*), or the administration of NMDA (*55*). Luciano *et al.* (*148*) did not observe EEG abnormalities in five human subjects during treatment with ibogaine in the dosage range of 20 to 25 mg/kg. There is apparently no reported human data on possible differences between the pre- and post-ibogaine treatment EEG, or effects persisting into extended periods of time after treatment.

3. Tremor

Ibogaine has been noted to produce tremor at dosages of 10 mg/kg i.p. in rats (*172*) and 12 mg/kg s.c. in mice (*173*). Glick *et al.* (*119*) evaluated ibogaine and several other *iboga* alkaloids, and found that their effects on drug self-administration and tendency to produce tremor were independent from one another. Studies of structure-activity relationships of the *iboga* alkaloids indicate that the tendency to cause tremor is enhanced by the presence of a methoxy group at position 10 or 11 and is diminished or eliminated by the presence of a carbomethoxy group at position 16 (*173,174*). Accordingly, tremors were not produced in rats administered noribogaine, which differs from ibogaine with respect to the absence of a methoxy group at position 10, at a dosage of 40 mg/kg i.p. (*124*). Likewise, tremors were not observed in rats administered a dosage of 18-MC as high as 100 mg/kg. 18-MC differs from ibogaine with respect to the absence of a methoxy group at position 10 and the presence of a carbomethoxy group at position 16 (*120*).

4. Observations in Humans

Concern over possible neurotoxicity led Mash *et al.* to quantitatively investigate ibogaine's effects on postural stability, body tremor, and appendicular tremor in humans (*36*). In U.S. FDA safety trials, nine subjects receiving 1 and 2 mg/kg of ibogaine showed only a statistically insignificant increase in body sway 6 hours after taking ibogaine. Ten patients evaluated 5 to 7 days after receiving doses of ibogaine ranging from 10 to 30 mg/kg showed no evidence of

abnormality on quantitative measures of static or dynamic posturography or hand accelometry, or on clinical neurologic exam.

A woman died in the United States in 1994 who had been previously treated with ibogaine 25 days earlier (*36*). This woman had undergone four separate treatments with ibogaine in dosages ranging from 10 to 30 mg/kg in the 15 months prior to her death. The cause of death was concluded to have been a mesenteric arterial thrombosis related to chronic cellulitis, and a role for ibogaine in causing the fatality was not suspected. Of interest with regard to concerns over potential neurotoxicity, was the absence of any neuropathological abnormality not associated with chronic IV drug use. Neuropathological examination revealed only slight medullary neuroaxonal dystrophy and an old focal meningeal fibrosis, which were explainable on the basis of chronic IV drug use (*36*). There was no evidence of cytopathology or neurodegenerative changes in the cerebellum or any other brain area, nor was there evidence of astrocytosis or microglial activation.

B. Cardiovascular Effects

Glick *et al.* (*45*) found no changes in resting heart rate or blood pressure at a dose of ibogaine of 40 mg/kg i.p., which has been used in that laboratory in drug withdrawal or self-administration studies. Higher doses of ibogaine (100 and 200 mg/kg) decreased the heart rate without an effect on blood pressure, and 18-MC had no apparent effect on heart rate or blood pressure at any of the above doses. Binieda *et al.* (*90*) found a significantly decreased heart rate in rats given ibogaine 50 mg/kg i.p.

Mash *et al.* (*175*) reported on intensive cardiac monitoring in 39 human subjects dependent on cocaine and/or heroin who received fixed p.o. doses of ibogaine of 500, 600, 800, or 1000 mg. Six subjects exhibited some significant decrease of resting pulse rate relative to baseline, one of whom evidenced a significant decrease in blood pressure, which was attributed to a transient vasovagal response. Monitoring revealed no evidence of EKG abnormalities appearing or intensifying during ibogaine treatment. No significant adverse events were seen under the study conditions, and it was concluded that the single dose of ibogaine was apparently well tolerated. In their chapter in this volume, Mash *et al.* comment further that random regression of vital signs showed no changes across time or by dosage in opiate-dependent subjects. They did however observe the occurrence of a hypotensive response to ibogaine in some cocaine-dependent subjects, which was responsive to volume repletion.

C. Fatalities

The LD50 of ibogaine is reportedly 145 mg/kg i.p. and 327 mg/kg intragastrically in the rat, and 175 mg/kg i.p. in the mouse (*158*).

In June 1990, a 44 year-old woman died in France approximately 4 hours after receiving a dose of ibogaine of about 4.5 mg/kg p.o. The cause of death was concluded to have been acute heart failure in an autopsy carried out at the Forensic-Medical Institute in Zurich (*176*). Autopsy revealed evidence of a prior myocardial infarction of the left ventricle, severe atherosclerotic changes, and 70 to 80% stenosis of all three major coronary artery branches. This patient had a history of hypertension, and inverted T waves were noted on EKG three months prior to the patient's death. The autopsy report concluded that the patients preexisting heart disease was likely to have caused the patient's death, and it specifically excluded the possibility of a direct toxic effect of ibogaine. The report acknowledged the possibility that an interaction between ibogaine and the patient's preexisting heart condition could have been a contributing factor in the fatal outcome.

The autopsy report, which included information obtained from the patient's family physician, and the psychiatrist who administered ibogaine, makes reference to the possibility that the patient might have taken other drugs. The autopsy report noted the presence of amphetamine in the enzyme immunocytochemical (EMIT) assay of a dialysate of the kidney tissue (urine was reported not to be obtainable). This finding, however, was regarded as artifactual and possibly attributable to a false positive EMIT result due to the presence of phenylethylamine.

A fatality occurred during a heroin detoxification treatment of a 24-year-old female in the Netherlands in June 1993. This incident was a significant factor in the NIDA decision not to fund a clinical trial of ibogaine in 1995. The patient received a total ibogaine dose of 29 mg/kg p.o. and suffered a respiratory arrest and died 19 hours after the start of the treatment. Forensic pathological examination revealed no definitive conclusion regarding the probable cause of death (*177*) and cited the general lack of information correlating ibogaine concentrations with possible toxic effects in humans. The high levels of noribogaine found in the deceased patient were possibly consistent with saturation of elimination kinetics. However, the higher levels of noribogaine in heart, relative to femoral blood, also suggested significant postmortem redistribution of noribogaine. The potential artifact associated with a high volume of distribution and postmortem release of drug previously sequestered in tissue (*51,139,158*) limits the interpretability of postmortem levels of noribogaine.

Some evidence suggested the possibility of surreptitious opioid use in this case, which was noted in the Dutch inquiry (*178*) and which is another source of uncertainty in this fatality. There is evidence suggesting that the interaction of opioids and ibogaine potentiates opioid toxicity (*68,179*). Analysis of gastric contents for heroin or morphine, which might have confirmed recent heroin smoking, and analysis of blood for 6-monoacetyl morphine, a heroin metabolite whose presence indicates recent use (*180*), were not performed. This incident

underscores the need for the security and medical supervision available in a conventional medical setting, and for completion of dose escalation studies to allow systematic collection of pharmacokinetic and safety data.

In London, in January 2000, a 40-year-old heroin addict died after having allegedly taken 5 g of *iboga* alkaloid extract 40 hours prior to his death (*38*, see the chapter by Alper *et al.* in this volume). The extract was said to have contained approximately five times the alkaloid content of the dried rootbark. The official British inquest regarding this matter is still in progress as of the time of the writing of this book.

D. Abuse Liability

The available evidence does not appear to suggest that ibogaine has significant potential for abuse. The 5-HT_{2A} receptor, the primary mediator of responding for LSD and other commonly abused drugs classified as "hallucinogenic" or "psychedelic," does not appear to be essential to discriminability of the ibogaine stimulus (*84,96*). Ibogaine is reportedly neither rewarding or aversive in the conditioned place preference paradigm (*132*). Rats given either 10 or 40 mg/kg ibogaine daily for 6 consecutive days did not show withdrawal signs (*129*). Animals do not self-administer 18-MC, an ibogaine analog, in paradigms in which they self-administer drugs of abuse (*45*). None of the consultants to NIDA in the 1995 Ibogaine Review Meeting identified the possible abuse of ibogaine as a potential safety concern.

VII. Learning, Memory, and Neurophysiology

A. Learning, Memory, and Addiction

Drug abusers may be viewed as having a disorder involving excess attribution of salience to drugs and drug-related stimuli (*181*), which suggests the possibility of a role of processes subserving learning and memory in the acquisition of the pathological motivational focus in addiction (*182-185*). Learning, in the most general sense, can be viewed as the modification of future brain activity, of which thought, motivation, consciousness, or sensory experience are emergent properties, on the basis of prior experience. This broad definition subsumes everything from social behavior to learning to read, to the neuroadaptations of drug tolerance and dependence.

Addiction can be argued to involve the pathological acquisition or "learning" of associations of drug related stimuli with motivational states corresponding to

valuation and importance (*181,183,184*). The pathological learning of addiction differs from that of normal learning in at least two important respects. First, the acquisition of drug salience in addiction does not involve learned associations between drug-related external cues or internal representations, and the experience of external events as they actually occur. Instead, the "imprinting" or "stamping in" of drug incentives appears to involve alterations of neural plasticity in processes that subserve motivation, memory and learning, resulting in neural behavior that to a significant extent has escaped the constraint of validation by experience with external reality (*183-186*). Dopamine and glutamate transmission are thought to be involved in the modulation of neural plasticity of both normal learning and the neuroadaptations of drug salience (*184*). Second, drug-related "learning" does not apparently habituate (*184*). Unlike normal learning, the drug stimulus appears to be experienced as perpetually novel and continues to command attention and be attributed with salience unattenuated by habituation (*53,182*).

B. Effects of Ibogaine on Learning and Memory

Ibogaine appears to have significant effects on brain events involved in learning and the encoding of drug salience. Ibogaine interacts significantly with the NMDA receptor (*39,58,179*), which is involved in long term potentiation (LTP), a process thought to be important in neural plasticity, memory, and learning (*182,184,187*). Experiences apparently involving memory, such as panoramic recall, are prominent in descriptions by individuals who have taken ibogaine (*14*).

The observation of an effect of ibogaine on the expression of behavioral sensitization to amphetamine, but not a conditioned place preference (*188*), raises the interesting possibility of a relatively selective effect of ibogaine on the pathological encoding of drug salience, distinguished from learning involving non-drug incentives. Ibogaine reportedly attenuates the acquisition of place preference for morphine or amphetamine (*131,132*). A general effect of interference with learning has been suggested (*189*), but studies on spatial learning show an actual enhancement by ibogaine (*102,190*). Consistent with a selective effect on neuroadaptations acquired from drug exposure are ibogaine's effects on locomotor activity and dopamine efflux in the NAc, which are relatively more evident in animals with prior experience with morphine (*112,113*) or cocaine (*111*).

C. Ibogaine and the EEG

Studies of animals treated acutely with ibogaine report a desynchronized EEG with fast low amplitude activity, a state described as "activated" or "aroused"

(*89,90,191*). Binienda *et al.* (*90*) noted a decline in delta amplitude and interpreted this as consistent with activation of dopaminergic receptors. However, observations on the interaction of atropine and ibogaine with respect to the EEG suggest the involvement of ascending cholinergic input. Depoortere (*191*) found that ibogaine enhanced an atropine-sensitive theta frequency EEG rhythm in rats. Schneider and Sigg (*89*) observed a shift toward high-frequency low-voltage EEG activity following the administration of ibogaine to cats, and they noted that this effect was blocked by the administration of atropine. Luciano *et al.* (*148*) observed no changes in the visually evaluated EEG in humans administered 20 to 25 mg/kg ibogaine.

D. Goutarel's Hypothesis

The French chemist Robert Goutarel (*14*) hypothesized that ibogaine treatment involves a state with functional aspects shared by the brain states of REM sleep, with important effects on learning and memory. During the REM state, there is believed to be reconsolidation of learned information in a state of heightened neural plasticity, with the reprocessing of previously learned information and the formation of new associations (*192,193*). Goutarel suggested that a REM-like state may be induced by ibogaine, which corresponds to a window of heightened neural plasticity, during which there may be weakening of the pathological linkages between cues and representations of the drug incentive and the motivational states with which they have become paired (*14*). Analogous to the reconsolidation of learned information that is thought to occur during the REM state (*192,193*), Goutarel theorized that the pathological learning of addiction was modified during ibogaine treatment. He appears to have based his theoretical formulation mainly on reports of the phenomenological experiences of awake ibogaine-treated subjects that share features in common with dreams. Goutarel's hypothesis is speculative, but nonetheless has an interesting apparent consistency with the literature on the relationship of learning and addiction and the physiologic function of the REM EEG state with regard to the consolidation of learned information.

There is some evidence that may be viewed as consistent with Goutarel's hypothesis. Goutarel's belief in a relationship of the ibogaine-treated EEG state to that of REM is supported by studies in animals treated with ibogaine that report an apparently activated or desynchronized EEG state consistent with arousal, vigilance, or REM sleep (*90,191*). The observation that ibogaine enhanced an atropine-sensitive theta frequency rhythm (*191*) suggests the possible involvement of ascending cholinergic input, which is an essential determinant of EEG desynchronization during REM sleep (*192*). The possible reconsolidation of learned information due to heightened plasticity during both the REM and ibogaine-induced desynchronized EEG states is suggested by the observation that

EEG dyssynchrony is associated with an increased facilitation of Hebbian covariance (*194*), which is believed to be an important determinant of the neural plasticity involved in consolidation of learning and memory. Also, with regard to a possible analogy of the REM and ibogaine induced brain states, some ibogaine treatment guides have anecdotally mentioned that they have observed REM-like eye movements in awake patients during treatments (*195,196*).

VIII. Anthropological and Sociological Perspectives

As discussed in various aspects by this volume by the Fernandezes, Frenken, and Lotsof and Alexander, ibogaine's use appears to involve distinctive interactions of psychopharmacologic effects with set and setting in both the subcultures of the United States and Europe, and the centuries older, sacramental context of the use of iboga in Bwiti, the religious movement in West Central Africa. In the Bwiti religious subculture, and arguably to some extent in the European ibogaine subculture, there is the common attribute of a group of initiates that seek to facilitate healing through the affiliation of the collective with the individual. In both the African and U.S./European contexts, the ibogaine experience has been attributed to serving the objective of facilitating personal growth and change. Use of ibogaine in both contexts has been criticized as involving the use of an "addictive" or "hallucinogenic" agent, and it appears to some extent to involve the formation of a subculture among individuals confronted with marginalizing social circumstances such as colonialism, or the state of addiction (*197-199*, see also Fernandez and Fernandez in this volume).

Galanter (*200*) identifies three important psychological features that he regards as descriptive of the process of charismatic groups or zealous self-help movements such as 12-step programs that appear to also be relevant to Bwiti. These three processes are group cohesiveness, shared belief, and altered consciousness, such as that of religious ecstasy or insight to which the group can attribute a new construction of reality in their life. An understanding of these powerful behavioral influences could be useful in optimizing the clinical milieu and interpersonal dynamics of present conventional treatment settings, or of future treatment settings, if ibogaine or a congener should receive official approval.

The application of ethnographic techniques to the analysis of the phenomenological features of the acute treatment experience could be informative from a neuropsychiatric, as well as from a cultural perspective. For example, similar subjective phenomena are frequently described in both ibogaine treatment and near death experiences (NDEs) (*14,152,199,201*) such as panoramic memory;

calm, detached emotional tone; specific experiences, such as passage along a long path or floating; "visions" or "waking dream" states featuring archetypal experiences such as contact with transcendent beings; and the frequent attribution of transcendent significance to the experience. Such shared features between ibogaine and NDEs suggest a common transcultural phenomenology of transcendent or religious experience or, alternatively, the possibility of a similar subjective experience due to the influence of a common underlying neurobiological mechanism such as NMDA transmission (*202*).

IX. Economic and Political Perspectives

A. Economic Incentives and the Development of Ibogaine

The academic research community working in the public sector has a crucial role in studying ibogaine as a paradigm for the development of new treatment approaches. The strategy of relying on the pharmaceutical industry to underwrite the cost of drug development works extremely well in many instances, but appears to present some limitations with regard to the development of pharmacotherapy for addiction in general, and specifically ibogaine.

In the public sector, the major economic incentives for the development of addiction treatment are the saved costs associated with preventing lost economic productivity, medical morbidity, or crime. In the private sector, decisions are based on weighing the expense of development against the expected profit, and not the magnitude of saved economic or social costs. Owing to limited financial incentives in the form of insurance reimbursements and a perceived lack of "breakthrough" compounds, the U.S. pharmaceutical industry has not generally viewed addiction as an attractive area for development (*203*), and expenditures for the development of medications for addiction are small relative to those to develop drugs for other indications. Ibogaine is particularly unattractive to industry for several reasons: its mechanism of action is apparently complex and incompletely understood, it may present significant safety issues, it is a naturally occurring alkaloid whose structure cannot itself be patented, and some of its use patents are close to expiration.

There is arguably an important role for academic/public-sector development in the case of a theoretically interesting drug with a limited profit potential and significant developmental expense such as ibogaine. However, the entire annual expenditures for medications development in NIDA, which accounts to about 90% of U.S. public sector spending on developing addiction pharmacotherapy, is on the order of approximately $60 million, a fraction of the average cost of

successfully developing a drug to market, which is estimated to exceed $300 million (*204*). Opportunities to fund research on ibogaine are limited by factors that generally affect the development of other drugs to treat addiction: a limited public sector budget in the presence of disproportionately low private-sector expenditures on the development of pharmacotherapies for addiction relative to other indications (*203*).

B. Political Issues

The chapter by Alper *et al.* in this volume describes the medical subculture of the informal ibogaine treatment scene and the political subculture of advocacy for the development and availability of ibogaine. These scenes are a distinctive and significant aspect of ibogaine's history, which arguably have impacted on decisions regarding its development. From a clinical standpoint, the informal treatment subculture has been an important source of information on human experience with ibogaine (*32*).

From a political or historical standpoint, the informal treatment subculture has viewed itself as a form of activism or civil disobedience on the part of its participants seeking a treatment, despite a lack of official approval (*34*). Ibogaine has been associated with a vocal activist subculture, which views its mission as making controversial treatments available to a stigmatized minority group of patients suffering from a life-threatening illness, and has utilized tactics intended to engage the attention of the press (*34*). These confrontational media-oriented tactics may well have provoked negative reactions at times, but may also have influenced Curtis Wright, the former FDA ibogaine project officer, to write in 1995 that ". . . a significant portion of the public we serve believes the drug merits investigation" (*205*).

X. Conclusions

Evidence that supports the possible efficacy of ibogaine as a treatment for addiction includes case reports in humans, and effects in preclinical models of drug dependence. The case report evidence has mainly involved the indication of acute opioid withdrawal, and there appears to be consistency between earlier observations derived from informal treatment contexts (*32-36,146-150*) and more recent work from a setting that appears to conform to a conventional medical model (*123*, Mash *et al.* in this volume). The continued existence of informal treatment scenes parallels case report evidence indicating possible efficacy. Animal work has provided observations of attenuation of opiate withdrawal signs

and reductions of self-administration of a variety of drugs including morphine, cocaine, alcohol, and nicotine. Preclinical models have also yielded evidence that with respect to certain abused drugs, ibogaine may dampen responses that may be associated with dependence, such as dopamine efflux in the NAc or locomotor activation.

Ibogaine's pharmacologic profile includes interactions with multiple neurotransmitter systems that could plausibly be related to addiction, including NMDA, nicotinic, mu- and kappa-opioid, and serotonergic systems. The putative efficacy of ibogaine does not appear fully explainable on the basis of interactions with any single neurotransmitter system, or on the basis of currently utilized pharmacologic strategies such as substitution therapies, or monoamine reuptake inhibition. Ibogaine's effects may result from interactions between multiple neurotransmitter systems, and might not be attributable to actions at any single type of receptor. The apparently persistent effect of ibogaine has been suggested to involve a long-lived metabolite. Some evidence suggests effects on second messenger signal transduction, an interesting possibility that could conceivably result from interactions between multiple neurotransmitter systems and produce persistent effects lasting beyond the duration of occupancy at receptor sites. Work with ibogaine congeners suggests that other *iboga* alkaloids can be developed that might minimize unwanted toxic, or possibly behavioral effects, while retaining apparent efficacy in drug dependence. In summary, the available evidence suggests that ibogaine and the *iboga* alkaloids may have efficacy in addiction on the basis of mechanisms that are not yet known and which can possibly be dissociated from toxic effects, and may present significant promise as a paradigm for the study and development of pharmacotherapy for addiction.

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Effect of Ibogaine on Naloxone-Precipitated Withdrawal Syndrome in Chronic Morphine-Dependent Rats

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Abstract—Ibogaine, an indole alkaloid, administered intracerebroventricularly 4–16 μg, attenuated a naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. It appears that ibogaine has a more consistent effect on certain selective withdrawal signs related to the locomotion. This might explain an attenuating effect of ibogaine on some withdrawal signs. However, due to complex interaction of ibogaine with serotonin and other neurotransmitter systems, the mechanism of ibogaine antiwithdrawal effect remains unknown and requires further elucidation.

Introduction

There is some evidence that brain serotonin (5-HT) can modulate an opiate withdrawal syndrome. Way *et al.* (1968) and Ho *et al.* (1973) demonstrated that inhibition of 5-HT synthesis or destruction of 5-HT system might significantly attenuate a naloxone-precipitated withdrawal syndrome. Because some of these results were not confirmed (Cheney and Goldstein, 1971; Johnson *et al.*, 1978), the relationship between the brain serotonin system and the opiate withdrawal syndrome has become controversial. Interest in this problem had increased again since recent data have shown a decrease or increase of severity of morphine withdrawal in animals after pretreatment with 5-HT_2 antagonists (Cervo *et al.*, 1981; Neal and Sparber, 1986) or with 5-HT releaser (Samanin *et al.*, 1980), respectively.

Ibogaine is an indole alkaloid of the family apocynaceae, with central

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stimulant (Schneider and Sigg, 1957; Singbartl *et al.*, 1973) and serotonin-like properties (Sloviter *et al.*, 1980), which can be antagonized by 5-HT blocking drug methysergide (Dhahir, 1971). Therefore, an interaction between 5-HT system and opiate withdrawal in ibogaine pretreated animals might be of importance. In addition, it has been demonstrated that ibogaine potentiated morphine analgesia in mice and humans (Schneider and McArthur, 1956). Ibogaine, however, exerts a complex activity interfering with many other neurotransmitter systems. It has been reported that ibogaine is a strong inhibitor of serum cholinesterase (Hamet and Rothlin, 1956) and its arousal-inducing effects can be blocked by atropine (Schneider and Sigg, 1957). Gershon and Lang (1962) found that ibogaine potentiated the pressor response to both adrenaline and noradrenaline. Ibogaine has been described as a hallucinogenic substance (Farnsworts, 1968; Sloviter *et al.*, 1980). Unpublished results by H. Lotsof (New York) suggested that ibogaine given per os may interrupt the physiological and psychological aspects of the opiate withdrawal syndrome in humans. Given these tentative conclusions, it seems useful to examine the effect of ibogaine on the naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. These experiments elucidate the effect of ibogaine on the opiate withdrawal syndrome and might provide some basis for clinical trials of ibogaine for the treatment of opiate dependence. However, the mechanism of the antiwithdrawal action of ibogaine remains unknown and requires further clarification.

Methods

Adult male Wistar rats (190–200 g) were used. The animals were housed singly. Food and water were available *ad libitum*. Ibogaine was administered intracerebroventricularly (i.c.v.). All animals were chronically implanted with stainless steel cannula in the left lateral cerebral ventricle as described in an earlier paper (Dzoljic *et al.*, 1979). An implantation of cannula was carried out under hypnorm anaesthesia (fluanison/fentanyl base, 0.15 ml/100 g, s.c.). The i.c.v. administration of drugs was performed by using a stainless steel cannula stereotaxically directed 1 mm in the left lateral ventricle (König and Klippel, 1963). Drug solutions were injected into the ventricle with needle gauge 30, attached to a Hamilton microsyringe by polyethylene tube. The needle was protruded 1 mm into the lateral ventricle. Correct ventricular cannulation was verified before each experiment, using a modification of the technique previously described by Paakkari (1980). In this procedure a polyethylene tube was attached to the injection needle and filled with saline. To test the correct placement of i.c.v. cannula during surgery, the tube was raised above the head of the animal on the stereotaxic apparatus, and a rapid inflow of saline denoted a

correct placement of cannula. At least 6 days recovery were allowed before the experiments. Each rat was tested only once.

For inducing chronic opiate dependence, morphine pellets (85 mg) were implanted subcutaneously (s.c.) on the back of the animals under ether anaesthesia (Blasig *et al.*, 1973). The opiate withdrawal syndrome was precipitated 72 hr after the pellets implantation by naloxone (5 mg/kg, i.p.) dissolved in saline. The withdrawal syndrome was induced only once in each rat. The behavior of the rat was observed in a plastic box (base area: 25 × 40 cm, height: 15 cm). Ibogaine, in a dose range 4–16 μg, was administered i.c.v. 15 min prior to naloxone. Doses referred to the salts and pH was kept about 6.6–7.0. The control group received the same volume of artificial cerebrospinal fluid (CSF). The composition of the CSF/l is as follows: NaCl 8.10 g; KCl 0.25 g; CaC_2 0.14 g; $MgCl_2$ 0.11 g; $NaHCO_3$ 0.18 g; NaH_2PO_4 0.07 g; urea 0.13 g; glucose 0.61 g. The observation period started at the time of i.c.v. injection of CSF or ibogaine and lasted up to 30 min after naloxone administration. Withdrawal signs were counted and checked. Jumping consisted of animals leaping on the metal rack, which covered the box, with all 4 feet off the ground. Other withdrawal signs have clear meaning.

Drugs used were naloxone-hydrochloride (SIGMA), morphine-sulphate (Diosynth) and ibogaine-hydrochloride (kindly donated by H. Lotsof, NDA, New York). Ibogaine and naloxone were dissolved in CSF or saline, respectively.

Statistical analysis was conducted to explore the difference in the 3 experimental groups and the control group over 21 distinct withdrawal signs. To ascertain significant difference a nonparametric analysis of variance (Kruskal-Wallis) was performed. The significance of the difference between the significantly different group means were evaluated by Mann-Whitney U-test (Saxena, 1985). Significance was accepted at $p < 0.05$.

Results

Effect of ibogaine on morphine-dependent animals

A tremor observed in mice by Singbartl *et al.* (1973) was not found in the ibogaine-treated rats within a dose range of 4–16 μg. The behavioral signs of addicted naive rats common with the opiate withdrawal syndrome (but in low frequency) have not been significantly altered by i.c.v. administration ibogaine. Thus, no marked changes were found in behavioral signs such as: digging, head shakes, scratching, grooming, drinking, eating, penile licking and ejaculation. However, rearing was significantly decreased (not shown).

Effect of ibogaine on morphine withdrawal syndrome

Administration of naloxone (5 mg/kg, i.p.) 15 min after CSF (i.c.v.) in chronic morphine-dependent rats induced a withdrawal syndrome (Table I). The i.c.v. administration of ibogaine (4–16 μg), 15 min prior to naloxone (5 mg/kg, i.p.), significantly reduced, in a dose-related manner, the frequency of the following symptoms: rearing, digging, head hiding, chewing, teeth chattering, writhing, jumping and salivation. The frequency of other withdrawal signs were nonsignificantly decreased or not altered: "wet-dog" shakes, head shakes, stretching, grooming, scratching,

TABLE I

The effects (mean ± S.E.M. or response ratios ([1])) of i.c.v. injection of increasing doses of ibogaine (4, 8 and 16 μg/rat) on withdrawal syndrome precipitated by naloxone (5 mg/kg, i.p.) in chronic morphine-dependent rats

		Ibogaine (μg/rat, i.c.v.)		
Withdrawal sign	CSF (n = 10)	4 (n = 10)	8 (n = 10)	16 (n = 10)
Rearing	20.30 ± 1.28	15.70 ± 1.86*	14.50 ± 1.17*	14.70 ± 1.43*
Digging	10.20 ± 2.67	5.20 ± 1.05*	4.20 ± 0.76*	4.20 ± 1.02*
Head hiding	6.60 ± 0.70	4.10 ± 2.14*	2.70 ± 1.26*	2.00 ± 0.70*
"Wet-dog" shakes	11.70 ± 1.85	10.20 ± 0.99	9.60 ± 1.27	9.20 ± 1.64
Head shakes	3.10 ± 0.55	2.70 ± 0.65	2.60 ± 0.65	2.60 ± 0.52
Chewing	55.50 ± 5.17	35.10 ± 5.13*	23.50 ± 1.81*	31.40 ± 4.31*
Teeth chattering	48.60 ± 7.26	24.50 ± 6.90*	23.50 ± 1.81*	23.70 ± 5.69*
Writhing	5.60 ± 1.06	3.20 ± 0.39*	1.80 ± 0.39*	1.60 ± 0.99*
Stretching	0.70 ± 0.30	0.60 ± 0.27	0.60 ± 0.22	0.70 ± 0.30
Grooming	6.70 ± 1.58	6.00 ± 0.88	4.60 ± 1.49	4.80 ± 0.51
Scratching	1.20 ± 0.61	1.10 ± 0.53	0.90 ± 0.50	0.40 ± 0.15
Penile licking	1.90 ± 0.46	4.00 ± 0.85*	4.10 ± 0.63*	6.80 ± 1.25*
		(mean ± S.E.M. – counted signs)		
Jumping	0.7	0.5	0.2*	0.2*
Vocalization on touch	0.7	0.7	0.5	0.6
Ptosis	0.7	0.6	0.4	0.4
Diarrhea	0.7	0.6	0.4	0.4
Urination	0.2	0.2	0.1	0.1
Rhinorrhea	0.5	0.2	0.1	0.1
Salivation	0.7	0.5	0.2*	0.2*
Paw tremor	0.1	–	–	0.1
Ejaculation	0.3	0.4	0.6	0.6
		(response ratio – checked signs)		

The control group received artificial cerebrospinal fluid (CSF) instead of an ibogaine.
([1]) = The number of rats displayed checked sign per number tested.
* = Significant difference compared to control ($p < 0.05$).

vocalization on touch, ptosis, diarrhea, urination, rhinorrhea, paw tremor and ejaculation. However, the frequency of penile licking was significantly increased (Table I). In addition, all animals pretreated with ibogaine demonstrated during withdrawal a decreased locomotion occasionally associated with tremor.

Discussion

Ibogaine decreased the motor activity in addicted animals during a naloxone-precipitated withdrawal syndrome. Since ibogaine is a rather strong central stimulant (Schneider and Sigg, 1957) a lack of increased motor activity following ibogaine is in contrast to observations of many other central stimulants. A reduction of locomotion induced by ibogaine might explain an inhibitory effect of this drug on the rearing, digging and jumping during naloxone-precipitated withdrawal.

In addition, this study shows that i.c.v. injection of ibogaine attenuates several other withdrawal symptoms in chronic-morphine-dependent rats such as: head hiding, chewing, teeth chattering, writhing and salivation. In general, rats had less desire to hide or to escape. A tremor, described in male mice after s.c. administration of ibogaine (Zetler *et al.*, 1972), was not observed after i.c.v. administration of this drug in morphine-dependent rats. However, a tremor was occasionally registered during naloxone-precipitated withdrawal of ibogaine pretreated (i.c.v.) rats.

The mechanism of attenuation of morphine-withdrawal signs in the rats following ibogaine administration is not clear. This indole alkaloid possesses a very complex activity on neurotransmitter systems, affecting both the noradrenergic (Gershon and Lang, 1962) and the cholinergic systems (Hamet and Rothlin, 1956). It has also been found that ibogaine, as hallucinogen, shares the common mechanism of action as lysergic acid (LSD), activating central 5-HT receptors (Sloviter *et al.*, 1980). Accordingly, methysergide, a 5-HT_2 receptor blocking drug, reduced the hypotensive effects of both ibogaine of 5-HT (Dhahir, 1971). These interactions of ibogaine and the 5-HT system might explain the stimulatory effect of this indole alkaloid on sexual phenomena in opiate withdrawal syndrome. In these experiments, ibogaine significantly increased penile licking. This confirms the recent data which have shown that activation of 5-HT_1 receptors facilitates the expression of sexual behaviour in male rats (Mendelson and Gorzalka, 1986). However, the intimate interaction of ibogaine with different 5-HT receptor populations is still unknown. The situation is further complicated by the fact that the intimate mechanism of opiate withdrawal is also unknown. However, this study shows that ibogaine has a consistent effect on certain selective withdrawal signs having to do with

locomotion. It seems promising to conduct more experimentation on the specific effects of ibogaine on the serotonin system and on selective parts of the opiate withdrawal syndrome.

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Inhibitory effects of ibogaine on cocaine self-administration in rats

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In order to determine the potential anti-addictive properties of ibogaine, we used the cocaine self-administration model in rats. The results indicate that a single injection of ibogaine (40 mg/kg i.p.) produced a significant decrease of cocaine intake, which remained unaltered for more than 48 h. Since the half-life time of ibogaine is short, this might suggest the involvement of one or several active metabolites of ibogaine in cocaine intake. Repetitive administration of ibogaine on three consecutive days also induced a pronounced decrease of cocaine intake. However, a more prominent inhibitory effect on cocaine intake was observed in animals treated repeatedly with ibogaine (40 mg/kg i.p.), once each week for 3 consecutive weeks. These results indicate that ibogaine or its metabolite(s) is a long-lasting interruptor of cocaine dependence, which supports similar observations from uncontrolled clinical studies.

Cocaine; Cocaine dependence; Ibogaine; Self-administration; (Rat)

1. Introduction

Ibogaine, an indole alkaloid found in the root bark of the African shrub *Tabernanthe iboga*, has been used in Gabon (West Central Africa) in low doses as a stimulant (combat fatigue, hunger and thirst) and in high doses for its hallucinogenic properties (religious rituals).

Recent animal studies and non-controlled observations in humans indicate that ibogaine may significantly affect drug dependence phenomena such as drug withdrawal and intake of addictive drugs. Accordingly, it has been demonstrated that ibogaine (i.c.v.) attenuated many (but not all) symptoms of naloxone-precipitated withdrawal in morphine-dependent rats (Dzoljic et al., 1988). A similar anti-withdrawal effect of ibogaine has been observed in morphine-dependent monkeys (Aceto et al., 1990) and rats (Glick et al., 1992).

Related to the intake of addictive drugs, it has been shown that ibogaine pretreatment decreases intravenous morphine self-administration in rats for several days (Glick et al., 1991). These results of animal experiments are in accordance with the long-lasting interruption of heroin abuse by ibogaine in humans (Lotsof, 1985). Ibogaine is also claimed to interrupt cocaine and amphetamine abuse and it was suggested that series of four treatments may be effective for several years (Lotsof, 1986). Other claims are that ibogaine attenuates alcohol and nicotine/tobacco dependency syndromes (Lotsof, 1989, 1991). The aim of the present experiments was to determine whether an interrupting effect of ibogaine on cocaine intake could be demonstrated in cocaine-dependent animals. We examined the effects of single and repeated injections of ibogaine on the cocaine self-administration model in rats.

2. Materials and methods

2.1. Animals

Male wistar rats (TNO Zeist) were used, weighing 200–250 g at the start of the experiments. The animals were housed in groups with water and food ad libitum. Artificial light was supplied on a 12-h light/dark cycle.

2.2. Operation procedure

All animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and surgically implanted with

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a chronic i.v. jugular catheter (0.5 mm inside diameter, 1.0 mm outside diameter, polyethylene tubing). The catheter was passed subcutaneously to a small incision at the back of the neck. After the operation the animals were housed individually with food and water ad libitum. Two days before the start of the experiments (i.e. 5–6 days after operation), the animals were brought to the test room and were deprived of food in order to obtain a weight reduction of about 20%. Weight reduction was introduced in order to facilitate acquisition of self-administration (Takahashi et al., 1978). A reversed 12-h light/dark cycle (lights out 8.00–20.00 h) was maintained during the whole experiment.

2.3. Apparatus

The experiments were performed in operant conditioning chambers. Cocaine infusions (1.2 mg/kg), consisting of 0.25 ml fluid (pH 7.30–7.35) delivered in 20 s, occurred when the reinforcement lever was depressed. During the infusion, the stimulus light was turned off and pressing the same lever had no programmed consequences.

2.4. Test procedure

Following 5–6 days of postoperative recovery, the rats were connected to an infusion pump (Braun Perfusor Secura MRD) by polyethylene tubing and a fluid swivel, which permitted unlimited movement of the animal during the session. Session length was 3 h each day (during the dark period of the cycle), 5 days per week with 2 days of no testing (during weekends, between each 5-day block of testing). The study of the effect of ibogaine began when the baseline rate of cocaine self-administration stabilized (< 10% variation between 3 consecutive sessions) after 12–16 days (sessions). These animals were randomly divided into vehicle and ibogaine-treated groups. The experiments lasted about 6 weeks (including the first 2 weeks used for stabilization of cocaine intake).

2.5. Experimental groups

Vehicle (1.0 ml/kg i.p.) or ibogaine was given 30 min prior to self-administration testing and the behaviour of animals was monitored for the subsequent 3 h.

2.5.1. Single administration of ibogaine (10–40 mg/kg i.p., n = 6–7 per dose)

Our preliminary experiments showed that administration of 80 mg/kg ibogaine caused severe locomotor disturbances (ataxia, jumping when touched and tremor for about 60 min). Therefore, in further experiments, this dose was omitted and 40 mg/kg ibogaine was constantly used. This dose had less prominent and shorter lasting behavioural effects than the higher dose (see Results).

2.5.2. Repetitive administration of ibogaine (40 mg/kg i.p.)

In one group of animals (n = 5) ibogaine was administered once on each of 3 consecutive days, while the other group (n = 5) received ibogaine once at the beginning of each of 3 consecutive weeks.

2.6. Drugs

Cocaine hydrochloride (OPG, Utrecht, Netherlands) was dissolved in saline and the pH was adjusted to 7.30–7.35. Ibogaine hydrochloride (kindly donated by H. Lotsof, NDA, New York) was dissolved in distilled water.

2.7. Data analysis

Responses were summed over the 3-h test period and subjected to two-way analysis of variance (ANOVA) with repeated measurements on days. Individual comparisons of means were made with Student's t-test (between baseline and treated groups and between vehicle and ibogaine-treated groups) with significance at $P < 0.05$ level.

3. Results

3.1. Single administration of ibogaine (10–40 mg/kg i.p., n = 6–7 per dose)

3.1.1. Behaviour

Administration of ibogaine in cocaine-dependent rats induced within 4 min stiffness of the hind legs, tremor, ataxia and hypersensitivity (jumping or violent locomotion when touched). The severity of this behavioural syndrome was dose dependent and, in the case of the highest dose of ibogaine (40 mg/kg), the effect lasted for a maximum of 30 min. Thereafter, animals showed normal behaviour and were used for the self-administration procedure.

3.1.2. Cocaine intake

The baseline cocaine intake was 5.0 ± 0.5 mg/kg (fig. 1). A single injection of 40 mg/kg ibogaine produced a significant depression of cocaine intake, while 10 and 20 mg/kg were ineffective (fig. 1). The inhibitory effect of a single administration of ibogaine on cocaine intake became more prominent on the next day and remained below the control level for the 24 h following (48 h after drug administration, fig. 1). Further studies were performed with the 40 mg/kg dose of ibogaine.

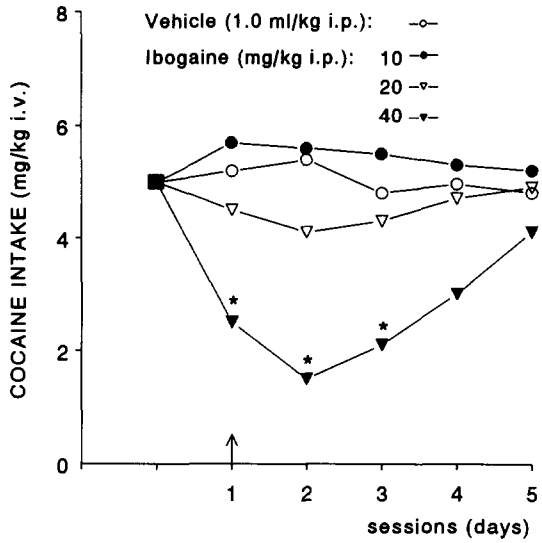

Fig. 1. Effect of a single dose of ibogaine (10–40 mg/kg i.p., n = 6–7 per dose) on cocaine intake in rats. The baseline cocaine intake (■ 5.0 ± 0.5 mg/kg) was calculated as the average rate of three consecutive sessions (< 10% variation) preceding treatment with vehicle (distilled water 1.0 ml/kg i.p., n = 7) or ibogaine. Vehicle (↑) or ibogaine (↑) were administered 30 min before the session started. The data are expressed as means of cocaine intake per session. * Significant decrease of cocaine intake (ANOVA and t-test $P < 0.05$) compared to the baseline intake and the vehicle-treated group. Note that a single injection of ibogaine (40 mg/kg) exerted a long-lasting (48 h) inhibition of cocaine intake.

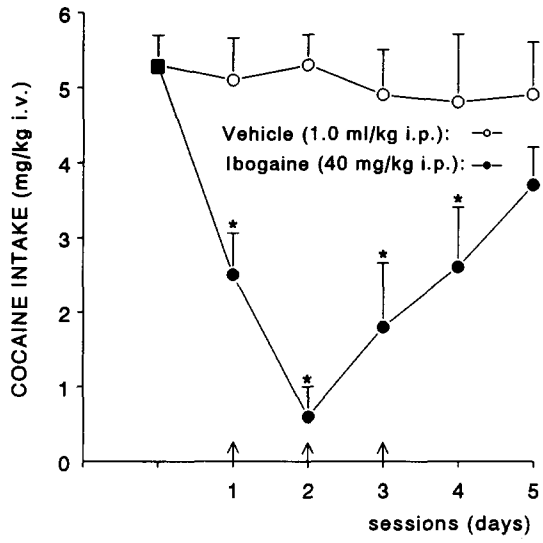

Fig. 2. Effect of repeated administration of ibogaine (40 mg/kg i.p., n = 5, given once on each of three consecutive sessions) on cocaine intake in rats. The baseline cocaine intake (■ 5.3 ± 0.4 mg/kg) was calculated as the average rate of three consecutive sessions (< 10% variation) preceding treatment with vehicle (distilled water 1.0 ml/kg i.p., n = 5) or ibogaine. Vehicle (↑) or ibogaine (↑) was administered 30 min before the session started. The data are expressed as means ± SEM cocaine intake per session. * Significant decrease of cocaine intake (ANOVA and t-test $P < 0.05$) compared to the baseline intake and the vehicle-treated group. Note that each injection of ibogaine significantly decreased cocaine intake.

3.2. Repeated (3 times) administration of ibogaine (40 mg/kg i.p.)

3.2.1. Ibogaine administered on each of 3 consecutive days

Compared to the baseline (5.3 ± 0.4), administration of vehicle (1.0 ml/kg i.p., n = 5) on each of 3 consecutive days did not induce significant changes in cocaine intake (fig. 2). However, a significant decrease of the cocaine intake (n = 5) occurred on the second day of ibogaine treatment. After the third injection of ibogaine, the inhibitory effect on cocaine intake lasted for the next 24 h (fig. 2). This effect on cocaine intake was not significantly different from that of a single injection of ibogaine, but was shorter (24 h versus 48 h).

3.2.2. Ibogaine administered at the beginning of each of 3 consecutive weeks

The baseline cocaine intake (4.9 ± 0.5) was not significantly affected by vehicle (1.0 ml/kg i.p., n = 5) administered at the beginning of each of 3 consecutive weeks (fig. 3). However, a significant decrease of cocaine intake was observed following each ibogaine injection. Compared to that after the first injection of ibogaine, the decrease of cocaine intake was more sustained after the second and third administration of ibogaine (fig. 3).

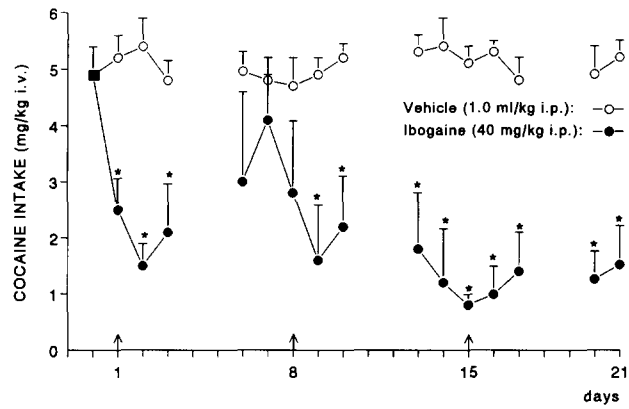

Fig. 3. Effect of repeated administration of ibogaine (40 mg/kg i.p., n = 5, given once at the beginning of each of 3 consecutive weeks) on cocaine intake in rats. The baseline cocaine intake (■ 4.9 ± 0.5 mg/kg) was calculated as the average rate of three consecutive sessions (< 10% variation) preceding treatment with vehicle (distilled water 1.0 ml/kg i.p., n = 5) or ibogaine. Vehicle (↑) or ibogaine (↑) was administered 30 min before the session started. The data are expressed as means ± SEM cocaine intake per session. The animals were not tested during weekends. * Significant decrease of cocaine intake (ANOVA and t-test $P < 0.05$) compared to the baseline intake and the vehicle-treated group. Note a gradual and long-lasting decrease of cocaine intake following second and third injection of ibogaine.

4. Discussion

A single dose or repeated doses (on each of 3 consecutive days) of ibogaine (40 mg/kg i.p.) in rats induced a decrease of cocaine intake lasting 1–2 days. This effect could be potentiated and prolonged by three injections of ibogaine, given once each week (but not once each day). This was rather surprising, as the half-life time of ibogaine in rodents is about 1 h, and a day after administration, the ibogaine levels in the body were undetectable (Dhahir, 1971, cited by Glick et al., 1991). This might indicate that the depression of cocaine intake could be ascribed to an active and long-lasting metabolite(s) of ibogaine or to irreversible interruption of the biological mechanism of cocaine dependence.

Related to the mechanism of anti-addictive properties of ibogaine several possibilities could be considered:

4.1. Disturbed locomotion

Ibogaine enhanced the amphetamine-induced increase of motor activity (Maisonneuve et al., 1992). Additional disturbances of motility, such as tremor and ataxia observed in this and other studies (Glick et al., 1991), might further affect the self-administration of cocaine. However, this possibility is unlikely, since in our experiments the ibogaine-induced locomotor disturbances such as ataxia and tremor lasted about 30 min, while the anti-addictive effect of a single dose of this drug remained for at least 2 days. A long-lasting effect of ibogaine (several days) on morphine intake in rats was also observed in other studies (Glick et al., 1991). Ibogaine pretreatment of rats (40 mg/kg i.p. 19 h prior) had no effect on the increased locomotion induced by various doses of cocaine (5, 10 and 40 mg/kg), while the locomotion after administration of 20 mg/kg cocaine was potentiated for only 1 h (Maisonneuve and Glick, 1992). Evidently, an effect of ibogaine on motor activity in rats is of marginal importance for understanding the long-lasting anti-addictive properties of ibogaine.

4.2. Dopaminergic system

The rewarding effects of drugs of abuse have been associated with their ability to increase dopamine release, particularly in the nucleus accumbens (Di Chiara and Imperato, 1988). It is of importance to note that ibogaine reduced the cocaine-induced dopamine release in the nucleus accumbens (Broderick et al., 1992). Thus, an anti-addictive effect of ibogaine might be explained by its inhibitory effect on dopaminergic neurotransmission, which seems of importance for rewarding processes. However, the interaction between ibogaine and dopamine neurotransmission has not been shown conclusively, mainly due to controversial data. For example, a recent study indicated that ibogaine (40 mg/kg i.p.) potentiates the cocaine-induced increase in extracellular dopamine levels in striatum and nucleus accumbens (Maisonneuve and Glick, 1992). Thus, in contrast to the previous data, this might indicate a stimulatory effect of ibogaine on the reinforcing properties of cocaine.

4.3. Serotonergic system

Stimulation of the serotonergic system by the 5-HT uptake inhibitor, fluoxetine, attenuates cocaine self-administration in animals (Richardson and Roberts, 1991). It has been shown that ibogaine inhibits the enzymic oxidation of 5-HT in the periphery (Barras and Coult, 1972). However, it is not known whether such a relationship exists in the CNS. There seems to be no direct evidence that an ibogaine-induced derangement of 5-HT systems might affect the rewarding properties of cocaine. This possibility remains to be examined.

4.4. Central neuronal excitability

Ibogaine increases arousability (Schneider and Sigg, 1957), which might affect behaviour. The proconvulsant effect of ibogaine (40 mg/kg i.p.) lasting several hours that we observed in our EEG study (unpublished data), supports the idea that ibogaine significantly affects the responsiveness of central neurons. A proconvulsant state is probably incompatible with self-administration behaviour. However, it is less clear why cocaine intake is decreased in the absence of a proconvulsant EEG pattern, more than 48 h after ibogaine administration.

In conclusion, these experiments indicate that ibogaine inhibits cocaine intake in rats. This effect could be potentiated by repeated injections of ibogaine, once each week. Although the mechanism of action of ibogaine remains to be established, the present results suggest the presence of an anti-addictive and long-lasting metabolite(s) of ibogaine or its irreversible/long-lasting derangement of an addictive mechanism in cocaine-dependent animals.

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BRAIN RESEARCH

Research report

Effects of *iboga* alkaloids on morphine and cocaine self-administration in rats: relationship to tremorigenic effects and to effects on dopamine release in nucleus accumbens and striatum

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Abstract

Ibogaine, a naturally occurring alkaloid, has been claimed to be effective in treating addiction to opioid and stimulant drugs and has been reported to decrease morphine and cocaine self-administration in rats. The present study sought to determine if other *iboga* alkaloids, as well as the chemically related *harmala* alkaloid harmaline, would also reduce the intravenous self-administration of morphine and cocaine in rats. Because both ibogaine and harmaline induce tremors, an effect that may be causally related to neurotoxicity in the cerebellar vermis, the temorigenic activities of the other *iboga* alkaloids were assessed. Lastly, in view of the involvement of the dopaminergic mesolimbic system in the actions of drugs of abuse, the effects of some of the *iboga* alkaloids on extracellular levels of dopamine and its metabolites in the nucleus accumbens and striatum were determined. All of the tested alkaloids (i.e., ibogaine, tabernanthine, R- and S-coronaridine, R- and S-ibogamine, desethylcoronaridine, and harmaline) dose-dependently (2.5–80 mg/kg) decreased morphine and cocaine intake in the hour after treatment; decreases in morphine and cocaine intake intake were also apparent the day after administration of some but not all of these alkaloids (i.e., ibogaine, tabernanthine, desethylcoronaridine, and the R-isomers of coronaridine and ibogamine). In some rats, there were persistent decreases in morphine or cocaine intake for several days after a single injection or after two or three weekly injections of one or another of these alkaloids; R-ibogamine produced such effects more consistently than any of the other alkaloids. At the doses used to assess effects on drug self-administration, ibogaine, tabernanthine, desethylcoronaridine and harmaline all induced tremors for at least 2–3 h; both enantiomers of both coronaridine and ibogamine induced very weak or no tremors. Using in vivo microdialysis, the effects of the R- and S-enantiomers of coronaridine and ibogamine on extracellular dopamine levels in the nucleus accumbens and striatum were compared. The R-enantiomers decreased dopamine levels in both brain regions whereas the S-enantiomers produced no significant changes in dopamine levels in either region. The results of this study indicate that the 'anti-addictive' and tremorigenic effects of the *iboga* alkaloids can be dissociated and that long-term effects of these alkaloids on drug self-administration appear to be related to initial decreases in dopaminergic activity in specific brain areas.

Key words: Ibogaine; Harmaline; Tabernanthine; Coronaridine; Ibogamine; Desethylcoronaridine; Morphine; Cocaine; Drug self-administration

1. Introduction

Ibogaine, one of several alkaloids found in the root bark of the African shrub *Tabernanthe iboga*, has been claimed, in two United States patents (number 4,499,096, Feb. 12, 1985 and number 4,587,243, May 6, 1986), to be effective in treating opiate (heroin) addiction and stimulant (cocaine and amphetamine) abuse, respectively. The treatment supposedly interrupts the 'physiological and psychological aspects' of addiction and eliminates the desire to use drugs. In both opiate and stimulant syndromes, a single oral treatment of

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ibogaine or its salts in dosages of 6 to 19 mg/kg is said to be effective for about 6 months; a series of four treatments is claimed to eliminate addictive behavior for approximately 3 years. Using an animal model of drug addiction, we have sought to determine whether these claims can be substantiated under controlled conditions. In our initial study [12], ibogaine dose-dependently decreased morphine self-administration in the hour after ibogaine treatment (acute effect) and, to a lesser but significant extent, a day later (aftereffect). In some rats there was a persistent decrease in morphine intake for several days or weeks after a single injection of ibogaine whereas other rats began to show such persistent changes after two or three weekly injections, and a few rats appeared to be entirely resistant to prolonged aftereffects. Similar effects of ibogaine on cocaine self-administration in rats were recently reported by Cappendijk and Dzoljic [1].

In humans, as in rats, ibogaine's efficacy as an anti-addictive therapy appears to vary substantially from one individual to another; even the most ardent supporters of ibogaine's usefulness would probably concede that at least 30% of treated addicts do not decrease their drug intake. Ibogaine also exhibits several side effects that may limit its therapeutic utility. In addition to having stimulant and hallucinogenic properties, ibogaine induces tremors. Very similar tremors are induced by harmaline, another natural alkaloid that is chemically related to ibogaine but is derived from a different plant (*Peganum harmala*). Both ibogaine- and harmaline-induced tremors appear to be due to activation of an olivo-cerebellar pathway [3,14,20], and in rats, high doses of both agents have recently been shown to produce damage to the cerebellar vermis, presumably a result of overstimulation of cerebellar Purkinje cells [19,20]. It has been suggested that ibogaine-induced cerebellar damage may mediate ibogaine's anti-addictive effects [28]. In the present study, the effects of several *iboga* alkaloids as well as harmaline on morphine and cocaine self-administration in rats were assessed. The primary objective was to determine if the anti-addictive (i.e., decreased drug self-administration) and temorigenic activities of ibogaine-like compounds could be dissociated and, as a corollary, to determine if an *iboga* alkaloid could be identified that was less temorigenic (i.e., probably safer) yet more effective as an anti-addictive agent than ibogaine.

2. Materials and methods

2.1. Drugs

Ibogaine hydrochloride and harmaline hydrochloride were purchased from the Sigma Chemical Company (St. Louis, MO). The R- and S-enantiomers of ibogamine and coronaridine (structures shown in [2]) as well as racemic desethylcoronaridine were synthesized by M.E. Kuehne, T.E. Wilson and D. Larson at the University of Vermont. Tabernanthine was supplied by P. Potier, CNRS, Institute of Chemistry of Natural Substances, Gif-sur-Yvette, France. All drugs were administered intraperitoneally; doses are expressed as the hydrochloride salts. Different drugs and doses (or saline) were administered to different groups of rats; rats were injected fifteen minutes before a morphine or cocaine self-administration session. Drug injections were usually made on Wednesdays and, in some cases, repeated injections were made at weekly intervals.

2.2. Subjects and apparatus

The subjects were naive female Sprague–Dawley (Taconic, Germantown, NY) rats approximately 3 months old and weighing 230–250 g at the beginning of the experiment; female rats were used because they grow at a much slower rate than males and are less likely than males to outgrow their intravenous cannulas. Rats were housed singly in Wahmann hanging cages and maintained on a normal light/dark cycle (lights on/off at 7:00 a.m./7:00 p.m.). All self-administration testing was conducted in twelve BRS/LVE operant test cages, each enclosed in a sound attenuated cubicle. Responses on either of two levers (mounted 15 cm apart on the front wall of each test cage) were recorded on an IBM compatible 386 computer with a Med Associates, Inc. interface. The intravenous self-administration system consisted of polyethylene-silicone cannulas constructed according to the design of Weeks [30], BRS/LVE harnesses and commutators, and Harvard Apparatus infusion pumps (no. 55–2222).

2.3. Self-administration procedures

Shaping of the bar-press response was initially accomplished by training rats to bar-press for water. Cannulas were then implanted in the external jugular vein according to procedures described by Weeks [30]. Self-administration testing began with a single 24-h session followed by daily 1-h sessions, 5 days (Monday-Friday) a week; rats were tested about the same time each day, during the middle of the light cycle. Depending upon the group, a lever-press response produced either a 20 μl (morphine) or 50 μl (cocaine) infusion of drug solution (0.01 mg of morphine sulfate or 0.1 mg of cocaine hydrochloride) in about 0.2 (morphine) or 0.5 (cocaine) second. Since all rats generally weighed 250 ± 20 g, each response delivered approximately 0.04 mg/kg of morphine or 0.4 mg/kg of cocaine; these doses are about two to four times the threshold doses required for maintaining self-administration behavior (e.g. [8,10]). One non-contingent drug infusion was administered at the beginning of each session. Experiments to assess the effects of the *iboga* and *harmala* alkaloids were begun when baseline self-administration rates stabilized ($\leq 10\%$ variation from one day to the next across 5 days), usually after 2 weeks of testing.

2.4. Microdialysis and HPLC procedures

Under pentobarbital anesthesia, rats (female Sprague–Dawley) were implanted stereotaxically with guide cannulas over the nucleus accumbens and striatum so that, when inserted, the tips of the dialysis probes would be located in the nucleus accumbens (rostral, +1.6 mm from bregma; lateral, ±1.5 mm; ventral, −8.6 mm from the skull surface) and in the striatum (rostral, +0.5 mm; lateral, ±2.9 mm; ventral, −7.0 mm) [21]. The two cannulas were fixed firmly in the skull with dental cement. One cannula was implanted in the left side of the brain, and the other in the right side of the brain; the side (left or right) assigned to each region (nucleus accumbens or striatum) was alternated from animal to animal.

At least 4 days after surgery, a rat was placed in a dialysis chamber, a cylindrical (30 cm diameter) Plexiglas cage providing free access to food and water. Probes (3 mm; BAS/CMA MF-5393) were then lowered into the guide cannulas. The dialysis probes were continuously perfused with a solution containing 146 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 0.05 mM ascorbic acid at a flow rate of 1 μl/min. On the next morning (15–20 h later), the dialysis experiment was carried out on a freely moving animal. Twenty minute fractions were collected in vials containing 2 μl of 5 N perchloric acid solution (containing 5 mg/l EDTA and 5 mg/l sodium metabisulfite). Upon completion of an experiment, rats were killed and histological analysis [26] of each brain was performed to verify the locations of the two probes.

Perfusate samples were analyzed by HPLC with electrochemical detection. The HPLC consisted of a Waters pump (model 510), a WISP autosampler (model 712), a Phase Separation Spherisorb column (S3 ODS2; 10 cm×4.6 mm) and a Waters detector (model 464). The mobile phase consisted of 6.9 g/l sodium monobasic phosphate, 495 mg/l heptane sulfonic acid, 80 mg/l disodium EDTA, and 100 ml/l methanol; the solution was adjusted with HCl to pH 3.7 and was pumped at a rate of 1.2 ml/min. Chromatograms were processed using Maxima 820 software.

Concentration estimates were expressed as uM or nM concentrations of the compounds in the dialysate fluid. Previous work has demonstrated that dialysate concentrations of dopamine (DA) and DOPAC are highly correlated ($r \geq 0.9$) with extracellular levels of these compounds as determined in 'no net flux' studies [9].

Prior to use in vivo, in order to identify 'bad' probes [9], each probe was calibrated in vitro at room temperature in an artificial CSF solution gassed with argon and containing DA (15 pm/ml), DOPAC (1.5 nm/ml) and HVA (0.75 nm/ml). In vitro recoveries averaged 30–35% and probes having in vitro recoveries of less than 20% were not used in vivo.

2.5. Tremor testing procedures

Whole body tremors were assessed in two ways. Direct visual observations were made of rats confined in a Plexiglas cylindrical (9 inches in diameter) enclosure; videotapes were sometimes made so that initial observations could be confirmed at a later time. Tremors were rated as absent, moderate or intense on a minute to minute basis for 30 min, beginning 15 min after drug administration. An automated and quantitative technique, based on a method originally designed for mice by other investigators [22], was also developed and utilized. Briefly, a Plexiglas enclosure was mounted on an audio speaker, the output of which was connected to a Hewlett-Packard 3392A integrator; the sensitivity of the integrator was adjusted such that random locomotor activity was generally ignored while large peaks representing tremors could be readily identified. Tremors were recorded and counted for 30 min, beginning 15 min after drug administration.

3. Results

3.1. Drug self-administration

Fig. 1 shows the initial acute effects of all of the alkaloids on morphine and cocaine self-administration. Each drug produced a dose-related depression of morphine and cocaine intake (ANOVA, $P < 0.001$ in every case). The potencies of the various alkaloids were very similar with the exception that desethylcoronaridine was approximately twice as potent as any of the other

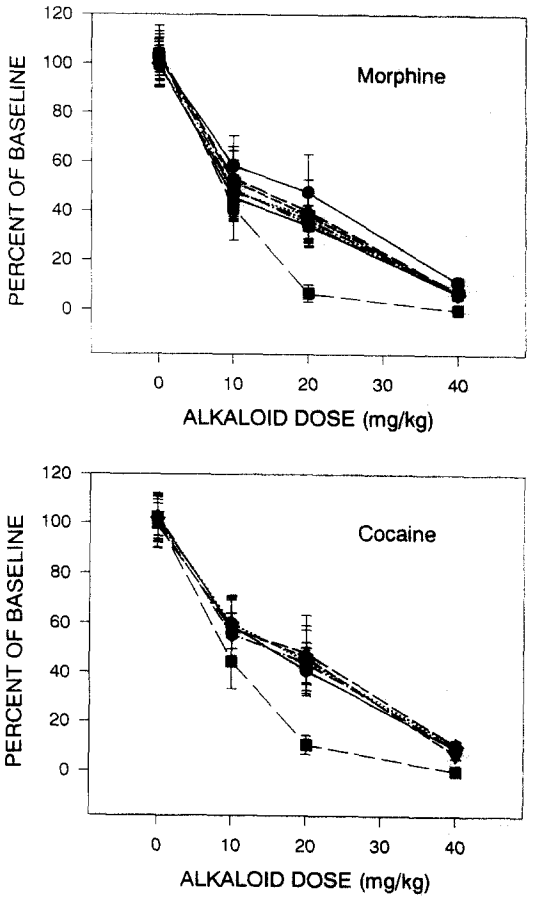

Fig. 1. Acute effects of ibogaine, harmaline, tabernanthine, desethylcoronaridine, R-coronaridine, S-coronaridine, R-ibogamine and S-ibogamine on morphine and cocaine self-administration. Each data point is the mean (±S.E.) from 3–8 rats. Baseline was calculated as the average rate for the three sessions preceding drug or saline (0 mg/kg) treatment. All doses of all drugs had significant effects (ANOVA and t-tests, $P < 0.05$–0.001); the only drug that differed significantly from any other was desethylcoronaridine (dashed line with square data points), which was approximately twice as potent as all of the other drugs.

drugs. Figs. 2 and 3 show that ibogaine (40 mg/kg), R-ibogamine (40 mg/kg), R-coronaridine (40 mg/kg), tabernanthine (40 mg/kg) and desethylcoronaridine (20 mg/kg), each administered for the first time, depressed morphine and cocaine intake for at least a day afterwards. In each of these cases, a group × days interaction was significant ($P < 0.05$ in a two-way ANOVA), and paired t-tests with baseline values were significant ($P < 0.05$–0.01) for days 1 and 2 in the indicated treatment groups. The extent of these aftereffects (one or more days later) on drug self-administration varied substantially from rat to rat; responses beyond a day later (Day 2) ranged from no further effect to a prolonged depression of morphine or cocaine intake, lasting up to several weeks in a few rats. In general, the aftereffects on cocaine intake were more variable than those on morphine intake; R-iboga-

mine produced the most consistent aftereffects on both morphine and cocaine self-administration.

Fig. 4 shows individual examples of the aftereffects of several of the *iboga* alkaloids. When such aftereffects were not apparent for particular rats, repeated injections of the same alkaloids were made, usually at weekly intervals. Fig. 5 shows examples of data from rats that were administered an *iboga* alkaloid three times: on the first two occasions there were no obvious effects beyond the day of injection whereas, after the third injection, morphine or cocaine intake was clearly depressed for at least several days afterwards. Some rats showed prolonged aftereffects following a second injection whereas others showed no aftereffects lasting more than a day even following three injections. There was one exception to the latter generality: prolonged aftereffects always appeared to occur by the third injection of R-ibogamine.

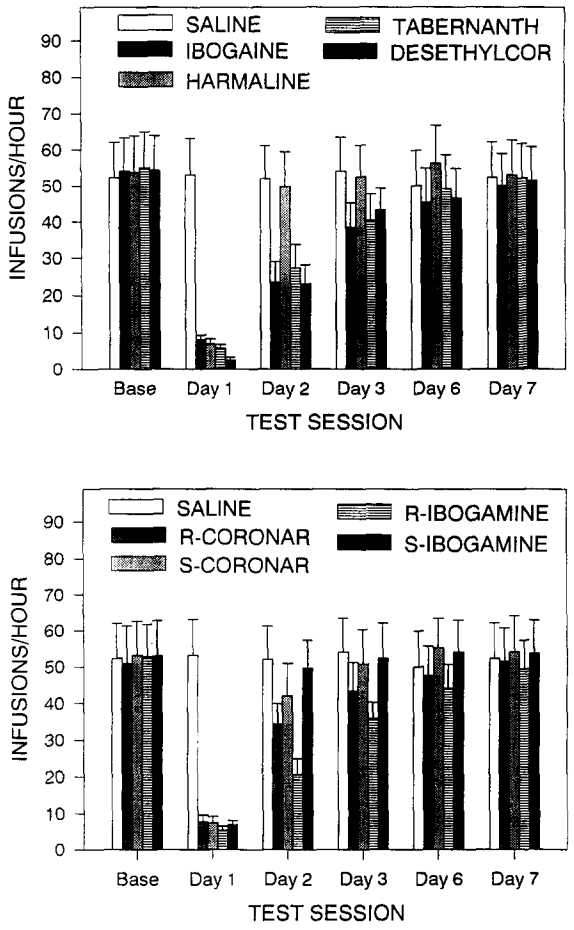

Fig. 2. Aftereffects of alkaloids (20 mg/kg for desethylcoronaridine and 40 mg/kg for all others) on morphine self-administration. Each data point is the mean (±S.E.) from 5–8 rats. 'Base' refers to the baseline rate of responding, calculated as the average for the three sessions preceding drug or saline treatment. There were significant effects on Day 1 for all drugs (see Fig. 1) and on Day 2 for ibogaine, tabernanthine, desethylcoronaridine, R-coronaridine and R-ibogamine (ANOVA and *t*-tests, $P < 0.05$–0.001).

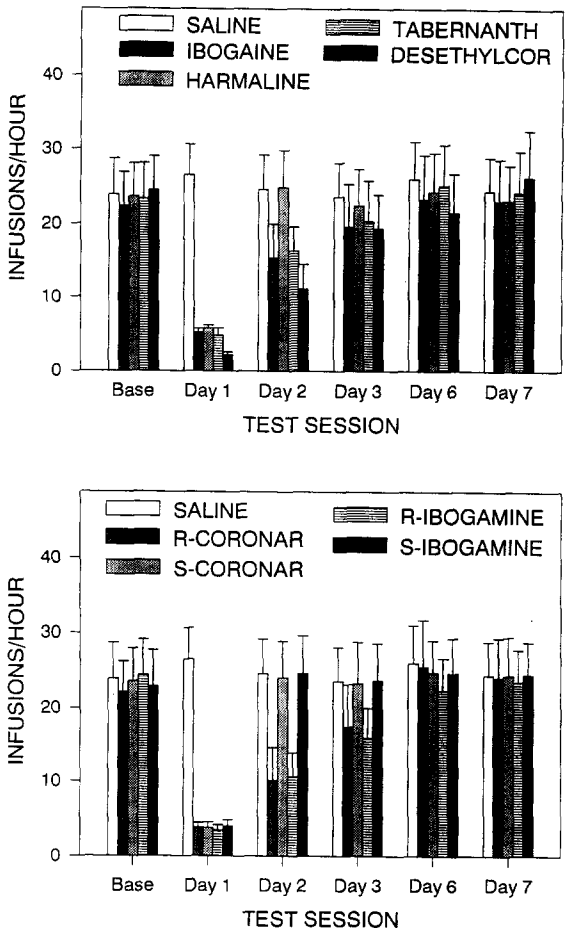

Fig. 3. Aftereffects of alkaloids (20 mg/kg for desethylcoronaridine and 40 mg/kg for all others) on cocaine self-administration. Each data point is the mean (±S.E.) from 4–8 rats. 'Base' refers to the baseline rate of responding, calculated as the average for the three sessions preceding drug or saline treatment. There were significant effects on Day 1 for all drugs (see Fig. 1) and on Day 2 for ibogaine, tabernanthine, desethylcoronaridine, R-coronaridine and R-ibogamine (ANOVA and *t*-tests, $P < 0.05$–0.001).

The Med Associates interface and software provided cumulative and event records of responding for each self-administration test session. For both morphine and cocaine, normal patterns of responding were characterized by an initial burst of drug intake at the beginning of each session followed by regularly spaced responding thereafter. Two kinds of alkaloid-induced effects were clearly apparent. Acutely, on the day of administration, the dose-related depressions of morphine and cocaine intake induced by all of the alkaloids were characterized by complete suppression of responding early in a session followed by irregularly spaced and sporadic responding thereafter; the highest dose of each of the alkaloids suppressed responding almost entirely in most animals. In contrast, the prolonged aftereffects on morphine and cocaine intake induced by some of the alkaloids were characterized by quite normal patterns of responding; that is, although

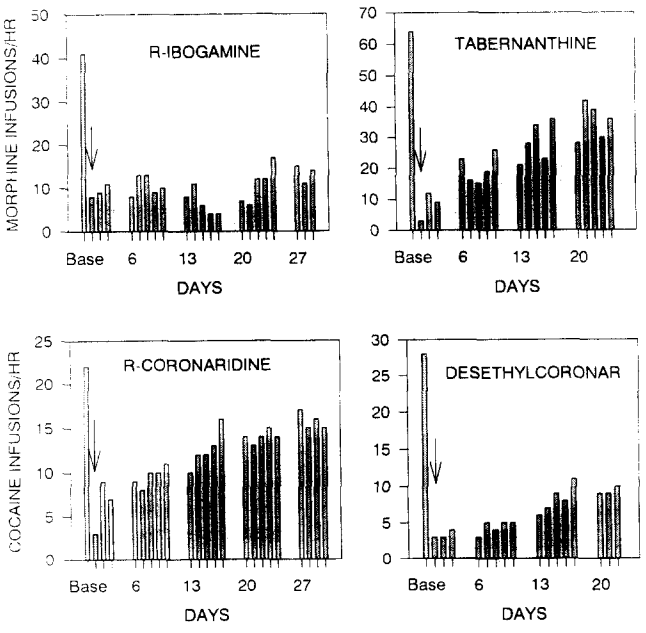

Fig. 4. Individual examples of long-term aftereffects of *iboga* alkaloids on morphine and cocaine self-administration; the doses administered (indicated by arrow) were 20 mg/kg for desethylcoronaridine and 40 mg/kg for the other drugs.

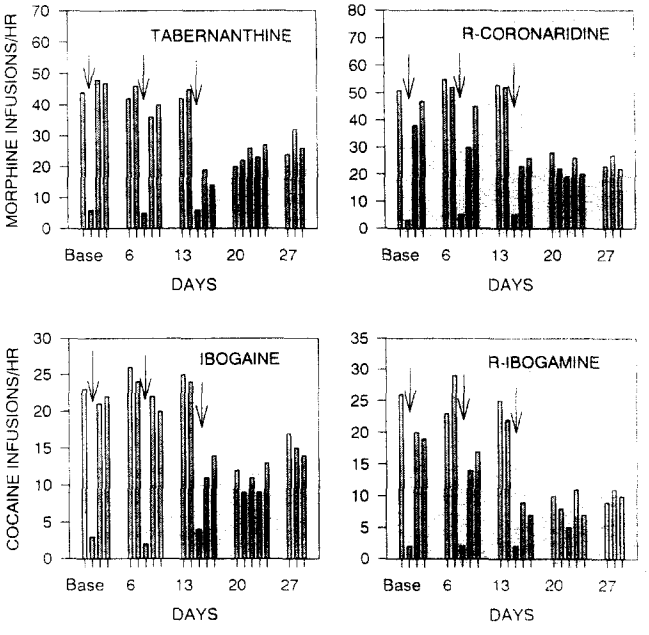

Fig. 5. Individual responses to repeated injections (indicated by arrows) of *iboga* alkaloids (40 mg/kg for all drugs); in each case, prolonged aftereffects only became apparent after the third injection.

responding was depressed, the effects were distributed uniformly throughout a test session such that the initial burst in responding was shorter and subsequent responses were more widely spaced than during baseline conditions. Fig. 6 shows typical cumulative records of cocaine self-administration by a rat treated with R-ibogamine (single injection of 40 mg/kg); the upper panel is a record of baseline responding on the day prior to R-ibogamine treatment while the bottom panel is the record of responding on the fifth day after R-ibogamine treatment.

3.2. *In vivo microdialysis*

Figs. 7 and 8 show the acute effects of R- and S-ibogamine and R- and S-coronaridine, respectively, on extracellular levels of dopamine (DA) in the nucleus accumbens (NAC) and striatum (STR). The R-

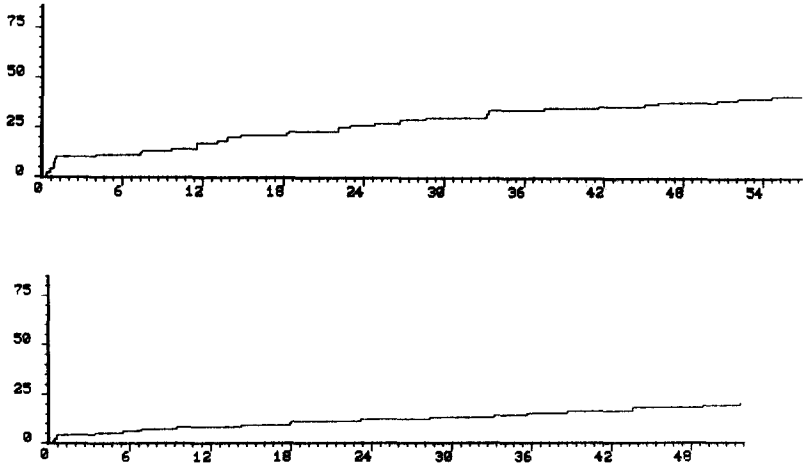

Fig. 6. Cumulative response records (ordinate, responses; abscissa, minutes) of intravenous cocaine self-administration (each response delivered 0.4 mg/kg cocaine) illustrating prolonged aftereffect of R-ibogamine. The upper panel is a record of baseline responding on the day prior to R-ibogamine (40 mg/kg, i.p.) treatment while the bottom panel is the record of responding on the fifth day after R-ibogamine treatment.

enantiomers of ibogamine and coronaridine significantly decreased DA levels (ANOVA, $P < 0.05$–0.01) while the S-enantiomers had no significant effects. There were no significant changes in DOPAC or HVA.

3.3. Tremorigenic effects

Ibogaine (20–40 mg/kg), harmaline (10–40 mg/kg) and desethylcoronaridine (10–40 mg/kg) were obviously tremorigenic for 3–4 h and no attempt was made to compare these drugs quantitatively. However, visual observations and videotape recordings of both R- and S-enantiomers of both ibogamine (40 mg/kg) and coronaridine (40 mg/kg) indicated very little if any tremorigenic activity. The effects of these latter drugs were therefore assessed using the automated testing procedure developed to quantitate tremors. Ibogaine (40 mg/kg) and saline (1 ml/kg) were used as positive and negative controls, respectively. While ibogaine produced a significant increase in movements indicative of tremors, none of the ibogamine and coronaridine enantiomers had effects that differed significantly from the effects of saline.

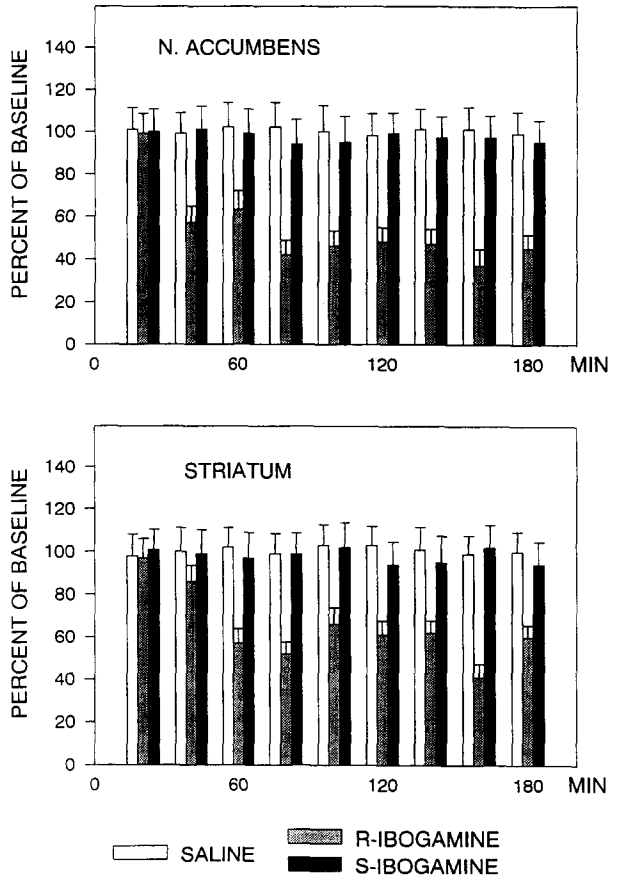

Fig. 7. Time course of extracellular dopamine levels in the nucleus accumbens and striatum after administration of saline ($n = 6$), R-ibogamine (40 mg/kg, $n = 6$), and S-ibogamine (40 mg/kg, $n = 7$). Samples were collected at 20 minute intervals. Data are expressed as a percent (±S.E.) of baseline dialysate values.

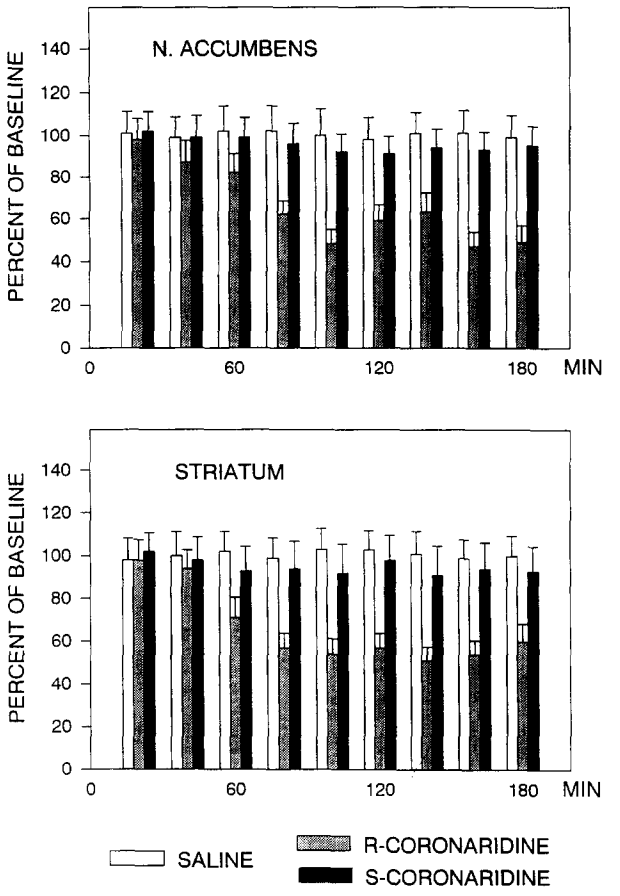

Fig. 8. Time course of extracellular dopamine levels in the nucleus accumbens and striatum after administration of saline ($n = 6$), R-coronaridine (40 mg/kg, $n = 5$), and S-coronaridine (40 mg/kg, $n = 6$). Samples were collected at 20 min intervals. Data are expressed as a percent (±S.E.) of baseline dialysate values.

4. Discussion

All of the *iboga* alkaloids as well as harmaline produced acute reductions in both morphine and cocaine self-administration. These effects may have occurred for a variety of reasons; while specific increases or decreases in the reinforcing efficacies of morphine and cocaine may have occurred, the alkaloids may also have induced non-specific malaise and/or motor deficits incompatible with performance of the operant response. In particular, it would appear that the whole body tremors induced by ibogaine and some of the other alkaloids may have made it difficult or impossible to execute coordinated motor responses. It should be noted, however, that aside from tremors, no other signs of overt toxicity were observed, and that with the ibogamine and coronaridine enantiomers, even tremors were hardly evident.

The aftereffects of the alkaloids on drug self-administration were clearly dissociated from their tremorigenic effects. Alkaloid-induced tremors generally disappeared within 4 h after administration while, for several of the alkaloids, rates of morphine and cocaine self-administration were significantly decreased for one or more days afterwards. Moreover, the efficacies of the alkaloids to induce tremors were unrelated to their efficacies to induce aftereffects on drug self-administration. Thus harmaline, which produces tremors that are very comparable to those produced by ibogaine [20,33], had no significant aftereffects on drug self-administration; and whereas the R-enantiomers of both ibogamine and coronaridine had significant aftereffects on drug self-administration, neither the R- nor the S-enantiomers of these agents produced any significant tremorigenic activity.

Although the acute rate-depressant effects of the alkaloids may have been non-specific, there are several indications that the aftereffects resulted from persistent modulatory actions of the alkaloids on the reinforcing efficacies of morphine and/or cocaine. The same dose of ibogaine that had persistent effects on morphine and cocaine self-administration was shown, in a previous study [12], to have no effect beyond the day of administration in rats bar-pressing for water. In the present study, the aftereffects of the 'active' alkaloids differed depending upon whether the reinforcer was morphine or cocaine. When percent changes on 'Day 2' data (cf. Figures 2 and 3) were compared, both ibogaine and tabernanthine were more effective (t-tests, $P < 0.05$) on morphine self-administration than on cocaine self-administration, R-coronaridine was more effective ($P < 0.05$) on cocaine self-administration than on morphine self-administration whereas R-ibogamine and desethylcoronaridine appeared to be equally effective on morphine and cocaine self-administration.

It should be noted that, in addition to the present and previous [12] results from this laboratory, other investigators have reported comparable aftereffects of ibogaine on intravenous cocaine self-administration in rats [1] as well as on oral cocaine self-administration in mice [25]; however, a lack of any aftereffects on either heroin or cocaine self-administration in rats has also been observed [5]. There were many procedural differences among these studies, including sex and strain of rats, schedule of reinforcement, and day-night cycle. The importance of each of these variables should be systematically assessed.

In a previous study [16], acute administration of ibogaine decreased extracellular levels of DA in the NAC and STR; however, the relevance of such changes to persistent effects of ibogaine on drug self-administration was unclear. In the present study, this issue was addressed by comparing the effects of the R- and S-enantiomers of ibogamine and coronaridine on NAC and STR DA. Since only the R-enantiomers of ibogamine and coronaridine had significant aftereffects on drug self-administration, it was of interest to determine if the neurochemical effects of the R- and S-enantiomers would differ as well. And, indeed, they did differ: R-ibogamine and R-coronaridine significantly decreased DA in the NAC and STR whereas S-ibogamine and S-coronaridine had no significant effects. Although it remains unclear how an acute change in extracellular DA levels can be associated with or responsible for a persistent behavioral effect, the data suggest that further investigation of such a relationship is warranted. It is conceivable, for example, that a decrease in DA release is the first step in a sequence of neurochemical changes that directly mediates a prolonged change in drug self-administration behavior.

Long-term decreases in morphine or cocaine intake lasting for several days and in some cases for several weeks after an *iboga* alkaloid treatment occurred in some rats. It was not possible to predict which rats would respond in this way, although, as noted in a previous report [12], there were again non-significant trends for rats having low baseline rates of drug intake to be less likely to exhibit such effects. When *iboga* alkaloid treatments were repeated at weekly or bi-weekly intervals, some rats that were initially resistant began to show long-term aftereffects; this suggests that there is a continuum of individual differences in sensitivity to these agents and that, with some dosage regimen of some of these agents, most or all rats would show long-term depressions of morphine or cocaine intake. It should be noted that persistent aftereffects after repeated treatment only occurred with those alkaloids producing significant 'day-after' effects and that, of all the alkaloids tested, R-ibogamine produced the most consistent prolonged aftereffects.

The mechanisms underlying the aftereffects of the *iboga* alkaloids are unknown although several possibilities have been suggested regarding ibogaine. One possibility is that the *iboga* alkaloids may persist in the body for long periods of time. In an early study using spectrofluorometry [4], the half-life of ibogaine in rodents was reported to be about one hour, and ibogaine levels in the body were undetectable a day after its administration. However, more recently, using gas chromatography mass spectrometry (GCMS), ibogaine has been detected a day after its administration in rat plasma and brain [7]; and other data indicate that the latter concentrations may be pharmacologically active [13]. Very little is known about the metabolism of ibogaine or other *iboga* alkaloids, and as suggested previously [12,16], it is possible that there are one or more active metabolites having long half-lives.

Ibogaine and related drugs (e.g., tabernanthine) have been suggested to have several neuropharmacological actions, including interactions with serotonergic [27],

muscarinic [4], and benzodiazepine receptors [29]); however, in radioligand binding studies [2], ibogaine and other *iboga* alkaloids were found to have no appreciable affinities for these receptors, although an affinity for kappa opioid receptors was demonstrated for ibogaine, tabernanthine and the racemic forms of coronardine and ibogamine. Ibogaine has been reported to potentiate the analgesic action of morphine [24], and perhaps this effect is attributable to a kappa-mu (ibogaine-morphine) opioid interaction [e.g., 6]. None of the receptor mechanisms have been shown or have even been proposed to be operative for more than a few hours after *iboga* alkaloid administration. In contrast, as reported previously ibogaine induces prolonged (at least 19 h) decreases in the extracellular [16] and tissue [17] levels of dopamine metabolites (DOPAC and HVA) in the NAC and STR; although the cellular basis for these effects are also not understood, the data are certainly consistent with the present self-administration data in terms of the well documented role of dopaminergic systems in morphine and cocaine reinforcement (e.g. 31,32]).

As noted earlier (see Introduction), Molliver [28] has proposed a novel theory that ibogaine-induced cerebellar damage may be responsible for its putative anti-addictive effects. Inasmuch as harmaline has been shown to produce comparable cerebellar damage [20], the lack of persistent effects of harmaline on drug self-administration, as observed in the present study, would appear to disprove Molliver's theory. Ibogaine-induced cerebellar damage has been most closely linked to its tremorigenic activity (cf. [20]). In the present study, the clear dissociation between the tremorigenic efficacies of other *iboga* alkaloids and their persistent effects on drug self-administration is a further indication that the cerebellum is unlikely to underlie or modulate a mechanism of addiction. Furthermore, in a recent histological study [18],we have found that ibogaine-induced cerebellar damage occurs after a high dose (100 mg/kg, i.p.), as reported by O'Hearn and Molliver [18], but not after a lower dose (40 mg/kg, i.p.) used in this and other self-administration studies [1,12].

Assuming that treatment with the 'active' *iboga* alkaloids alters the reinforcing efficacies of morphine and cocaine, the treatment-induced decreases in morphine and cocaine intake could result from either antagonism or enhancement of morphine's and cocaine's actions (e.g. [11]). That is, if an *iboga* alkaloid antagonized a self-administered drug's actions, it would be expected that rats might transiently self-administer more drug in an attempt to compensate for the reduced effect but then self-administer less drug as extinction occurred (i.e., analogous to decreasing the drug infusion dose to below threshold); if an *iboga* alkaloid enhanced a self-administered drug's actions, it would be expected that rats would also self-administer less drug but, in this case, as a way of compensating for the increased effect (i.e., analogous to increasing the drug infusion dose). Although the observed response patterns underlying the alkaloid-induced aftereffects (e.g., Fig. 6) favor the latter interpretation, and there was no evidence of a biphasic extinction pattern of responding that would support the 'antagonist' interpretation, other treatments (e.g., lesions) that disrupt drug self-administration by reducing reinforcing efficacy frequently do so by just decreasing response rates and without producing an extinction pattern of responding [23]. It is therefore not possible to discriminate between these two interpretations on the basis of the present data alone. Furthermore, in other studies involving measurement of locomotor activity, ibogaine pretreatment inhibited morphine-induced stimulation [17] and enhanced cocaine-induced stimulation [15]. Thus the effects of the *iboga* alkaloids on morphine and cocaine self-administration could even represent different kinds of drug interactions that are mediated by different mechanisms.

Acknowledgements

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Antagonism of α3β4 nicotinic receptors as a strategy to reduce opioid and stimulant self-administration

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Abstract

The *iboga* alkaloid ibogaine and the novel *iboga* alkaloid congener 18-methoxycoronaridine are putative anti-addictive agents. Using patch–clamp methodology, the actions of ibogaine and 18-methoxycoronaridine at various neurotransmitter receptor ion-channel subtypes were determined. Both ibogaine and 18-methoxycoronaridine were antagonists at α3β4 nicotinic receptors and both agents were more potent at this site than at α4β2 nicotinic receptors or at NMDA or 5-HT_3 receptors; 18-methoxycoronaridine was more selective in this regard than ibogaine. In studies of morphine and methamphetamine self-administration, the effects of low dose combinations of 18-methoxycoronaridine with mecamylamine or dextromethorphan and of mecamylamine with dextromethorphan were assessed. Mecamylamine and dextromethorphan have also been shown to be antagonists at α3β4 nicotinic receptors. All three drug combinations decreased both morphine and methamphetamine self-administration at doses that were ineffective if administered alone. The data are consistent with the hypothesis that antagonism at α3β4 receptors is a potential mechanism to modulate drug seeking behavior. 18-Methoxycoronaridine apparently has greater selectivity for this site than other agents and may be the first of a new class of synthetic agents acting via this novel mechanism to produce a broad spectrum of anti-addictive activity.

Keywords: 18-Methoxycoronaridine; Dextromethorphan; Mecamylamine; Morphine; Methamphetamine; Nicotine receptor; Drug addiction

1. Introduction

18-Methoxycoronaridine is a novel *iboga* alkaloid congener that is being studied as a potential treatment for multiple forms of drug abuse. In rats, 18-methoxycoronaridine (10–40 mg/kg) decreases the self-administration of morphine (Glick et al., 1996; Maisonneuve and Glick, 1999), cocaine (Glick et al., 1996), methamphetamine (Glick et al., 2000a), nicotine (Glick et al., 2000a) and ethanol (Rezvani et al., 1997) but does not affect responding for a non-drug reinforcer (water; Glick et al., 1996). Like its parent compound ibogaine (Glick and Maisonneuve, 1998), the precise mechanism of action of 18-methoxycoronaridine has remained elusive despite evidence that it modulates dopamine release in the nucleus accumbens (Glick et al., 1996) and binds, with low affinity, to several types of receptors (Glick and Maisonneuve, 2000; Glick et al., 2000b). In functional assays ($^{86}Rb^+$ efflux from KXα3β4R2 cells) conducted by Dr. Kenneth Kellar (Georgetown University) as part of the NIMH Psychoactive Drug Screening Program, 18-methoxycoronaridine was found to be an antagonist at α3β4 nicotinic receptors. However, this work did not establish whether 18-methoxycoronaridine's nicotinic antagonist action was specific to the α3β4 subtype or whether other nicotinic subtypes were also affected, for example, the α4β2 subtype that is most prevalent in the brain (e.g., Flores et al., 1992). Hence, as part of the present study, the actions of both 18-methoxycoronaridine and ibogaine at both α3β4 and α4β2 receptors were determined using patch–clamp methodology.

Other studies from the Kellar laboratory have shown that the antitussive dextromethorphan and its metabolite dextrorphan are also antagonists at α3β4 receptors (Hernandez et al., 2000), and we have recently found that both dextromethorphan and dextrorphan reduce morphine, methamphetamine and nicotine self-administration in rats (Glick et al., 2001). Although dextromethorphan and dextrorphan are also antagonists at NMDA glutamate receptors (Murray and Leid, 1984; Ebert et al., 1998), the relative potencies of dextromethorphan and dextrorphan in our drug self-admin-

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istration studies (Glick et al., 2001) were more consistent with actions at α3β4 receptors than at NMDA receptors. Furthermore, mecamylamine, a nonspecific nicotinic antagonist, has been reported to decrease craving for cocaine in humans (Reid et al., 1999) and to reduce cocaine self-administration in rats (Levin et al., 2000); and Papke et al. (2001) recently reported that mecamylamine has preferential affinity for α3β4 receptors versus other nicotinic subtypes (e.g., α4β2). All of these findings together suggest that antagonism of acetylcholine's actions at α3β4 nicotinic receptors may constitute an important mechanism for reducing the rewarding effects of multiple drugs; α3β4 receptors are localized in brain areas that are well suited to modulate mesolimbic activity, both directly (e.g., Klink et al., 2001) and indirectly (e.g., Nishikawa et al., 1986; Quick et al., 1999).

Totally selective antagonists of α3β4 receptors are unavailable, and hence it is difficult to directly test our hypothesis that α3β4 receptor antagonists will reduce drug self-administration. However, we reasoned that if two agents had the common action of blocking this site but also had other actions that were unique to each agent, the combination of low doses of such agents (doses of each agent that would be ineffective if administered alone) might produce additive effects at the α3β4 site and reduce drug self-administration without the involvement of other actions contributing to side effects. In the present study, as an initial test of this idea, the effects of three such combined treatments (18-methoxycoronaridine plus dextromethorphan, 18-methoxycoronaridine plus mecamylamine, and dextromethorphan plus mecamylamine) on morphine and methamphetamine self-administration were assessed.

2. Materials and methods

2.1. Receptor functional analyses

Human embryonic kidney 293 (HEK293) fibroblasts (ATCC CRL1573) were cultured in minimal essential medium supplemented with 10% fetal bovine serum and 2 mM glutamine (Life Technologies, Rockville, MD). Cells were plated on poly-D-lysine-coated 35 mm nunc dishes, transfected by the LipofectaminePLUS method (Life Technologies), and examined functionally between 18 and 48 h post transfection. The following receptor subunit cDNAs were used (accession no.): nAChR-α3 (nicotinic acetylcholine receptor-α3; L31621), nAChR-α4 (L31620), nAChR-β2 (L31622), nAChR-β4 (U42976), 5-HT_3R-A (5-HT_{3A} receptor; M74425), NR1 (*N*-methyl-D-aspartate receptor 1; X63255), NR2A (X91561), and NR2B (M91562). The nAchR and NR clones were rats; the 5-HT_3R-A clone was mouse. Co-transfection of enhanced green fluorescent protein (EGFP, 10% of total cDNA) provided a marker to identify transfected cells. Transfected cells were selected for EGFP expression and examined by voltage-clamp recording in the whole-cell configuration using an Axopatch 200B patch–clamp amplifier (Axon Instruments, Foster City, CA). Thin-walled borosilicate glass microelectrodes (TW150F, World Precision Instruments, Sarasota, FL) had resistances of 3–5 MΩ when filled with an internal solution containing (in mM): 135 CsCl, 10 CsF, 10 HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]), 5 EGTA (ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid), 1 $MgCl_2$, 0.5 $CaCl_2$, pH 7.2. Whole-cell capacitance and series resistance were recorded and adequately compensated using the available circuitry of the amplifier. Current responses were filtered at 1 kHz with an 8-pole Bessel filter (Cygnus Technologies, Delaware Water Gap, PA), digitized at 3 kHz, and stored on a Macintosh PowerPC-G3 computer using an ITC-16 interface (Instrutech, Great Neck, NY) under control of the data acquisition and analysis program Synapse (Synergy Research, Gaithersburg, MD). Cells were continuously superfused with extracellular solution containing (in mM): 150 NaCl, 3 KCl, 5 HEPES, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 0.1 mg/ml phenol red, pH 7.3 ($MgCl_2$ was omitted from all solutions used for the study of NMDA receptors). Drug stocks (10 mM) were made up in DMSO (dimethylsulfoxide) and diluted in extracellular solution immediately prior to use; final concentration of DMSO was 0.2% or lower. Control, agonist, and drug solutions were applied to individual cells by rapid perfusion. Solutions were driven by a syringe pump through a flowpipe having four inputs that converge at a single common output of approximately 100 μm diameter. Rapid switching between inputs was achieved using a set of upstream solenoid valves (Lee, Westbrook, CT) under computer control; the solution exchange rate was ~ 5 ms as measured from liquid junction currents.

2.2. Chemicals used in vivo

18-Methoxycoronaridine hydrochloride (1–2 mg/kg; Albany Molecular Research, Albany, NY) was dissolved in phosphate buffer and injected intraperitoneally 15 min before behavioral testing. Dextromethorphan hydrobromide (5 mg/kg; Sigma/RBI, St. Louis, MO) was dissolved in saline and injected subcutaneously 20 min before testing. Mecamylamine hydrochloride (1 mg/kg; Sigma/RBI) was dissolved in physiological saline and injected intraperitoneally 30 min before testing. All rats received two injections (for rats that received a single drug, half of them also received the appropriate saline/vehicle injection corresponding to each of the other two drugs).

2.3. Animals

Naïve female Long–Evans derived rats (250 g; Charles River, NY) were maintained on a normal 12 h light cycle (lights on at 7:00 a.m., lights off at 7:00 p.m.). For all experiments the "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1985) were followed.

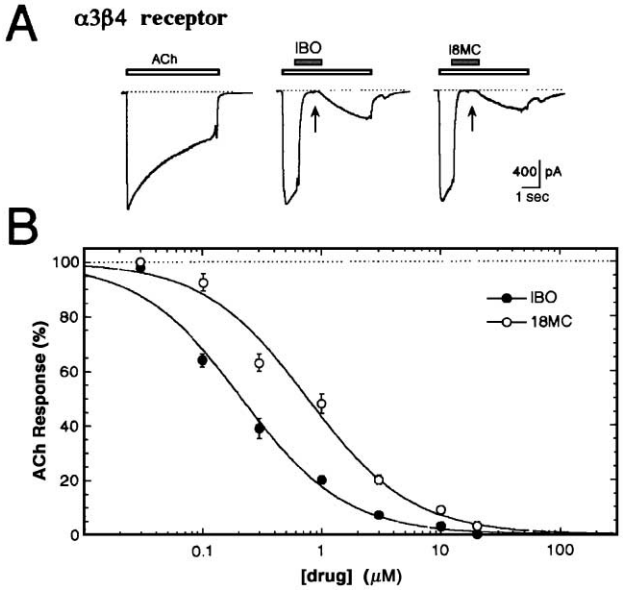

Fig. 1. Inhibition of α3β4 nicotinic receptors by ibogaine (IBO) and 18-methoxycoronaridine (18MC). Recombinant receptors were expressed in HEK293 cells and examined by whole-cell patch clamp recording with rapid application of agonist and drug solutions. (A) Whole-cell currents evoked by 1 mM ACh in transfected cells. Open bars depict the timing of ACh application. Filled bars depict the timing of co-application of 20 μM IBO or 20 μM 18MC. Inhibition was measured relative to control at the end of the drug application (arrows). (B) Inhibition of 1 mM ACh-evoked currents by various concentrations of IBO and 18MC. Data are mean ± SEM for 3–14 cells per point. Curve fits are given for the logistic equation: $I = I_{max}/(1+([drug]/IC_{50}))$. Best fitting IC_{50} values were 0.22 μM for IBO and 0.75 μM for 18MC.

2.4. Self-administration procedure

The intravenous self-administration procedure has been described previously (e.g., Glick et al., 1996, 2000a). Briefly, responses on either of two levers (mounted 15 cm apart on the front wall of each operant test cage) were recorded on an IBM compatible computer with a Med Associates interface. The intravenous self-administration system consisted of polyethylene-silicone cannulas constructed according to the design of Weeks (1972), Instech harnesses and swivels, and Harvard Apparatus infusion pumps (no. 55-2222). Shaping of the bar-press response was initially accomplished by training rats to bar-press for water. Cannulas were then implanted in the external jugular vein according to procedures described by Weeks (1972). Self-administration testing began with a 16-h nocturnal session followed by daily 1-h sessions, 5 days (Monday–Friday) a week. A lever-press response produced a 10-μl infusion of drug solution (0.01 mg of morphine sulfate) in about 0.2 s or a 50-μl infusion of drug solution (0.025 mg of methamphetamine sulfate) in about 1 s. Since all rats generally weighed 250 ± 20 g, each response delivered approximately 0.04 mg/kg of morphine or 0.1 mg/kg of methamphetamine. Experiments to assess the effects of experimental treatments were begun when baseline self-administration rates stabilized (< 10% variation from 1 day to the next across 5 days), usually after 2 weeks of testing. Each rat typically received two or three different treatments spaced at least 1 week apart. In order to provide an indication of the specificity of treatment effects on drug self-administration, all treatments were also administered to other rats bar-pressing for water (0.01 ml orally) on a comparable schedule (continuous reinforcement; 1-h sessions).

3. Results

3.1. Drug actions at neurotransmitter receptor ion-channels

Transfected HEK293 cells expressing various receptor subunit cDNAs were examined by whole-cell patch–clamp recording with fast perfusion of agonist and drug solutions. We began with α3β4 nicotinic acetylcholine receptors as these are the principal ganglionic nicotinic acetylcholine receptor subtype for which inhibition by ibogaine had previously been demonstrated (Badio et al., 1997; Mah et al., 1998; Fryer and Lukas, 1999). Transfected HEK293 cells expressing α3β4 nicotinic acetylcholine receptors were voltage-clamped to − 70 mV and stimulated with 1 mM acetylcholine at 30 s intervals. Acetylcholine alone evoked a large inward current not seen in untransfected cells. Application of 20 μM ibogaine or 20 μM 18-methoxycoronaridine alone did not produce any response. Co-application of either 20 μM ibogaine or 20 μM 18-methoxycoronaridine nearly abolished the ACh-evoked responses in all cells tested ($N = 15$) (Fig. 1A). The inhibition developed rapidly in the presence of acetylcholine and reversed more slowly following the removal of drug. The inhibition was concen-

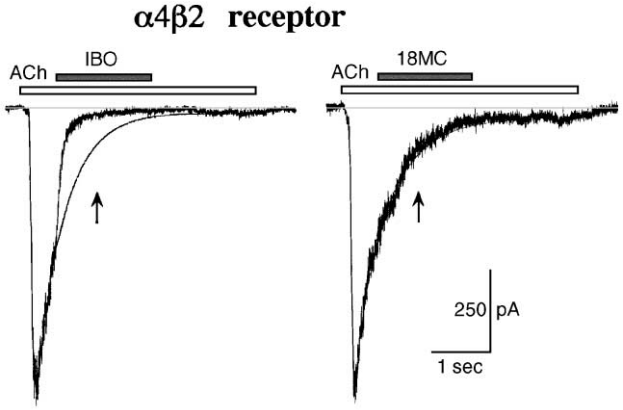

Fig. 2. Inhibition of α4β2 nicotinic receptors by ibogaine (IBO) but not by 18-methoxycoronaridine (18MC). Whole-cell currents in cells expressing recombinant α4β2 receptors were evoked by 300 μM ACh. Open bars depict the timing of ACh application. Filled bars depict the timing of co-application of 20 μM IBO or 20 μM 18MC. Because of the relatively rapid desensitization of the α4β2 response, inhibition was measured relative to control (superimposed curve fits) during drug application (arrows).

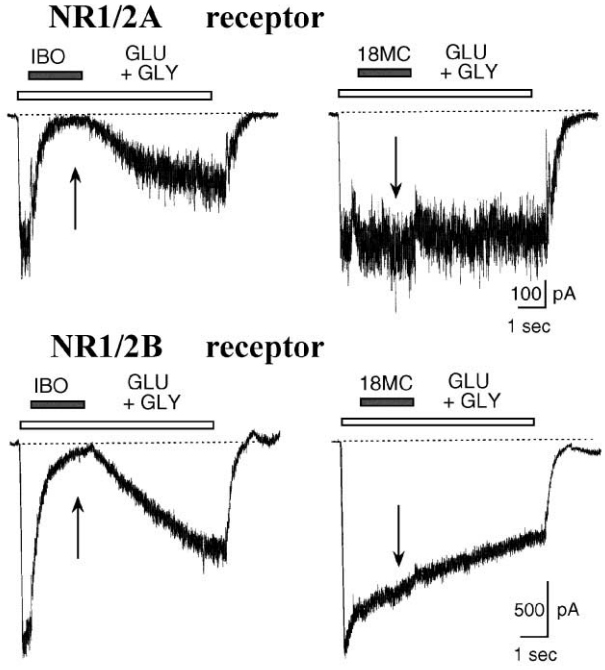

Fig. 3. Inhibition of NMDA receptors by ibogaine (IBO) but not by 18-methoxycoronaridine (18MC). Whole-cell currents in cells expressing recombinant NR1/2A and NR1/2B receptors were evoked by 100 μM glutamate plus 10 μM glycine. Open bars depict the timing of agonist application. Filled bars depict the timing of co-application of 20 μM or 20 μM 18MC. Inhibition was measured relative to control at the end of the drug application (arrows).

tration-dependent; IC_{50} values were 0.22 μM for ibogaine versus 0.75 μM for 18-methoxycoronaridine, and the concentration–response relationship had a Hill slope of unity, which is consistent with a single site of action (Fig. 1B). These data confirm previous reports of the actions of ibogaine and indicate that 18-methoxycoronaridine has similar actions at ganglionic nicotinic acetylcholine receptors. Results were somewhat different, however, at neuronal nicotinic acetylcholine receptors. In this case, transfected HEK293 cells expressing α4β2 nicotinic acetylcholine receptors were voltage-clamped to −70 mV and stimulated with 300 μM acetylcholine at 30-s intervals. Acetylcholine alone evoked an inward current whereas application of 20 μM ibogaine or 20 μM 18-methoxycoronaridine alone did not produce any response. Co-application of 5 μM ibogaine inhibited the ACh-evoked response by 61 ± 4% ($N=5$) whereas 5 μM 18-methoxycoronaridine produced no apparent inhibition ($N=6$). At higher drug concentrations (20 μM) (Fig. 2), ibogaine inhibition reached 93 ± 3% ($N=7$) versus only 8 ± 4% by 18-methoxycoronaridine ($N=6$). These data suggest the IC_{50} for ibogaine at α4β2 nicotinic acetylcholine receptors is on the order of 1–5 μM and that 18-methoxycoronaridine is considerably less potent ($IC_{50}>20$ μM) at this neuronal nicotinic acetylcholine receptor subtype.

Another known action of ibogaine involves the inhibition of NMDA-type glutamate receptors (Popik et al., 1995; Chen et al., 1996) presumably by interaction with the PCP/MK-801 (phencyclidine/dizocilpine) binding site (Sweetnam et al., 1995; Chen et al., 1996). Indeed, it has been suggested that the NMDA receptor-mediated actions of ibogaine may be central to its putative anti-addictive properties (Popik et al., 1995). To study these, transfected HEK293 cells expressing NR1/2A or NR1/2B receptors were voltage-clamped to −70 mV and stimulated with 100 μM glutamate plus 10 μM glycine at 30 s intervals. Agonist application evoked a large inward current not seen in untransfected cells. Application of 20 μM ibogaine or 20 μM 18-methoxycoronaridine alone did not produce any response. Co-application of 20 μM ibogaine reduced the agonist-evoked response of NR1/2A receptors by 98 ± 3% ($N=3$) and of NR1/2B receptors by 95 ± 2% ($N=3$); inhibition by 10 μM ibogaine was 51 ± 9% ($N\pm3$) and 82 ± 3% ($N\pm3$), respectively (Fig. 3). These data are consistent with IC_{50} values of 3–5 μM obtained in hippocampal neurons (Popik et al., 1995; Chen et al., 1996). However, we also found that co-application of 18-methoxycoronaridine failed to inhibit either NR1/2A ($N=3$) or NR1/2B receptors ($N=5$) at concentrations up to 20 μM (Fig. 3). This result necessarily calls into question any role of NMDA receptors in the putative anti-addictive actions of these drugs.

There are no published studies examining the effects of ibogaine on 5-HT_3 receptor function. Although some action may be expected based on competition binding studies (Sweetnam et al., 1995), it remains to be seen whether ibogaine binding to these receptors activates, inhibits, or otherwise alters channel function. Likewise, the effects of 18-methoxycoronaridine on 5-HT_3 receptor function have

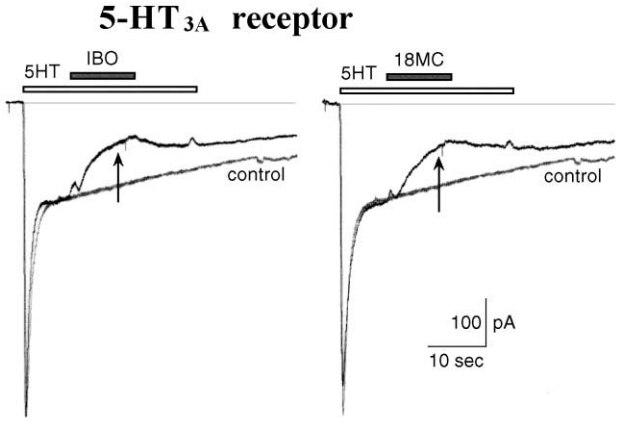

Fig. 4. Inhibition of 5-HT_3 receptors by ibogaine (IBO) and 18-methoxycoronaridine (18MC). Whole-cell currents in cells expressing recombinant 5-HT_{3A} receptors were evoked by 100 μM serotonin (5HT). Open bars depict the timing of agonist application. Filled bars depict the timing of co-application of 20 μM IBO or 20 μM 18MC. Inhibition was measured relative to control at the end of the drug application (arrows).

not been examined. Transfected HEK293 cells expressing 5-HT_{3A} receptors were voltage-clamped to −70 mV and stimulated with 100 μM serotonin at 30-s intervals. Serotonin alone evoked an inward current not seen in untransfected cells. Application of 20 μM ibogaine or 20 μM 18-methoxycoronaridine alone did not produce any response, indicating that neither is an agonist at 5-HT_3 receptors. Co-application of 20 μM ibogaine or 20 μM 18-methoxycoronaridine inhibited serotonin-evoked responses by 53 ± 3% (N=8) versus 50 ± 3% (N=4), respectively (Fig. 4). Thus, it appears that these drugs have similar potencies at 5-HT_3 receptors with IC_{50} values of approximately 20 μM.

3.2. In vivo treatment effects

Figs. 5–7 show the effects of drug treatments on morphine and methamphetamine self-administration and on responding for water. All three drug combinations, but none of the drugs administered alone, significantly decreased morphine and methamphetamine self-administration while having no effect on responding for water. The particular doses of 18-methoxycoronaridine, dextromethorphan and mecamylamine selected for study were, in each instance, based on knowledge of the respective dose–response functions. The doses of 18-methoxycoronaridine (1 and 2 mg/kg) were approximately one-fifth of those required to decrease morphine (Glick et al., 1996) and methamphetamine (Glick et al., 2000a) self-administration, respectively, when administered alone. The dose of dextromethorphan (5 mg/kg) was one-half to one-fourth of that necessary to decrease morphine and methamphetamine self-administration (Glick et al., 2001), respectively, when administered alone. The dose of mecamylamine (1 mg/kg) was one third of that required to decrease either morphine or methamphetamine self-administration, and at a dose of 3 mg/kg, mecamylamine also decreases responding for water (data not shown). Lastly, although Fig. 7 only shows results with the 2 mg/kg dosage of 18-methoxycoronaridine, virtually identical results were found with 1 mg/kg.

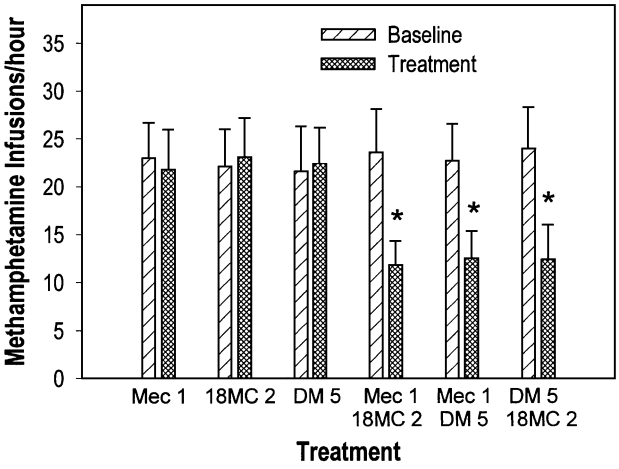

Fig. 6. Effects of drug combinations on methamphetamine self-administration. Rats were administered two of the following treatments before testing: mecamylamine (MEC; 1 mg/kg i.p., 30 min), 18-methoxycoronardine (18MC; 2 mg/kg i.p., 15 min), dextromethorphan (DM; 5 mg/kg s.c., 20 min), or vehicle (saline for MEC and DM; phosphate buffer for 18MC). Each data point represents the mean (± SEM) responses per hour of 6–7 rats. * Significant differences between baseline and treatment (paired t-test, $P<0.01$).

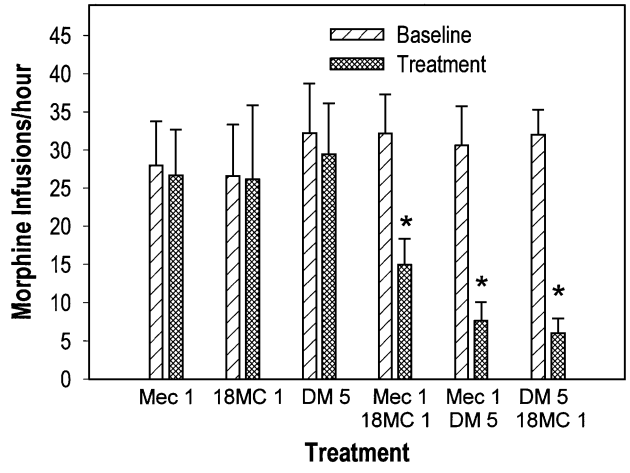

Fig. 5. Effects of drug combinations on morphine self-administration. Rats were administered two of the following treatments before testing: mecamylamine (MEC; 1 mg/kg i.p., 30 min), 18-methoxycoronardine (18MC; 1 mg/kg i.p., 15 min), dextromethorphan (DM; 5 mg/kg s.c., 20 min), or vehicle (saline for MEC and DM; phosphate buffer for 18MC). Each data point represents the mean (± SEM) responses per hour of 6–8 rats. * Significant differences between baseline and treatment (paired t-test, $P<0.01$–0.001).

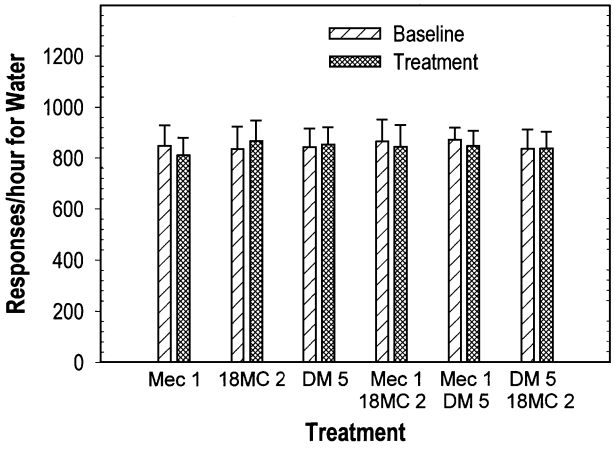

Fig. 7. Effects of drug combinations on responding for water. Rats were administered two of the following treatments before testing: mecamylamine (MEC; 1 mg/kg i.p., 30 min), 18-methoxycoronardine (18MC; 2 mg/kg i.p., 15 min), dextromethorphan (DM; 5 mg/kg s.c., 20 min), or vehicle (saline for MEC and DM; phosphate buffer for 18MC). Each data point represents the mean (± SEM) responses per hour of six rats.

4. Discussion

Previous studies have reported inhibition by ibogaine of both nicotinic acetylcholine receptors (Badio et al., 1997; Mah et al., 1998; Fryer and Lukas, 1999) and NMDA receptors (Popik et al., 1995; Chen et al., 1996). Competitive binding to both NMDA receptors and 5-HT_3 receptors has also been demonstrated (Sweetnam et al., 1995). However, the selectivity of ibogaine for the various neurotransmitter receptor ion-channel subtypes has been little explored and the activity of 18-methoxycoronaridine at these receptors has not been tested. Therefore, we sought to compare the actions of ibogaine and 18-methoxycoronaridine at nACh, NMDA, and 5-HT_3 receptors.

The present results are consistent with previous reports of the antagonist action of ibogaine at $\alpha3\beta4$ nicotinic receptors; and the data also indicate that 18-methoxycoronaridine has a similar action. Furthermore, both ibogaine and 18-methoxycoronaridine show some selectivity for this site in that much higher drug concentrations were required to block other sites. Ibogaine was at least five times less potent at $\alpha4\beta2$ than at $\alpha3\beta4$ sites, and even more times less potent at NMDA and 5-HT_3 receptors. 18-Methoxycoronaridine was approximately 25 times less potent at 5-HT_3 than at $\alpha3\beta4$ receptors and, up to at least 20 μM, was inactive at $\alpha4\beta2$ and NMDA receptors. Hence, the data suggest that antagonism at $\alpha3\beta4$ receptors is a potentially important mechanism of action for both ibogaine and 18-methoxycoronaridine.

Low dose combinations of 18-methoxycoronaridine with either mecamylamine or dextromethorphan or of mecamylamine with dextromethorphan all significantly reduced morphine and methamphetamine self-administration without affecting responding for water. It should be noted that although baseline rates of responding for water were much higher than baseline rates of drug self-administration, previous work (e.g., Glick et al., 1991) has established that higher rates are more rather than less sensitive to nonspecific treatment effects. Due to the inverted U shape that is characteristic of drug self-administration dose-effect functions, treatment-induced decreases in drug self-administration at a single infusion dosage could conceivably reflect either potentiation (leftward shift of dose-effect function) or antagonism (rightward shift of dose-effect function) of the self-administered drug. Although this issue was not directly addressed here, previous work involving the effects of 18-methoxycoronardine on morphine self-administration (Maisonneuve and Glick, 1999) and of dextromethorphan on methamphetamine self-administration (Jun and Schindler, 2000) indicated that, in both instances, the infusion dose–effect function was shifted downward without displacement to the left or right. This suggests that these treatments reduced the reinforcing efficacy of the self-administered drugs. Because the effects of the combination treatments assessed in the present study closely mimicked the effects produced by higher doses of each component of the treatments (e.g., Glick et al., 2000a,b, 2001), it is likely that the reinforcing efficacies of the self-administered drugs were similarly reduced here.

Antagonism at $\alpha3\beta4$ sites is the only known action that 18-methoxycoronaridine, mecamylamine and dextromethorphan have in common. The self-administration results are therefore consistent with our original hypothesis implicating antagonism at $\alpha3\beta4$ receptors as a potential mechanism to modulate drug seeking behavior. However, this antagonism at $\alpha3\beta4$ receptors may not directly involve the dopaminergic mesolimbic pathway presumed to mediate drug reward. Although low densities of $\alpha3\beta4$ receptors reside in the dopaminergic nuclei of the ventral tegmental area, $\alpha3\beta4$ nicotinic receptors are mainly located in the medial habenula and the interpeduncular nucleus (e.g., Klink et al., 2001; Quick et al., 1999). While the interpeduncular nucleus receives its main input from the medial habenula, forming the habenulointerpeduncular pathway, there are multiple avenues for interaction between this pathway and the mesolimbic pathway. Thus, for example, the medial habenula receives input from the nucleus accumbens and has efferents to the ventral tegmental area; and the interpeduncular nucleus has efferent connections to the brainstem raphe nuclei and the medial dorsal thalamic nucleus, both of which directly or indirectly (e.g., via the prefrontal cortex) connect to the ventral tegmental area. Functional interactions between the habenulointerpeduncular and mesolimbic pathways have in fact been demonstrated (Nishikawa et al., 1986).

Aside from accounting for other preclinical effects of dextromethorphan (Pulvirenti et al., 1997; Jun and Schindler, 2000; Glick et al., 2001) and mecamylamine (Levin et al., 2000) on drug self-administration, the mechanism proposed here may also underlie effects already reported in the human literature. Administration of mecamylamine was found to reduce cue-induced craving in patients addicted to cocaine (Reid et al., 1999), and in short-term clinical trials, dextromethorphan reduced heroin intake (Koyuncuoglu, 1995), craving (Koyuncuoglu and Saydam, 1990), and signs of opioid withdrawal (Koyuncuoglu and Saydam, 1990; Koyuncuoglu, 1995). 18-Methoxycoronaridine, having apparently greater selectivity for $\alpha3\beta4$ sites than either mecamylamine or dextromethorphan, or the prototypical *iboga* alkaloid ibogaine, may represent the first of a new class of synthetic agents acting via a novel mechanism and having a broad spectrum of activity to diminish multiple forms of addictive behavior. This hypothesis will be pursued in further studies.

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Cellular/Molecular

Glial Cell Line-Derived Neurotrophic Factor Mediates the Desirable Actions of the Anti-Addiction Drug Ibogaine against Alcohol Consumption

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Alcohol addiction manifests as uncontrolled drinking despite negative consequences. Few medications are available to treat the disorder. Anecdotal reports suggest that ibogaine, a natural alkaloid, reverses behaviors associated with addiction including alcoholism; however, because of side effects, ibogaine is not used clinically. In this study, we first characterized the actions of ibogaine on ethanol self-administration in rodents. Ibogaine decreased ethanol intake by rats in two-bottle choice and operant self-administration paradigms. Ibogaine also reduced operant self-administration of ethanol in a relapse model. Next, we identified a molecular mechanism that mediates the desirable activities of ibogaine on ethanol intake. Microinjection of ibogaine into the ventral tegmental area (VTA), but not the substantia nigra, reduced self-administration of ethanol, and systemic administration of ibogaine increased the expression of glial cell line-derived neurotrophic factor (GDNF) in a midbrain region that includes the VTA. In dopaminergic neuron-like SHSY5Y cells, ibogaine treatment upregulated the GDNF pathway as indicated by increases in phosphorylation of the GDNF receptor, Ret, and the downstream kinase, ERK1 (extracellular signal-regulated kinase 1). Finally, the ibogaine-mediated decrease in ethanol self-administration was mimicked by intra-VTA microinjection of GDNF and was reduced by intra-VTA delivery of anti-GDNF neutralizing antibodies. Together, these results suggest that GDNF in the VTA mediates the action of ibogaine on ethanol consumption. These findings highlight the importance of GDNF as a new target for drug development for alcoholism that may mimic the effect of ibogaine against alcohol consumption but avoid the negative side effects.

Key words: **addiction; alcohol; growth factor; neurotrophic; self-administration; ventral tegmental area**

Introduction

Drug abuse and alcoholism are serious and costly health problems. Unfortunately, few medications have proven effective for treating the disease states of addiction and dependence. Ibogaine, a natural alkaloid extracted from the root bark of the African shrub *Tabernanthe Iboga*, has attracted attention because of its reported ability to reverse human addiction to multiple drugs of abuse, including alcohol (Popik et al., 1995; Mash et al., 1998; Vastag, 2002). Human anecdotal reports assert that a single administration of ibogaine reduces craving for opiates and cocaine for extended periods of time and reduces opiate withdrawal symptoms (Sheppard 1994; Mash et al., 1998; Alper et al., 1999). Studies also suggest that ibogaine attenuates drug- and ethanol-induced behaviors in rodents. For example, ibogaine reduces operant self-administration of heroin in rats, as well as naloxone-precipitated withdrawal in morphine-dependent rats (Glick et al., 1992; Dworkin et al., 1995). Administration of ibogaine decreases cocaine-induced locomotor activity and reduces cocaine self-administration in rats (Cappendijk and Dzoljic, 1993) and mice (Sershen et al., 1994). Rezvani et al. (1995) reported that ibogaine reduces ethanol self-administration in alcohol-preferring selected lines of rats; however, the effects of ibogaine have not been tested in an operant procedure in which oral ethanol reinforces lever press behavior. Our first aim was to extend the characterization of the effects of ibogaine on ethanol self-administration to the operant procedure, including a test of the effects of ibogaine on reinstatement of ethanol self-administration after a period of extinction.

Despite its attractive properties, ibogaine is not approved as an addiction treatment because of the induction of side effects such as hallucinations. In addition, ibogaine at high doses causes degeneration of cerebellar Purkinje cells (O'Hearn and Molliver, 1993, 1997) and whole-body tremors and ataxia (Glick et al., 1992; O'Hearn and Molliver, 1993) in rats. In an attempt to dif-

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ferentiate between the desirable and undesirable actions of ibogaine, we set out to elucidate the molecular pathway that specifically mediates the effect of the drug on ethanol consumption. Our hope was that identification of this pathway would reveal possibilities for new pharmacological approaches to treat alcoholism that would not have the undesirable side effects of ibogaine.

All drugs of abuse, including alcohol, activate the "reward" neurocircuitry (Koob et al., 1998; Spanagel and Weiss, 1999). One of the major brain regions in this pathway is the ventral tegmental area (VTA). The VTA is the brain site at which many biochemical neuroadaptations induced by repeated drugs of abuse, including ethanol, have been observed. For example, long-term ethanol exposure increases levels of tyrosine hydroxylase (TH), glial fibrillary acidic protein, and the NR1 subunit of the NMDA receptor and decreases levels of neurofilament protein and the α1 subunit of the $GABA_A$ receptor in the VTA (Ortiz et al., 1995; Charlton et al., 1997). Furthermore, several studies suggest that the VTA may also be the site of action for reversal of the neuroadaptations that lead to the development of addiction. For example, infusion of brain-derived neurotrophic factor (BDNF) into the VTA reverses morphine-induced elevations of TH (Berhow et al., 1995). In addition, Messer et al. (2000) reported that chronic exposure to cocaine and morphine decreases the level of Ret phosphorylation, and thus activation, of the glial cell line-derived neurotrophic factor (GDNF) pathway in the VTA, whereas infusion of GDNF into the VTA dose-dependently reverses the increase in TH immunoreactivity observed after morphine injection. Furthermore, intra-VTA GDNF treatment blocks the behavioral effects of repeated exposure to cocaine (Messer et al., 2000). Thus, we postulated that the VTA may be, at least in part, the site of action of ibogaine. Therefore, we tested the effects of intra-VTA ibogaine administration on ethanol consumption and set out to determine the molecular effects of ibogaine in the midbrain and in SHSY5Y cells, a dopaminergic neuroblastoma cell line (Biedler et al., 1978). Our studies suggest that GDNF in the midbrain region mediates the ibogaine-induced reduction in ethanol consumption.

Materials and Methods

Materials

Ibogaine-HCl, phosphatidylinositol phospholipase C (PI-PLC), Wortmannin and Latrunculin B were purchased from Sigma (St. Louis, MO). Human and rat GDNF and anti-GDNF monoclonal neutralizing antibodies were purchased from R & D Systems (Minneapolis, MN). The inhibitors U0126 and PD98059 were purchased from Calbiochem (La Jolla, CA). Protease inhibitor mixture was purchased from Roche Applied Science (Indianapolis, IN). Anti-phosphotyrosine antibodies were purchased from BD Transduction Laboratories (San Diego, CA). Anti-Ret, anti-GDNF family receptor α1 (anti-GFRα1), anti-extracellular signal-regulated kinase 2 (ERK2), anti-phosphoERK1/2, anti-tyrosine kinase (Trk) B, anti-phospho-Trk antibodies, and mouse IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Trizol reagent was purchased from Invitrogen (Carlsbad, CA). GDNF Emax Immunoassay System, Reverse Transcription System, and PCR Master Mix were purchased from Promega (Madison, WI). Primers for PCR were synthesized by BioSource International (Camarillo, CA). Fluoro-Jade was purchased from Histo-Chem (Jefferson, AZ). Artificial CSF (aCSF) was purchased from CMA Microdialysis (North Chelmsford, MA).

Animals

Adult male Long–Evans rats were purchased from Harlan (Indianapolis, IN); C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Rodents were housed in ventilated polycarbonate cages with food and water available *ad libitum* except as noted below, on a standard 12 hr light/dark schedule with lights on at 6 A.M. Subjects were group housed for all studies, except for the ethanol two-bottle and operant self-administration studies, for which they were housed singly. All studies were conducted with approval by the Gallo Center Institutional Animal Care and Use Committee and were in accordance with *PHS Policy on Humane Care and Use of Laboratory Animals*, Office of Laboratory Animal Welfare, National Institutes of Health, revised 2002.

Ibogaine preparation and treatment for in vivo *studies in mice and rats*

Ibogaine-HCl was dissolved in water to create a stock solution. The stock solution was diluted further by appropriate vehicle solution (saline or aCSF) as needed. Systemic injections were intraperitoneal and given in injection volumes of 2 ml/kg for rats and 1 ml/100 gm for mice at a concentration of 20 or 40 mg/kg. The 40 mg/kg dose of ibogaine has been reported previously to reduce cocaine and heroin self-administration (Glick et al., 1992; Cappendijk and Dzoljic, 1993; Dworkin et al., 1995). This dose of ibogaine is not toxic to cells because it was shown not to produce Purkinje cell death (Molinari et al., 1996).

Ibogaine effects on gene expression in vivo

Male C57BL/6 mice or Long–Evans rats were habituated with three saline injections over 3 d followed by intraperitoneal administration of 40 mg/kg ibogaine. The animals were killed at the indicated time point, and brain regions were excised and stored at −80°C until use for analysis of *GDNF* expression by RT-PCR or by microarray.

Microarray analysis

RNA was isolated using the RNeasy protocol (Qiagen, Chatworth, CA) as per manufacturer protocols. In each case the specific brain regions (e.g., midbrain) from five mice were pooled together to form a single sample. After isolation, the RNA was reverse-transcribed using cytokine-specific primers, avian myeloblastosis virus reverse transcriptase, and ^{33}P-labeled dCTP to generate radio-labeled cDNA. This cDNA was then column purified and hybridized to mouse cytokine expression arrays (R & D Systems) for 16 hr at 65°C as per manufacturer protocols. These arrays contain ~500 different cDNAs related to cytokine signaling. The blots were then washed, and the hybridization signal was visualized using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The hybridization signal for each gene was normalized to the mean level of expression of four housekeeping genes (*β-actin*, *GPDH*, *α-tubulin*, and *cyclophilin*). Genes that showed a minimum change of 40% in at least three of five comparisons (ibogaine vs vehicle) were flagged as either an increase or a decrease.

RT-PCR

Total RNA was isolated using Trizol reagent and reverse transcribed using the Reverse Transcription System kit with the oligo (dT)15 primer at 42°C for 30 min. *GDNF, actin*, or *GPDH* expression was analyzed by RT-PCR. The *GDNF* primers were based on the coding frame of the human *GDNF* gene: upstream, 5′-TGC CAG AGG ATT ATC CTG ATC AGT TCG ATG-3′; downstream, 5′-TTG TCG TAC GTT GTC TCA GCT GCA TCG CAA-3′. The *actin* primers were based on the human *actin* gene: upstream, 5′-TCA TGA AGT GTG ACG TTG ACA TC-3′; downstream, 5′-AGA AGC ATT TGC GGT GGA CGA TG-3′. The *GPDH* primers were based on the rat *GPDH* gene: upstream, 5′-TGA AGG TCG GTG TCA ACG GAT TTG GC-3′; downstream, 5′-CAT GTA GGC CAT GAG GTC CAC CAC-3′. PCR conditions were optimized to specifically ensure that the amplification reactions were within the linear range by testing a range of 25–40 PCR cycles. The optimal numbers of cycles used were as follows: 30 cycles for controls *GPDH* and *actin* and 35 cycles for *GDNF*. After completion of PCR, 10 μl of each product was separated by 1.8% agarose gel in Tris/acetic acid/EDTA buffer with 0.25 μg/ml ethidium bromide, photographed by Eagle Eye II (Stratagene. La Jolla, CA), and quantified by NIH Image 1.61.

Cell culture

SHSY5Y human neuroblastoma cells were grown in DMEM containing 10% fetal bovine serum (FBS) plus 100 U/ml penicillin and 100 μg/ml streptomycin. Before experiments, the medium was replaced with DMEM containing 1% FBS.

Effects of ibogaine on cell toxicity in vivo *and* in vitro

For *in vivo* testing, male C57BL/6 mice were injected with ibogaine (40 mg/kg, i.p.). The mice were subsequently anesthetized using carbon dioxide and perfused intracardially with 4% paraformaldehyde 12 hr after ibogaine administration. The skull was then removed and the brain post-fixed in paraformaldehyde. After sucrose impregnation, brains were blocked and 25-μm-thick coronal sections were generated, placed on gelatinized slides, and stained with Fluoro-Jade. Briefly, sections were dehydrated in 100% ethanol for 3 min followed by 1 min in 70% ethanol. After a 1 min wash in distilled water, the sections were treated with 0.06% $KMnO_4$ with constant agitation for 15 min. After a 1 min wash in distilled water, the sections were placed in a solution composed of 0.001% Fluoro-Jade in 0.1% acetic acid for 15 min. The slides were washed three times with distilled water, dried, and coverslipped with a mixture of distyrene, tricresyl phosphate, and xylene (DPX). Sections were visualized with a Leica fluorescent microscope using an FITC filter and a 10× and 20× objective.

To assay cell death *in vitro*, SHSY5Y cells were treated with 10 μM ibogaine for 24 hr. Parallel cultures were treated with 4 μM Wortmannin (PI3 kinase inhibitor) or 2 μM Latrunculin B (actin polymerization inhibitor) for 90 min. After treatment, cells were fixed in 4% paraformaldehyde for 3 min and placed in 100% ethanol for 1 min, followed by a 1 min distilled water wash. The cells were then treated in $KMnO_4$ for 3 min followed by 5 min incubation in a 0.001% acetylated Fluoro-Jade solution. After three washes in distilled water, the cells were cleared in xylene for 1 min, coverslipped with DPX, and visualized using a Leica fluorescent microscope with a FITC filter and 10× and 20× objectives.

Immunoprecipitation

Cells were collected and lysed in RIPA buffer (50 mM Tris-Cl, pH 7.6, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 2 mM EDTA, protease inhibitor mixture, and 10 mM sodium orthovanadate). Homogenates were incubated with 5 μg anti-Ret antibody in TBS-T buffer (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) overnight at 4°C, followed by 2 hr incubation with Protein G agarose. Samples were separated on an SDS-PAGE gel for Western blot analysis.

ELISA

GDNF secretion in the medium was detected using the GDNF Emax Immunoassay System according to Balkowiec and Katz (2000). GDNF concentration was interpolated from the standard curves (linear range of 15.6–1000 pg/ml).

Behavioral studies

Effects of ibogaine on ethanol and sucrose self-administration in the two-bottle preference test. Male Long–Evans rats were allowed continuous access to two bottles, one containing 10% ethanol (10E) in tap water and the other containing tap water. Two months later, 20 or 40 mg/kg ibogaine or vehicle was injected intraperitoneally at 3 P.M., 3 hr before the start of the dark cycle; ethanol and water intake were measured 24 hr later. Each subject received each dose, with one injection per week. Next, subjects were exposed to two bottles, one containing 10% sucrose (10S) in tap water and the other containing tap water. Four days later, the effects of 40 mg/kg ibogaine were determined as above.

Effects of ibogaine and GDNF on ethanol operant self-administration: operant ethanol self-administration training. The self-administration chamber contained two levers: an active lever, for which presses resulted in delivery of 0.1 ml of fluid reward, and an inactive lever, for which responses were counted but no programmed events occurred. Rats were water restricted and then trained to lever press for a 10S/10E reinforcer solution in three overnight sessions. On the fourth day, rats were allowed *ad libitum* access to water in the home cage and were transferred to 1 hr daily sessions, wherein responses were reinforced with 5S/10E on a fixed-ratio 3 (FR3) schedule. Sucrose was phased out of the reinforcer solution over the next 7 d until the solution consisted of 10E only. Experimental manipulations began after 30 sessions when stable 10E intake of ≥0.4 gm/kg ethanol was attained.

Effects of systemic ibogaine on ethanol self-administration. The mean intake by the group receiving intraperitoneal ibogaine was 0.45 ± 0.13 gm/kg. Intakes at these levels produce measurable levels in blood (Weiss et al., 1993) and brain (Ferraro et al., 1991). Half of the subjects received ibogaine (40 mg/kg, i.p.), whereas the other half received vehicle, 3 hr before the start of an ethanol self-administration session. This time interval was chosen to mimic the time interval between ibogaine injection and the start of the dark cycle in the two-bottle preference study. One week later, the drug treatments were reversed, such that each subject received both ibogaine and vehicle treatments.

Effects of systemic ibogaine administration on ethanol self-administration after a period of extinction (ethanol reinstatement). The eight rats used in the operant self-administration study were also used to study ethanol self-administration after a period of extinction. After a period of stable responding for ethanol, rats underwent extinction in which no ethanol was delivered after active lever responses. After 12–15 extinction sessions, rats met the extinction criteria (fewer than five presses per session). Half of the rats were then injected with ibogaine (40 mg/kg, i.p.) or vehicle 3 hr before the reinstatement test session, in which lever presses on the ethanol-associated lever resulted in the delivery of 0.1 ml of 10E (FR1 schedule). After an additional 2 weeks of extinction sessions, a second reinstatement test session was conducted with the drug treatments reversed, such that every subject received both a vehicle and an ibogaine treatment.

Effects of intra-VTA microinjection of ibogaine on ethanol self-administration. Ethanol self-administration was established as described above in a separate group of rats; these subjects attained a mean daily intake of ethanol of 0.62 ± 0.07 gm/kg. Bilateral guide cannulas (26 gauge, Plastics One, Roanoke, VA) were implanted into the VTA (5.6 mm posterior to bregma, 1.0 mm mediolateral, 8.0 mm ventral to the skull surface). After 7–10 d recovery, sham injections were conducted before ethanol self-administration sessions to habituate subjects to the microinjection procedure. Next, ibogaine [0.1 μM/0.5 μl (0.05 pmol), 1 μM/0.5 μl (0.5 pmol), and 10 μM/0.5 μl (5.0 pmol)] or aCSF (0.5 μl) was injected into the VTA of gently restrained rats at a rate of 0.1 μl/min via an internal injection cannula extending 0.5 mm beyond the guide cannula tip. Injection cannulas were left in place for an additional 1 min to allow for diffusion of the drug. Rats were placed in the self-administration chambers 3, 24, and 48 hr after injection for a 1 hr self-administration session. All subjects received each concentration, with the order of injections counterbalanced across subjects. Subjects received one injection per week.

Effects of intra-substantia nigra microinjection of ibogaine on ethanol self-administration. The effects of 10 μM/0.5 μl (5.0 pmol) ibogaine microinjected into the substantia nigra were determined as described above for the VTA in a separate group of subjects trained to self-administer ethanol as described above. This group attained a mean daily intake of ethanol of 0.67 ± 0.09 gm/kg. The surgical coordinates for cannula placement were 5.2 mm posterior to bregma, 2.8 mm mediolateral, 6.7 mm ventral to the skull surface; the injector tip extended 0.5 mm beyond the guide cannula tip. Three hours after injection, rats were placed in the self-administration chambers for a 1 hr self-administration session.

Effects of intra-VTA microinjection of GDNF on ethanol self-administration. Ethanol self-administration was established as described above in a separate group of rats. These subjects attained a mean daily intake of ethanol of 0.24 ± 0.05 gm/kg. Bilateral guide cannulas (26 gauge, Plastics One) were implanted in the VTA (5.6 mm posterior to bregma, 1.0 mm mediolateral, 8.0 mm ventral to the skull surface). After 7–10 d recovery, sham injections were conducted before ethanol self-administration sessions to habituate subjects to the microinjection procedure. Next, GDNF (5 μg/1 μl per side) or aCSF (1 μl) was injected into the VTA of gently restrained rats at a rate of 0.5 μl/min via an internal injection cannula extending 0.5 mm beyond the guide cannula tip. Injection cannulas were left in place for an additional 1 min to allow for diffusion of the drug. Ten minutes after injection, rats were placed in the self-administration chambers for a 1 hr self-administration session. All subjects received both aCSF and GDNF treatment. Subjects received one injection per week.

Effects of intra-VTA administration of anti-GDNF neutralizing antibodies on the reduction of ibogaine reduction in ethanol self-administration. In an additional group of rats, ethanol self-administration was established as described above. Next, bilateral osmotic pump guide cannulas (Plastics One) were implanted in the VTA (5.6 mm posterior to bregma, 1.0

mm mediolateral, 8.5 mm ventral to the skull surface). After 7 d recovery, subjects were given 10–15 training sessions to allow recovery of baseline levels of ethanol self-administration (rats in the control group consumed 0.65 ± 0.11 gm/kg, whereas rats in the experimental group consumed 0.66 ± 0.08 gm/kg). An osmotic minipump (Alzet, Palo Alto, CA) was then connected to each of the two guide cannulas and implanted subcutaneously for continuous infusion of either anti-GDNF neutralizing antibodies (600 ng/12 μl per side per day) or mouse IgG (600 ng/12 μl per side per day). Two days later, 1 hr daily ethanol self-administration sessions resumed for a 10 d testing period. On the eighth day of the 10 day self-administration testing period, rats received an intraperitoneal injection of ibogaine (40 mg/kg).

Histology

Locations of cannulas were verified in 50 μm sections stained with thionin. Only data from subjects with cannulas located in the region of interest were included in the analyses.

Data analysis

Student's *t* test was used to evaluate differences in *in vivo* gene expression and for the biochemical data. Two-bottle preference measures and ethanol self-administration data were analyzed by one- or two-way ANOVA with repeated measures. *F* values attaining significance were evaluated further by Student–Newman–Keul's method. Latency data were analyzed by the Wilcoxon matched-pairs test. $p < 0.05$ was regarded as statistically significant for all tests.

Results

Systemic and intra-VTA administration of ibogaine decrease self-administration of ethanol

We first set out to confirm that ibogaine administration attenuates ethanol self-administration by testing the effects of systemic injection of 40 mg/kg ibogaine on voluntary ethanol consumption in rats allowed continuous access to ethanol in the home cage. The 40 mg/kg dose was chosen because it has been reported to reduce cocaine and heroin self-administration (Glick et al., 1992; Cappendijk and Dzoljic, 1993; Dworkin et al., 1995) without evidence of neurotoxicity (Molinari et al., 1996). Under conditions of continuous access to ethanol in the home cage, the acute administration of ibogaine reduced both intake of (Fig. 1*A*) and preference for (Fig. 1*B*) ethanol. Ibogaine did not decrease water intake; as has been reported (Rezvani et al., 1995), there was an increase in water intake after ibogaine, but this increase was significant only for the 20 mg/kg dose. Total volume of fluid consumed was significantly increased for the 20 mg/kg dose but was not significantly changed after 40 mg/kg ibogaine (vehicle, 31.44 ± 1.6 ml; 20 mg/kg, 43.11 ± 4.9 ml; 40 mg/kg, 27.5 ± 2.2 ml). In addition, no effect of ibogaine on preference for sucrose was observed (Fig. 1*C*).

Next, we tested the effects of ibogaine in an ethanol operant self-administration paradigm. Ibogaine (40 mg/kg, i.p.) injection 3 hr before the behavioral session decreased responding at the ethanol-paired lever and had no effect on inactive lever responding (Fig. 1*D*). We also tested the effect of ibogaine in an ethanol reinstatement paradigm in which subjects were allowed access to ethanol after a period of extinction. This procedure resulted in an enhancement of ethanol intake relative to the amount obtained before extinction (Fig. 1*E*, baseline vs saline). Enhanced intake after a period of abstinence is used as an animal model of drinking after relapse and may model the high intakes observed after relapse in humans (Koob, 2000; McBride and Li, 1998). As shown in Figure 1*E*, ibogaine reduced the enhanced ethanol intake observed after a period of extinction. This effect was also apparent when the number of lever presses was examined during the reinstatement test, as shown in Figure 1*F*. Ibogaine significantly decreased responding for ethanol when made available after a period of extinction but had no effect on inactive lever responding (Fig. 1*F*). Importantly, the latency to the first lever press after ibogaine treatment was not different from that after vehicle for the findings presented in Figure 1, *D* and *F* (Table 1), indicating

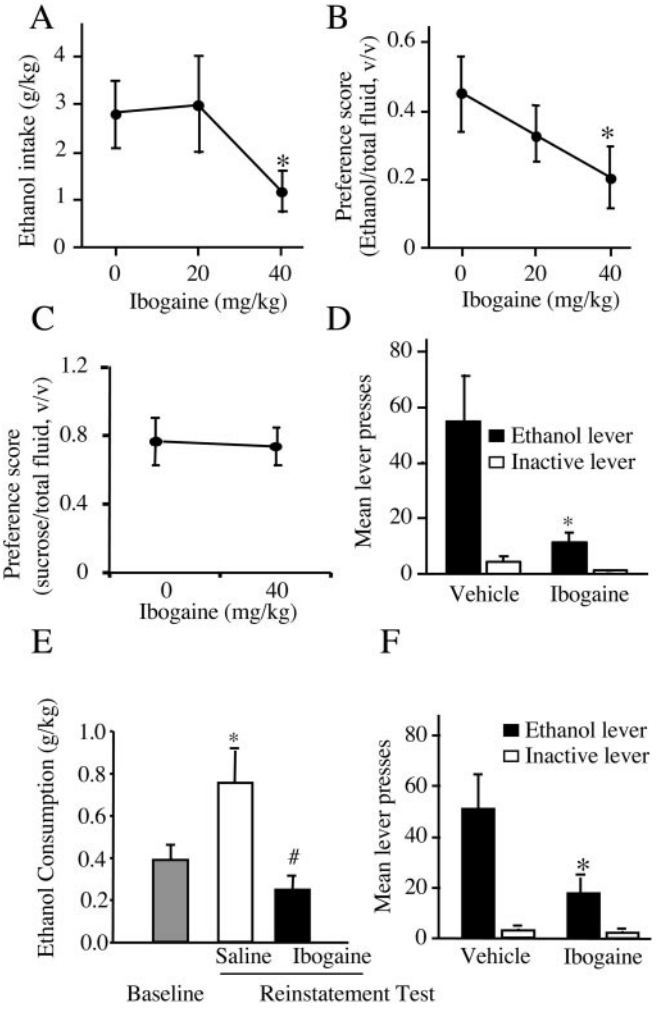

Figure 1. Ibogaine decreases ethanol consumption. *A*, Ibogaine decreased ethanol consumption expressed as mean ± SEM grams of ethanol per kilogram body weight during continuous access to both ethanol and water when intake was measured 24 hr after an acute injection ($F_{(2,16)} = 5.12$; $p < 0.02$). *$p < 0.05$ compared with vehicle treatment ($n = 9$). *B*, Ibogaine treatment decreased ethanol preference expressed as mean milliliters of ethanol/(ml ethanol + ml water) ± SEM ($F_{(2,16)} = 7.83$; $p < 0.005$). *$p < 0.05$ compared with vehicle treatment ($n = 9$). *C*, Ibogaine did not affect sucrose preference, expressed as mean milliliters of sucrose/(ml sucrose + ml water) ± SEM, measured 24 hr after injection ($F_{(1,8)} = 0.02$; $p = 0.88$) ($n = 9$). *D*, Ibogaine attenuated operant ethanol self-administration in rats. Systemic ibogaine injected 3 hr before the session reduced responding for oral ethanol at the ethanol-paired lever but not the inactive lever (main effect of treatment: $F_{(1,7)} = 5.77$, $p < 0.05$; main effect of lever: $F_{(1,7)} = 15.83$, $p < 0.006$; treatment × lever interaction: $F_{(1,7)} = 5.78$, $p < 0.05$]. Ethanol was delivered on an FR3 reinforcement schedule. Data are shown as mean ± SEM. *$p < 0.05$ compared with active lever responding after vehicle treatment ($n = 8$). *E*, Enhanced ethanol intake after a period of extinction was reduced by ibogaine injected 3 hr before the reinstatement test session (main effect of treatment: $F_{(2,12)} = 10.07$, $p < 0.03$). Data are shown as mean ± SEM. *$p < 0.02$ compared with baseline responding before extinction; #$p < 0.002$ compared with vehicle injection ($n = 8$). *F*, Data from *E* expressed as number of lever presses on the ethanol and inactive levers at reinstatement test. Ibogaine injected 3 hr before the reinstatement test session reduced responding for ethanol at the active lever but not the inactive lever (main effect of treatment: $F_{(1,6)} = 11.16$, $p < 0.02$; main effect of lever: $F_{(1,6)} = 12.63$, $p < 0.02$; treatment × lever interaction: $F_{(1,6)} = 11.08$, $p < 0.02$). Ethanol was delivered on an FR1 reinforcement schedule. Data are shown as mean ± SEM. *$p < 0.02$ compared with active lever responding after vehicle injection ($n = 7$).

Table 1. Latency (in seconds) to first press is not significantly altered by ibogaine or GDNF treatment[a]

Experiment	Mean ± SEM	Median (minimum, maximum)
Ethanol self-administration		
Intraperitoneal vehicle	113.4 ± 48.3	67.4 (7.5, 439.4)
Intraperitoneal ibogaine	86.6 ± 17.8	86.6 (13.6,191.4)
Ethanol relapse		
Intraperitoneal vehicle	411.6 ± 139.4	427.5 (21.8,248.7)
Intraperitoneal ibogaine	163.0 ± 54.7	106.0 (27.6,461.9)
Ethanol self-administration		
Intra-VTA ACSF	102.95 ± 19.23	98.78 (33.7,207.1)
Intra-VTA ibogaine (10 μM)	92.69 ± 22.14	63.71 (17.1,205.5)
Ethanol self-administration		
Intra-VTA ACSF	176.43 ± 27.10	153.98 (27.6,295.6)
Intra-VTA GDNF	169.70 ± 30.23	145.75 (64.8,312.7)

[a]Wilcoxon matched-pairs test; all $p > 0.05$.

no ibogaine-induced impairment in initiating or completing the lever-press response.

Because the VTA is both a major component of the brain reward circuit (Koob et al., 1998) and a site at which numerous adaptations to ethanol have been noted (Ortiz et al., 1995), we tested whether ibogaine acts directly within the VTA to inhibit ethanol self-administration. Microinjection of ibogaine [0.1–10.0 μM (0.05–5 pmol)] into the VTA 3 hr before the self-administration session dose-dependently decreased responding at the ethanol-paired lever (Fig. 2*A*) but did not affect inactive lever responding (data not shown). The behaviorally effective concentration of 10 μM did not affect latency to the first press (Table 1), indicating no suppressant effect of intra-VTA ibogaine on locomotor activity. Interestingly, intra-VTA activities of ibogaine were found to be long-lasting because lever pressing for ethanol did not return to baseline levels within 48 hr of injection of the 10 μM concentration of ibogaine (Fig. 2*B*). To determine the site specificity of the consequences of intra-VTA ibogaine, the compound was microinjected into the neighboring dopaminergic cell group of the substantia nigra. There was no effect of 10 μM ibogaine on ethanol self-administration measured 3 hr after treatment (Fig. 2*C*); hence the decrease in ethanol intake observed after microinjection of ibogaine into the VTA is mediated by this region and not by diffusion into the nearby substantia nigra.

Systemic administration of ibogaine increases *GDNF* mRNA levels in the midbrain

To identify the molecular mechanism that mediates the effects of ibogaine on ethanol intake, we used a small-scale expression array to study gene expression in the midbrain of mice that were injected with ibogaine. Administration of ibogaine (40 mg/kg, i.p.) significantly increased the mRNA expression of *GDNF* with an approximate twofold increase at the 1 and 12 hr time points after injection (data not shown). Next, using RT-PCR, we confirmed these results by measuring the mRNA level of *GDNF* in mice and rats at various time points after systemic administration of ibogaine. Ibogaine (40 mg/kg) administration caused an increase in the expression of *GDNF* in the midbrain in both mice (Fig. 2*D*) and rats (Fig. 2*E*) up to 24 hr after injection; hence, in the rat, the same dose of ibogaine that caused a reduction in ethanol intake (Fig. 1*A–D*) and ethanol-induced reinstatement (Fig. 1*E*,*F*) also caused an increase in the mRNA expression of *GDNF*.

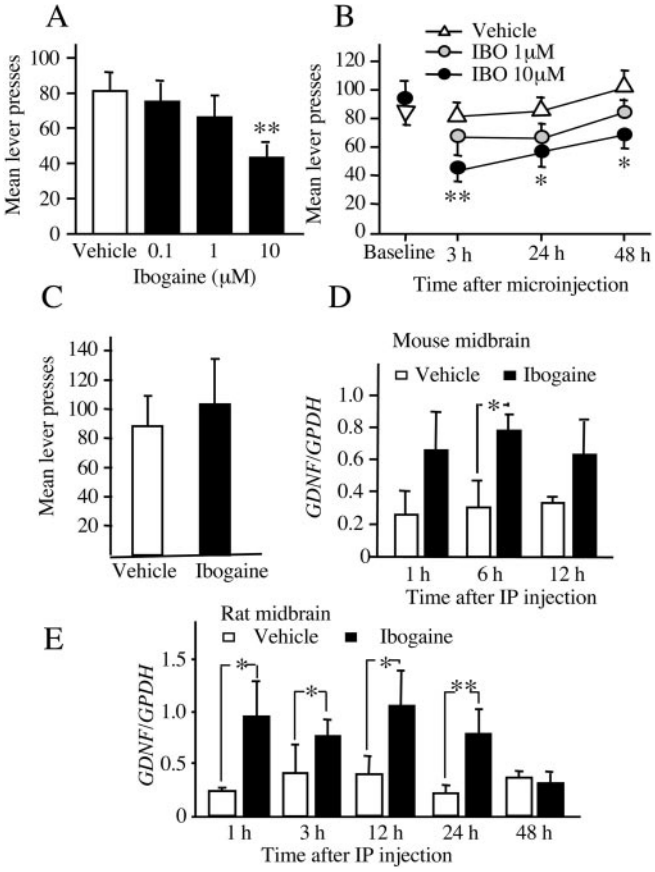

Figure 2. Intra-VTA microinjection of ibogaine decreases ethanol self-administration, and systemic ibogaine increases *GDNF* expression in a midbrain region that contains the VTA. *A*, *B*, Intra-VTA ibogaine decreased ethanol self-administration by rats. *A*, Ibogaine (0, 0.1, 1, 10 μM; equivalent to 0, 0.05, 0.5, 5.0 pmol) microinjected into the VTA 3 hr before an ethanol self-administration session dose-dependently decreased lever press responding ($F_{(3,27)} = 5.90$; $p < 0.002$). Data are shown as mean ± SEM. $^{**}p < 0.001$ compared with active lever responding after vehicle injection ($n = 10$). *B*, Time course of effect of intra-VTA ibogaine. The same subjects in *A* were tested 24 and 48 hr after ibogaine (IBO) treatment. There was no difference in responding on the day before treatment (Baseline) ($F_{(3,27)} = 0.36$; $p = 0.78$). Ibogaine reduced responding for ethanol, and this reduction was long lasting (main effect of concentration: $F_{(3,54)} = 8.62, p < 0.001$; main effect of time: $F_{(2,54)} = 6.76, p < 0.006$; concentration × time interaction: $F_{(6,54)} = 0.26, p = 0.95$). The 10 μM dose significantly reduced responding at all time points. The 0.1 μM dose was omitted for clarity; responding after this dose did not differ from vehicle at any tested time point. Data are shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.005$ compared with active lever responding after vehicle injection ($n = 10$). *C*, Ibogaine microinjected into the substantia nigra 3 hr before an ethanol self-administration session did not affect ethanol lever press responding relative to vehicle microinjection (main effect of treatment: $F_{(1,7)} = 0.59, p = 0.47$; main effect of lever: $F_{(1,7)} = 13.37, p < 0.007$; treatment × lever interaction: $F_{(1,7)} = 0.38, P = 0.56$). Data are shown as mean ± SEM ($n = 8$). *D*, *E*, The midbrain region was excised 1, 6, or 12 hr (mouse) and 1, 3, 24, and 48 hr (rat) after intraperitoneal injection of 40 mg/kg ibogaine. The expression of *GDNF* and control *GPDH* in mouse (*D*) and rat (*E*) was analyzed by RT-PCR. Histogram depicts the mean ratio (*GDNF*/*GPDH*) ± SD of $n = 3$ (*D*), $n = 6$ (1, 3, and 12 hr), and $n = 5$ (24 and 48 hr) (*E*). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with saline injection.

Ibogaine treatment does not result in cell death

To confirm that the effects of ibogaine are not attributable to a neurotoxic effect of the drug, we examined the brains of mice ($n = 2$) injected with 40 mg/kg ibogaine and compared them with uninjected control mice ($n = 2$) using the Fluoro-Jade technique, a technique that has been used to identify ibogaine-induced cerebellar toxicity (Schmued and Hopkins, 2000). We scanned serial coronal sections from anterior forebrain to hindbrain of ibogaine-treated and control brains for brightly fluorescing (dy-

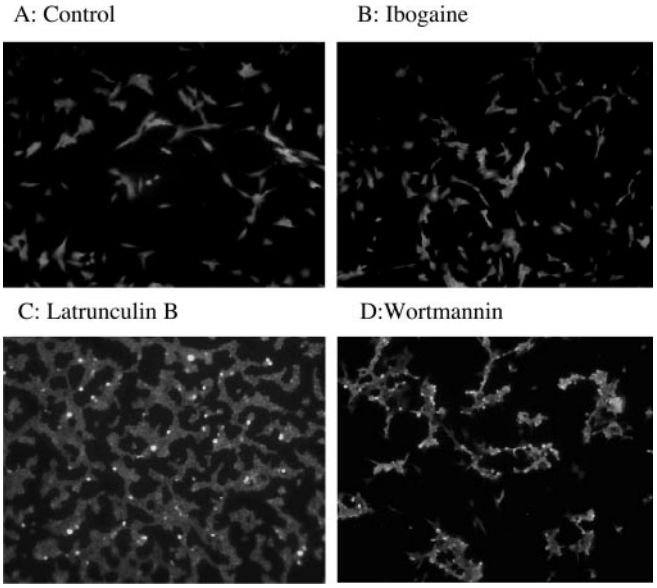

Figure 3. Ibogaine is not neurotoxic to cells in culture. *A–D*, SHSY5Y cells were treated with vehicle (*A*), 10 μM ibogaine for 24 hr (*B*), 2 μM Latrunculin B (*C*), or the PI3 kinase inhibitor Wortmannin (4 μM) for 90 min (*D*). Cell death was measured with Fluoro-Jade as described in Materials and Methods.

ing) neurons. Although myelin, ependymal cells, and choroid plexus were lightly stained, we observed no brightly fluorescing dying neurons in either control or ibogaine-treated brains (data not shown). Particular attention was paid to the midbrain, the region in which we found the increase in *GDNF* mRNA after ibogaine, and to the cerebellum, because cell death has been reported in the cerebellum after administration of high doses of ibogaine (O'Hearn and Molliver, 1993, 1997). The lack of staining that we observed is in concordance with previous data (Molinari et al., 1996; Schmued and Hopkins, 2000). The lack of ibogaine neurotoxicity was also confirmed in SHSY5Y cells treated with 10 μM ibogaine for 24 hr. As positive controls, cells were treated with Wortmannin (PI3 kinase inhibitor) or Latrunculin B (actin polymerization inhibitor), which cause SHSY5Y cells to retract their processes, become rounded, and die. As shown in Figure 3, cell death is observed in both the Latrunculin B- and Wortmannin-treated cells as detected by the brightly fluorescing dying cells; however, there was no difference in Fluoro-Jade staining in control or ibogaine-treated cultures, indicating that Fluoro-Jade is an effective cell-death marker.

The GDNF signaling pathway is activated in SHSY5Y cells after exposure to ibogaine

Because we found that ibogaine increased the expression of *GNDF* mRNA in the rodent midbrain, we tested whether ibogaine reduces ethanol consumption by activating the GDNF pathway within neurons in this brain region. GDNF promotes the survival of dopaminergic midbrain neurons (for review, see Airaksinen and Saarma, 2002), and the GDNF receptors GFRα1 and Ret are expressed in dopaminergic neurons, including within the VTA (Glazner et al., 1998; Sarabi et al., 2001). Therefore, we used the dopaminergic SHSY5Y human neuroblastoma cell line to determine the activities of ibogaine on GDNF expression, secretion, and signaling. Ibogaine induced dose-dependent (data not shown) and time-dependent (Fig. 4*A*) increases in *GDNF* expression that lasted up to 12 hr. Next, we examined whether the increase in *GDNF* mRNA levels leads to an increase in GDNF

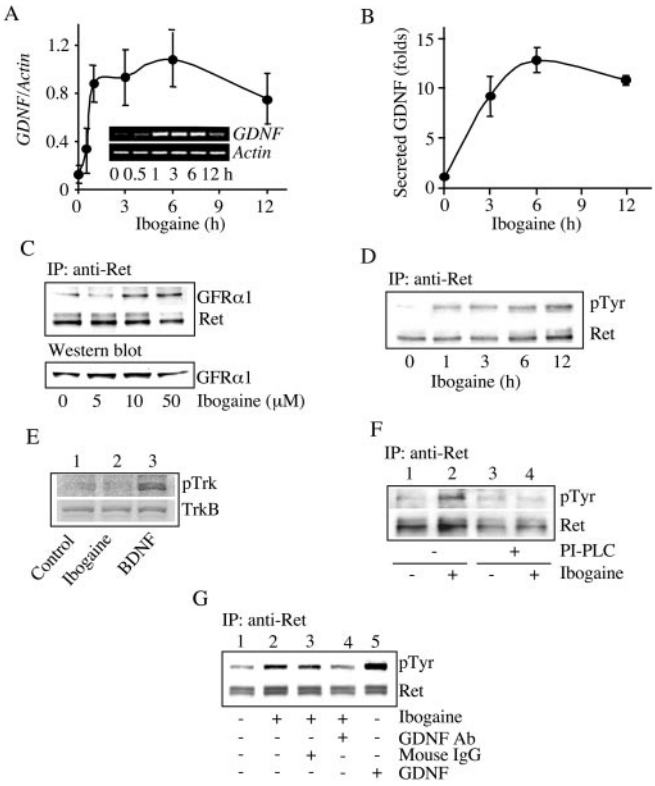

Figure 4. Ibogaine activates the GDNF pathway in SHSY5Y cells. *A*, Cells were treated with 10 μM ibogaine for the indicated times and lysed for total RNA isolation. Expression of *GDNF* and control *actin* was analyzed by RT-PCR ($n = 4$). *B*, Cells were treated with 10 μM ibogaine for the indicated times. GDNF in the media was detected by an ELISA assay. Histogram depicts the mean ± SD of GDNF secretion in three experiments. *C*, Cells were treated with the indicated concentrations of ibogaine for 3 hr. Ret was immunoprecipitated with anti-Ret antibodies, followed by Western blot analysis with anti-GFRα1 antibodies. The levels of GFRα1 in the homogenates were determined by Western blot analysis ($n = 3$). *D*, Cells were treated with 10 μM ibogaine for the indicated times. Ret was immunoprecipitated followed by Western blot analysis with anti-phosphotyrosine or anti-Ret antibodies ($n = 3$). *E*, Cells were treated with vehicle (lane 1) or 10 μM ibogaine (lane 2) for 3 hr or with 50 ng/ml BDNF for 10 min (lane 3). Trk phosphorylation was analyzed by Western blot analysis with anti-phospho-Trk antibodies. The levels of TrkB were also determined by Western blot analysis ($n = 3$). *F*, Cells were preincubated with 0.3 U/ml PI-PLC for 1 hr (lanes 3 and 4). Cells were then washed and treated without (lanes 1 and 3) or with (lanes 2 and 4) 10 μM ibogaine for 3 hr. Ret phosphorylation was determined as described above ($n = 4$). *G*, Cells were treated for 3 hr with vehicle (lane 1), 10 μM ibogaine (lane 2), 10 μM ibogaine plus 10 μg/ml of mouse IgG (lane 3), or 10 μM ibogaine plus anti-GDNF neutralizing antibodies (lane 4). Treatment with 50 ng/ml GDNF was used as a positive control (lane 5). The cells were lysed and Ret phosphorylation was analyzed as described above ($n = 3$).

secretion. To do so, we measured GDNF levels in the media of cells treated with ibogaine and found that GDNF accumulated in the media in a time-dependent manner consistent with the time course for mRNA increases (Fig. 4*B*).

Activation of the GDNF pathway is initiated after ligation of GDNF with GFRα1 leading to the association of Ret with GFRα1, and the consequent autophosphorylation, and thus activation, of Ret (Jing et al., 1996; Treanor et al., 1996); hence, we assessed whether ibogaine induces the association of Ret with GFRα1. Ibogaine treatment increased Ret association with GFRα1 (Fig. 4*C*, top), and this increased association was not caused by increased GFRα1 protein levels (Fig. 4*C*, bottom). Next, we determined whether the ibogaine-induced association of Ret with GFRα1 leads to the activation of the GDNF pathway by measuring the levels of phospho-Ret in the absence or presence of ibogaine. Ibogaine induced Ret phosphorylation in dose-dependent

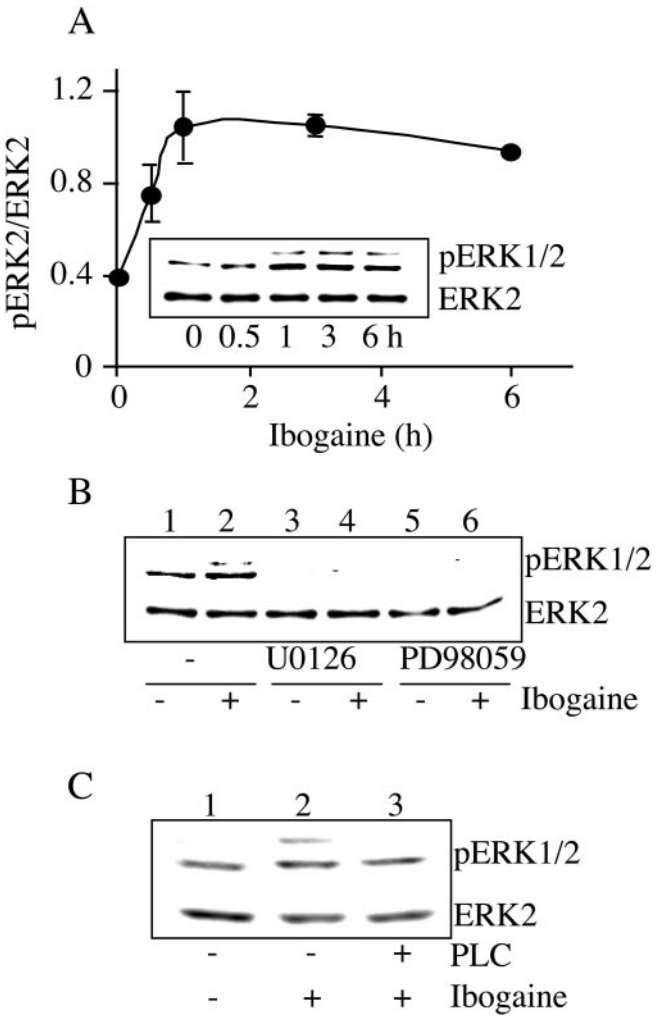

Figure 5. Ibogaine activates the MAPK signaling pathway. *A*, SHSY5Y cells were treated with 10 μM ibogaine for the indicated times. ERK2 and phosphoERK 1/2 (pERK1/2) were detected by Western blot analysis with anti-ERK2 and anti-pERK1/2 antibodies, respectively. Line graph depicts the mean ratio (pERK2/ERK2) $\pm$ SD of three experiments. *B*, Cells were preincubated with the inhibitors U0126 (20 μM) (lanes 3 and 4) and PD58089 (40 μM) (lanes 5 and 6) for 30 min and then treated without (lanes 1, 3, and 5) or with (lanes 2, 4, and 6) 10 μM ibogaine for 3 hr ($n = 3$). *C*, Cells were preincubated with 0.3 U/ml PI-PLC for 1 hr (lane 3). Cells were then washed and treated without (lanes 1) or with (lanes 2 and 3) 10 μM ibogaine for 3 hr ($n = 3$).

(data not shown) and time-dependent manners (Fig. 4*D*). We found no change in the phosphorylation state of another growth factor receptor tyrosine kinase, Trk, in the presence of ibogaine (Fig. 4*E*). GFRα1 is a glycosyl-phosphatidylinositol-anchored protein that can be hydrolyzed by PI-PLC (Jing et al., 1996; Treanor et al., 1996). If ibogaine activates Ret by inducing the association of Ret with GFRα1, then ibogaine-induced Ret phosphorylation should be prevented by PI-PLC. Preincubation of the cells with PI-PLC abolished ibogaine-induced Ret phosphorylation (Fig. 4*F*). Ret phosphorylation induced by ibogaine was also inhibited in the presence of anti-GDNF neutralizing antibodies (Fig. 4*G*, lane 2 vs 4). Finally, we determined whether downstream signaling cascades known to be activated by GDNF (Trupp et al., 1999; Hayashi et al., 2000) also were activated in the presence of ibogaine. We measured the phosphorylation, and thus activation state, of ERK1/2, also named p42/44 mitogen-activated protein kinases (p42/44 MAPK). We found that ibogaine induced the phosphorylation of ERK1/2, and the activation lasted up to 6 hr (Fig. 5*A*). Furthermore, U0126 and PD98059, two inhibitors specific for MAPK/ERK kinase 1, a kinase upstream of ERK1/2, blocked ERK1/2 phosphorylation induced by ibogaine (Fig. 5*B*). Finally, preincubation with PI-PLC abolished ibogaine-induced ERK1/2 phosphorylation (Fig. 5*C*), suggesting that ibogaine-induced ERK1/2 phosphorylation is mediated by the association of the GDNF receptors GFRα1 and Ret.

Ibogaine treatment reduces ethanol self-administration via GDNF

Because ibogaine administration increased *GDNF* expression in the midbrain, we tested whether direct microinjection of GDNF into the VTA would itself decrease ethanol self-administration. Microinjection of 5 μg/μl GDNF into the VTA 10 min before placement in the operant chamber significantly reduced responding for ethanol (Fig. 6*A*), without affecting the latency to the first press (Table 1). Next, we tested whether the behavioral effects of ibogaine would be inhibited by anti-GDNF neutralizing antibodies infused into the VTA (Messer et al., 2000). Two subcutaneous osmotic minipumps were attached to bilateral cannulas aimed at the VTA to continuously infuse either anti-GDNF neutralizing antibodies or control mouse IgG for 2 weeks. During the infusion of the antibody, ethanol intake was measured in daily operant self-administration sessions. Neither the infusion of vehicle (data not shown) nor the infusion of control mouse IgG altered ethanol self-administration (Fig. 6*B*); however, infusion of anti-GDNF neutralizing antibodies significantly attenuated the ability of ibogaine to decrease ethanol self-administration both 3 and 24 hr after ibogaine administration (Fig. 6*B*). Specifically, 3 hr after injection of ibogaine (40 mg/kg, i.p.), control subjects decreased responding for ethanol by 79.1% ($\pm$0.05), whereas only a 39.1% ($\pm$0.13) decrease was observed in animals receiving the anti-GDNF neutralizing antibodies (Fig. 6*B*); however, inactive lever responding was unchanged (data not shown). Together, these results suggest that the effects of ibogaine on ethanol consumption are mediated by GDNF.

Discussion

We found that ibogaine reduces ethanol consumption and that this effect is likely caused by the actions of ibogaine within the VTA, because microinjection of ibogaine into the VTA, but not the substantia nigra, also decreased ethanol self-administration. Furthermore, systemic administration of ibogaine increased expression of *GDNF* in the midbrain. In a dopaminergic neuroblastoma cell line, ibogaine-induced increases in the expression of *GDNF* led to increased secretion of GDNF and subsequent activation of the GDNF pathway. Finally, the *in vivo* effects of ibogaine were mimicked by intra-VTA microinjection of GDNF and were diminished when ibogaine was given in the presence of anti-GDNF neutralizing antibodies infused into the VTA. These findings collectively suggest that ibogaine decreases ethanol intake by increasing the level of GDNF in the VTA.

Ibogaine attenuates ethanol self-administration

A previous study found that ibogaine decreased ethanol consumption in ethanol-preferring rats in the two-bottle preference paradigm (Rezvani et al., 1995). Our initial studies were designed to replicate these findings in an outbred line of rats and to extend them to the operant self-administration procedure. In agreement with Rezvani et al. (1995), we found that ibogaine decreases ethanol intake whether the ethanol is available continuously on the home cage or after lever-press responding for a limited time each day. We also tested the effects of acute ibogaine on responding for ethanol in a model of relapse, in which subjects were granted

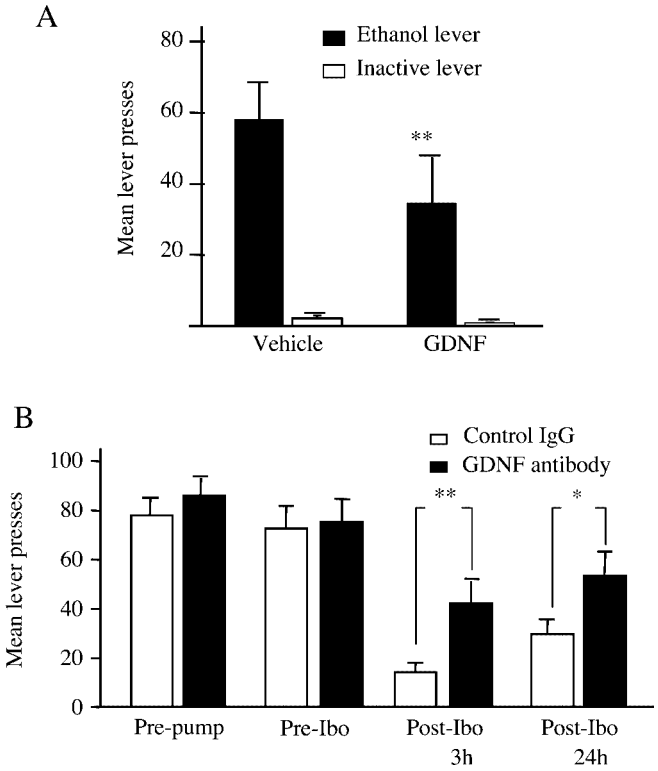

Figure 6. Intra-VTA infusion of GNDF mimics the effects of ibogaine, and anti-GDNF neutralizing antibodies attenuate the effects of ibogaine on ethanol self-administration. *A*, GDNF (5 μg/μl) microinjected into the VTA 10 min before an ethanol self-administration session decreased mean lever press responding relative to vehicle (main effect of treatment: $F_{(1,7)} = 8.38$, $p < 0.03$; main effect of lever: $F_{(1,7)} = 17.10$, $p < 0.005$; treatment × lever interaction: $F_{(1,7)} = 5.88$, $p < 0.05$). **$p < 0.003$. The data are shown as mean lever presses ± SEM ($n = 8$). *B*, Rats received continuous infusion for 14 d of anti-GDNF neutralizing antibodies or mouse IgG (600 ng/12 μl per side per day) into the VTA via osmotic minipumps. Responding for ethanol reinforcement was measured before and after antibody infusion in daily 1 hr ethanol self-administration sessions. The effect of ibogaine (40 mg/kg, i.p.) was tested on the 10th day of antibody infusion, and self-administration behavior was measured for three additional daily sessions. "Pre-pump" represents the mean of the last three training sessions before antibody infusion; "Pre-Ibo" represents the mean of the last three training sessions after antibody infusion and before ibogaine injection. There was no effect of intra-VTA anti-GDNF antibody infusion on baseline levels of ethanol self-administration (Pre-pump vs Pre-Ibo) ($F_{(1,15)} = 0.244$; $p = 0.63$). When the Pre-Ibo baseline was compared with data obtained 3 and 24 hr after ibogaine injection (Post-Ibo), there was a significant effect of antibody treatment ($F_{(1,30)} = 5.15$; $p < 0.04$) and a significant effect of time ($F_{(2,30)} = 23.1$; $p < 0.001$). Although ibogaine treatment decreased responding in both groups, the decrease in responding is significantly greater in the control mouse IgG group ($n = 9$) than in subjects treated with anti-GDNF neutralizing antibodies ($n = 8$). **$p < 0.02$; *$p < 0.05$. The data are shown as mean lever presses ± SEM.

access to ethanol after 2 weeks of extinction. This protocol is similar to alcohol deprivation procedures used to study the neurobiology of ethanol relapse and craving, in which rats demonstrate enhanced intake after a period of abstinence (Koob et al., 1998; McBride and Li, 1998). This enhanced intake may be analogous to the avid ethanol consumption seen among human alcoholics after relapse. We found that ethanol intake was increased significantly after 2 weeks of extinction, compared with pre-extinction baseline levels: ibogaine reduced the enhanced ethanol intake to baseline levels. Interestingly, human anecdotal reports also suggest a decrease in craving and relapse to addictive drugs after ibogaine intake (Mash et al., 2000). Together, these findings indicate that ibogaine reduces ethanol intake, suggesting that the identification of its molecular mechanism of action for this effect may be beneficial.

For several reasons, it is unlikely that the reduction of ethanol intake observed after 40 mg/kg ibogaine is caused by nonspecific activities of the drug. First, we found that ibogaine did not reduce preference for a sucrose solution, in agreement with a previous study that found no effect of 40 mg/kg ibogaine on intake of a sucrose/saccharin solution in rats (Blackburn and Szumlinski, 1997). Second, it is unlikely that changes in locomotor activity can explain the decrease in ethanol consumption. In our experiments, we tested the effects of ibogaine on ethanol intake 3 hr after ibogaine injection; a previous report in rats found that ibogaine does not affect locomotor activity 3–4 hr after injection (Maisonneuve et al., 1997). In addition, we found no effect of ibogaine on inactive lever responding in the operant self-administration procedure. Although low responding on the inactive lever precludes examination of activity decreases, these findings suggest that ibogaine did not produce nonspecific activity increases. We also found no effect of ibogaine on the latency to the first lever press, indicating that ibogaine-treated subjects were not impaired in the initiation or production of lever-press responding. Third, there is no evidence that ibogaine-induced decreases in ethanol intake are subsequent to a neurotoxic effect. We found no evidence for ibogaine-induced neurotoxcitiy in the brain or in a neuronal cell line, in concordance with Molinari et al. (1996); hence, it is unlikely that the reduction of ethanol intake by ibogaine here resulted from nonspecific actions on consumption or motor activity or from neurotoxic effects.

The VTA is the site of action for the reduction of ethanol intake and induction of *GDNF* mRNA by ibogaine

Although attenuation of ethanol self-administration by 40 mg/kg ibogaine in rats is not likely to reflect nonspecific alterations in behavior, the use of ibogaine for treatment of humans is problematic given the uncertainties surrounding the doses that might induce neurotoxicity and the fact that it is a hallucinogen. Therefore, in the second part of our study we set out to determine a molecular mechanism of ibogaine action against ethanol self-administration. We reasoned that ibogaine might alter signaling pathways within the "addiction" neurocircuitry. We found that microinjection of ibogaine into the VTA reduced ethanol intake, suggesting that the VTA is a primary site of action for systemic administration of ibogaine. This suggestion was strengthened by the lack of effect of ibogaine microinjected into the neighboring dopaminergic cells comprising the substantia nigra.

The VTA was further implicated as the site of action of ibogaine by the finding that ibogaine increased *GDNF* expression in the midbrain, a region that includes the VTA. This result suggests that increased *GDNF* expression in the midbrain mediates the effects of ibogaine on ethanol intake. Because the increase in *GDNF* was not observed 48 hr after ibogaine injection but ethanol consumption was still reduced, activation of the pathway downstream to GDNF likely persists longer than the changes in *GDNF* expression.

Ibogaine increases *GDNF* expression and signaling in a dopaminergic cell line

Ibogaine also increased expression of *GDNF* in SHSY5Y cultured cells, suggesting that this dopaminergic cell line is an appropriate model for examining the effects of ibogaine on GDNF signaling. In SHSY5Y cells, the ibogaine-induced increase in *GDNF* expression led to increased GDNF secretion and activation of the GDNF pathway. Specifically, ibogaine increased the activity of the MAPK pathway, which has been reported to be activated via GDNF (Besset et al., 2000; Hayashi et al., 2000). Interestingly,

activation of the MAPK pathway was shown previously to result in the inhibition of A-type potassium channels (Yuan et al., 2002), and GDNF has been reported to enhance the excitability of midbrain dopaminergic neurons by inhibiting the A-type potassium channels (Yang et al., 2001). Chronic ethanol inhibits the excitability of VTA neurons (Bailey et al., 1998), and the firing rates of dopaminergic cells are markedly reduced in the early abstinence period after chronic ethanol consumption (Bailey et al., 2001). These findings suggest that ibogaine, via GDNF, may reverse the actions of ethanol in the VTA by inhibiting the activity of A-type potassium channels, thus enhancing VTA firing.

Although ibogaine injection induced expression of *GDNF* mRNA in rodent midbrain, the cellular source of the *GDNF* mRNA is unknown. Because *GDNF* is expressed in both glia and neurons (Airaksinen and Saarma, 2002), at this point we cannot exclude the possibility that ibogaine increases the expression of *GDNF* in astrocytes; however, the finding that ibogaine also induced expression of *GDNF* in SHSY5Y cells, a dopaminergic neuroblastoma line (Biedler et al., 1978), indicates that the source of GDNF may be neuronal.

The actions of ibogaine are long lasting

Ibogaine induction of *GDNF* expression lasted for at least 12 hr in cultured cells and *in vivo*. In addition, the effects of ibogaine on ethanol self-administration were long lasting. The half-life of ibogaine is reported to be ~1–2 hr, whereas its metabolite, noribogaine, is detected at high concentrations within 24 hr after administration (Mash et al., 1998; Glick and Maisonneuve, 2000). It is therefore possible that noribogaine initiates and/or maintains *GDNF* expression in the brain up to 24 hr after injection. Interestingly, noribogaine reduces cocaine and morphine self-administration (Glick et al., 1996), suggesting that the efficacy of ibogaine against drugs of abuse, including alcohol, may result from this metabolite. Another possible mechanism for the long-lasting effects of ibogaine is that the initial increase in *GDNF* expression induced by ibogaine is followed by autocrine regulation of the expression of the growth factor (D.-Y. He, D. Ron, unpublished results).

GDNF counters the effects of ethanol and other drugs of abuse

Our finding that intra-VTA GDNF reduces ethanol self-administration agrees with previous findings that GDNF counteracts the effects of drugs of abuse. For example, GDNF prevents ethanol-induced apoptosis in a neuroblastoma cell line (McAlhany et al., 2000). In addition, intra-VTA infusion of GDNF reverses morphine-induced increases in protein levels of TH (Messer et al., 2000). Intra-VTA GDNF treatment also blocks and reverses the biochemical effects of cocaine and blocks the rewarding effects of cocaine measured by the conditioned place preference test (Messer et al., 2000). Conversely, chronic exposure to drugs of abuse and ethanol decreases GDNF levels. Prolonged exposure to ethanol decreases GDNF secretion in developing cerebellum (McAlhany et al., 1999). Chronic exposure to cocaine and morphine decreases Ret phosphorylation in the VTA (Messer et al., 2000). Because exogenous administration of GDNF blocks the effects of drugs of abuse, including ethanol, it is plausible that endogenous GDNF systems may counter the effects of these drugs and that ibogaine acts to inhibit intake of stimulants, opiates, and ethanol by enhancing this protective pathway.

Conclusions

In conclusion, we have identified GDNF as a candidate molecule that mediates, at least in part, the effects of ibogaine on ethanol consumption. Therefore, this study suggests that the development of agents that upregulate the GDNF pathway may be useful in the treatment of drug and alcohol abuse.

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Autoregulation of glial cell line-derived neurotrophic factor expression: implications for the long-lasting actions of the anti-addiction drug, Ibogaine

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ABSTRACT **We recently showed that the up-regulation of the glial cell line-derived neurotrophic factor (GDNF) pathway in the midbrain, is the molecular mechanism by which the putative anti-addiction drug Ibogaine mediates its desirable action of reducing alcohol consumption (1). Human reports and studies in rodents have shown that a single administration of Ibogaine results in a long-lasting reduction of drug craving (humans) and drug and alcohol intake (rodents). Here we determine whether, and how, Ibogaine exerts its long-lasting actions on GDNF expression and signaling. Using the dopaminergic-like SHSY5Y cell line as a culture model, we observed that short-term Ibogaine exposure results in a sustained increase in *GDNF* expression that is mediated via the induction of a long-lasting autoregulatory cycle by which GDNF positively regulates its own expression. We show that the initial exposure of cells to Ibogaine or GDNF results in an increase in *GDNF* mRNA, leading to protein expression and to the corresponding activation of the GDNF signaling pathway. This, in turn, leads to a further increase in the mRNA level of the growth factor. The identification of a GDNF-mediated, autoregulatory long-lasting feedback loop could have important implications for GDNF's potential value as a treatment for addiction and neurodegenerative diseases.—He, D.-Y., Ron, D. Autoregulation of glial cell line-derived neurotrophic factor expression: implications for the long-lasting actions of the anti-addiction drug, Ibogaine. *FASEB J.* 20, E1820–E1827 (2006)**

Key Words: growth factor · SHSY5Y · gene expression

Ibogaine is a psychoactive indole alkaloid extracted from the root bark of the African shrub *Tabernanthe Iboga* that has been used for decades in Africa in folklore medicine and rituals. Human anecdotal reports and several studies indicated that Ibogaine reduces craving and withdrawal symptoms for multiple drugs of abuse, including heroine, psychostimulants and cocaine (2–4), The potential anti-addictive properties of Ibogaine were confirmed in rodent models: Ibogaine was shown to attenuate cocaine, nicotine, morphine, heroine, and alcohol-seeking behaviors (5). For example, Ibogaine was found to reduce cocaine and morphine self-administration (6–8). The drug was also found to alleviate morphine withdrawal symptoms (9). Finally, we and others reported that systemic administration of Ibogaine reduces voluntary ethanol intake in a 2-bottle choice paradigm (1, 10, 11). In addition, we recently reported that Ibogaine inhibits operant ethanol self-administration in rats and reduces ethanol intake in an ethanol reinstatement paradigm (1). Despite its potential use as an anti-addiction agent, Ibogaine is not used in the US to treat addiction because of its undesirable side effects, which include hallucination, bradycardia, and tremor (5). In addition, administration of high concentrations of Ibogaine in rats produced cerebellar Purkinje cell death (12, 13).

In an attempt to separate the desirable anti-addiction actions of the drug from the undesirable side effects, we set out to identify the molecular mechanism mediating Ibogaine's effects on voluntary ethanol consumption. We found that systemic administration of Ibogaine increased the expression of *GDNF* in the dopaminergic ventral tegmental area (VTA), as well as in a dopaminergic-like cell culture model, the SHSY5Y cell line (1). Furthermore, we found that in SHSY5Y cells, Ibogaine incubation resulted in a time- and dose-dependent activation of the GDNF signaling pathway. When the GDNF pathway was inhibited in the VTA, Ibogaine was significantly less effective in reducing ethanol intake (1). Finally, similar to Ibogaine, GDNF administered into the VTA reduced ethanol consumption (1). Together, our results suggest that up-regulation of the GDNF pathway in the VTA mediates the Ibogaine-induced reduction in voluntary ethanol consumption.

Anecdotal reports have suggested that a single treatment of Ibogaine reduced drug craving in humans for a period of weeks or even up to six months (14). These possible long-lasting actions of Ibogaine have also been reported in animal studies. Single or multiple injections of the drug produced a long-lasting reduction of cocaine self-administration (7, 8), and we observed that a single systemic injection of Ibogaine in rats reduced

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ethanol consumption for up to 48 h after injection (1). We also found that incubation of SHSY5Y cells with Ibogaine produced sustained changes in the *GDNF* pathway; Ibogaine-mediated increase in *GDNF* expression and secretion, as well as the subsequent phosphorylation (and thus, activation) of the *GDNF* receptor, Ret, lasted for at least 12 h (1). We hypothesized that the long-lasting actions of Ibogaine are mediated, at least in part, via initiation of prolonged activation of the *GDNF* pathway. To test this hypothesis we used SHSY5Y cells as a model system and found that the initial activation of the *GDNF* pathway by Ibogaine leads to the induction of a cycle in which secreted *GDNF* induces expression of itself, leading to the prolonged action of Ibogaine on this signaling pathway.

MATERIALS AND METHODS

Materials

Ibogaine-HCl, phosphatidylinositol phospholipase C (PI-PLC) and cycloheximide (CHX) were purchased from Sigma. Human *GDNF*, anti-phospho-tyrosine antibodies (anti-pTyr), and pUSE vector were purchased from Upstate Cell Signaling Solutions. Anti-*GDNF* monoclonal neutralizing antibodies were purchased from R&D Systems. The inhibitors U0126 and actinomycin D (A/D) were purchased from Calbiochem. The protease inhibitor cocktail was purchased from Roche Applied Science. Anti-Ret antibodies were purchased from Santa Cruz Biotechnology. pGEM-T vector, Reverse Transcription System and 2× polymerase chain reaction (PCR) Master Mix were purchased from Promega Corp. Lipofectamine 2000, Geneticin (G418) and protein G agarose were purchased from Invitrogen. Primers for PCR were synthesized by Sigma-Genosys.

Cell culture

SHSY5Y human neuroblastoma cells were cultured according to the method described in He *et al.* (1). All experiments were carried out in cells that were incubated in a low serum medium containing 1% FBS for 2 days.

pUSE-*GDNF* stable cell line

Human GDNF cDNA was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from total RNA of SHSY5Y cells, and cloned into pGEM-T vector. *GDNF* primers were as follows: upstream 5′-G AAG CTT ATG AAG TTA TGG GAT GTC GTG GCT GTC-3′ and downstream 5′-AAG CTT CTC GAG TCA GAT ACA TCC ACA CCT TTT AGC-3′. pGEM-*GDNF* was digested with restriction endonucleases *Hind*III and *Xho*I to produce the GDNF cDNA insert, which was then recombined into pUSE vector. The integrity of the *GDNF* cDNA was confirmed by sequencing. Lipofectamine 2000 was used to transfect pUSE-GDNF (pGDNF) and pUSE empty vector (control) into SHSY5Y cells, the transfected cells were maintained in growth media containing 800 μg/ml G418 and were subcultured every 5–6 days. Stable cells expressing pGDNF or pUSE were obtained after a one-month culture selection, then maintained in growth media containing 500 μg/ml G418. Prior to experiments, the pGDNF cells and the pUSE cells were grown in media without G418 for 2 days and incubated in low serum media containing 1% FBS for another 2 days. Conditioned media was collected from an overnight culture (15 h) of pGDNF cells (CM-GDNF). The pGDNF cells overexpressed *GDNF* (Supplemental Fig. S1A) and secreted high levels of the GDNF polypeptide (Supplemental Fig. S1B). These cells maintained constitutively high levels of Ret (Supplemental Fig. S2A) and ERK (Supplemental Fig. S2B) phosphorylation.

Treatments

Ibogaine was dissolved in water to make a stock solution of 10 mM and was used at a final concentration of 10 μM as described in He *et al.* (1). Cells were incubated with Ibogaine for the indicated time periods. To study the persistence of Ibogaine's effects following washout, cells were treated with Ibogaine for 3 h, washed, and incubated in fresh media for the indicated times, as shown in the figure legends. PI-PLC was used to hydrolyze GFRα1 from the cell surface; treatment involved incubation of cells with 0.3 u/ml PI-PLC for 1 h followed by washes with fresh media before treatment with conditioned media. Anti-GDNF neutralizing antibodies (anti-GDNF antibodies) were dissolved in PBS as a stock solution of 500 μg/ml, and were used at a concentration of 10 μg/ml.

Reverse transcription-polymerase chain reaction

Cells were treated in 6-well plates followed by total RNA isolation using 1 ml/well of Trizol reagent, as described in the manufacturer's protocol. The RNA samples were dissolved in 40 μl of water. RT-PCR was then performed to analyze *GDNF* expression level, with *Actin* as an internal control. Briefly, 1–2 μg of total RNA was used in a 10 μl reverse transcription (RT) reaction, using the oligo(dT)15 primer and the Reverse Transcription System kit, except in experiments with the inhibitors A/D and CHX. In those experiments, 4 μl of each RNA sample was used in the 10 μl RT reaction. The RT reaction was carried out at 42°C for 30 min followed by heating at 99°C for 6 min, then diluted to a final volume of 50 μl. 6 μl of the RT product was used for PCR in a 40 μl PCR mix; the PCR reaction was run for 35 cycles using *GDNF* primers or for 30 cycles using *Actin* primers. In parallel, PCR was performed with *GDNF* and *Actin* primers using the RNA samples as the template to confirm that results were not due to genomic DNA contamination. Signals of PCR products were visualized by electrophoresis in Tris/acetic acid/EDTA (TAE) buffer containing 0.25 mg/ml ethidium bromide, photographed by Eagle Eye II (Stratagene, La Jolla, CA, USA), and quantified by NIH Image 1.61.

Immunoprecipitation

Cells were treated in T75 flasks and lysed in radio-immunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 2 mM EDTA, protease inhibitor complete minitab and 10 mM sodium orthovanadate). 500 μg of homogenate was incubated with 5 μg anti-Ret antibodies in TBS-T buffer (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) overnight at 4°C, followed by 2 h incubation with Protein G agarose. Samples were separated on an SDS-PAGE gel for Western blot analysis with anti-pTyr and anti-Ret antibodies.

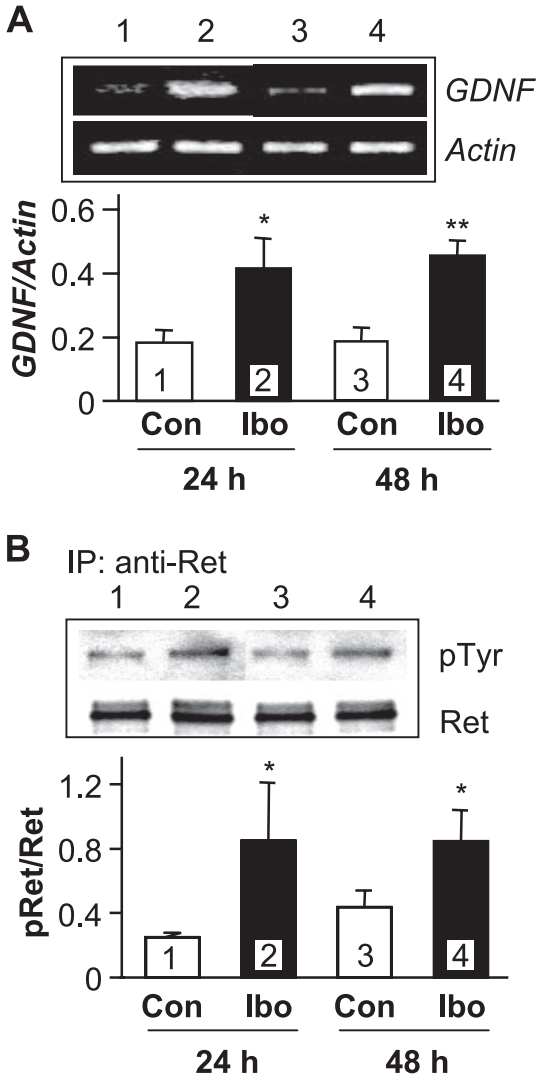

Figure 1. Ibogaine induces long-lasting expression and signaling activity of GDNF. SHSY5Y neuroblastoma cells were incubated in Dulbecco's modified Eagle medium (DMEM) containing 1% FBS for 2 days, then treated without (Con, lanes 1 and 3) or with (Ibo, lanes 2 and 4) 10 μM Ibogaine for 24 h and 48 h. *A) GDNF* expression was analyzed by RT-PCR with *Actin* as control. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. *B)* Ret was immunoprecipitated using anti-Ret antibodies, followed by immunoblotting with anti-p-Tyr and anti-Ret antibodies. Histogram depicts the mean ratios of pTyr (pRet) to Ret ± SD of five experiments. $*P < 0.05$; $**P < 0.01$, compared with control.

RESULTS

Ibogaine treatment induces a persistent increase in GDNF expression and Ret phosphorylation

First, we tested the effect of long-term incubation of SHSY5Y cells with Ibogaine on the expression of *GDNF,* and on the activation of the GDNF receptor, Ret. Treatment of cells with Ibogaine resulted in a sustained increase in *GDNF* mRNA for 24 and 48 h (**Fig. 1*A***, lanes 2, 4), as well as an increase in Ret phosphorylation (and thus, activation), which also persisted for 48 h after Ibogaine incubation (Fig. 1*B*, lanes 2, 4).

Next, we determined whether an acute treatment with Ibogaine leads to a sustained increase in *GDNF* mRNA levels. Cells were treated with Ibogaine for 3 h, at which time media containing the drug was removed, the cells were washed extensively, and new, Ibogaine-free media was added for the indicated times (**Fig. 2*A***). We found that 3 h incubation of cells with Ibogaine resulted in a persistent increase in *GDNF* mRNA levels even 12 h after Ibogaine removal (Fig. 2*A*). Next, we tested whether the long-term increase in *GDNF* message after acute exposure of cells to the drug was due to an increase in the stability of *GDNF* mRNA. Cells were treated with Ibogaine for 3 h, washed, and fresh media was added with or without actinomycin D (A/D), an inhibitor of transcription. As shown in Fig. 2*B*, A/D inhibited the long-lasting increase in *GDNF* mRNA, suggesting that Ibogaine does not affect the stability of the growth factor's message. We also tested whether the initial increase in *GDNF* expression on Ibogaine exposure, which leads to activation of the GDNF pathway (1), is required for the long-lasting increase in *GDNF* mRNA levels. To test this possibility, cells were treated with Ibogaine for 3 h and subsequently, following Ibogaine washout, the cells were incubated with fresh media containing anti-GDNF neutralizing antibodies. Incubation of cells with anti-GDNF antibodies, which sequester GDNF and thus inhibit the GDNF signaling pathway, abolished the increase in the expression of *GDNF* (Fig. 2*C*). These results suggest that Ibogaine triggers the long-lasting increase in *GDNF* expression in a GDNF-dependent manner.

Autoregulation of GDNF expression

Neurotrophins such as the brain-derived neurotrophic factor (BDNF) have been reported previously to self-regulate their expression and secretion (15–17). We, therefore, speculated that Ibogaine mediates its long-lasting activities via autoregulation of GDNF expression and function. First, we tested the effects of GDNF on its own transcription. We found that incubation of SHSY5Y cells with recombinant GDNF induced a sustained increase in *GDNF* expression (**Fig. 3*A***), suggesting a positive feedback mechanism in which activation of the GDNF pathway results in an increase in the message of the growth factor itself. To confirm this possibility, we incubated cells stably expressing the empty pUSE vector with conditioned media from cells that stably overexpress GDNF and secrete high levels of the growth factor (CM-GDNF; see Supplemental Fig. 1), to test whether a prolonged increase in *GDNF* mRNA was observed. As shown in Fig. 3*B*, incubation of cells with CM-GDNF induced a continuous increase in *GDNF* expression. This increase was not observed when the same cells were incubated with media of cells expressing the empty vector pUSE (Fig. 3*B*, CM-C). Next, we determined whether the induction of *GDNF* expression requires the ligation of the secreted

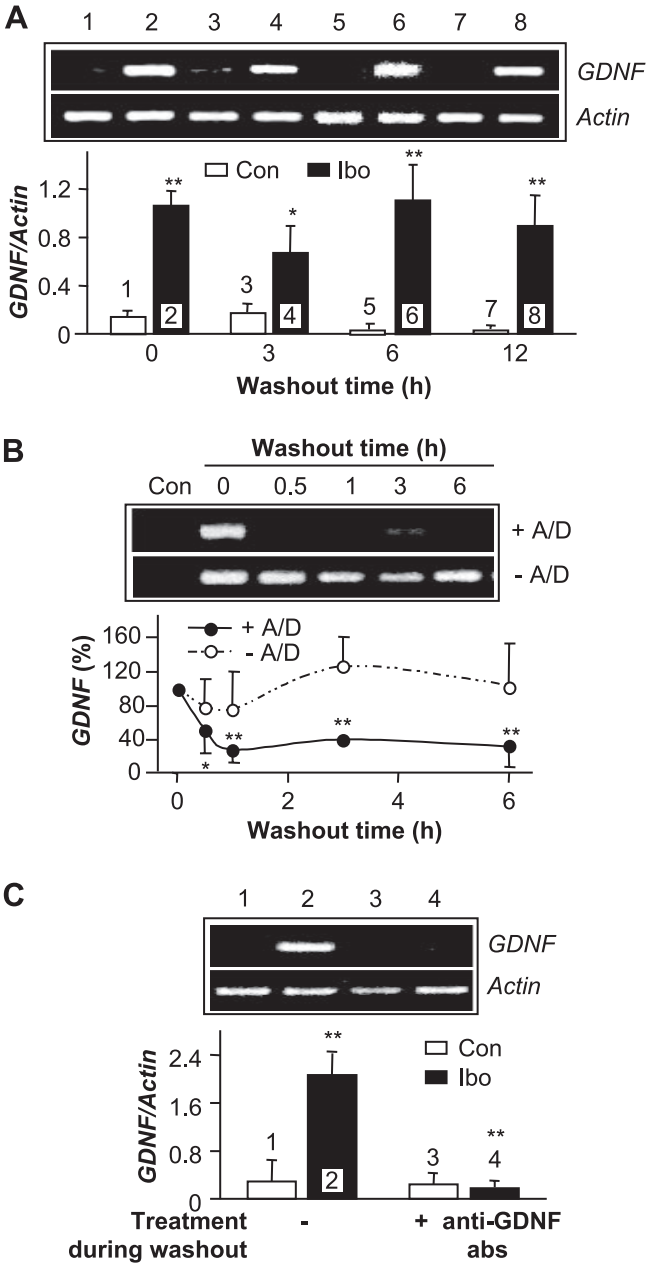

Figure 2. 3 h treatment with Ibogaine leads to a long-lasting increase in *GDNF* expression that is mediated by the GDNF polypeptide. *A)* Cells were treated without (Con, lanes 1, 3, 5, and 7), or with (lanes 2, 4, 6, and 8) 10 μM Ibogaine for 3 h. Cells were then extensively washed and incubated in fresh media for the indicated time period. *GDNF* expression was analyzed by RT-PCR. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $*P < 0.05$; $**P < 0.01$, compared with control. *B)* Cells were treated without (Con) or with 10 μM Ibogaine for 3 h, then washed extensively and incubated in fresh media for the period indicated without (lower panel) or with (upper panel) 5 μg/ml actinomycin D (A/D). Histogram depicts the mean percentages of *GDNF* ± SD of three experiments. $*P < 0.05$; $**P < 0.01$, compared with 0 time. *C)* Cells were treated without (lanes 1 and 3), or with (lanes 2 and 4) 10 μM Ibogaine for 3 h. Cells were then extensively washed and incubated with fresh media in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 10 μg/ml anti-GDNF neutralizing antibodies for 3 h. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $**P < 0.01$, lane 2 *vs.* 1, or lane 4 *vs.* 2.

polypeptide to its receptors. Cells were, therefore, treated with PI-PLC, which hydrolyzes the glycosylphosphatidylinositol (GPI) link of the GDNF coreceptor GFRα1 and thus blocks GDNF signaling, or anti-GDNF neutralizing antibodies, followed by addition of CM-GDNF. As shown in Fig. 3*C, D* (lanes 2 *vs.* 4), both the inhibitory reagents prevented the increase in *GDNF* expression. In addition, similar to Ibogaine's effect, short treatment with the CM-GDNF induced a persistent increase in the message of the growth factor, even after the CM-GDNF was removed (Fig. 3*E*). Finally, we found that, similar to the long-lasting actions of Ibogaine, the long-term increase in the expression of *GDNF* induced by CM-GDNF was inhibited in the presence of A/D, suggesting that GDNF does not increase the stability of its own message (Fig. 3*F*).

Ibogaine- and GDNF-induced *GDNF* expression are mediated via the activation of MAP kinase

GDNF-mediated autophosphorylation of the Ret receptor initiates activity in several different downstream signaling pathways, including activation of the mitogen-activated protein (MAP) kinases (ERKs), leading to alteration in gene expression (18). To determine whether activation of the MAP kinase pathway is required for Ibogaine-mediated long-term induction of *GDNF* mRNA, we investigated the effects of Ibogaine and CM-GDNF on *GDNF* expression in cells that were preincubated with the MEK-specific inhibitor U0126. We found that blockade of ERK activity by U0126 inhibited the induction of *GDNF* expression in cells given 3 h exposure to either Ibogaine (**Fig. 4*A***) or CM-GDNF (Fig. 4*B*); this effect was also observed when the MEK inhibitor was added after Ibogaine washout (Fig. 4*C*).

Long-term but not short-term up-regulation of *GDNF* expression depends on protein synthesis

Our data suggest that acute exposure of cells to Ibogaine results in an increase in *GDNF* mRNA levels, followed by subsequent translation of the polypeptide, which is then secreted to up-regulate its own message, initiating a GDNF-mediated autoregulatory cycle. If our model is correct, then increases in *GDNF* message on short-term Ibogaine exposure should not be dependent on protein synthesis. As predicted, a brief (0.5 h) incubation of cells with Ibogaine was insensitive to the protein synthesis inhibitor, cycloheximide (**Fig. 5*A***, lane 3; Fig. 5*B*, lane 3), whereas longer (≥1 h) Ibogaine-mediated induction of *GDNF* expression was sensitive to cycloheximide treatment (Fig. 5*A*, lanes 5, 7, 9; Fig. 5*B*, lane 6).

DISCUSSION

Based on our results, we propose a model (**Fig. 6**) in which Ibogaine exposure leads to an increase in *GDNF*

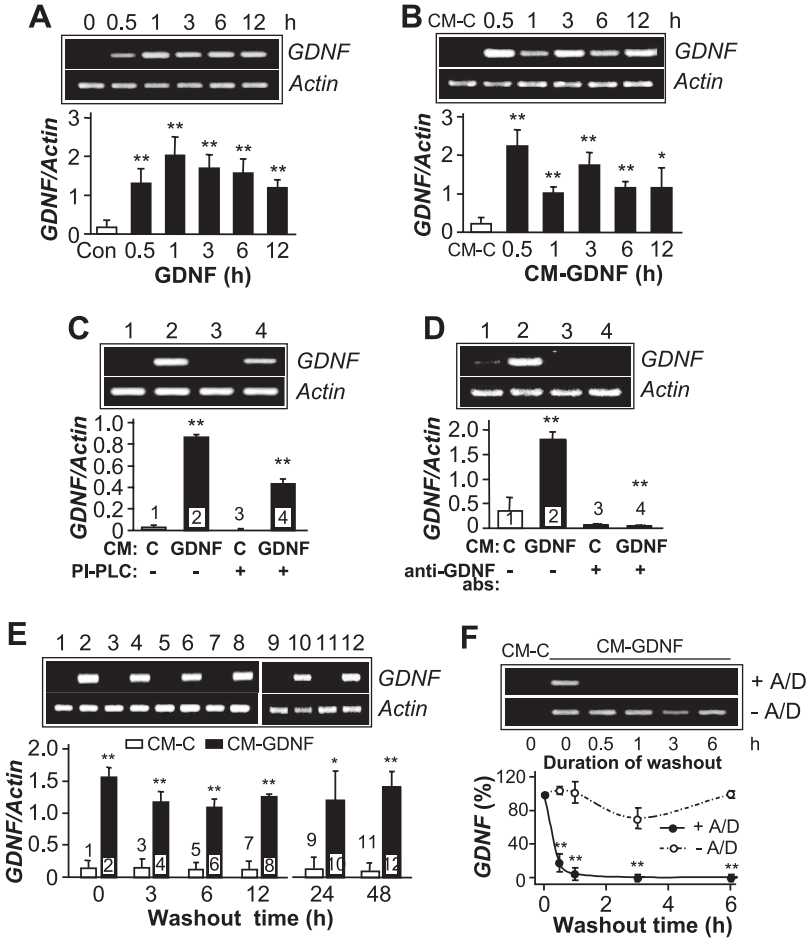

Figure 3. GDNF induces *GDNF* expression. *A)* Cells were treated without (Con) or with 50 ng/ml of recombinant GDNF polypeptide for the indicated time period. *GDNF* expression was analyzed by RT-PCR. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{**}P < 0.01$, compared with control. *B)* SHSY5Y cells stably transfected with the pUSE empty vector were treated with conditioned media from the empty vector cells (CM-C) or media from the pUSE-GDNF stable cells (CM-GDNF) for the indicated length of time. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with control (CM-C). *C)* pUSE cells were preincubated without (lanes 1 and 2) or with (lanes 3 and 4) 0.3 u/ml PI-PLC for 1 h. Cells were washed and incubated with media from pUSE cells (CM-C, lanes 1 and 3) or from pUSE-GDNF cells (CM-GDNF, lanes 2 and 4) for 3 h. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{**}P < 0.01$, lane 2 *vs.* 1, or lane 4 *vs.* 2. *D)* CM-C (lanes 1 and 3), and CM-GDNF (lanes 2 and 4) were preincubated for 1 h without (lanes 1 and 2), or with (lanes 3 and 4) with 10 μg/ml anti-GDNF neurtralizing antibodies prior to incubation of pUSE cells with the indicated media for an additional 3 h. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{**}P < 0.01$, lane 2 *vs.* 1, or lane 4 *vs.* 2. *E)* pUSE cells were treated without (lanes 1, 3, 5, 7, 9, and 11) or with (lanes 2, 4, 6, 8, 10 and 12) CM-GDNF for 3 h. Cells were then washed and incubated in fresh media as indicated. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with control (CM-C). (F) pUSE cells were treated with CM-C or with CM-GDNF for 3 h. Cells were then extensively washed and incubated in fresh media for the indicated time period without (lower panel) or with (upper panel) 5 μg/ml actinomycin D (A/D). Histogram depicts the mean percentages of *GDNF* ± SD of three experiments. $^{**}P < 0.01$ compared with 0 time.

message, followed by the translation and subsequent secretion of the polypeptide, resulting in the activation of the GDNF receptor tyrosine kinase Ret, which then activates the MAP kinase pathway to further up-regulate the message of the growth factor. This GDNF-mediated autoregulatory positive feedback mechanism may explain the long-lasting actions of Ibogaine to reduce drug and alcohol self-administration, which have been shown in rodent models to last 24 h or longer (1, 6, 7). In addition, this mechanism may account for anecdotal human reports suggesting that a single treatment of Ibogaine reduces craving for various drugs of abuse for up to six months (14); however, these observations need further investigation.

Autoregulation of growth factors has been previously documented. For example, BDNF has been reported to positively regulate its own expression (17), secretion (15), and dendritic targeting of its own mRNA (16). In addition, various endogenous and pharmacological agents have been shown to control *GDNF* expression (19–21); however, to our knowledge this is the first report of the up-regulation of *GDNF* expression via GDNF itself. The mechanism for GDNF regulation of its own message needs to be determined, however, analysis of the promoter region of the human *GDNF* gene revealed putative Sp-1 and activating protein (AP)-2 transcription factor binding sites (22). The MAP kinase pathway has been shown to up-regulate the transcription of another growth factor, the vascular endothelial growth factor (VEGF), via the Sp1 and AP-2 biding sites (23). We found that the autoregulatory increase in *GDNF* expression is inhibited on incubation of cells with a MEK inhibitor, suggesting that activation of the MAP kinase pathway contributes to the long-lasting increase in *GDNF* expression. Therefore, it is possible that the Sp1 and AP-2 transcription factor binding sites within the *GDNF* promoter contribute to a GDNF-mediated increase in its own mRNA.

An intriguing possibility is that autoregulation of GDNF expression and sustained activation of the GDNF pathway contribute to such long-term processes as neuronal survival, as GDNF has been shown to be a critical mediator of the development and survival of midbrain dopaminergic neurons (18). This positive cycle may also account for GDNF's actions on dopamine synthesis (24) via increasing the phosphorylation

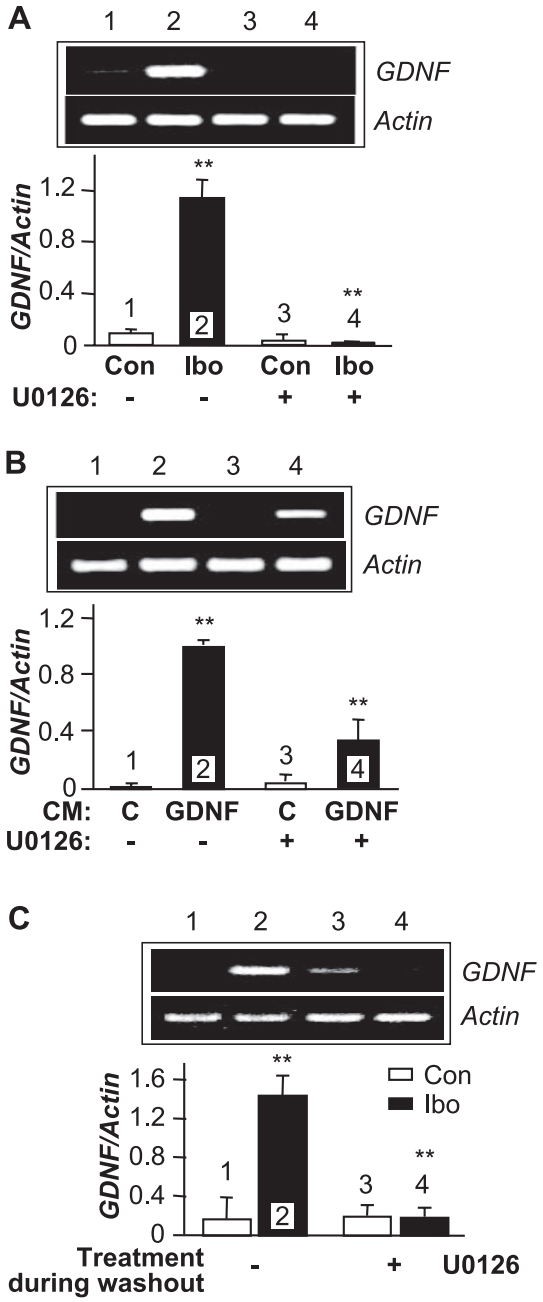

Figure 4. Ibogaine- and GDNF-induced *GDNF* expression are mediated via the MAP kinase pathway. *A, B)* Wild-type (WT) SHSY5Y cells (*A*) or pUSE cells (*B*) were preincubated without (lanes 1, 2) or with (lanes 3, 4) 20 μM U0126 for 0.5 h before the addition of 10 μM Ibogaine (*A*, lanes 2, 4) or CM-GDNF (*B*, lanes 2, 4) for 3 h. *C)* WT SHSY5Y cells were treated without (lanes 1, 3) or with (lanes 2, 4) 10 μM Ibogaine for 3 h. Cells were then washed and incubated in fresh media in the absence (lanes 1, 2) or presence (lanes 3, 4) of 20 μM U0126 for 3 h. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of three experiments. $^{**}P < 0.01$, lane 2 *vs.* 1, or lane 4 *vs.* 2.

and activity of tyrosine hydroxylase, the enzyme controlling the rate-limiting step in dopamine biosynthesis (25). Finally, GDNF has been shown to contribute to synaptic plasticity processes and learning and memory (26–29). One of the intracellular signaling cascades initiated via GDNF-mediated activation of the Ret receptor is the MAP kinase pathway (18), and MAP kinase has been shown to be a critical player in long-term potentiation and learning and memory (30–32). Together our results suggest a mechanism for GDNF involvement in synaptic plasticity.

Importantly, this autoregulatory positive feedback in the GDNF pathway may have implications for the treatment of addiction. Various studies have suggested that GDNF acts as a negative regulator of biochemical and behavioral adaptations to drugs of abuse and alcohol. For example, infusion of GDNF into the VTA of rats blocks and/or reverses cocaine-induced increases in the NR1 subunit of the NMDA receptor, alters ΔFosB and PKA Ca in the nucleus accumbens, and blocks the behavioral effects of repeated exposure to cocaine, as measured by the conditioned place preference procedure (33). Green-Sadan *et al.* reported that transplantation of simian virus-40 glial cells, which produce and secrete GDNF, or delivery of GDNF-conjugated nanoparticles into the dorsal and ventral striatum impaired the acquisition of cocaine self-ad-

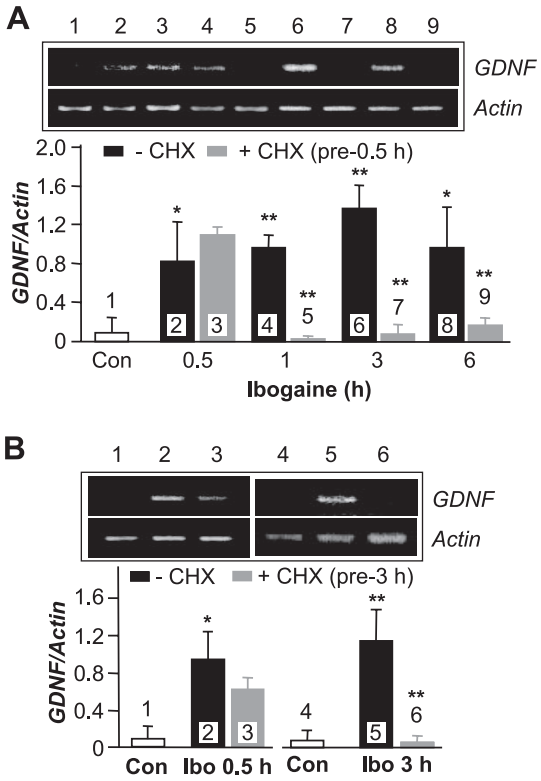

Figure 5. Long-lasting Ibogaine-mediated induction of *GDNF* expression depends on protein synthesis. *A)* SHSY5Y cells were preincubated without (lanes 1, 2, 4, 6, and 8) or with (lanes 3, 5, 7, and 9) 30 μg/ml cycloheximide (CHX) for 0.5 h before the addition of 10 μM Ibogaine (lanes 2 – 9) for the indicated time periods. *B)* Cells were preincubated without (lanes 1, 2, 4, and 5) or with (lanes 3 and 6) 30 μg/ml CHX for 3 h before the addition of 10 μM Ibogaine for 0.5 h (lanes 2 and 3) or for 3 h (lanes 5 and 6). Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD of 3 experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, Ibogaine alone (–CHX) *vs.* control, or + CHX *vs.* –CHX.

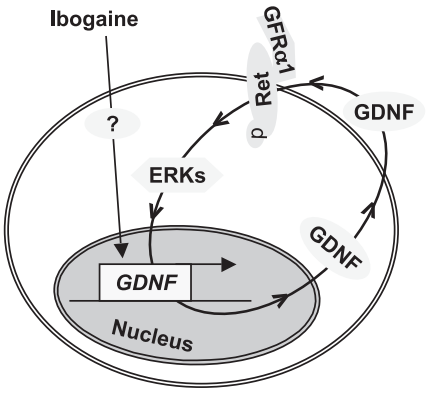

Figure 6. Diagram of Ibogaine- and GDNF-mediated long-lasting induction of GDNF expression and signaling. The data presented suggest a model in which GDNF or Ibogaine up-regulates *GDNF* expression leading to the translation of the polypeptide, which is secreted and consequently activates the Ret receptor and its downstream target ERK. This, in turn, results in further increases in *GDNF* expression. This positive feedback loop induces a sustained long-lasting activation of the pathway.

ministration (34, 35). Finally, we found that intra-VTA infusion of GDNF reduced rats' operant self-administration of ethanol (1). Taken together, these results suggest that agents which activate the GDNF pathway and/or increase GDNF message may be useful drugs to treat addiction, and our current work implies that short-term treatment with such agents may result in long-lasting changes in addictive phenotypes. Finally, the identification of a GDNF-mediated autoregulatory feedback loop may have implications for its potential therapeutic value as treatment for neurodegenerative diseases such as Parkinson's disease. FJ

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Addiction Biology

PRECLINICAL STUDY

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Noribogaine, but not 18-MC, exhibits similar actions as ibogaine on GDNF expression and ethanol self-administration

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ABSTRACT

Ibogaine is a naturally occurring alkaloid that has been reported to decrease various adverse phenotypes associated with exposure to drugs of abuse and alcohol in human and rodent models. Unfortunately, ibogaine cannot be used as a medication to treat addiction because of severe side effects. Previously, we reported that the desirable actions of ibogaine to reduce self-administration of, and relapse to, alcohol consumption are mediated via the upregulation of the expression of the glial cell line-derived neurotrophic factor (GDNF) in the midbrain ventral tegmental area (VTA), and the consequent activation of the GDNF pathway. The ibogaine metabolite, noribogaine, and a synthetic derivative of ibogaine, 18-Methoxycoronaridine (18-MC), possess a similar anti-addictive profile as ibogaine in rodent models, but without some of its adverse side effects. Here, we determined whether noribogaine and/or 18-MC, like ibogaine, increase *GDNF* expression, and whether their site of action to reduce alcohol consumption is the VTA. We used SH-SY5Y cells as a cell culture model and found that noribogaine, like ibogaine, but not 18-MC, induces a robust increase in *GDNF* mRNA levels. Next, we tested the effect of intra-VTA infusion of noribogaine and 18-MC on rat operant alcohol self-administration and found that noribogaine, but not 18-MC, in the VTA decreases responding for alcohol. Together, our results suggest that noribogaine and 18-MC have different mechanisms and sites of action.

Keywords 18-Methoxycoronaridine, addiction, ethanol self-administration, GDNF, ibogaine, noribogaine.

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INTRODUCTION

Alcohol and drug addiction are chronic relapsing diseases characterized by compulsive drug use, and are major societal issues. However, available medications to treat drug addiction in general, and alcoholism in particular, are very limited. Additionally, the two leading FDA-approved medications to treat alcoholism, Naltrexone and Acamprosate, have limited efficacy and success rates (Johnson 2008; Garbutt 2009). One drug that initially showed great promise as a possible treatment for multiple types of addiction is ibogaine (Maciulaitis *et al.* 2008). Ibogaine is a naturally occurring indole alkaloid claimed to reverse addiction to opiates, stimulants, alcohol, and nicotine (Popik, Layer & Skolnick 1995; Mash *et al.* 1998; Vastag 2002; Alper, Lotsof & Kaplan 2008). Ibogaine is extracted from the root bark of the West African shrub *Tabernanthe iboga* and is used by indigenous people in low doses to keep hunters awake and motionless, and in higher doses for religious rituals because of its psychostimulant and hallucinogenic properties (Alper *et al.* 2008; Maciulaitis *et al.* 2008). The initial discovery that ibogaine eliminates signs and symptoms of opioid withdrawal and diminishes craving for heroin was made in the 1960s by a group of heroin addicts (Mash *et al.* 1998; Maciulaitis *et al.* 2008). Since that time, several human anecdotal reports and studies have shown that ibogaine possesses many attractive properties as an anti-addiction agent. First, the compound crosses the blood brain barrier and can be given to addicts orally (Mash *et al.* 1998). Second, a single dose of ibogaine is claimed to abolish drug craving for up to 6

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months, and repeated ibogaine treatment (a series of four oral administrations) was reported to be effective in blocking craving and relapse for up to 3 years in cocaine and opiate addicts (Sheppard 1994; Mash *et al.* 1998; Alper *et al.* 2000). Since compliance is a significant problem in medication development to treat addiction, the fact that ibogaine has very long lasting effects (months to years) is very desirable.

Studies in animal models recapitulate human observations. Ibogaine reduces self-administration and withdrawal symptoms for alcohol and various drugs of abuse in mice, rats and monkeys (Glick *et al.* 2000). For example, single and repeated intraperitoneal (i.p.) injections of a moderate dose of ibogaine (40 mg/kg) can reduce self-administration of cocaine or morphine in rats and cocaine in mice for several days (Cappendijk & Dzoljic 1993; Glick *et al.* 1994; Sershen, Hashim & Lajtha 1994). Ibogaine decreases heroin self-administration and naloxone/naltrexone-precipitated withdrawal in morphine-dependent rats (Glick *et al.* 1992; Cappendijk, Fekkes & Dzoljic 1994; but see Sharpe & Jaffe 1990). Finally, ibogaine reduces alcohol (ethanol) intake in several strains of alcohol-preferring rats in a dose-dependent manner, using concentrations ranging from 10 to 60 mg/kg, with no changes in blood ethanol concentration or food intake (Rezvani, Overstreet & Lee 1995). We replicated and extended the findings of Rezvani *et al.* (1995) on different ethanol-drinking behaviors. We found that an acute injection of ibogaine (40 mg/kg, i.p.) in male Long-Evans rats 3 hours before the test session significantly reduced ethanol intake, but not the concurrent water or sucrose intake, using a two-bottle preference procedure, as well as operant ethanol self-administration (He *et al.* 2005). We were also interested in testing whether ibogaine would have efficacy in a model of relapse, in which rats are allowed to self-administer ethanol in operant chambers following 3 weeks of extinction training. Ibogaine blocked the excessive intake produced by such forced abstinence period (He *et al.* 2005), suggesting a potent action of ibogaine to prevent relapse. In summary, human anecdotal reports, as well as several preclinical studies in rodents, suggest that ibogaine is a long-lasting and selective anti-addiction medication for alcohol, opioids, psychostimulants and nicotine.

Unfortunately, despite its attractive properties, ibogaine is not used in the US to treat addiction because of severe side effects, which include hallucination, bradycardia, whole-body tremors and ataxia (Popik *et al.* 1995; Glick *et al.* 2000; Maas & Strubelt 2006; Alper *et al.* 2008; Maciulaitis *et al.* 2008). In addition, while a moderate dose of ibogaine (40 mg/kg) that is effective to reduce self-administration of several drugs of abuse is not neurotoxic in rodents (Molinari, Maisonneuve & Glick 1996; He *et al.* 2005), cerebellar Purkinje cell death has been reported in rats after administration of a higher concentration (100 mg/kg, i.p.) (O'Hearn & Molliver 1993, 1997). Moreover, although ibogaine is not addictive, the FDA classified the drug in the schedule I category of narcotics because of its stimulant and hallucinogenic properties.

In an attempt to separate the desirable anti-addictive properties of the drug from the undesirable side effects, we set out to identify the molecular mechanism mediating ibogaine's effects on voluntary ethanol consumption. We found that systemic administration of ibogaine induced a long-lasting increase in the expression of the glial-derived neurotrophic factor (GDNF) in the dopaminergic ventral tegmental area (VTA) of rodents and that the effect of ibogaine to reduce ethanol self-administration is localized in the VTA (He *et al.* 2005). Importantly, when the GDNF pathway was inhibited in the VTA, ibogaine was significantly less effective in reducing ethanol self-administration (He *et al.* 2005). More recently, we observed that upregulation of tyrosine hydroxylase levels resulting from long-term exposure of ethanol in a dopaminergic-like cell line is blocked by ibogaine in a mechanism that requires GDNF (He & Ron 2008). Finally, similarly to ibogaine, GDNF administered into the VTA resulted in a long-lasting inhibition of ethanol-seeking and -drinking behaviors in models of moderate and excessive drinking of ethanol, as well as in a model of relapse (He *et al.* 2005; Carnicella *et al.* 2008; Carnicella & Ron 2009; Carnicella, Amamoto & Ron 2009b). Together, our results suggest that the upregulation of the GDNF pathway in the VTA mediates, at least in part, the desirable actions of ibogaine to reduce voluntary ethanol consumption and relapse.

Ibogaine is metabolized by cytochrom P4502D6 into a major metabolite noribogaine (12-hydroxyibogamine) (Obach, Pablo & Mash 1998). Interestingly, while ibogaine levels in the plasma rapidly decline, noribogaine levels remain high for at least 24 hours after oral administration of ibogaine in humans (Mash *et al.* 1995; Mash *et al.* 1998; Mash *et al.* 2000). Similarly, it has been shown in rats that peak blood levels of noribogaine were reported to exceed those of ibogaine, and a small, but significant portion of the administered noribogaine was still detected in the bloodstream 24 hours after an acute i.p. administration (Baumann *et al.* 2001). Behavioral studies show that noribogaine acts as an active metabolite (Baumann *et al.* 2001) and possesses a similar anti-addictive profile as ibogaine (Glick *et al.* 1996b). For example, systemic administration of noribogaine induces a long-lasting decrease of morphine and cocaine self-administration (Glick *et al.* 1996b). Importantly, noribogaine does not produce tremors and ataxia (Glick *et al.* 1996b; Baumann *et al.* 2001), suggesting that it is less

neurotoxic than its parent compound. Moreover, noribogaine was recently found to be more than two times less toxic than its parent compound in mice (Kubiliene *et al.* 2008).

18-MC is a synthetic derivative of ibogaine (Glick *et al.* 1996a). 18-MC shares ibogaine's desirable properties but, interestingly, the derivative lacks some of the adverse effects of ibogaine (Glick *et al.* 2000). Like ibogaine, 18-MC administration results in a long-lasting decrease in ethanol, morphine, cocaine, methamphetamine and nicotine self-administration, and attenuation of opioid withdrawal symptoms (reviewed in Glick *et al.* 2000). However, unlike ibogaine, 18-MC does not induce bradycardia, whole body tremor and cerebellar toxicity in rats, even at high doses (Glick *et al.* 2000). Moreover, 18-MC is probably not hallucinogenic as it does not affect serotonin levels (Glick *et al.* 2000).

Because of similarities in structure and anti-addictive actions of ibogaine, noribogaine and 18-MC, we hypothesized that like ibogaine, the ibogaine derivatives upregulate *GDNF* expression and that their mechanism of action to reduce ethanol self-administration is localized in the VTA. Therefore, we set out to determine whether noribogaine and 18-MC increase *GDNF* expression in SH-SY5Y cells and whether the ibogaine derivatives affect operant ethanol self-administration in rats when infused into the VTA.

METHODS

Reagents

Noribogaine hydrochloride was a generous gift from the Addiction Research Institute (Austin, Texas), and 18-MC hydrochloride was obtained from Albany Molecular Research, Inc. (Albany, NY). Ibogaine was purchased from Sigma (St. Louis, MO). Growth medium Dulbecco's modified Eagle's medium (DMEM) and Trizol reagent were purchased from Invitrogen (Carlsbad, CA). The Reverse Transcription System kit was purchased from Promega (Madison, WI).

Animals

Male Long-Evans rats (280–300 g at the beginning at the experiment) were obtained from Harlan (Indianapolis, IN). Rats were housed on a 12-hour light/dark cycle, with lights on 7:00 a.m., and food and water available *ad libitum*. All animal procedures in this report were approved by the Gallo Center Institutional Animal Care and Use Committee and were conducted in agreement with the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996.

Cell culture and drug treatment

SH-SY5Y human neuroblastoma cells were cultured in the growth medium DMEM containing 10% fetal bovine serum (FBS) plus 1 × MEM non-essential amino acid solution. Cells were incubated in a low-serum medium containing 1% FBS for 2 days before experiments. Cells were then treated for 3 hours with different concentrations of ibogaine (5, 10 or 50 μM), noribogaine (5, 10 or 50 μM) or 18-MC (10, 20, 50 or 100 μM) dissolved in the medium. This time point was chosen because we previously observed that 3 hours incubation of SH-SY5Y cells with ibogaine or cabergoline was sufficient time to upregulate GDNF pathway (He *et al.* 2005; Carnicella *et al.* 2009b).

Reverse transcription—polymerase chain reaction (RT-PCR)

Total RNAs were isolated using Trizol reagent and reversely transcribed using a Reverse Transcription System kit at 42°C for 30 minutes. *GDNF* was analyzed by PCR with temperature cycling parameters consisting of initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 2 minutes, and a final incubation at 72°C for 7 minutes. PCR for *actin*, an internal control, was run with the same temperature cycling parameters for 30 cycles. The primers used in RT-PCR are as follows: human *GDNF*, upstream 5′-TGC CAG AGG ATT ATC CTG ATC AGT TCG ATG-3′ and downstream 5′-TTG TCG TAC GTT GTC TCA GCT GCA TCG CAA-3′; human *actin*, upstream 5′-TCA TGA AGT GTG ACG TTG ACA TC-3′ and downstream 5′-AGA AGC ATT TGC GGT GGA CGA TG-3′. PCR products were separated on 1.8% agarose gels in Tris/acetic acid/EDTA buffer with 0.25 μg/ml ethidium bromide, photographed by Eagle Eye II (Stratagen, La Jolla, CA) and quantified by NIH Image 1.61.

Operant ethanol self-administration after a history of high voluntary ethanol consumption

High levels of voluntary ethanol consumption were obtained in an intermittent access two-bottle choice drinking paradigm of 20% ethanol, as previously described (Carnicella *et al.* 2009b). Briefly, animals were given 24-hour concurrent access to one bottle of 20% (v/v) ethanol in tap water and one bottle of water on Monday, Wednesday and Friday, with 24- or 48-hour ethanol deprivation periods in between the ethanol-drinking sessions. After achieving a stable baseline of ethanol consumption of ~6 g/kg/24 hours and preference for the 20% ethanol solution over water (see Fig 2a–c), rats were trained to self-administer a 20% ethanol

solution in operant self-administration chambers. The chambers contained two levers: an active lever, for which presses result in delivery of 0.1 ml of the ethanol solution, and an inactive lever, for which responses are counted but no programmed events occur. After two nights in the chambers to allow acquisition of a lever-press response for ethanol under a fixed ratio 1 (FR1), operant sessions were conducted 5 days per week, with the schedule requirement increased to FR2 and the length of session shortened from 60 to 30 minutes over the first 2 weeks. Because the level of presses on the inactive lever was extremely low after acquisition of the self-administration procedure (< 10 presses), and the activity on this lever was not affected by any of the experimental treatments, this measure was excluded from the figures and the analysis for better clarity. After 1 month of training, surgery to implant cannulae was conducted.

Surgery and intra-VTA infusions

Rats were anesthetized continuously with isoflurane (Baxter Health Care Corporation, Deerfield, IL). Bilateral guide cannulae (C235G-1.5, 26 ga, Plastics One, Roanoke, VA) were aimed dorsal to the VTA (5.6 mm posterior to bregma, 0.75 mm mediolateral, 8.0 mm ventral to the skull surface), according to Paxinos & Watson (2007). The coordinates were chosen according to previous studies (He *et al.* 2005; Carnicella *et al.* 2008). After 3 to 5 days of recovery, rats returned to self-administration training and intra-VTA infusions began when operant responding returned to a stable baseline. Noribogaine and 18-MC were dissolved in PBS containing 2% DMSO. Three hours before the beginning of the session, 0.8 µl of noribogaine (1, 10, 100 µM; equivalent to 3, 30, 300 ng/µl), 18-MC (1, 10, 100 µM; equivalent to 3.7, 37, 370 ng/µl) or vehicle were infused over 2 minutes to gently restrained rats via injection cannula extending 0.5 mm beyond the guide cannula tip. The time point of infusion and concentration were chosen according to He *et al.* (2005), in which we found that intra-VTA infusion of ibogaine 3 hours before the beginning of the session decreased ethanol self-administration. The experiment was performed using a within-subject, Latin Square Design, with an interval of at least 1 week between two infusions, allowing lever responding for ethanol to return to baseline (e.g. at least three sessions with similar operant performance as the pre-infusion period) between treatments. This repeated procedure of infusions does not produce significant physical damage in the VTA (Carnicella *et al.* 2008) and does not affect the VTA function. Indeed, baseline operant performance stayed the same throughout the experiment without any adverse effects in response to repeated infusion of the vehicle. Moreover, the efficacy of the agents infused into the VTA to reduce operant ethanol self-administration remained similar throughout the experiment. It should be noted that responding for ethanol in the vehicle group decreased somewhat over the 4 days of self-administration. This effect is due to the fact that rats have access to ethanol 5 days a week. Therefore, a small deprivation effect is detected at the beginning of the week (after 2 days of ethanol withdrawal) that disappears over the self-administration session. Importantly, we also observe this effect without manipulations, indicating that it is independent of the intra-brain region infusion.

Histology

Locations of cannulae were verified in 60-µm coronal sections of paraformaldehyde-fixed tissue stained with thionine. Only data from subjects with cannulae located in the region of interest were included in the analysis (see Fig 2d).

Statistical analysis

Data were analyzed using a one-way or two-way ANOVA with repeated measures, followed by Student-Newman-Keuls test or the method of contrasts (Keppel 1991) when indicated.

RESULTS

Noribogaine, but not 18-MC, is a potent inducer of *GDNF* expression

First, we determined whether exposure of the dopaminergic-like SH-SY5Y cell line to noribogaine or 18-MC results in an increase in the expression of *GDNF*. We, therefore, treated cells for 3 hours with different concentrations of noribogaine or 18-MC and compared the level of *GDNF* expression to those obtained upon incubation of cells to ibogaine. As shown in Fig. 1a and b, noribogaine induced a dose-dependent increase in *GDNF* expression similar to the one observed upon exposure of cells to ibogaine [$F(3, 12) = 14.64$, $P < 0.001$ and $F(3, 20) = 10.39$, $P < 0.001$, for ibogaine and noribogaine, respectively]. In contrast, incubation of SH-SY5Y cells with 18-MC produced a very small, albeit significant, increase in *GDNF* expression, even at high concentrations [Fig 1c, $F(4 \text{ and } 34) = 4.02$, $P < 0.01$]. Noribogaine and 18-MC were also tested at other time points (0.5 to 6 hours). Noribogaine increased *GDNF* expression at 1 hour and 6 hours; however, no significant effects were observed when cells were treated with 18-MC (data not shown), indicating that the absence of effect of 18-MC on *GDNF* expression is not due to an issue of the time

window of action. These results, therefore, suggest that the ibogaine metabolite, noribogaine, but not its synthetic derivative, 18-MC, is a potent inducer of *GDNF* expression.

Intra-VTA infusion of noribogaine, but not 18-MC, reduces operant ethanol self-administration in rats

Next, we tested whether infusion of noribogaine or 18-MC into the VTA, the site of action of both ibogaine and GDNF (He *et al*. 2005; Carnicella *et al*. 2008; Carnicella *et al*. 2009b), decreases operant ethanol self-administration in rats with a history of high levels of ethanol consumption. Specifically, intermittent access to a 20% ethanol solution in a two-bottle choice drinking paradigm led to an escalation of ethanol consumption (Fig. 2a), a progressive decrease in the drinking of the water solution (Fig. 2b) and, consequently, to an increase in preference for the 20% ethanol solution over water (Fig. 2c). Rats were then trained to self-administer a 20% ethanol solution in operant self-administration chambers and noribogaine, 18-MC or vehicle was infused into the VTA (Fig. 2d) after acquisition of a stable baseline of responding. As shown in Fig. 3a and b, noribogaine—but not 18-MC—significantly reduced operant responding for ethanol 3 hours after the intra-VTA infusion [$F(3, 36) = 6.72$, $P < 0.001$ and $F(3, 36) = 1.23$, $P = 0.31$, for noribogaine and 18-MC, respectively], leading to a substantial decrease in ethanol intake only for the rats that received noribogaine [$F(3, 36) = 7.66$, $P < 0.001$ and $F(3, 36) = 1.85$, $P = 0.16$, for noribogaine and 18-MC, respectively] (Fig. 3c & d).

To ensure that the absence of behavioral effects in response to intra-VTA administration of 18-MC was not due to the use of too low of a dose, the dose of 10 µg/µl of 18-MC, which has been shown to efficiently reduce morphine and methamphetamine self-administration when infused into the medial habenula or the interpeduncular nucleus (Glick *et al*. 2006; Glick, Sell & Maisonneuve 2008), was also tested in the VTA and did not affect operant ethanol self-administration (data not shown).

Interestingly, as shown in Fig. 4a and c, noribogaine at a concentration of 10 µM produced a decrease in operant ethanol self-administration that was long-lasting and persisted for more than 48 hours [Treatment, $F(1, 36) = 10.63$, $P < 0.01$, Session, $F(3, 36) = 2.46$, $P = 0.08$, Treatment–Session interaction, $F(3, 36) = 3.26$, $P < 0.05$ and Treatment, $F(1, 36) = 11.26$, $P < 0.01$, Session, $F(3, 36) = 2.18$, $P = 0.11$, Treatment–Session interaction, $F(3, 36) = 3.13$, $P < 0.05$, for ethanol deliveries and ethanol intake, respectively]. Similar results were obtained with the 100 µM concentration of noribogaine (data not shown). In contrast, and consistent with

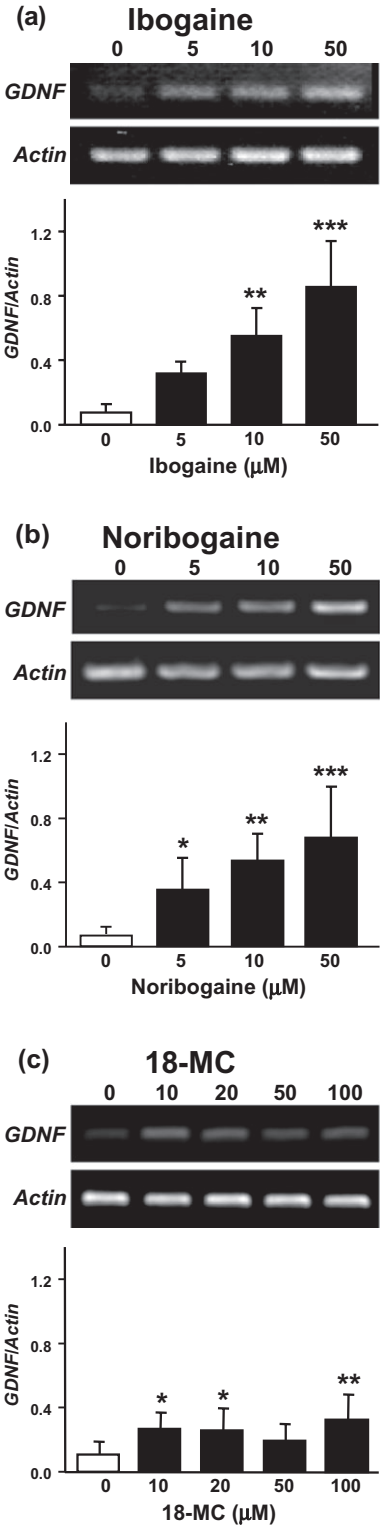

Figure 1 Ibogaine and noribogaine, but not 18-MC, dose-dependently increase *GDNF* expression in the dopaminergic-like SH-SY5Y cell line. SH-SY5Y cells were treated for 3 hours with ibogaine (5, 10 or 50 µM; *a*), noribogaine (5, 10 or 50 µM; *b*) or 18-MC (10, 20, 50 or 100 µM; *c*), and *GDNF* expression was analyzed by RT-PCR. Data are expressed as mean ± SD of the *GDNF*/*Actin* ratios. $n = 6$–8. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control

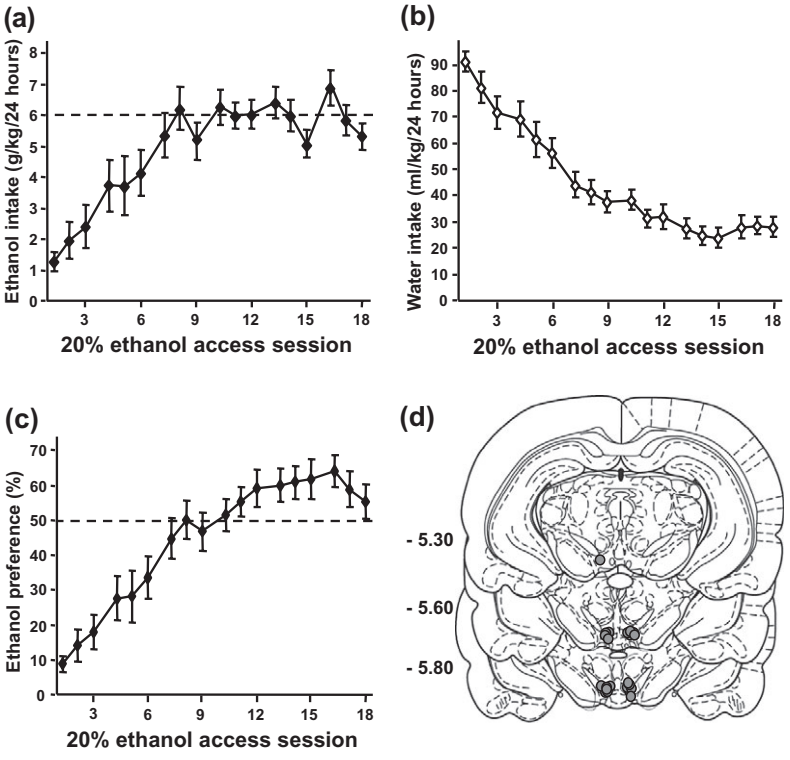

Figure 2 Escalation of ethanol intake and preference in an intermittent access two-bottle choice paradigm and representation of the cannulae placement. (*a–c*), Mean ± standard error of the mean (SEM) of ethanol (*a*), water (*b*) intake and ethanol preference (*c*) during acquisition of voluntary consumption of a 20% ethanol solution. (*d*), Schematic representation of the cannulae placement on coronal section (Paxinos & Watson 2007). The location of the injector tips is represented by *gray circles*. Numbers on the left side indicate the distance posterior to bregma in millimeters. $n = 13$

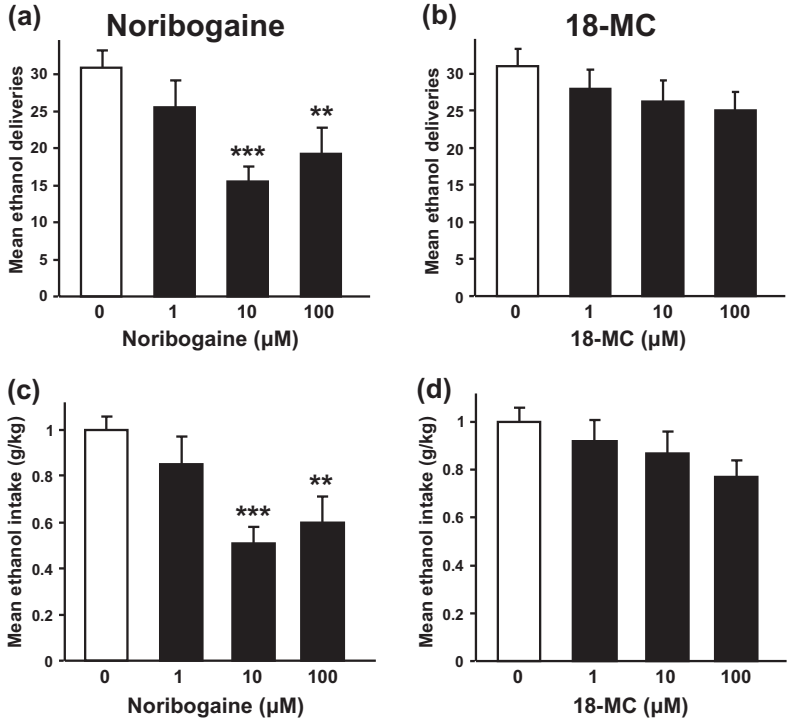

Figure 3 Intra-VTA infusion of noribogaine, but not 18-MC, decreases operant ethanol self-administration in rats. Rats were infused into the VTA with noribogaine (1, 10 or 100 μM; a, c), 18-MC (1, 10 or 100 μM; b, d) or vehicle 3 hours before the beginning of the test session. (*a&b*), Mean ± SEM of the number of ethanol deliveries for noribogaine (*a*) and 18-MC (*b*). (*c&d*), Mean ± SEM of the ethanol intake for noribogaine (*c*) and 18-MC (*d*). $n = 13$. $^{**}P < 0.01$, $^{***}P < 0.001$

the absence of effect 3 hours post-infusion, no effect of 18-MC on level of response and intake was observed at any of the test sessions [Fig. 4b&d; Treatment, $F(1, 36) = 0.07$, $P = 0.80$, Session, $F(3, 36) = 2.67$, $P = 0.06$, Treatment-Session interaction, $F(3, 36) = 2.26$, $P = 0.10$ and Treatment, $F(1, 36) = 0.01$, $P = 0.92$, Session, $F(3, 36) = 2.32$, $P = 0.09$, Treatment-Session interaction, $F(3, 36) = 1.87$, $P = 0.15$, for ethanol deliveries and ethanol intake, respectively]. Taken together, these data suggest that the VTA is an important site of action of noribogaine, but not 18-MC, to reduce operant ethanol self-administration.

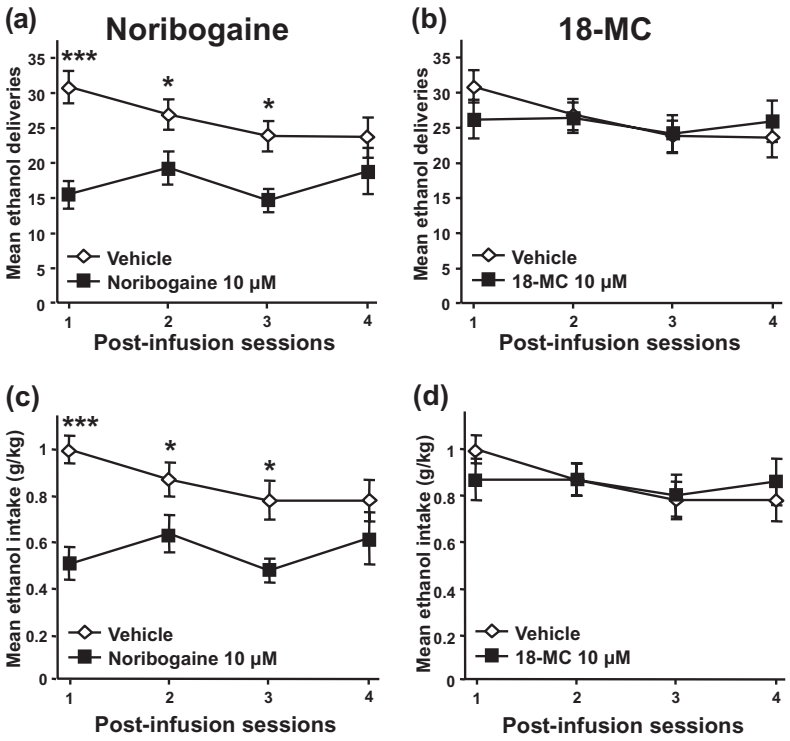

Figure 4 Intra-VTA infusion of noribogaine, but not 18-MC, induces a long-lasting decrease in operant ethanol self-administration in rats. Noribogaine or 18-MC (10 μM) was infused into the VTA and operant ethanol self-administration was monitored over 4 consecutive sessions. The same subjects as in Fig. 2 were tested 24 hours (session 2), 48 hours (session 3) and 72 hours (session 4) after the intra-VTA infusion of the drugs. Session 1 recapitulates the results obtained 3 hours after the infusion and represented in Fig. 2. *(a&b)*, Mean ± SEM of the number of ethanol deliveries for noribogaine (*a*) and 18-MC (*b*). (*c&d*), Mean ± SEM of the ethanol intake for noribogaine (*c*) and 18-MC (*d*). $n = 13$. $^{*}P < 0.05$, $^{***}P < 0.001$

DISCUSSION

Here, we show that noribogaine, like ibogaine, but not 18-MC, potently increases *GDNF* expression in a dopaminergic-like cell culture model. In addition, we found that administration of noribogaine, but not 18-MC, into the VTA results in a significant and long-lasting decrease in operant self-administration of ethanol.

Different factors can contribute to a decrease in operant performance, including potential suppressant effects of the pharmacological agent, such as sickness, sedation or locomotor deficits. It is, however, unlikely that such factors contribute to the decrease in operant behavior induced by noribogaine as systemic administration of noribogaine does not induce tremor or ataxia (Glick *et al.* 1996b; Baumann *et al.* 2001), and does not alter locomotor activity in rats (Baumann *et al.* 2001). While intra-VTA infusion of noribogaine, and not systemic administration, was used in the present study, the present data suggest that noribogaine in the VTA reduces the motivation to self-administer ethanol.

Our results suggest that the ibogaine metabolite noribogaine exhibits similar activities as ibogaine in both increasing the level of *GDNF* and reducing ethanol operant self-administration after infusion of the drug into the VTA, the site of action of both ibogaine (He *et al.* 2005) and GDNF (Carnicella *et al.* 2008; Carnicella & Ron 2009). Interestingly, noribogaine is as potent as ibogaine in increasing *GDNF* expression in SH-SY5Y cells and in reducing operant ethanol self-administration (He *et al.* 2005). Also, like ibogaine, the decrease in ethanol self-administration induced by noribogaine is long-lasting, and although in this study we performed a 72-hour (four sessions) time course for noribogaine and previously we tested only a 48-hour (three sessions) time course for ibogaine (He *et al.* 2005), the results for both drugs are likely to be similar.

Ibogaine is metabolized to noribogaine (Obach *et al.* 1998) and the levels of noribogaine were reported to remain high for a prolonged period of time following oral administration of ibogaine in humans (Mash *et al.* 1995; Mash *et al.* 1998; Mash *et al.* 2000). In rats, however, in which metabolism of drugs in the liver and in the brain is generally much faster (Smith 1991; Woodland *et al.* 2008), only a small fraction of the noribogaine injected systemically is detected 24 hours after the administration (Baumann *et al.* 2001). Therefore, it seems unlikely that the decrease in operant ethanol self-administration observed 24 hours or more after the infusion results from a persistent level of noribogaine in the brain. However, this possibility cannot be excluded. We previously showed that ibogaine blocks or reverses biochemical and behavioral adaptations obtained in response to exposure of SH-SY5Y cells and rodents to ethanol via GDNF (He *et al.* 2005; He & Ron 2008). We also obtained evidence to suggest that the long-lasting increase in *GDNF* expression induced by ibogaine is due to the induction of a positive autoregulatory loop in which GDNF increases its own expression (He & Ron 2006). As GDNF in the VTA has been shown to be a

potent inhibitor of ethanol-drinking and -seeking behaviors (Carnicella *et al*. 2008; Carnicella *et al*. 2009b), these data strongly suggest that the long-term action of noribogaine on operant ethanol self-administration, as with ibogaine, is mediated by GDNF via the activation of the same positive feedback loop.

In contrast, we did not observe a significant increase in *GDNF* expression upon treatment of cells with the synthetic derivative of ibogaine, 18-MC. In line with these results, infusion of 18-MC into the VTA did not lead to a reduction of operant self-administration of ethanol, even at high concentrations. Interestingly, systemic administration of 18-MC in selectively bred alcohol-preferring rats was found to dose-dependently decrease ethanol intake and preference in a two-bottle choice paradigm (Rezvani *et al*. 1997). These results thus suggest that the site of action of 18-MC is not the VTA, and that this synthetic derivative of ibogaine does not mediate its actions on ethanol consumption via the upregulation of the GDNF pathway. 18-MC has been reported to act as an antagonist of nicotinic α3β4 receptors (Glick *et al*. 2002b; Maisonneuve & Glick 2003). Indeed, low dose combinations of 18-MC with other pharmacological agents that antagonize the nicotinic α3β4 acetylcholine receptors (mecamylamine, dextromethorphan or burproprion), decrease morphine, methamphetamine and nicotine self-administration at doses that were ineffective when administered alone (Glick, Maisonneuve & Kitchen 2002a; Glick *et al*. 2002b). Moreover, 18-MC reduces morphine and methamphetamine self-administration after infusion into the medial habenula or interpeduncular nucleus (Glick *et al*. 2006; Glick *et al*. 2008), two diencephalon structures with a high level of nicotinic α3β4 receptors (Quick *et al*. 1999; Perry *et al*. 2002) that are known to influence the activity of the mesocorticolimbic system (e.g. Nishikawa, Fage & Scatton 1986; Jhou *et al*. 2009). In contrast, and in line with the present data, 18-MC is not effective in decreasing morphine and methamphetamine self-administration when infused into the VTA (Glick *et al*. 2006; Glick *et al*. 2008), in which the expression of the nicotinic α3β4 is low (Klink *et al*. 2001; Perry *et al*. 2002). Therefore, while the two ibogaine congeners noribogaine and 18-MC reduce drug self-administration when administered systemically, our data suggest that their site and mechanism of action are different. Like 18-MC, ibogaine is a potent antagonist of the nicotinic α3β4 receptors (Glick *et al*. 2002b). However, ibogaine also has a plethora of mechanisms of action in the brain, and it is proposed that this variety of effects accounts for its anti-addictive properties (Sweetnam *et al*. 1995; Glick & Maisonneuve 1998). It is, therefore, likely that both the effects of ibogaine on *GDNF* expression in the VTA, as well as its antagonist activity at the nicotinic α3β4 receptors, are likely to contribute significantly to its anti-addictive activities.

As described in the introduction, ibogaine's side effects preclude its use as a medication. Noribogaine, as suggested by the present study and others (e.g. Glick *et al*. 1996b), shares the same desirable long-lasting actions and anti-addictive mechanisms as ibogaine. Importantly, however, noribogaine is safer than its parent compound and, for example, has no tremorigenic effects (Glick *et al*. 1996b; Baumann *et al*. 2001). As such, noribogaine may be considered to have a greater therapeutic profile than ibogaine. The present data also suggest that the effects of noribogaine on *GDNF* expression greatly contribute to its potential anti-addictive profile. We recently showed that another molecule, cabergoline, reduces ethanol consumption and relapse in rodent preclinical models by the upregulation of the GDNF pathway in the VTA (Carnicella *et al*. 2009a). Together, these results put forward the use of GDNF inducers as a valuable strategy to combat alcohol use and abuse disorders.

In summary, the present data strongly suggest that activation of the GDNF pathway in the VTA is a mechanism of action of noribogaine, but not 18-MC, to reduce ethanol taking. Taken with other studies, it emphasizes an important role of the GDNF pathway within the VTA to modulate ethanol-drinking behaviors. Understanding how ibogaine derivatives decrease drug intake and delineating the different signaling events mediating the anti-addictive properties of ibogaine derivatives may lead to the development of a new generation of drugs to treat addiction.

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Authors' Contribution

SC and DR designed research. SC, DYH and QVY performed research. SC, DYH and DR assisted with data analysis and interpretation of findings. SC and DR wrote the manuscript and SDG provided material consultation

and discussion. All authors critically reviewed content and approved final version for publication.

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Behavioural Pharmacology

Induction of energy metabolism related enzymes in yeast *Saccharomyces cerevisiae* exposed to ibogaine is adaptation to acute decrease in ATP energy pool

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ABSTRACT

Ibogaine has been extensively studied in the last decades in relation to its anti-addictive properties that have been repeatedly reported as being addiction interruptive and craving eliminative. In our previous study we have already demonstrated induction of energy related enzymes in rat brains treated with ibogaine at a dose of 20 mg/kg i.p. 24 and 72 h prior to proteomic analysis. In this study a model organism yeast *Saccharomyces cerevisiae* was cultivated with ibogaine in a concentration of 1 mg/l. Energy metabolism cluster enzymes glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase and alcohol dehydrogenase were induced after 5 h of exposure. This is a compensation of demonstrated ATP pool decrease after ibogaine. Yeast in a stationary growth phase is an accepted model for studies of housekeeping metabolism of eukaryotes, including humans. Study showed that ibogaine's influence on metabolism is neither species nor tissue specific. Effect is not mediated by binding of ibogaine to receptors, as previously described in literature since they are lacking in this model.

1. Introduction

Ibogaine, an indole alkaloid present in the root bark of African plant *Tabernanthe iboga* has been extensively studied in the last decades in relation to its anti-addictive properties that have been repeatedly reported as being addiction interruptive and craving eliminative for opiates, stimulants, alcohol and nicotine (Alper et al., 1999; Maciulaitis et al., 2008). Although controlled clinical trials haven't yet been done, both lay and scientific literature suggest a certain level of ibogaine's efficacy (Lotsof, 2007; Alper et al., 2008). Claims are supported with results from *in vitro* studies and proven in animal models of drug addictions (Alper, 2001).

Multiple ligand binding and activity modulation actions of ibogaine on receptors, transporters and enzymes have been described in the scientific literature, in particular: 5-Hydroxytryptamine (5-HT), opioid, nicotinic and N-methyl-D-aspartate (NMDA) receptors, dopaminergic and 5-HT transporters and monoamine oxidase enzyme (MAO) (Alper, 2001; Glick et al., 2002; Leal et al., 2003).

Besides the effects on receptors, transporters and enzymes, the molecular aspects of ibogaine's influence on drug addictions concerning signal transduction and modulation of gene expression are becoming increasingly recognized (Ali et al., 1999; Onaivi et al., 2002). Consequent biochemical, neuroendocrine, structural and functional changes in terms of brain plasticity have been suggested (Ali et al., 1996; He et al., 2005; Carnicella et al., 2008).

Our recent work (Paškulin et al., 2006) showed the stimulating influence of ibogaine at a dose of 20 mg/kg i.p. on rat brain energy metabolism. We have observed changes in proteome at 24 and 72 h after i.p. application with induction of glycolysis and TCA cycle enzymes (glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase).

In the present study analysis of changes in proteome using 2-D electrophoresis was done again, this time on yeast *Saccharomyces cerevisiae* in stationary growth phase, at a concentration of 1 mg/l ibogaine in the media, which represents local bioavailability in brain tissue in previous experiment (Mash et al., 2000; Kontrimaviciute et al., 2006).

This cell suspension model doesn't show any cell differentiation, nor organization in tissue, and it lacks the influence on metabolism due to synaptic intercellular communication, as is the case in *in vivo* experiments. In spite of that, yeast is an accepted model for studies of basic metabolic pathways of higher eukaryotes, including mammalian cells (Ma, 2001; Menacho-Marquez and Murguia, 2007).

The aims of this study were to investigate if the effect of ibogaine is species and/or tissue specific and to find the cause for the induction of energy related enzymes.

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2. Materials and methods

2.1. Yeast strain and cultivation

Yeast strain S288C (*MATα mal gal2*) was used in this study. Cells were cultivated in YEPD medium with the following composition: 10 g/l glucose (Kemika), 5 g/l yeast extract (Biolife), and 5 g/l pepton (Oxoid), at 28 °C and 220 rpm to the stationary growth phase. Then cells were centrifuged for 5 min at 4000 rpm, washed once with 50 mM potassium phosphate buffer, pH 7.0 and suspended in the same buffer at a concentration of $1 \cdot 10^8$ cells/ml.

A fresh ibogaine stock solution (10 mg/ml) was added to the cell suspension to reach different concentrations: 0, 1, 4, and 20 mg/l. After 0.25, 0.5, 1, 2 and 5-h incubation at 28 °C and 220 rpm, samples were taken to measure cell energy metabolic status and intracellular oxidation of treated and non-treated cells. Protein profile of yeast cell extract was analyzed only at 5 h of exposure to the lowest concentration of 1 mg/l.

2.2. Two-dimensional electrophoresis

Cells were sedimented by centrifugation from 20 ml samples of the cultures and washed twice with 50 mM potassium phosphate buffer, pH 7.0. 0.1 g of cells (wet weight) was suspended in 0.5 ml extraction buffer (40 mM Tris–HCl, pH 8.0; 2% (w/v) 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 65 mM dithiothreitol (DTT)) containing protease inhibitor cocktail (Complete, Mini; Roche) — 1 tablet per 10 ml of buffer. The cells were disrupted by vortexing with glass beads five times, 1 min each with 1-min intervals for cooling the mixture on ice. The cell homogenate was centrifuged at 20 000*g* for 20 min at 4 °C.

The protein content in the cell extracts was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Two-dimensional (2-D) electrophoresis was performed according to Görg (1991) with minor modifications. Samples (150 µg protein) were mixed with rehydration solution (9 M urea, 2% (w/v) CHAPS, 2% (v/v) immobilized pH gradient (IPG) buffer, 18 mM DTT, a trace of bromophenol blue) and applied on 13-cm immobilized pH 3 to 10 gradient (IPG) strips (GE Healthcare). After rehydration (13 h) isoelectric focusing (IEF) as first dimension was carried out at 20 °C on a Multiphor II (GE Healthcare). The following voltage program was applied: 300 V (gradient over 1 min), 3500 V (gradient over 1.5 h), and 3500 V (fixed for 4 h). Prior to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the IPG strips were equilibrated in SDS equilibration buffer (50 mM Tris–HCl, pH 8.8; 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, a trace of bromophenol blue) containing 1% DTT for 15 min, and containing 4.8% iodoacetamide for an additional 15 min. SDS-PAGE as the second dimension was carried out with 12% running gel on the vertical discontinuing electrophoretic system SE 600 (Hoeffer Scientific Instruments) at constant 20 mA/gel 15 min and then at constant 40 mA/gel until the bromophenol blue reached the bottom of the gel. 2-D gels were stained with Sypro Ruby (Invitrogen). For each sample two 2-D gels were run at the same conditions.

2.3. Protein visualization and image analysis

2-D gels were recorded using the CCD camera G.BOX_HR (Syngene). Gel image analysis was done with the 2-D Dymension software version 2.02 (Syngene) and included spot detection, spot quantification, pattern aligning and matching. For all spot intensity calculations, normalized volume values were used. The results are expressed as a ratio of the normalized volume of protein spot in ibogaine-treated cells divided by normalized volume of matched protein spot in untreated control cells at the same time of exposure. Differences by a fold change >2 between treated and untreated cells were considered as significant.

2.4. Protein identification

The protein spots of interest were excised from the gels and analyzed by LC-MS/MS using an ESI-TRAP instrument. The Mascot software was used to search SwisProt 54.7 database. The following search parameters were applied: *S. cerevisiae* as species; tryptic digest with a maximum number of one missed cleavage. The peptide mass tolerance was set to $\pm$ 1.5 Da and fragment mass tolerance to $\pm$0.5 Da. Additionally, carbamidomethylation and oxidation of methionine were considered as possible modifications. Mascot protein scores greater than 29 were considered statistically significant ($P<0.05$).

2.5. Determination of cell energy metabolic status

Cell energy metabolic status was determined *via* the ATP pool by measuring luminescence with the commercially available kit BacTiter-Glo™ Microbial Cell Viability Assay (Promega) according to the manufacturer's instructions. Briefly, 100 µl of BacTiter-Glo reagent was added to 100 µl sample of cell culture and after 5 min luminescence was recorded using the microplate reader Safire II (Tecan). The results were expressed as a difference in ATP pool regarding the control.

2.6. Estimation of intracellular oxidation

Intracellular oxidation was estimated by using 2′,7′-dichlorofluorescein (H_2DCF), which is able to react with oxidant-reactive oxygen species. It is given as 2′,7′-dichlorofluorescein diacetate (H_2DCFDA), which easily penetrates the plasma membrane and is hydrolysed inside the cells by non-specific esterases. Non-fluorescent H_2DCF is oxidized to fluorescent 2′,7′-dichlorofluorescin (DCF), which is determined fluorimetrically (Jakubowski and Bartosz, 1997).

Cells were sedimented by centrifugation from 2 ml samples of the cultures, washed twice with 50 mM potassium phosphate buffer, pH 7.8, resuspended in the same buffer at a concentration of 1% (v/v) and preincubated at 28 °C for 5 min. H_2DCFDA was added as stock of 1 mM ethanol solution to a final concentration of 10 µM. After incubation (28 °C, 220 rpm, 20 min) 200 µl of cell suspension was transferred to the microplate and fluorescence was measured using the Tecan microplate reader Safire II (excitation and emission wavelengths of 488 and 520 nm, respectively). The results were expressed as a difference in reactive oxygen species level regarding the control.

3. Results

We have repeated our previous *in vivo* experiment with rats, this time on yeast *S. cerevisiae* cell culture in stationary growth phase with 1 mg/l of ibogaine in media, which mirrors peak mammalian brain tissue concentration after 20 mg/kg i.p.

Proteins from 5 h ibogaine-treated and control yeast cells were separated with 2-D electrophoresis and analyzed using the 2-D Dymension software. Of all protein spots that showed a significant change in intensity compared to control samples 12 spots that fall in the category of interest were excised and analyzed by LC-MS/MS, which gave sufficient confirmation of protein identity for five spots.

Proteins that were induced in yeast cells treated with ibogaine relative to control samples were identified as metabolic enzymes involved in glycolysis: glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase and alcohol dehydrogenase (6.3-, 4.6-, 3.8-, and 3.2-fold, respectively); and one as a member of antioxidant defence: superoxide dismutase (2.2-fold) (Fig. 1, Table 1).

To find the background of enzyme induction, ATP pool was measured in dose and time escalation manner. Results showed that immediately after exposure of yeast cells to ibogaine, the ATP pool measured by luciferine/luciferase test significantly falls in a dose dependent manner (Fig. 2A). It reaches the minimum at 30 min and then gradually returns towards control.

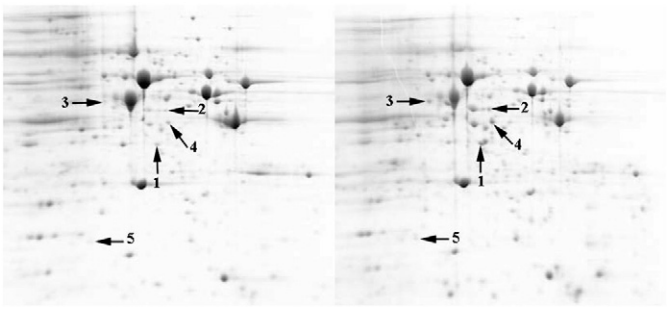

Fig. 1. Representative partial 2-D gel of proteins extracted from *Saccharomyces cerevisiae* cells: left — control cells, right — cells treated with 1 mg/l ibogaine after 5-h exposure. Labeled proteins were found to be upregulated and were identified by LC-MS/MS. Details are listed in Table 1.

Samples grown for 5 h in the presence of ibogaine at 1 mg/l concentration, also used for 2-D proteomic analysis, were washed with phosphate buffer. Eliminating the ibogaine from culture gradually brings ATP levels above the control ones (Fig. 2B).

Reactive oxygen species levels were simultaneously measured and we have observed immediate elevation of reactive oxygen species production after the ibogaine lasting up to 1 h, then inverted to decrease reaching the minimum at 2 h and this was followed by gradual return towards control (Fig. 3).

Additionally, enhanced synthesis of numerous unidentified types of low abundance proteins with relative shift of protein quantity towards low abundance fraction was observed and processed by computer analysis of existing 2-D gels, where a normalized volume value of 0.5% was a distinguishing criterion for a spot to enter low vs. high abundance group. Quantity of low abundance group was relatively enriched by a factor of 1.27 in treated sample compared to control while high abundance group being relatively impoverished. While high abundance group can be considered as a representative of structural proteins being constant at non-growing yeast cells in stationary growth phase, elimination of its relative diminution gives an increase factor of 1.76 for low abundance group (Table 2).

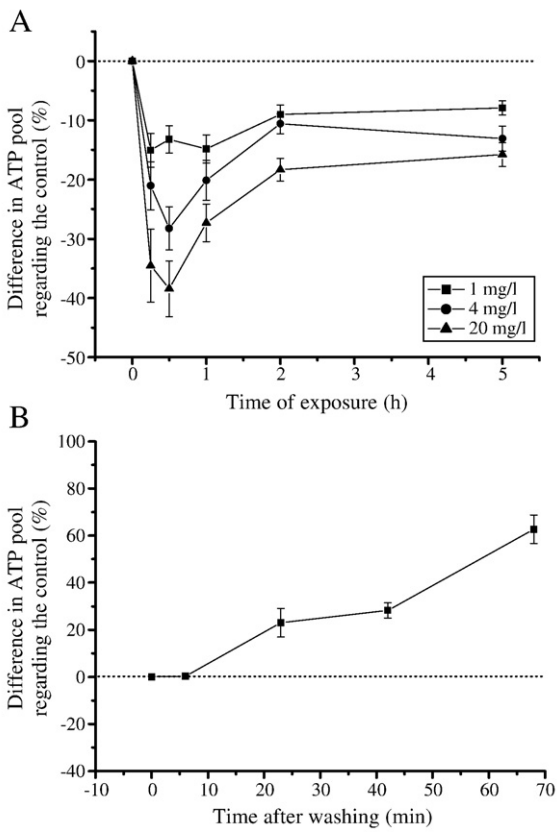

Fig. 2. A. Measuring of ATP pool during exposure of cells to different concentrations of ibogaine. Results are expressed as difference in ATP pool regarding the control and were measured in duplicate from two independent cultures for each concentration. The bars represent the averages ± S.E. B. Measuring of ATP pool after elimination of ibogaine from culture treated for 5 h at a concentration of 1 mg/l. Results are expressed as difference in ATP pool regarding the control and were measured in duplicate from two independent cultures. The bars represent the averages ± S.E.

4. Discussion

Molecular aspects of drug addictions are becoming increasingly recognized and findings suggest involvement of adaptation changes in gene expression patterns with the influence on cellular metabolism; to the very fundamental and ubiquitous housekeeping metabolism (Li et al., 2008). Reversibility of such changes is the platform for future anti-addiction treatments.

The induction of energy metabolism related enzymes due to ibogaine, previously triggered *in vivo* on rat model, was repeated on yeast *S. cerevisiae*, which is an accepted model for studies of primary metabolic pathways of higher eukaryotes (Ma, 2001; Menacho-Marquez and Murguia, 2007). A group of catabolism related enzymes was found to be induced after 5 h of exposure to ibogaine in a concentration of 1 mg/l (Fig. 1, Table 1); in higher proportion of induction and after shorter exposure compared to our previous study, assuming presently used concentration to be representative of brain tissue concentration in our previous experiment (Paškulin et al., 2006; Mash et al., 2000; Kontrimaviciute et al., 2006).

Ibogaine is known to affect numerous receptors and enzymes that are lacking in yeast cell. Therefore it is proved that enhanced expression of energy metabolism related enzymes is not mediated through receptor bindings, as previously described in the literature and it is not linked to cell differentiation or organization in tissue.

In search for a cause of enzyme induction, ATP pool and reactive oxygen species levels were investigated. ATP level falls to the minimum at 30 min of ibogaine's presence and then gradually returns towards control.

Table 1
List of *Saccharomyces cerevisiae* identified proteins whose expressions were stimulated by 1 mg/l ibogaine.

Spot enzyme	Swiss-Prot accession number	Fold ibogaine/control	Theor. M_r(Da)/pI	Matched peptides	Mascot score
1 Glyceraldehyde-3-phosphate dehydrogenase 3	P00359	6.3	35747/6.46	16	381
2 Phosphoglycerate kinase	P00540	4.6	44738/7.11	19	492
3 Enolase 2 (2-phosphopyruvate dehydratase 2)	P00925	3.8	46914/5.67	22	933
4 Alcohol dehydeogenase 1	P00330	3.2	36823/6.26	29	694
5 Superoxide dismutase (Cu–Zn)	P00445	2.2	15855/5.62	7	125

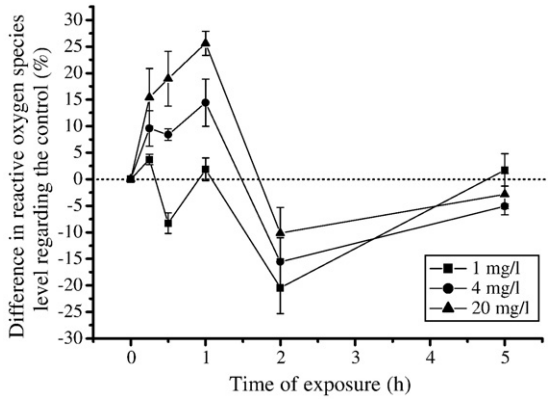

Fig. 3. Measuring of reactive oxygen species level during exposure of cells to different concentrations of ibogaine. Results are expressed as difference in reactive oxygen species level regarding the control and were measured in duplicate from two independent cultures for each concentration. The bars represent the averages ± S.E.

Table 2
Comparison of relative contents of low and high abundant protein fractions.

	Treated Σ normalized volumes (%)	Control Σ normalized volumes (%)	Ratio (treated/control)	Ratio (corrected)
High abundance proteins (normalized volume ≥0.5%)	36.398	50.103	0.726	1
Low abundance proteins (normalized volume <0.5%)	63.602	49.897	1.275	1.756

Restoration of energy status is due to induced energy metabolism related enzymes being translated. Besides, some immediate alosteric feed-back stimulation of regulatory glycolytic enzyme activity due to low ADP/ATP ratio could be recognized, indicated by immediate elevation of reactive oxygen species as byproducts of energy metabolism. Latter fall of reactive oxygen species level compared to control is due to efficient endogenous antioxidative systems with quick onset, as shown by the induction of Cu–Zn SOD (Fig. 1). This shows a typical exercise rebound effect — reactive oxygen species load provocation exerts protective role by "alarming and awakening" of antioxidative defence (Jamnik et al., 2006; Hakkiwell and Gutteridge, 2007).

It was shown that the induction of energy metabolism related enzymes is not an event per se, but compensation to a transient dose dependent fall in ATP level in the first hours of exposure to ibogaine (Fig. 2A). Whether this fall is a consequence of lower ATP production or results from higher ATP consumption is answered by immediate increase in intracellular reactive oxygen species levels after the ibogaine (Fig. 3). Since reactive oxygen species are mainly products of ATP synthesis in mitochondria, results negate decreased production. Rather, they suggest enhanced consumption of ATP pool, which is insufficiently buffered by an immediate increase of production due to alosteric feed-back modulation of glycolytic enzymes. Only after sufficient translation of additional quantity of enzymes, levels of ATP approach control values.

Induction i.e. bigger amount of enzymes being the cause of elevated specific activity is additionally confirmed by rebound effect, when eliminating the ibogaine from milieu brings ATP levels of samples, previously grown in the presence of ibogaine, above the control ones (Fig. 2B).

In which processes the consumption of ATP is increased remains unclear. We have excluded ibogaine's toxicity to cells and consequent energy cost of repair (data not shown). Neither, energy is consumed for metabolism of ibogaine itself, since yeast does not have cytochromes P450 that are known to be responsible for degradation of ibogaine in human (Walker, 1998; Maciulaitis et al., 2008).

Enhanced synthesis of numerous low abundance proteins with relative shift to low abundance fraction (Table 2) was observed. This is by itself an anabolic process that requires energy, which additionally suggests that enlarged energy demands are the primary trigger for induction of enzymes.

What is the exact mechanism and purpose of this wide non-specific activation of transcription, translation and consequent metabolic changes remain unclear, but metabolic turnover acceleration with even further energy demands is suspected. Shifts in quantities of energy related enzymes with subsequent elevated energy availability affect all metabolic processes inside and outside of the cells of any type and functional state; directly by fuelling ATP dependent reactions and indirectly by facilitating the synthesis of functional units. This could facilitate different healing processes, including restoration of physiological homeostasis in functionally remodeled cells after the development of tolerance to drugs of abuse, termed detoxification.

It should be pointed out here that substance-related disorders are not just a matter of neuronal circuits, being tuned on a drug seeking, craving-reward cycles, but are also a matter of a single cell, being habituated to the presence of drug of abuse and missing it, when it is gone. Interference with energy supply might be the crucial meeting point of these diverse adaptations to different types of drugs. Briefly, ibogaine has the opposite effect on energy metabolism than most of the drugs of abuse, which after chronic use downcast cellular energy status (Ryman and Walsh, 1952; Sadava et al., 1997; Sharma et al., 2003). On the contrary, after acute deprivation the ibogaine's induction of enzymes supplies additional energy; the effect once triggered, not being dependent to the presence of the drug and thereafter lasting for a prolonged period of time (Fig. 2B).

The proposed mechanism of action extends indications of ibogaine for medical use beyond syndrome of addiction, since induced catabolism enzymes with accelerated metabolism turnover facilitates detoxification and renewal of tissues after numerous pathological conditions like reconvalescence after infectious diseases, recovery after trauma, general exhaustion of chronic systemic diseases, cancer cachexia, depression etc. Ibogaine could be an adjuvant, non-specific therapy in synergism with disease targeting drug.

Besides, rebound effect of elevated energy availability after washing ibogaine from culture represents human individual after treatment, elevated in mood, strength and will, being capable of exerting resistance to their addiction for a prolonged period of time. These life changing, mind opening properties are exactly what the ibogaine medical subculture votes for.

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Metabolic plasticity and the energy economizing effect of ibogaine, the principal alkaloid of *Tabernanthe iboga*

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ABSTRACT

Ethnopharmacological relevance: The root bark of iboga plant—*Tabernanthe iboga* has been used traditionally in Central Africa as a psychoactive substance in religious rituals, while in smaller doses it is appreciated due to its stimulant properties. The iboga root bark, iboga extract or pure ibogaine are being recognized in the West as an anti-addiction remedy and their use is increasing.
Aim of the study: Our previous studies have demonstrated a transient ATP pool reduction under ibogaine accompanied by the induction of energy metabolism related enzymes. The present study aimed to find the cause of this energy deprivation and to foresee its immediate and long-term impact on metabolism.

The overall project is designed to disclose the common mechanism of action at these seemingly diverse indications for iboga use, to predict eventual adverse effects and to build the grounds for its safe and beneficial utilization.
Materials and methods: The rate of carbon dioxide (CO_2) as a marker of energy metabolism in stationary yeast model under aerobic conditions in the presence of ibogaine at concentration of 1, 4 and 20 mg/l was measured for 5 h by gas chromatography. The overall oxidative load was determined fluorimetrically by 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) and *in vitro* antioxidant properties of ibogaine were defined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test.
Results: The CO_2 production under ibogaine was temporarily increased in a dose dependent manner.

The increased energy consumption as an early effect of ibogaine was proven by the fact that in spite of energy mobilization, the ATP pool has been simultaneously decreased.

Although increased cellular respiration co-produces reactive oxygen species (ROS), the overall oxidative load was significantly lowered by ibogaine. Since ibogaine does not show any significant *in vitro* antioxidant properties, the results indicate its stimulating influence on physiological oxidative stress defence system.
Conclusion: Ibogaine triggers remodeling of the housekeeping metabolism. Under the initial energy cost it results in increased efficacy of physiological antioxidative systems, which reduce oxidative damage and lowers basal metabolic needs. Together with induced catabolic enzymes they set a new metabolic equilibrium that saves energy and makes it easily available in case of extra needs. While healthy organism profits from improved fitness and mental performance and can withstand higher stress without risking a disease, due to the same principle ibogaine provides beneficial support at the recovery after diseases including addiction syndrome.

1. Introduction

Ibogaine is an indole alkaloid naturally found in the root bark of tropical rainforest shrubby plant iboga—*Tabernanthe iboga* Baill. (Apocynaceae family) and to a lesser extend in some other species of Tabernaemontana tribe. Iboga (tabernanthe radicis

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cortex) has been traditionally used in tribes of the Congo basin in Central Africa as a psychoactive sacrament used in the ceremony of initiation into adulthood. It induces trance and is considered to reveal one's purpose of life and his role in a society (Fernandez, 1982). In smaller doses it is appreciated due to its stimulant and aphrodisiac properties (Naranjo, 1969; Schultes, 1970). Hunters use it to promote vigilance while stalking pray (Fernandez, 1982). Its use is highly valued on long, tiring marches, on lengthy canoe voyages, and on difficult night watches (Schultes et al., 2001).

In the former century iboga extract under trademark Lambarene was sold in France and recommended as a tonic against fatigue, asthenia and depression and for recovery after infectious diseases (Goutarel et al., 1993). Other compositions containing ibogaine had been on the marked named Bogadin, Iperton, Endabuse (Ratsch, 1998).

In the last four decades the urban traditional use of iboga root bark, iboga extract or pure ibogaine is on the increase as an anti-addiction therapy (Alper et al., 2008). In the so called Ibogaine medical subculture it is used to ease the detoxification of drugs, for abstinence syndrome alleviation and to speed up the tolerance reversion. In long-term abstinence, it reduces craving for drugs by anxiety reduction and improvement of mood (Mash et al., 2000) and one of the explanations for this is psychoanalytical catharsis with resolution of inner conflicts (Naranjo, 1973). Existential insights resulting in social (re)integration of an individual are recognized as important consequence of iboga initiation in both native and Western societies (Fernandez and Fernandez, 2001). Besides, descriptions as spiritual revelation and religious redemption are not uncommon (www.ibogaine.co.uk/experience.htm) and interest for bare psycho-spiritual and religious use of iboga is so taking roots also in the West (www.sacrament.kibla.si). On the other hand *in vitro* and *in vivo* studies in animal models expose diverse biochemical impacts of ibogaine application (Alper, 2001; Maciulaitis et al., 2008).

Our recent work (Paškulin et al., 2010) showed that the induction of energy related enzymes in the yeast *Saccharomyces cerevisiae* accompanies the dose dependant decrease in ATP energy pool caused by ibogaine at concentrations of 1, 4 and 20 mg/l during 5 h. Yeast in stationary growth phase under aerobic conditions is an accepted model for studies of basic metabolic pathways of higher eukaryotes, including mammalian cells (Ma, 2001).

The aim of present study was to identify the cause and to foresee the consequences of ATP energy pool deprivation observed under ibogaine exposure, especially to confirm whether this energy shortage is a consequence of increased ATP consumption or it might be due to its silenced production. The rate of carbon dioxide (CO_2) production in yeast *Saccharomyces cerevisiae* in aerobic stationary growth phase was measured to define the level of oxidative catabolism and ATP production, under the concentrations of ibogaine that mirror those in the blood at different use—up to 1 mg/l corresponds to moderate stimulant effect, raising the dose brings psychoactive range and approaching 4 mg/l relates to the anti-addictive properties, while above are the traditional initiation doses (Fernandez and Fernandez, 2001; Mash et al., 2000). Parallel work on potential energy consumers like toxicity, oxidative stress and kinetics of ibogaine were conducted.

Our hypothesis was that ibogaine triggers energy consuming process and that there is a common denominator at diverse outcomes of iboga use.

2. Material and methods

2.1. Material

Ibogaine HCL was donated by Sacrament of Transition, Maribor, Slovenia. Ibogaine was used in our series of experiments since it is directly related to the iboga plant as its principal alkaloid. Besides, majority of literature concerns this pure form. The effect and after-effect of iboga root bark, its extract or pure ibogaine is except for kinetics reported as subjectively indiscriminative.

2.2. Yeast cultivation

Yeast *Saccharomyces cerevisiae* was cultivated in YEPD growth medium with the following composition: 10 g/l glucose (Kemika), 5 g/l yeast extract (Biolife), 5 g/l peptone (Oxoid), at 28 °C and 220 rpm to the stationary growth phase. Then cells were centrifuged for 5 min at 4000 rpm, washed with and resuspended in 50 mM potassium phosphate buffer, pH 7.0 to density of 1×10^8 cells/ml. The yeast culture was incubated at 28 °C and 220 rpm.

2.3. Cell CO_2 production

To determine cell respiration, 5 ml of 1×10^8 cells/ml yeast culture in 50 mM potassium phosphate buffer were transferred in sterile 15-ml serum bottles covered with airtight rubber stoppers. The suspension was incubated with ibogaine in concentrations of 0, 1, 4 and 20 mg/l at 150 rpm at 28 °C in the dark. The amount of CO_2 produced was measured at 0, 0.25, 0.5, 1, 2, 3, 4, and 5 h of incubation with gas chromatograph Hewlett Packard HP5890, as described by Odić et al. (2007). The chromatograph settings were as follows: column Porapak R mesh 100/120 (180 cm/1.8 in), oven temperature 50 °C, injector temperature 100 °C, TCD detector temperature 100 °C, carrier gas helium (180 ml min^{-1}), integrator HP3392A. The chromatograph was calibrated with an external standard having known CO_2 concentration. For each time point the results are expressed as relative difference in production of CO_2 by yeast cells under ibogaine compared to the control.

2.4. Estimation of oxidative stress

Intracellular oxidation was defined by using 2′,7′-dichlorofluorescein (H_2DCF), which is able to react with oxidants—reactive oxygen species (ROS) (Jakubowski and Bartosz, 1997).

Stationary phase yeast cells at concentration of 1×10^8 cells/ml were added H_2DCFDA as a stock of 1 mM ethanol solution to the final concentration of 10 μM. After incubation for 20 min at 28 °C, 220 rpm cells were treated with ibogaine in concentrations of 0, 1, 4 and 20 mg/l or ascorbic acid in *in-vitro* equipotent concentrations of 0, 1, 2 and 4 μM and samples were taken at the end of accelerated energy metabolism period. 200 μl of the cell suspension was transferred to the microplate and fluorescence was measured using Tecan microplate reader Safire II (excitation and emission wavelengths of 488 and 520 nm, respectively). The results are expressed as a relative difference in overall ROS load compared to the control:

$$\text{Ratio}\,[\%] = \left[E_{\text{treated}}/E_{\text{control}}\right] \times 100$$

where E is emission of ibogaine treated or control solution.

2.5. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 nm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment (Yamaguchi et al., 1998).

The reaction mixture (1 ml) contained 500 μl of daily prepared (1,1-diphenyl-2-picrylhydrazyl) DPPH solution (250 μM), 400 μl of Tris–HCl buffer pH 7.4 (100 mM) and 100 μl of various concentrations (10, 20, 40 and 80 μM) of ibogaine dissolved in distilled water. After thorough mixing, the solutions were kept in

the dark for 20 min at room temperature. Thereafter, the absorbance was measured at 517 nm. All tests were performed in triplicate, with trolox and ascorbic acid as physiological controls. The percent inhibition of the DPPH radical by ibogaine was calculated according to the formula:

$$\%\text{Inhibition} = \left[(A_{\text{blank}} - A_{\text{test}})/A_{\text{blank}}\right] \times 100$$

where A_{blank} is the absorbance of the DPPH in solution without test sample (antioxidant), and A_{test} is the absorbance of DPPH in solution with ibogaine.

2.6. Yeast viability

Cell viability was measured as cell-membrane integrity using LIVE/DEAD Funga Light™ Yeast Viability Kits (Molecular Probes), according to the manufacturer instructions. Briefly, the cells from 1 ml cell cultures were centrifuged ($14{,}000 \times g$, 5 min) and washed once with filtered PBS, and cell suspensions at 1×10^6 cells/ml were prepared in PBS. Then 1 μl SYTO® 9 and 1 μl propidium iodide were added in the dark and the samples incubated at 37 °C for 30 min. After the incubations, the fluorescence was measured using a microplate reader (Tecan). The excitation/emission wavelengths for these two dyes are 480/500 nm for SYTO® 9 and 490/635 nm for propidium iodide.

2.7. Ibogaine kinetics

Yeast cells at a concentration of 1×10^8 cells/ml were suspended in different ibogaine buffer solutions (0, 1, 4, 20 mg/l) and incubated at 28 °C. Samples were taken in 15 min intervals, prepared and adequately diluted for analysis according to a modified method which was initially developed for the determination of ibogaine and noribogaine (internal standard prazepam) in biological samples (Koželj, 2010). The compounds were separated on Zorbax XDB-CN (75 mm × 4.6 mm i.d, 3.5 μm) by using an Agilent 1100 HPLC system and detected in the tandem quadrupole mass spectrometer Quattro micro™ API from Waters, the software used was MassLynx 4.1. All samples and standards were treated adequately to prevent decomposition of ibogaine and noribogaine due to daylight exposure.

3. Results

We have proceeded with our previous *in vivo* experiments on Wistar rats and yeast *Saccharomyces cerevisiae* in stationary growth phase in relation to ibogaine's influence on energy metabolism. In the present experiment, yeast in the same metabolic state was treated with 0, 1, 4 and 20 mg/l of ibogaine and CO_2 production was measured at 0.25, 0.5, 1, 2, 3, 4 and 5 h time points. Immediately after addition of ibogaine there was a dose dependent raise in CO_2 production with peak values of 16, 67, 142% (1, 4 and 20 mg/l of ibogaine, respectively) relative to control, which ceased in an hour and raised again between 2 and 4 h with second peak of 15, 11, 27% (1, 4 and 20 mg/l of ibogaine, respectively) at 3 h time point. After 3 h there was a progressive decline of CO_2 production crossing the control values at 4 h and dropping further until the end of experiment at 5 h, when reduction of the energy metabolism of 10, 9 and 31% (1, 4 and 20 mg/l of ibogaine, respectively) relative to the control was observed and further diminution expected through extrapolation from late trends (Fig. 1).

The total oxidative load in the enhanced catabolism part of the experiment was decreased by 24, 23, 57% (1, 4 and 20 mg/l of ibogaine, respectively) relative to the control (Table 1). Influence of the ascorbic acid in *in vitro* equipotent concentrations

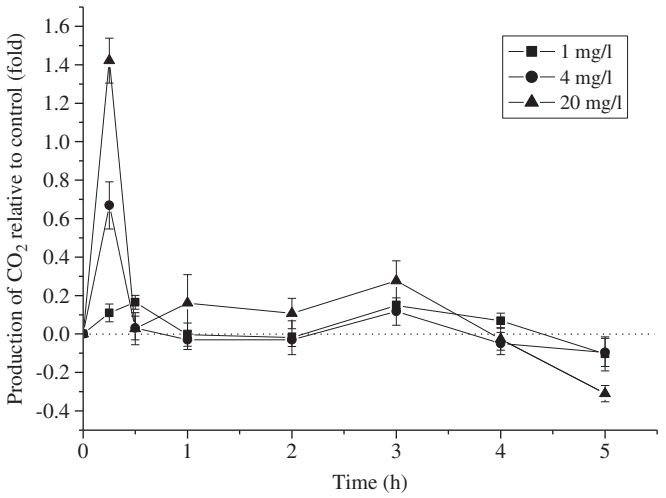

Fig. 1. Time dependent CO_2 production in yeast under 1, 4 and 20 mg/l of ibogaine in media. Results are expressed as the relative change in CO_2 production between exposed and control cells at each time point. They were collected from four independent cultures for each concentration. The data represent average values and standard errors.

Table 1
Concentrations of ibogaine in yeast cytosole after equilibrium, the influence of ibogaine at different concentrations on the total oxidative load and the influence of *in vitro* equipotent concentration of ascorbic acid are represented. The values represent averages and standard errors and are results of the experiment in triplicates.

Calculated ibogaine concentration [mg/l]	Ibogaine concentration in cytosol [mg/l]	Oxidative load ratio treated/control [%]	Ascorbic acid concentration [μM]	Oxidative load reduction by ascorbic acid
1	0.83 ± 0.03	76.26 ± 1.69	1	Non-significant
4	3.89 ± 0.06	76.67 ± 1.56	2	Non-significant
20	18.14 ± 0.34	43.45 ± 1.30	4	Non-significant

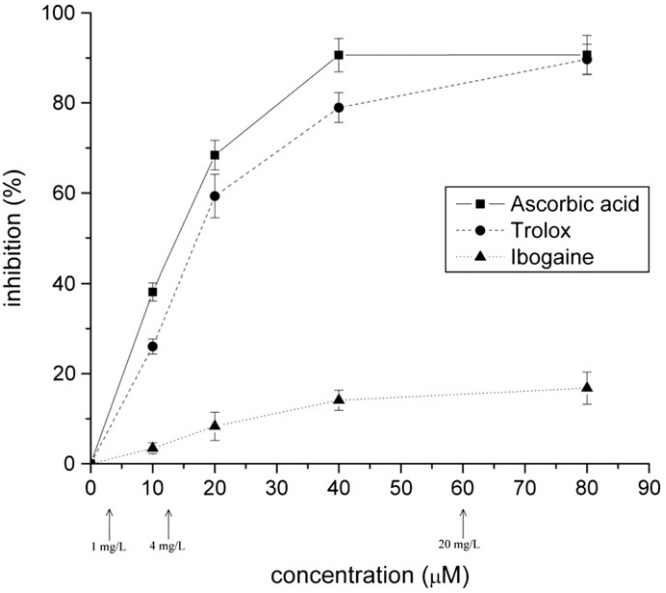

Fig. 2. *In vitro* anti-oxidant properties of ibogaine represented in molar concentrations. Results are expressed in percentage of DPPH reduction and were measured in triplicates. The bars represent averages and standard errors.

estimated in Fig. 2 to be 1, 2 and 4 μM was used as a positive control and no significant influence on oxidative load was found.

Ibogaine's intrinsic antioxidative potential was measured *in vitro* by DPPH test. Molar concentrations were used to compare with Trolox and ascorbic acid efficacy. No significant direct antioxidant properties of ibogaine were found in concentrations, relevant for experiment (Fig. 2).

The viability of yeast cells was examined by cell-membrane integrity test which excluded toxicity due to ibogaine presence at any concentration of concern (data not shown). Also, cell's morphology and growth after the treatment didn't show any deviations from control.

Ibogaine kinetics was examined in the yeast in phosphate buffer for different concentrations of ibogaine. Concentrations inside and outside the cells reached the balance in 2 h with a half of shift occurred in 15 min after beginning of the experiment. The results of measurements after equilibrium are shown in Table 1. Ibogaine as a highly lipid soluble molecule freely enters the cell and accumulates in the membranes causing a slight drop in concentration of water dissolved fraction. No noribogaine, a principal metabolite of ibogaine in humans was found neither in intracellular nor extracellular compartment at any time during the experiment.

4. Discussion

In our first work (Paškulin et al., 2006) induction of energy related enzymes in rat brain was demonstrated as a consequence of ibogaine administration. In our previous work (Paškulin et al., 2010) we have demonstrated similar results of ibogaine's influence on energy metabolism cluster in yeast *Saccharomyces cerevisiae*, while following the changes in ATP pool showed its transient reduction in a dose dependant manner. In the present experiment, the influence of ibogaine on metabolism was further studied on yeast in the stationary phase under aerobic conditions, this time by measuring the rate of CO_2 production and followed by search for energy consumers.

4.1. Energy metabolism acceleration

Transient oxidative energy metabolism acceleration was directly confirmed by increased CO_2 production after ibogaine exposure, in a dose dependant manner. Interestingly, this elevation is not permanent but shows rather interesting dynamics with biphasic elevation of CO_2 production, followed by the calming of catabolism at the end of the experiment.

Considering the fact that in spite of energy mobilization the ATP pool has been simultaneously decreased, the increased energy consumption as an early effect of ibogaine's presence was proven.

4.2. Possible energy consumers

4.2.1. Oxidative stress

Parallel to observation of the catabolism the level of oxidative stress as the sum of produced ROS was tracked under the same conditions. Surprisingly, we have observed that apart from undisputable increase in ROS formation due to stimulated ATP production (Halliwell and Gutteridge, 2007) there was a significant drop in the total oxidative load on the cell (Table 1). Since ibogaine doesn't show any significant *in vitro* antioxidant activity at the concentrations of concern and due to the failure of *in vitro* equipotent concentration of ascorbic acid to exert such effect *in vivo*, the impact on the physiological antioxidative systems must be responsible for such improvement, in a pro-antioxidant manner. Unlike the antioxidants that directly scavenge free radicals, pro-antioxidants act indirectly either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins (Dinkova-Kostova and Talalay, 2008; Stevenson, 2012; Vertuani et al., 2004). Indeed, in our previous work (Paškulin et al., 2010) we have observed the 2,2 fold induction of Cu–Zn SOD enzyme expression after ibogaine treatment. Therefore, the reduction of oxidative load lowers energy expenses for cellular maintenance and saves the energy. This correlates with the later reduction of metabolic turnover as seen in Fig. 1.

4.2.2. Ibogaine kinetics

Energy consumption due to the ibogaine uptake cannot be responsible for energy load since active transport by specialized ATP coupled transporters is highly improbable at simple yeast model (Walker, 1998). Besides, it would show constant inward pumping effort opposing the escape of highly lipid soluble molecule (Maciulaitis et al., 2008) that would be presented as a constant rise in CO_2 production, rather than being expressed as biphasic production acceleration with latter inversion.

Redox and energy linked metabolism of ibogaine was checked regarding transformation to noribogaine, in mammals being catalyzed by CYP 450 enzyme reduction. This reaction is not possible since yeast does not posses CYP 450 system (Walker, 1998). Also, we have not found any measurable levels of noribogaine in the yeast cells, treated with ibogaine. Other possible degradation products were not undoubtedly excluded but any kind of energy coupled degradation would again manifest itself by constant CO_2 production elevation, prolonged far beyond our experiment until all ibogaine being metabolized.

4.2.3. Ibogaine toxicity

The ibogaine's toxicity with potential energy cost of cellular repairs was excluded by the test of cellular membrane's integrity and even further excluded by observations of normal growth and morphology after ibogaine exposure (data not shown). Also, literature denies antimicrobial activity of indole alkaloids from Apocynaceae family against yeast (Verpoorte et al., 1983).

Since transient, intermittent effort of coupled oxidative energy catabolism to compensate ATP pool diminution is not a permanent effect of ibogaine's presence, its toxicity or kinetics are not expected to be the subject of energy expenditure. Rather, a trigger mechanism is suspected where ibogaine serves as an elicitor of some finite energy consuming process. In our recent work (Paškulin et al., 2010) we have found induction of low abundance, functional protein fraction in yeast (including energy metabolism and antioxidative system enzymes in question), whose synthesis seems to be responsible for these energy expenditures. Deeper insight into the systems biology of complete proteome changes needs to be done for profound understanding of the ibogaine effect.

4.3. Acute effect

The ibogaine initiates the energy consuming process and manifestation of the effects depends on capability of catabolism to compensate the energy outputs in this dynamic equilibrium. This might be the case with low doses quickly gaining moderate improvement of physical and mental performance in a stimulant manner, while higher doses initially overcome cellular catabolism buffering capacity with energy flux being exclusively occupied with metabolic plasticity, not leaving much of free energy for physical activities. This puts the user in a period of lethargy under high ritual doses while full invigorating effect appears later (Fernandez, 1982).

Mind altering property of iboga might also be mediated by this mechanism of reduced energy availability (Magistretti, 2006).

Brain activity modulation due to interference of ibogaine in neurotransmitter release, action and reuptake extensively described in literature is undoubtedly involved in perception and cognitive shifts, but also reduced disposable energy has its implication. Brain cortex as energetically most demanding tissue and a site of cognition as well as psychological inhibitions can in case of ATP pool reduction move into a different mode of action. Decreased effectiveness of K/Na-ATPase due to acute lack of ATP might cause changes in electroencephalogram (EEG) patterns. This includes observed theta and delta trance state that precipitate subconscious psychological material into the awareness (Binienda et al., 2011; Strubelt and Maas, 2008).

Many factors influence the efficacy and safety of ibogaine use and so the justification of its use. Detoxification from drugs of abuse or any other recovery after illness is a restitution of physiological balance that by itself represents considerable energy load. Although ibogaine quickens such adaptation, such aid and its justification have its limits in terms of energy overload. Accelerated detoxification of severe addiction with the use of high ibogaine dose can overcome the body's buffering capacities and result in complications, so medical surveillance during such treatments is highly recommended.

4.4. After-effect

Under the cost of transitional energy expenditures ibogaine enables changes in proteome with the shift to a more economical and cytoprotective metabolic equilibrium. Besides, induced energy metabolism related enzymes serve as an extended energy source for a prolonged period of time. Resulting metabolic state is of special value in state of elevated energy demands under different stress in an adaptogen manner.

4.5. Ibogaine and anti-addiction effect

Tolerance is adaptation of an organism to the presence of drugs and their withdrawal causes the abstinence syndrome. Physical weakness with lack of will is recognized as one of symptoms. Besides subjective descriptions as "run out of gas", literature describes choking influence of diverse drugs of abuse on the energy metabolism (Chen et al., 2007; Hargreaves et al., 2009; Ryman and Walsh, 1951, 1952; Sadava et al., 1997). Reversibility of such changes is the platform for future anti-addiction treatments.

The prolonged anti-addiction effect of ibogaine at least partially consists of improved energy accessibility that can be considered as stabile metabolic shift in the epigenetic landscape (Huang et al., 2009; Waddington, 1957). While escaping the genetic determinism (Noble, 2006) such recognition of ibogaine as a causal remedy puts a question mark upon the definition of addiction as a chronic and relapsing disorder.

Nevertheless, full benefit of iboga use arises only from conjunction of its invigorating quality with the spiritually initiated intent for a life change—either initiating adulthood or quitting addiction (as if there was any difference…).

5. Conclusions

The increased energy consumption is confirmed to be the cause of acute energy depletion due to ibogaine. The proteome changes including induction of energy metabolism and antioxidative enzymes are responsible for initial energy expenditures. After the shift is accomplished the new metabolic equilibrium results in improved fitness.

As the dose distinguishes remedy from poison, the same is true for beneficial eustress influence of the adaptogen that can be overcame by adverse distress of metabolic overload. Special attention must be paid to pace at which the adaptation from one metabolic equilibrium to another is conducted.

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Medication Development of Ibogaine as a Pharmacotherapy for Drug Dependence[a]

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ABSTRACT: The potential for deriving new psychotherapeutic medications from natural sources has led to renewed interest in rain forest plants as a source of lead compounds for the development of antiaddiction medications. Ibogaine is an indole alkaloid found in the roots of *Tabernanthe iboga* (Apocynaceae family), a rain forest shrub that is native to equatorial Africa. Ibogaine is used by indigenous peoples in low doses to combat fatigue, hunger and in higher doses as a sacrament in religious rituals. Members of American and European addict self-help groups have claimed that ibogaine promotes long-term drug abstinence from addictive substances, including psychostimulants and cocaine. Anecdotal reports attest that a single dose of ibogaine eliminates withdrawal symptoms and reduces drug cravings for extended periods of time. The purported antiaddictive properties of ibogaine require rigorous validation in humans. We have initiated a rising tolerance study using single administration to assess the safety of ibogaine for the treatment of cocaine dependency. The primary objectives of the study are to determine safety, pharmacokinetics and dose effects, and to identify relevant parameters of efficacy in cocaine-dependent patients. Pharmacokinetic and pharmacodynamic characteristics of ibogaine in humans are assessed by analyzing the concentration-time data of ibogaine and its desmethyl metabolite (noribogaine) from the Phase I trial, and by conducting *in vitro* experiments to elucidate the specific disposition processes involved in the metabolism of both parent drug and metabolite. The development of clinical safety studies of ibogaine in humans will help to determine whether there is a rationale for conducting efficacy trials in the future.

INTRODUCTION

The potential for deriving new psychotherapeutic medications from natural sources has led to renewed interest in rain forest plants for the development of anti-addiction medications. Ibogaine is an indole alkaloid found in the root bark of *Tabernanthe iboga* (Apocynaceae family), a shrub that grows in West Central Africa. The Pygmies attribute the discovery of the plant to the warthogs who it seems are very fond of it.[1] Ibogaine is used by indigenous peoples in low doses to combat fatigue, hunger and thirst, and in high doses for its psychoactive properties as a sacrament in religious rituals. Discussion of the central nervous system (CNS) and cardiovascular actions of ibogaine have appeared in the literature since the early 1900s.[2] In the 1950s, CIBA investigated

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ibogaine as an antihypertensive agent, but these studies were not continued because the company was unconvinced of its commercial potential. The pharmacology of ibogaine was studied extensively by the French pharmacologists Lambert, Heckel and Pouchet early in the 20th century (for review, see Ref. 3). After the introduction of the Rauwolfia and given the general interest in the Apocynaceae family of which *Tabernanthe iboga* is a member, French scientists pursued chemical, pharmacological and behavioral studies of ibogaine.[4-6] Ibogaine was marketed in France under the tradename Lambarene until 1970.[7]

The putative antiaddictive properties of ibogaine were first described by Howard Lotsof.[8] He reported that ibogaine administrations led to an active period of visualizations that were described as a "waking dream state," followed by an intense cognitive phase of "deep introspection." Drug-dependent individuals who had received ibogaine treatments reported that the visions usually centered on early childhood and other significant developmental events that occurred during formative periods of their life. In the cognitive phase, insights were gained into their addictive and self-destructive behaviors. Interestingly, at the end of the ibogaine treatment sessions, opiate- and cocaine-dependent subjects reported an alleviation or in some cases a complete cessation of drug 'craving' for extended periods of time, and a few patients remained drug-free for several years thereafter. Opiate-dependent patients reported that ibogaine blocked the symptoms of opiate withdrawal. An informal self-help network had provided ibogaine treatments from 1987 until 1993 to addicts in Europe[9] (International Coalition of Addict Self Help, ICASH). Based on his own experience and that of six of his friends, Howard Lotsof filed a series of use patents describing a method for treating narcotic, psychostimulant, nicotine, alcohol and polydrug dependence with ibogaine (US Patents 4,499,096; 4,587,243; 4,857,523). Our research team at the University of Miami were the first to request permission from the US Food and Drug Administration (FDA) to conduct a limited Phase 1 Pharmacokinetic and Safety Trial in male subjects (IND 39,680). This clinical protocol was initially limited to include only ibogaine veterans. In April 1995, the FDA approved a revised clinical protocol to conduct these studies in cocaine-dependent male volunteers. However, these dose-escalation studies have not progressed beyond 2 mg/kg oral doses due to a lack of research support for the clinical trial.

The anecdotal reports of ibogaine's purported efficacy require controlled clinical studies in order to validate the claims that either single or repeated ibogaine administrations are effective for treating substance abuse. However, ibogaine's actions may have specific pharmacological actions that target an underlying neurochemical etiology and/or neural adaptations associated with chronic cocaine or opiate abuse, along with the individual psychosocial disorders as discussed above. As with most pharmacological agents, it is important for ibogaine pharmacotherapy to be integrated with psychotherapy. This suggestion for drug development of ibogaine as a pharmacotherapy for substance abuse is consistent with the current advances in substance abuse treatment strategies, which indicate that outcomes can be enhanced and extended by combining the most effective forms of psychotherapy and pharmacotherapy.[10]

PROOF-OF-CONCEPT IN ANIMAL BEHAVIORAL MODELS

Ibogaine is reportedly not a substitute for narcotics or stimulants, it is not addicting, and it promotes long-term drug abstinence after a single-dose administration. The narrative claims of humans who were successfully treated with ibogaine have led to investigations in rodents and monkeys. Ibogaine administrations have been shown to reduce morphine self-administration[11,12] and decrease morphine-induced locomotor ac-

tivity.[13] Ibogaine eliminates some of the signs of opiate withdrawal precipitated by naloxone or naltrexone in morphine-dependent rats[14,11] and monkeys.[15] However, Sharpe and Jaffe[16] failed to demonstrate significant effects of ibogaine administrations on the signs of morphine-withdrawal in mice at either subtremorogenic (5 and 10 mg/kg subcutaneous (s.c.)) or tremorogenic (20 and 40 mg/kg s.c.) ibogaine doses. In the single-dose suppression test in monkeys, ibogaine reduced the total number of withdrawal signs, but failed to substitute completely for morphine (College on the Problems of Drug Dependence (CPDD) Drug Testing Program, 1989). Dzoljic *et al.*[14] reported that ibogaine administered intracerebroventricularly at 4–16 μg/kg attenuated the naloxone-precipitated withdrawal syndrome in chronic morphine-dependent male rats. In this study, ibogaine had more consistent effects on withdrawal signs that were related to locomotor behavior. Luxton *et al.*[17] using the place conditioning procedure showed that 40 mg/kg i.p. ibogaine reduced the rewarding effect of a single injection of morphine (2 mg/kg i.p.) in rats. The attenuation of the effect of morphine was seen even if the animals were pretreated with opiate 24 hr before the test. After the fourth morphine conditioning trial, ibogaine failed to modify the reward of morphine. Ibogaine by itself was neither reinforcing nor aversive, and it did not affect place conditioning motivated by drugs that are not rewarding.[18]

Cocaine-induced stimulation of locomotor activity was decreased by ibogaine in mice[19] and rats.[20] Ibogaine administrations also reduced cocaine intake in mice[21] and rats.[22,12] In mice, cocaine intake was reduced sharply by ibogaine administrations at doses of 40 mg/kg i.p.[19] However, Dworkin *et al.*[23] failed to demonstrate significant effects of ibogaine on cocaine self-administration in rats, but did show sensitive effects of ibogaine on responding maintained by heroin. In contrast to this work, cocaine self-administration was reportedly decreased for up to 48 hr after a single ibogaine dose in rats.[22] Repeated administration of ibogaine on three consecutive days produced a significant and marked decrease in cocaine intake in this study. Glick *et al.*[12] have reported reductions in cocaine self-administrations in rats that persisted for several days following three doses of ibogaine. The long-lasting *aftereffects* in rats and humans have led to the suggestion that ibogaine may persist in brain or that an active metabolite with a slow clearance rate may contribute to the actions of ibogaine. However, studies in rhesus monkeys demonstrated that dose-effect curves for ibogaine's effects on food- and cocaine-maintained behavior in individual monkeys did not show consistent dose dependency for either event.[24] While questions have been raised previously concerning the use of different chemical preparations of ibogaine across earlier studies,[25] one possible explanation for conflicting results is species or strain differences in the patterns of ibogaine metabolism and clearance rates.[26] For example, ibogaine is cleared from blood very rapidly in the primate as compared to either the rat or human subjects.[26,27]

Glick and co-workers[12] have described the effects of iboga alkaloids on extracellular levels of dopamine and its metabolites in the nucleus accumbens and striatum. Morphine and cocaine intake were dose-dependently decreased (2.5 to 80 mg/kg i.p.) in the hour after treatment. Some, but not all of the alkaloids (ibogaine, tabernanthine, desethylcoronaridine, and the R-isomer of ibogamine) reduced drug intake the day after they were administered. Interestingly, R-ibogamine produced the most consistent and persistent pattern of *aftereffects* showing decreased drug intake following two or three injections. At the doses used to assess effects on drug intake, most of the alkaloids were tremorogenic, while R-ibogamine and R-coronaridine induced very weak or no tremors. Using *in vivo* microdialysis, the effects of R- and S-enantiomers were compared for their effects on extracellular dopamine levels in the striatum and nucleus accumbens. The R-enantiomers decreased dopamine levels in both brain regions, while the S-enantiomer failed to produce a significant effect. These results indicate

that the putative 'antiaddictive' and tremorogenic effects of iboga alkaloids can be dissociated.

IDENTIFICATION OF A PRIMARY METABOLITE

The narrative reports of long-lasting effects in humans after a single dose, together with the demonstrated *aftereffects* of ibogaine on drug self-administration in rodents, have led to the suggestion that ibogaine may persist in the body or that there may be one or more active metabolites formed.[28,22] We have developed a procedure for quantifying ibogaine and have identified a single primary metabolite in blood samples from rats, primates and humans.[29,30] Our group was the first to identify the primary metabolite as 12-hydroxyibogamine (noribogaine), by full scan electron impact gas chromatography/mass spectrometry (GC/MS). The analytical procedure involved a solvent extraction under basic conditions with D_3-ibogaine as an internal standard. Urines taken from dosed monkeys and humans were extracted under strongly basic conditions (pH >10) with ethyl acetate. Extracts were evaporated to dryness, reconstituted with methanol, and analyzed by GC/MS in full scan electron impact ionization mode. Analysis of the resulting total-ion chromatograms revealed a peak identified as parent drug, ibogaine, by comparison with an authentic standard. In addition, all samples were found to contain a second major component eluting after ibogaine. Similar spectral characteristics of this peak to ibogaine's spectrum (*i.e.*, presence of M/Z 122, 135, 136 and 149 fragments) define it as an ibogaine metabolite (FIG. 1). The apparent molecular ion at M/Z 296 suggests that it is formed by a loss of a methyl group. In addition, the appearance of a fragment of mass 211 in place of the M/Z 225 fragment of the ibogaine spectrum indicates that the demethylation occurs on the indole end of the molecule. The most probable site for metabolic demethylation of ibogaine was the methoxy group, resulting in the compound 12-hydroxyibogamine (noribogaine). To confirm the identity of the desmethyl metabolite, an authentic standard of noribogaine (s.a. Omnichem, Belgium) was run in parallel with the experimental samples. This analysis of the authentic noribogaine standard gave a single peak at the same retention time and with the same electron impact fragmentation pattern as the endogenous compound isolated from monkey and human urines.[26,30]

Limited pharmacokinetic measurements have been obtained from human patient volunteers who had received single oral doses of ibogaine. The results have provided some important information about the metabolism and clearance of ibogaine. At 4 hr, the peak concentrations of ibogaine measured in blood ranged from 600 to 1250 ng/ml in two male subjects who had received 600 mg and 800 mg, respectively (FIG. 2). The time required to eliminate the majority of absorbed ibogaine (>90%) was 24 hr postdose (FIG. 2). The pharmacokinetic profiles determined in whole blood demonstrate that the concentrations of 12-hydroxyibogamine (noribogaine) measured at 24 hr remained elevated in agreement with previous findings.[30] The concentration of noribogaine measured at 24 hr postdose was in the range of 800 ng/ml. One female opiate-dependent subject treated with a single 500-mg oral dose of ibogaine had very low levels of ibogaine measured in blood (FIG. 2C). However, the peak levels of the metabolite were comparable to those seen in the male subjects that had received higher doses of ibogaine. Interestingly, in contrast to the two male subjects who had elevated levels of ibogaine measured in blood, this female subject reported no remarkable 'visionary' experience at this dose of ibogaine. Physical dependence to opiates is characterized by a distinctive pattern of signs and symptoms that make up the withdrawal syndrome. Physician-rated assessments demonstrated that there were no objective signs of opiate withdrawal seen in this female subject. These preliminary observations further suggest

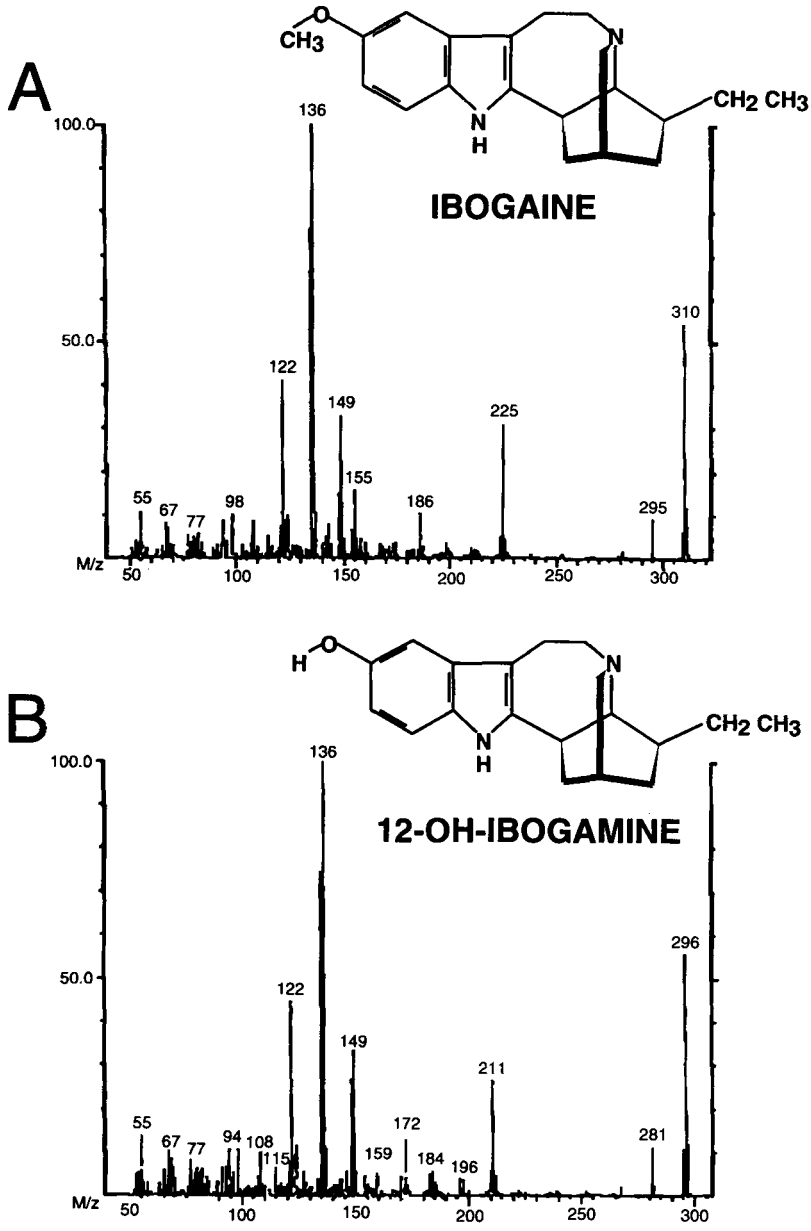


FIGURE 1. Identification of an ibogaine metabolite in human urine. Full scan electron impact mass spectra of **(A)** ibogaine and **(B)** 12-hydroxyibogamine propyl ether derivative.

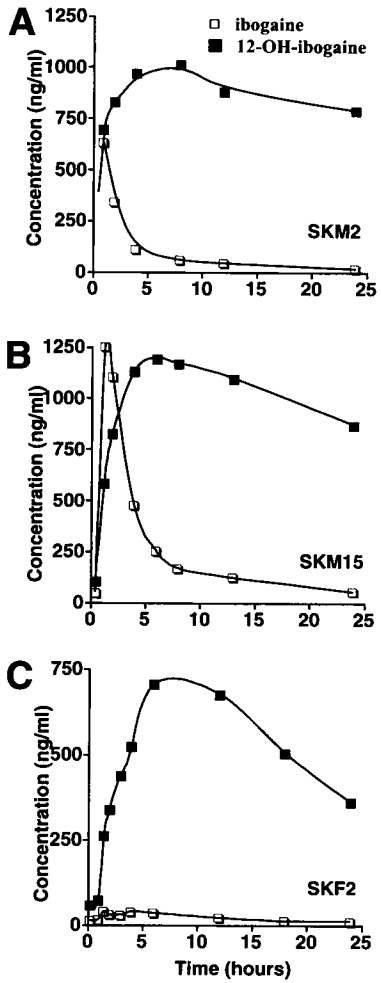


FIGURE 2. Pharmacokinetics of ibogaine and noribogaine over the first 24 hr after oral doses in human subjects. Data shown are from representative male and female subjects. Values for parent drug and the desmethyl metabolite were measured in whole blood samples at the times indicated. Demographic information for the subjects codes are as follows: SKM2 (W/M 39 yr, 800 mg); SKM15 (W/M 46 yr, 1000 mg); SKF2 (W/F 30 yr, 500 mg). *Abbreviations:* SK, St. Kitts.

that the action of the metabolite may account in part for ibogaine's ability to reduce the symptoms of spontaneous withdrawal in opiate-dependent humans.

IDENTIFYING MULTI-SITE TARGETS AND MECHANISM(S) OF ACTION

The concentrations of ibogaine and noribogaine have been measured in rat brain following oral and intraperitoneal administrations.[27,31] The significance of micromolar interactions of ibogaine and noribogaine with various radioligand binding sites was related to the concentration of parent drug and metabolite in brain. Regional brain levels of ibogaine and noribogaine were measured in rat cerebral cortex, striatum, brainstem and cerebellum at 15 min, and 1 and 2 hr post drug administration. The results demonstrate that ibogaine is rapidly detected in brain following oral administration.[27] The metabolite was detected at the earliest time point (15 min), consistent with first pass metabolism of the parent drug.[26] Administration of ibogaine (50 mg/kg per os (p.o.)) in rodents resulted in levels of ibogaine and noribogaine ranging from 4 to 17 μM and 1 to 17 μM, respectively. These results indicate that micromolar activities of ibogaine and the *O*-demethylated metabolite are relevant for defining binding site activities. At present, the metabolism and intracerebral disposition of ibogaine in the brain are not completely known. Both the parent drug and metabolite have high heptane/phosphate buffer partition coefficients,[32] indicating their ability to penetrate the blood-brain barrier and consistent with a rapid entry of ibogaine into the brain. Partitioning of the parent drug into lipid may serve as a slow release storage 'depot.'[33] Sequestration of ibogaine into lipophilic compartments in brain, may result in lower concentrations of the parent drug in the extracellular fluid. The more polar nature of the desmethyl metabolite may result in higher extracellular fluid concentrations of the metabolite. If ibogaine is *O*-demethylated in brain, it is reasonable to conclude that the slow elimination of a central nervous system (CNS) trapped polar metabolite may contribute to some of the reported *aftereffects* of single oral dose administration of ibogaine in humans.

The receptor binding site profile of ibogaine suggests that multiple mechanisms of action may contribute to ibogaine's putative antiaddictive activity.[34,27] Radioligand binding assays targeting 50 distinct neuroreceptors, ion channels and components of second messenger systems were used to establish a broad pharmacological profile for ibogaine. The results demonstrate that ibogaine interacts with a number of different molecular targets, including the mu and kappa opioid receptors and serotonin $(5\text{-HT})_2$ and 5-HT_3, and muscarinic (M1 and M2) receptors, and monoamine uptake sites. In addition, ibogaine interacted with the *N*-methyl-D-aspartate (NMDA) receptor-coupled ion channel[35,30] and sodium ion channel.[34] Ibogaine was inactive at γ-aminobutyric acid (GABA), benzodiazepine or chloride channel sites.[34] Many drugs acting on the CNS have pharmacological side effect profiles, which include weak actions at muscarinic receptors. Ibogaine has been shown to be a weak inhibitor at putative M1 and M2 sites.[34,27] Ibogaine was inactive at brain/gut peptide receptors, prostaglandins, and second messenger binding sites.[34] Deecher *et al.*[36] had previously reported a similar ligand binding profile with an exception at 5-HT_2 sites. The lack of affinity of either ibogaine or noribogaine at 5-HT_2 (brain membranes) and 5-HT_6 and 5-HT_7 (recombinant proteins) sites has been confirmed in radioligand binding assays from our laboratory, suggesting further that the hallucinogenic activity of ibogaine (or the metabolite) is not mediated by an action at these 5-HT receptor subtypes. Ibogaine and noribogaine have affinities in the low micromolar range at the k_1 and k_2 opioid receptor subtypes.[37,27] The potency of noribogaine at μ-opioid receptors labeled with [^{3}H][D-Ala^2, MePhe^4, Gly-

ol[5]enkephalin ([^{3}H]DAMGO was 200 nM.[38] These results suggest a more potent action for the metabolite than the parent drug at μ-opioid receptors.

The ability of ibogaine to inhibit [^{3}H]MK-801 binding to the NMDA receptor complex[35,34,39,40] may be of relevance to understanding the antiaddictive actions of ibogaine. MK-801 and the noncompetitive NMDA antagonist, ketamine, block the development of tolerance to the motor incoordinating actions of ethanol.[41,42] MK-801 has been reported to block sensitization (reverse tolerance) to the behavioral activating effects of cocaine and amphetamine.[43,44] The administration of MK-801 was shown to attenuate both the development of tolerance to the analgesic effect of morphine and morphine dependence.[45] Thus, ibogaine's ability to modify drug-seeking behavior may be due to a blockade of NMDA receptor-coupled cation channels. However, further studies are needed, since we have shown that the metabolite is less potent than ibogaine at inhibiting [^{3}H]MK-801 binding in human brain preparations.[39] We have determined that ibogaine and its metabolite noribogaine are competitive antagonists at the MK-801 binding site in the NMDA-receptor cation channel. Both compounds competitively displaced specific [^{3}H]MK-801 binding to caudate and cerebellar membranes from postmortem human brain with submicromolar and micromolar affinities. In addition, ibogaine and noribogaine blocked the ability of NMDA to depolarize frog motoneurons in the isolated frog spinal cord.[39] The block of NMDA-depolarizations in frog motoneurons showed use-dependency and was very similar to the block produced by MK-801. In view of the abilities of MK-801 to affect the responses to addictive substances in preclinical investigations, our results are compatible with the idea that some of the antiaddictive properties of ibogaine may result from an interaction with NMDA-coupled cation channels. In this regard it is interesting to point out that there is considerable evidence to support the involvement of NMDA receptor stimulation in the behavioral sensitization to psychostimulants and opiates.[46] However, the lower potency of the *O*-demethylated metabolite in these assays may indicate that noribogaine does not have a significant interaction at NMDA receptor-coupled cation sites. Ibogaine's interaction with NMDA receptor-coupled cation channels may contribute to the psychotropic and high-dose neurotoxic actions of ibogaine.[34] Given the lower potency of the metabolite for inhibiting [^{3}H]MK-801 binding, it is unlikely that the dwell time for noribogaine in the ion channel is additive to ibogaine's activity.

According to the model by Spanagel and Shippenberg,[47] opioids tonically regulate mesolimbic dopaminergic transmission through two opposing mechanisms that regulate dopamine release via actions at the cell body and terminal. Administration of the μ-opioid receptor agonist DAMGO into the nucleus accumbens and caudal ventral pallidum increases intracranial self-stimulation reward.[48] The selective k-1 agonist U-69593 attenuates cocaine-induced behavioral sensitization.[49] The nonselective k-opioid antagonist nor-binaltorphimine enhances morphine-induced sensitization.[47] Ibogaine decreased morphine self-administration[28] and inhibited some of the symptoms of naloxone-precipitated withdrawal in morphine-dependent rodents.[14,11] Taken together, these observations suggest that opioid receptors may be involved in the inhibitory actions of ibogaine on opiate withdrawal and drug-taking behaviors. Ibogaine and noribogaine bind with micromolar or lower affinities at μ- and k-opioid receptor sites.[34,27] Competition binding of ibogaine and [^{3}H]naloxone in a sodium shift assay demonstrated a complex interaction, suggesting that ibogaine may recognize the high-affinity agonist state of the μ-opioid receptor.[50] However, ibogaine failed to modify morphine-induced antinociception in mice, while noribogaine potentiated morphine's antinociceptive response.[51] Thus, the exact role of the parent drug or its active metabolite in ibogaine's inhibitory actions on morphine self-administration and the withdrawal syndrome remains unclear. The recent demonstration that noribogaine stimulated

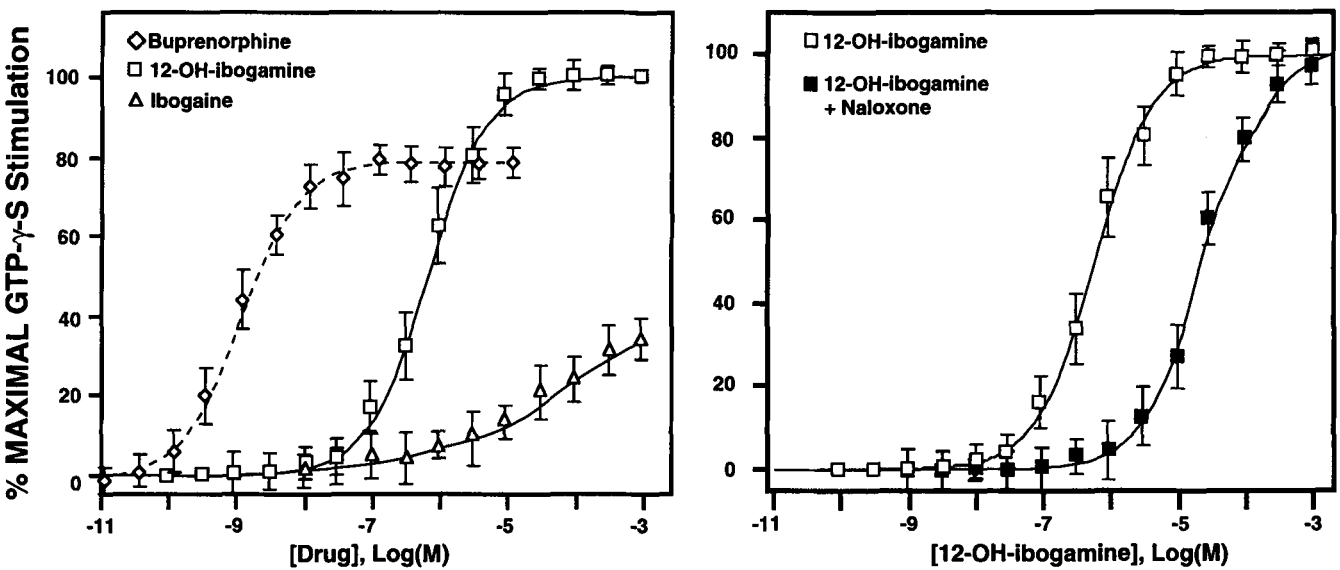


FIGURE 3. Stimulation of [^{35}S]GTPγS binding to rat thalamic membranes by various concentrations of buprenorphine (partial agonist), noribogaine (12-OH-ibogamine) and ibogaine *(left panel)*. Ibogaine failed to stimulate [^{35}S]GTPγS binding over the same dose range. Data represent percentage of basal [^{35}S]GTPγS binding measured in the presence of guanosine diphosphate (GDP) and absence of agonist. Effects of naloxone (0.1 μM) on various concentrations of noribogaine in rat thalamic membranes *(right panel)*. Values are means ± standard errors from three or more separate experiments performed in triplicate.

guanylyl-5′(γ[^{35}S]thio) triphosphate ($GTP_\gamma S$) binding to G proteins in a naloxone-sensitive manner indicates that the metabolite functions as a full μ-opioid agonist (FIG. 3). The functional response for noribogaine in rat thalamic membranes distinguished this compound from classic opiate drugs of differing intrinsic activities and binding site affinities.[38] The actions of noribogaine at μ-opioid receptors may account in part for the ability of ibogaine to reduce the symptoms of spontaneous withdrawal in opiate-dependent humans, since the long duration of action of noribogaine may produce a self-taper effect.[38]

RATIONALE FOR IBOGAINE AS A PHARMACOTHERAPY OF DRUG DEPENDENCE

Clinical and preclinical investigations are determining how psychological symptoms associated with drug withdrawal, including depressed mood states and drug cravings, maintain chronic patterns of drug use.[52] Chronic self-administration of cocaine and opiates results in a particularly intense euphoria and persistent drug dependence. Studies of cocaine and opiate dependence in animal models provide a rationale for pharmacotherapeutic agents with potential to attenuate withdrawal symptoms, to decrease drug craving, and to reduce relapse to previous patterns of drug use. The underlying assumption is that long-term substance abuse produces neurochemical adaptations in specific neural systems that regulate the capacity to experience pleasure (for review, see Ref. 53). Ibogaine may reestablish homeostasis in these neural systems, alleviating drug craving and diminishing the possibility for relapse to cocaine and opiate abuse.

Interventions with specific pharmacological agents should be guided by an understanding of the neurochemical derangements that underlie the clinical phases of drug abstinence.[54,55] However, not enough is known about the spectrum of human neurochemical alterations occurring with chronic use of cocaine and opiates. Nevertheless, a rational approach to pharmacotherapy would include the development of agents to: a) initiate and facilitate the acute phase of abstinence, b) diminish drug-specific withdrawal symptoms, and c) prevent cue-associated relapse to previous patterns of drug-taking behavior. Such pharmacotherapeutic agents may include drugs that facilitate initiation of abstinence through limited dopaminergic agonist activity.[55] A medication that facilitates the first phase of abstinence by reversing the neurochemical alterations that are induced by chronic psychostimulant or opiate use would include also agents that possess neurochemical activity at a number of different neurotransmitter systems. For example, pharmacologic intervention would attempt to correct the dual deficit in dopamine and serotonin neurotransmission that has been postulated to underlie the anhedonia and depression associated with withdrawal from cocaine.[56,57] The tendency to relapse to cocaine use that occurs during the early part of the 'crash' phase or during the later phases of withdrawal, could be treated with pharmacological agents to reduce drug craving.[58] Although drug craving is not easily quantified, the concept of craving is useful as a shorthand notation for all the external and internal cues that lead to drug-taking behavior.[53] The rationale for treatment of drug craving is based on the hypothesis that a dysregulation of neurotransmission of monoamines within the reward system is one of the causes of relapse. This change in brain function continues for months or years after the last use of the drug, and interacts with environmental factors such as social stress and situational triggers.[53]

The discovery that ibogaine eliminates the signs and symptoms of opioid withdrawal and diminishes craving for opioids was made in the 1960s by a group of self-treating heroin addicts.[59] A single oral dose administration of ibogaine (6 mg/kg to 19 mg/kg) was associated with a disruption of five addicts' use of opiates for up to six

months. Ibogaine had been administered to opiate and cocaine addicts in Europe and Central America through an informal self-help network.[9] Although promising, these anecdotal reports from addict self-help groups have not been verified in controlled clinical trials by established investigators. Although the precise neurochemical mechanism(s) of action for ibogaine have only begun to be studied, it is important to emphasize that many therapeutic successes have arisen empirically. The anecdotal reports of the effectiveness of ibogaine for the treatment of opioid and cocaine dependence provide a basis for further studies of ibogaine as a pharmacotherapy for drug dependence.

SAFETY CONSIDERATIONS

Ibogaine has a variety of dose-dependent pharmacological actions that may not be relevant to its effectiveness for opiate detoxification and psychostimulant and opiate dependence, but may influence considerations for safety. However, toxicological studies conducted in primates have demonstrated that oral ibogaine administrations, given at doses (5×25 mg kg^{-1}) recommended for the treatment of cocaine and opiate dependence, appear to be safe and free of behavioral or cerebellar toxicity.[60] The development of ibogaine as an antiaddiction drug has been hindered due to uncertainties over potential cerebellar neurotoxicity demonstrated in rat studies.[61,62] O'Hearn and Molliver[61] showed that high doses of ibogaine (100 mg kg^{-1} or 3×100 mg kg^{-1}) causes degeneration of the cerebellar purkinje cells in rats. Molinari *et al.*[63] reevaluated the dose effects of ibogaine. In this study, a lower dose (40 mg/kg) of ibogaine, one effective in reducing morphine and cocaine self-administration, displayed no degeneration above the level seen in saline-treated controls. These observations suggested that the degenerative and 'antiaddictive' properties of ibogaine reflect different actions of the drug.

Although the Phase I investigations by our group have not advanced recently, we have had the opportunity to obtain additional safety data in persons receiving ibogaine treatments abroad under controlled conditions. Baseline screening in these subjects included a medical evaluation, physical examination, electrocardiogram (ECG), blood chemistries, and hematological workup, as well as psychiatric and chemical dependency evaluations. A total of thirty (23 male, 7 female) drug-dependent subjects were assigned to one of three fixed-dose treatments under open label conditions: 500 mg, 600 mg, and 800 mg ibogaine. Adverse effects were assessed by clinician side-effect ratings and open-ended query. No significant adverse events were seen under these study conditions. The most frequent side effects observed were nausea and mild tremor at early time points after drug administration. Random regression of vital signs (respiration rate, systolic and diastolic blood pressures and pulse) revealed no significant changes across time or by treatment condition. White blood cell count, neurotrophil levels, sodium or potassium levels were in the normal range. No significant changes from baseline were seen for alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT). Intensive cardiac monitoring demonstrated that no electrocardiographic abnormalities were produced or exaggerated following ibogaine administration. These preliminary results demonstrate that single oral doses of ibogaine were well tolerated in drug-dependent subjects, and that there were no significant problems with safety within this dose range.

Concern over potential cerebellar toxicity[61] compelled us to examine ibogaine's effects on postural stability, body tremor and appendicular tremor (FIG. 4). In the FDA pharmacokinetic and safety trial studies, two doses of ibogaine (1 and 2 mg kg^{-1}) were administered to 9 volunteers with histories of recent cocaine abuse. Static posturogra-

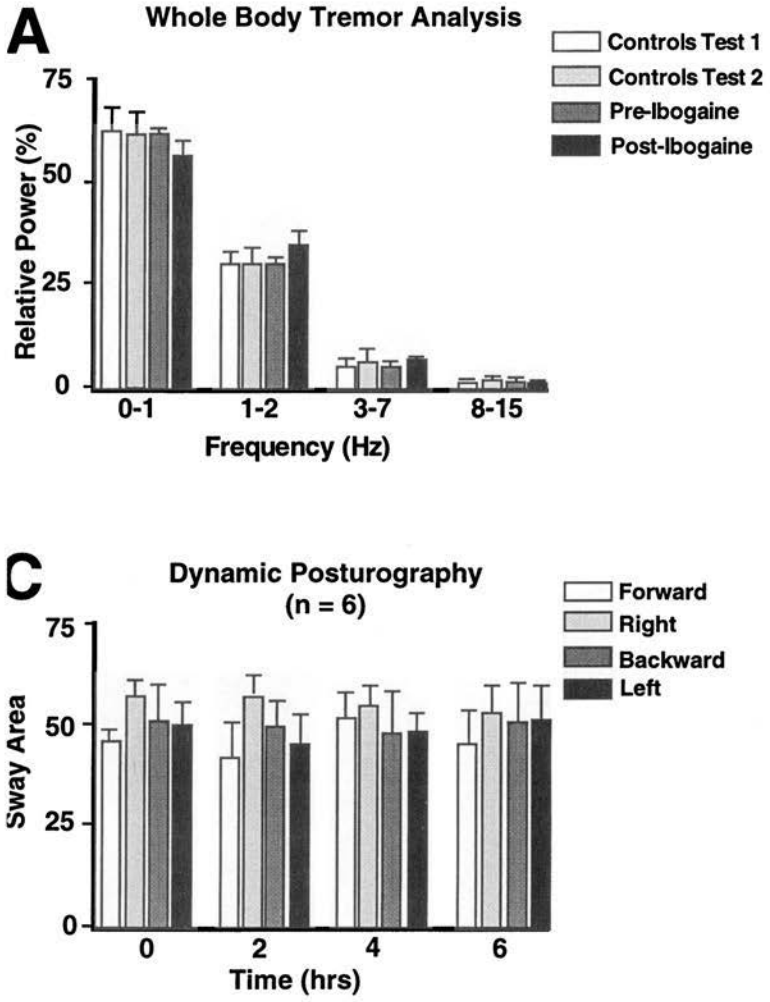
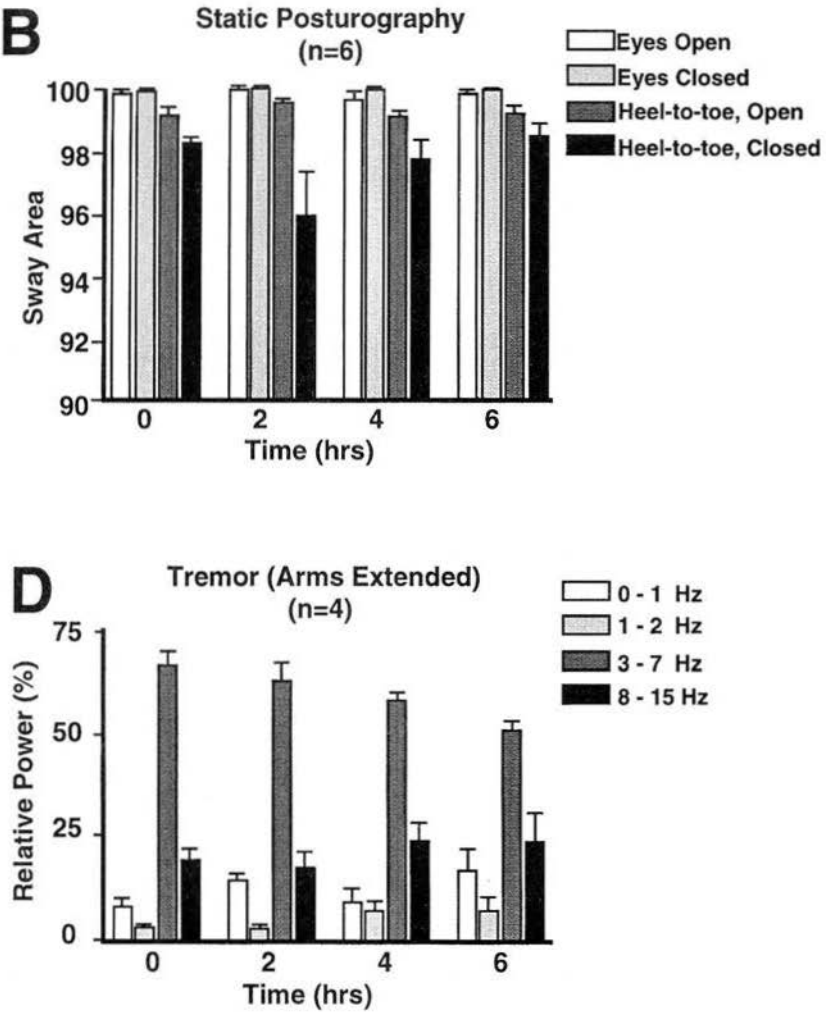


FIGURE 4. **(A)** 'Whole body tremor' analysis on NeuroCom platform. Mean relative power of involuntary movement expressed in 4 frequency bands. Normal age-matched and drug-free patient volunteers ($n = 15$) were tested at two different times (test and retest validation) and compared with patients ($n = 6$) tested before and 48 hr after an oral dose of ibogaine (2 mg kg^{-1}). **(B)** 'Static posturography' analysis on portable platform. Mean sway area for six subjects as a function of time after ingestion of 2 mg kg^{-1} dose of ibogaine. Sway area is determined by measuring actual area of shifts of center of gravity as subject stands on the force plate; the actual height and weight of the subject are expressed as (1-[actual area – theoretical area]) × 100%). One hundred percent represents the best score (*i.e.*, no shifting of weight beyond the theoretical limit). **(C)** 'Dynamic posturography' analysis on portable platform. Mean sway area as a function of time after 2 mg kg^{-1} dose of ibogaine. Area of purposeful sway is based on measuring actual area of shifts of center of gravity as the subject purposefully shifts his weight to intersect the target arc. (1-[actual areas covered – theoretical area within limit of stability]) × 100. **(D)** Extended hand tremor analysis by accelerometer ($n = 6$). The mean relative power of movement as a function of time after ibogaine administration. *Bars* are *shaded* to represent the frequency (Hz) separated into 4 bands.



phy with a portable bedside computerized platform was used to quantify body sway while standing normally and in a heel-to-toe position with eyes opened and eyes closed. Measurements were taken at baseline and every two hours following oral administrations of ibogaine. Dynamic posturography measured functional limits of stability over 6 hr. Accelerometry was used to measure tremor of the hands at rest and with arms extended over the same time period. Whole body tremor, akinesia, and retropulsion were measured with the Neurotest™ apparatus at baseline and 48 hr after drug administration. Both doses of ibogaine produced no clinically visible effects, but static posturography revealed a trend (albeit not significant) toward increased body sway when subjects stood in the heel-to-toe posture with eyes closed. Dynamic posturography and the Neurotest measurements revealed no changes from baseline. Hand accelerometry did

not show any effects of ibogaine on tremor (at rest or with arms extended). However, baseline measurements of tremor revealed quantitative differences between cocaine-dependent patients and age-matched and drug-free control subjects. Power spectrum analysis of these data revealed an increase in the 3–7-Hz range, supporting the hypothesis that early cocaine abstinence may reflect a hypodopaminergic state.[64] Similar observations on patients receiving oral doses of ibogaine in a range of 10 to 30 mg/kg in offshore clinical settings, failed to demonstrate any effects with Neurotest measurements taken at 5 to 7 days post dose ($n = 10$; 8 male, 2 female; data not shown). In addition to the lack of posturographic abnormalities, clinical neurologic exams demonstrated no evidence of permanent cerebellar ataxia in these subjects.

Our research group had the opportunity to conduct a neuropathological evaluation on a female subject who had received 4 doses of ibogaine ranging from 10 to 30 mg kg^{-1} over a period of 15 months. The last two administrations occurred in a Panamanian hospital, approximately 25 days prior to her death from natural causes. Before receiving these last ibogaine treatments, the subject received a series of clinical evaluations at the University of Miami School of Medicine. Her diagnoses at that time included: 1) opiate and cocaine dependence; 2) amenorrhea for 11 months; 3) a history of asthma; 4) a history of peptic ulcer disease; and 5) a history of hypertension. The general physical exam was normal. Neurological examination (including magnetic resonance imaging (MRI), electroencephalogram (EEG) and a Neurotest gait analysis for cerebellar signs were normal. Urine toxicology was positive for cocaine, opiates, and marijuana. She received two doses of ibogaine three days apart (10 mg/kg and 20 mg/kg). Approximately one week later, she returned to Miami for follow-up neurological evaluations. At that time, the patient was admitted to the hospital for the evaluation of tremors. The examination revealed temperature 98, pulse 92, respirations 22, and blood pressure 160/108. General physical exam was notable for "an ulcerative lesion on her right anterior thigh with 3 to 4 cm erythmatous area surrounding it and partially healed." Repeat neurological exam at that time was grossly normal. She was treated with clonidine and discharged. The patient went back to New York where she was treated with diazepam for anxiety and poor sleep. She returned to Miami three weeks later, where she complained to a friend that she had been having diarrhea and vomiting since eating raw fish the previous night. Her vomiting progressively worsened, but she did not seek medical attention. The subject died thereafter and was autopsied. The toxicology screen was positive for benzodiazepines only. Postmortem antinuclear antibody (ANA) and rheumatoid factor were negative. The postmortem autopsy revealed mesenteric artery thrombosis with small bowel infarction as the cause of death, left renal cortical hemorrhagic infarcts, splenic infarct, a capsular hemorrhage of the right ovary, and agonal aspiration of gastric contents. Comprehensive evaluations of the histopathology revealed multiple microscopic arterial thrombi in several tissues, although there was no evidence of arteritis whatsoever. These observations led to the conclusion that the pathological picture was that of a generalized hypercoaguable state. The pathological picture was most consistent with an infectious source (the leg ulcer) producing a thrombotic process resulting in mesenteric artery occlusion and death. This cause of death was more likely than an acute drug toxicity (which would have occurred at 25 days after ibogaine administration).

Neuropathological evaluation revealed slight medullary neuroaxonal dystrophy and an old focal meningeal fibrosis. There were no degenerative changes seen in the cerebellum; cerebellar Purkinje cells were normal and there was no evidence of any significant cytopathology or neurodegeneration in any other brain area (FIG. 5). There was no evidence of astrocytosis or microglial activation. The neuropathological analysis for a human subject (NH) was important in light of the observations of O'Hearn and Molliver,[61] which demonstrated that at high doses, ibogaine administrations result in the

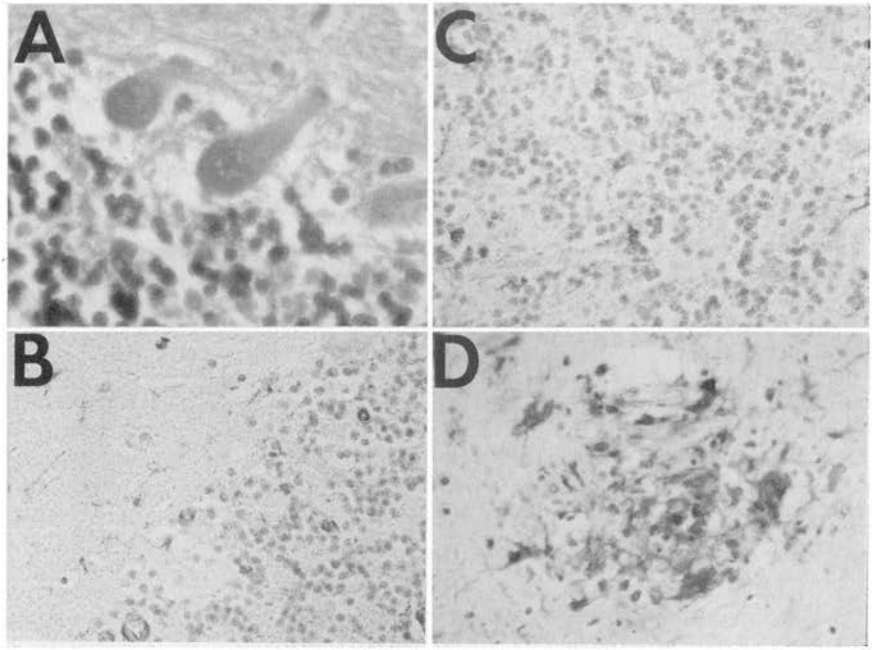


FIGURE 5. Postmortem neuropathological examination of the cerebellum from a female subject who had received four ibogaine treatments. **(A)** Section taken at the level of the anterior vermis. Note the normal cytoarchitecture of the cerebellar Purkinje cells. **(B)** Near adjacent section stained with an anti-ferritin antibody to reveal the presence of microglial cells. There is evidence of a small number of faintly stained microglial cells. **(C)** Near adjacent section labeled with a ricin A (RCA) lectin antibody to localize resting and activated microglia. A few scattered microglial cells are observed in the molecular cell layer. These appear to be in a resting state. **(D)** A positive control section taken from an HIV-infected brain shows robust staining of a microglial nodule for comparison.

degeneration of cerebellar Purkinje cells in rats. At a National Institute on Drug Abuse (NIDA)/Medical Development Division (MDD)/MDD Ibogaine Review Meeting, these investigators reported findings that the toxicity in monkeys was much less than in rats.[62] Our own studies conducted in African green monkeys (vervets) with routine histopathological evaluation (independently rated by two neuropathologists) failed to demonstrate any neuropathological damage caused by ibogaine following 5 days of repeat dosing at either 25 mg/kg p.o. or 100 mg/kg s.c.[60] Taken together, these results demonstrate further that the risk for cerebellar toxicity in human subjects in the dose range purported to be effective for opiate and cocaine detoxification is low.

FUTURE DIRECTIONS

The two major forms of treatment for drug abuse, psychotherapy and pharmacotherapy, have tended to develop along parallel but separate lines.[10] Ibogaine is an interesting example of a pharmacotherapeutic strategy that has the added benefit of it

being an adjuvant to psychotherapy. While much more clinical research is needed, ibogaine has shown preliminary efficacy for the management of opiate detoxification and for short-term stabilization and maintenance of drug users as they prepare to enter treatment. Ibogaine seems to have an added benefit in that it helps clients to establish their resolve to stop substance misuse and to gain self-control over their destructive behaviors. We have observed in offshore studies that ambivalent clients become motivated to seek long-term treatment, since the ibogaine experience seems to bolster the patient's own motivational resources for change.

Drug dependence results from distinct, but interrelated neurochemical adaptations, which underlie tolerance, sensitization and withdrawal. Ibogaine's ability to alter drug-taking behavior may be due to combined actions of either the parent drug and/or its active metabolite at key pharmacological targets that modulate the activity of dopaminergic circuits.[27,38] The active metabolite noribogaine has a unique spectrum of activities as compared to the parent compound. Recent studies have suggested that noribogaine's efficacy as a full μ-opioid agonist may explain ibogaine's ability to block the acute signs of opiate withdrawal and its suppressive effects on morphine self-administration.[38] In addition, a preclinical evaluation of noribogaine's anticocaine medication effects has been conducted in rat models. These results demonstrated that noribogaine antagonized cocaine-induced locomotor stimulation and reinforcement.[31] Ibogaine's interaction with NMDA receptor-coupled cation channels may contribute to the adverse effects of the drug, including the psychotropic (phencyclidine hydrochloride (PCP)-like actions) and the high-dose neurotoxic changes in cerebellar Purkinje cells.[34] Given the lower potency of the metabolite at this molecular target, it is likely that the channel activity of ibogaine may partly explain ibogaine's acute effects. Since ibogaine is cleared rapidly after oral administration, the observed *aftereffects* of ibogaine treatments on drug craving, mood and cognition may be related to the targeted actions of the metabolite noribogaine. The potential development of a slow release formulation of noribogaine as an anticraving medication for opiates and psychostimulants deserves further consideration.

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Acute toxicity of ibogaine and noribogaine

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Key words: *ibogaine; noribogaine; median lethal dose; toxicity; mice.*

Summary. *Objective. To evaluate acute toxic effect of ibogaine and noribogaine on the survival of mice and determine median lethal doses of the substances mentioned.*

Material and methods. White laboratory mice were used for the experiments. Ibogaine and noribogaine were administered intragastrically to mice via a stomach tube. Control animals received the same volume of saline. The median lethal dose was calculated with the help of a standard formula.

Results. To determine the median lethal dose of ibogaine, the doses of 100, 300, 400, and 500 mg/kg were administered intragastrically to mice. The survival time of mice after the drug administration was recorded, as well as the number of survived mice in each group. Upon administration of ibogaine at a dose of 500 mg/kg, all mice in this dose group died. Three out of four mice died in the group, which received 300 mg/kg of ibogaine. No mouse deaths were observed in the group, which received 100 mg/kg of ibogaine. The determined LD_{50} value of ibogaine equals to 263 mg/kg of body mass. In order to determine the median lethal dose of noribogaine, the doses of 300, 500, 700, and 900 mg/kg were administered to mice intragastrically. Noribogaine given at a dose of 500 mg/kg had no impact on the mouse survival. The increase of noribogaine dose to 700 mg/kg of mouse body mass led to the death of three out of four mice in the group. Upon administration of noribogaine at a dose of 900 mg/kg, all mice in this group died. The LD_{50} value of noribogaine in mice determined on the basis of the number of dead mice and the size of the doses used equals to 630 mg/kg of mouse body mass. The behavior of mice was observed upon administration of ibogaine or noribogaine. Low doses of ibogaine and noribogaine had no impact on the mouse behavior. External effects (convulsions, nervous behaviour, limb paralysis) were observed only when substances were administrated at higher doses.

Conclusions. It has been determined that the median lethal dose of ibogaine and noribogaine equals to 263 mg and 630 mg/kg of mouse body mass, respectively. The toxicity of ibogaine is 2.4 times higher than that of noribogaine.

Introduction

Looking for new medications for the treatment of drug and alcohol dependence encourages us to focus more attention on and investigate an indole alkaloid ibogaine. There are findings demonstrating its capability to attenuate craving for alcohol (1). However, its toxicity and lethal dose are still unknown. In addition, an active metabolite of ibogaine, noribogaine, has been identified and is currently being analyzed.

Naturally occurring ibogaine is a psychoactive alkaloid extracted from the *Tabernanthe iboga* shrub. For many years, extracts of *Tabernanthe iboga* have been used as central nervous system (CNS) stimulants at low doses or as hallucinogens at high doses (2). Preclinical studies have demonstrated that ibogaine reduces craving for cocaine and morphine, attenuates morphine withdrawal symptoms (3). Based on the clinical studies, a conclusion can be made that ibogaine has a certain antiaddictive action (4). However, its mechanism of action is still not clear enough. The identified antagonistic activity of ibogaine on *N*-methyl-*D*-aspartate receptors as well as its agonist activity on opioid receptors can be regarded as a possible mechanism of antiaddictive action (5). It should be mentioned that ibogaine interacts with several neurotransmitter systems, including serotonin uptake sites and sigma sites. Some of ibogaine actions can be attributed to its long-lasting metabolite, O-desmethyli-

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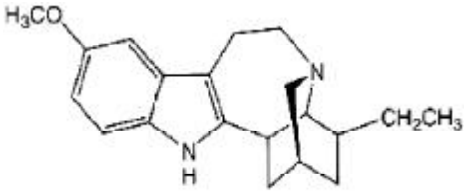
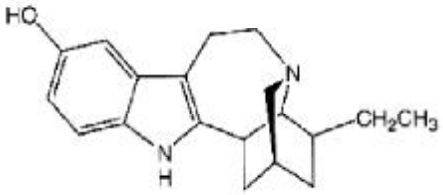


Ibogaine



12-Hydroxyibogamine
(Noribogaine)

Fig. 1. **Structure of ibogaine and noribogaine**

bogaine (other names are noribogaine or 12-hydroxyibogamine) (2). Noribogaine differs from ibogaine in that it contains a hydroxyl instead of a methyl group at position 12 (Fig. 1). Following ibogaine administration, noribogaine has been detected in human plasma (6) as well as in plasma and in the brain of ibogaine-treated rats (7), which proves once more that noribogaine is a metabolite of ibogaine. Experimental studies on rats have established that noribogaine is pharmacologically active and produces effects that mimic those of ibogaine: decrease in craving for morphine and cocaine, reduction in the locomotor effect of morphine (8). Other data presented in literature (9), however, demonstrate that noribogaine produces no positive action in respect of inhibition of the morphine withdrawal signs.

Ibogaine and noribogaine can evoke different behavioral effects despite having similar chemical structures (10, 11). Moreover, it appears that the mechanisms of antiaddictive effects of ibogaine and noribogaine may involve different patterns, which call for more detailed studies. In this connection, our objective was to evaluate acute toxic effect of ibogaine and noribogaine on the survival of mice and to determine median lethal doses (LD_{50}) of these substances.

Materials and methods

Experiments were done on 4–6-week-old outbred mice weighing 20–25 g. All experiments were performed according to the Law on the Care, Keeping and Use of Animals, Republic of Lithuania (License of State Veterinary Service for Working with Laboratory Animals, No. 0153). Before starting experiments, animals were acclimatized to laboratory conditions. Mice were randomly assigned to groups and weighed. Ibogaine and noribogaine are almost completely insoluble in water, so suspensions were used for their administration. Study substances were administered intragastrically to mice via a stomach tube. Control mice received the same amount of saline. The same method of administration was used.

LD_{50} was calculated with the help of the following formula (12):

$$\lg LD_{50} = \lg D_N - \delta(\Sigma L_i - 0.5),$$

where D_N is the highest dose of the study substance administered to mice; δ is the logarithm of the ratio between the doses of the substance administered; L_i is the ratio of the number of dead mice to the number of mice used to determine the dose effect.

Results

Toxicity studies of various drugs and comparison of toxic effects of different substances on the body require evaluation of LD_{50} of such drugs and substances. Determination of the LD_{50} value allows for the correct planning of an experiment not being afraid of overdosing the study drug. Moreover, this value allows for the comparison of the toxicity of various substances. LD_{50} is a calculated single dose of a substance expected to kill 50% of studied animals.

To determine the median lethal dose of ibogaine, we used the following substance concentrations: 500 mg/kg (working suspension concentration of 25 mg/mL), 400 mg/kg (20 mg/mL), 300 mg/kg (15 mg/mL), and 100 mg/kg (5 mg/mL). Each of these doses was administered intragastrically to four mice via a stomach tube. Afterwards, the survival time of mice after the drug administration was recorded, as well as the number of survived mice in each group. Upon administration of the highest ibogaine dose (500 mg/kg), all mice in this group died. No mouse deaths were observed in the last group only, which received the lowest ibogaine dose (100 mg/kg). In the preceding group, which received 300 mg/kg of ibogaine, three mice out of four in that experimental group died (Fig. 2). The LD_{50} of ibogaine was calculated according to the formula specified in the section "Materials and methods." The determined median lethal dose of this drug in mice is 263 mg/kg of body mass (Fig. 3).

To determine the median lethal dose of noribogaine, we used the following substance concentrations:

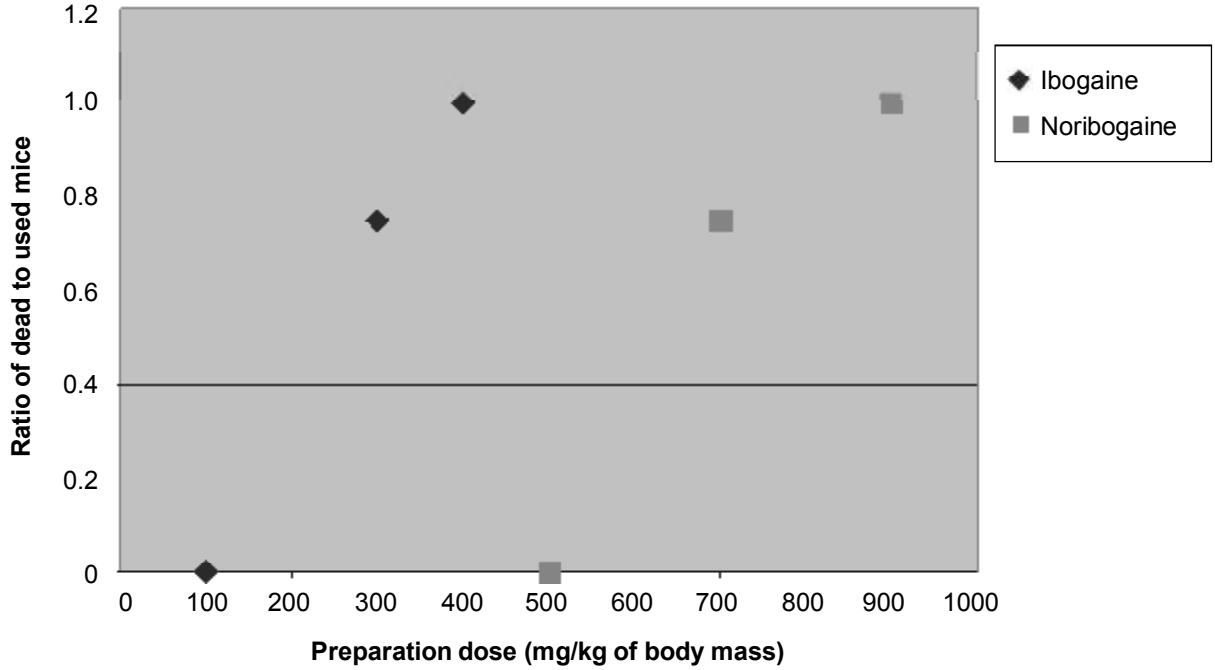


Fig. 2. **The dependence of the mouse survival on the dose of drug administered**
Four mice received the different dose of drug.

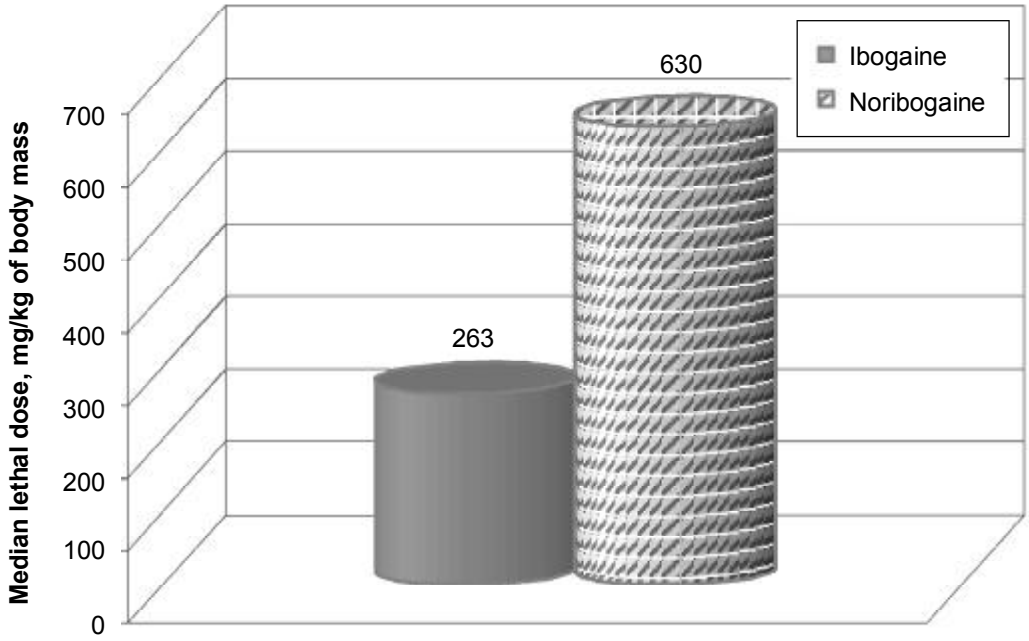


Fig. 3. **Median lethal doses of ibogaine (n=12) and noribogaine (n=16) for mice**

900 mg/kg (working suspension concentration of 45 mg/mL), 700 mg/kg (35 mg/mL), 500 mg/kg (25 mg/mL), and 300 mg/kg (15 mg/mL). The highest dose used in the ibogaine arm of the experiment (500 mg/kg) had no effect on the mouse survival in the noribogaine arm (Fig. 2). That is, all four mice in this experimental group survived. Therefore, we increased the dose of noribogaine to 700 mg/kg of mouse body mass. In this case, three out of four mice in the group died. Following the technique used, the noribogaine dose had to be increased to the higher level (to 900 mg/kg) in order to detect the group, in which all mice die. According to the determined number of dead mice and the doses used, we calculated the LD_{50}

for mice, which is 630 mg/kg of mouse body mass for noribogaine (Fig. 3).

The behaviour of mice was observed following administration of both ibogaine and noribogaine. Behaviour is one of the markers of the substance toxicity in animals. Upon administration of low ibogaine and noribogaine doses, no changes in the behavior of mice were observed. External effects (convulsions, nervous behavior, limb paralysis) of the drugs were observed only in case of administration of higher doses of substances: ibogaine at a dose of 400 mg/kg and noribogaine at a dose of 500 mg/kg of body mass.

Discussion

Data presented in literature show that LD_{50} varies depending on the animal. The route of administration into the body of laboratory animals is also of major importance. The LD_{50} of ibogaine has been determined in guinea pig (82 mg/kg intraperitoneally) and rat (327 mg/kg orally and 145 mg/kg intraperitoneally) (13, 14). No changes in rat liver, kidneys, heart, and brain have been established during the chronic ibogaine toxicity studies (10 mg/kg for 30 days and 40 mg/kg for 12 days) (13). No evidence of neurotoxicity has been found in monkeys given ibogaine at doses of 5–25 mg/kg orally for four consecutive days (15). Other investigations have revealed that ibogaine causes neurotoxic effects, i.e., induces degeneration of Purkinje cells (16) and that the neurotoxicity of ibogaine is dose-dependent (17). Based on the data presented in literature, after intraperitoneal and subcutaneous injection of ibogaine in rats, the highest level of the substance is achieved in brain and adipose tissue one hour after administration. Thus, it can be stated that the potent effect induced by ibogaine in the brain lasts for up to 12 hours following administration, and the further action is determined by active metabolite noribogaine (13). The study by Baumann et al. has demonstrated that in rats, the ratio of noribogaine to ibogaine in the bloodstream is much higher when ibogaine is injected by the intraperitoneal route rather than the intravenous route (11). In our study, we are also planning to study and compare distribution of these substances in internal organs (liver, kidneys, heart, spleen), brain, smooth muscles, and blood when ibogaine and noribogaine are administered directly into the stomach via the stomach tube.

Glick et al. (18) and O'Hearn with Molliver (19) have proved that ibogaine induces tremor and ataxia when administered intraperitoneally at the dose ranging from 40 to 100 mg/kg, meanwhile noribogaine does not cause such effects. In our study, both study substances had an impact on the mouse behavior: ibogaine at a dose of 400 mg/kg and noribogaine at a dose of 500 mg/kg. Since noribogaine shows lower toxicity, it can be more promising for the clinical use.

Conclusions

1. The median lethal dose of both drugs studied was determined. LD_{50} of ibogaine equals to 263 mg/kg and LD_{50} of noribogaine is 630 mg/kg of body mass.
2. The comparison of ibogaine and noribogaine toxicity for mice was performed. It was detected that the latter is 2.4 times lower than that of ibogaine.

Ūminis ibogaino ir noribogaino toksiškumas

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Raktažodžiai: ibogainas, noribogainas, vidutinė mirtina dozė, toksiškumas, pelės.

Santrauka. *Tyrimo tikslas*. Įvertinti ibogaino ir noribogaino toksinį poveikį pelių išgyvenimui bei nustatyti šių medžiagų vidutinę mirtiną dozę.

Tyrimo medžiaga ir metodai. Eksperimentai atlikti su baltomis laboratorinėmis pelėmis. Ibogaino ir noribogaino zondu suleista į pelių skrandį. Kontroliniams gyvūnams skirtas toks pat fiziologinio tirpalo tūris. Vidutinė mirtina dozė apskaičiuota pagal standartinę formulę.

Rezultatai. Vidutinei mirtinai ibogaino dozei nustatyti pelėms skirtos 100, 300, 400 ir 500 mg/kg dozės. Registruotas laikas, kurį išgyveno pelės po preparato suleidimo, ir kiek pelių kiekvienoje grupėje išgyveno. Suleidus 500 mg/kg ibogaino dozę visos grupėje buvusios pelės nugaišo. Grupėje, kur pelėms skirta 300 mg/kg ibogaino dozė, krito trys pelės iš keturių. Skyrus 100 mg/kg ibogaino dozę visos pelės išgyveno. Nustatyta

LD_{50} reikšmė ibogainui lygi 263 mg/kg kūno masės. Vidutinei mirtinai noribogaino dozei nustatyti pelėms per zondą suleistos 300, 500, 700 ir 900 mg/kg dozės. 500 mg/kg noribogaino dozė jokio poveikio pelių išgyvenimui neturėjo. Padidinus noribogaino dozę iki 700 mg/kg pelės kūno masės, krito trys iš keturių grupėje buvusių pelių. Skyrus 900 mg/kg ibogaino, nustatyta, kad šioje grupėje žuvo visos pelės. Pagal žuvusių pelių skaičių ir dozių dydį nustatyta noribogaino LD_{50} reikšmė pelėms lygi 630 mg/kg pelės kūno masės. Suleidus ibogaino ar noribogaino, stebėta pelių elgsena. Mažos ibogaino ir noribogaino dozės pelių elgesio neveikė. Išorinis poveikis (traukuliai, nervingas elgesys, kojų paralyžius) užfiksuotas tik suleidus didesnes medžiagų dozes.

Išvados. Nustatyta, kad vidutinė mirtina dozė yra 263 mg ibogaino ir 630 mg noribogaino kg pelės kūno masės. Ibogainas yra 2,4 karto toksiškesnis už noribogainą.

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Long-QT Syndrome Induced by the Antiaddiction Drug Ibogaine

TO THE EDITOR: Anecdotal evidence suggests that ibogaine alleviates drug craving and relapse of drug use in humans, as has been confirmed for animals.[1] Ibogaine is currently used as an antiaddiction drug in alternative medicine. In 1993, the Food and Drug Administration approved a clinical trial in humans to study those effects. The National Institute on Drug Abuse decided not to fund this study because of safety issues.[2,3]

Ibogaine is a naturally occurring alkaloid with hallucinogenic and psychedelic effects, derived from the bark of the root of the West African *Tabernanthe iboga* plant and is used in Gabonian initiation ceremonies. At least 11 sudden deaths were described after ibogaine use, in which the cause of death remained unclear even after autopsy.[4] It was hypothesized that ibogaine might dysregulate the autonomic nervous system, causing sudden death.[5]

A 31-year-old American woman was admitted to our emergency department because of a seizure-like attack after she had taken a single dose of 3.5 g of ibogaine 15% (usual dose, 2 to 6 g). She had not taken any other drugs or alcohol concurrently with the ibogaine. Her medical history was unremarkable, and there was no family history of cardiac-rhythm abnormalities. Besides nausea, she had no specific symptoms to report. She had come to the Netherlands to receive ibogaine as an alternative medicine for treatment-resistant alcohol addiction.

Electrocardiography showed a severe prolonged QT interval of 548 msec (QT interval corrected for the heart rate, 616 msec) and ventricular tachyarrhythmias during prolonged monitoring (Fig. 1). Laboratory findings revealed mild hypomagnesemia (magnesium level, 0.49 mmol per liter [1.2 mg per deciliter]; reference range, 0.70 to 1.00 [1.7 to 2.4]), hypokalemia (potassium level, 3.2 mmol per liter [12.5 mg per deciliter]; reference range, 3.8 to 5.0 [14.9 to 19.5]), and a normal serum osmolal gap (3.1 mOsm per kilogram; reference value, <10). Despite rapid correction of the electrolyte levels, the QT interval remained prolonged. During admission to the intensive care unit, with no further doses of ibogaine given, the QT interval normalized at 42 hours after presentation. The patient was subsequently discharged, in good condition.

In this case, ibogaine use was associated with severe lengthening of the QT interval and ventricular tachyarrhythmias, which normalized after 42 hours. These findings are suggestive of a causal relation. The electrolyte imbalance may also have played a role. The previously described sudden deaths may thus have been caused by

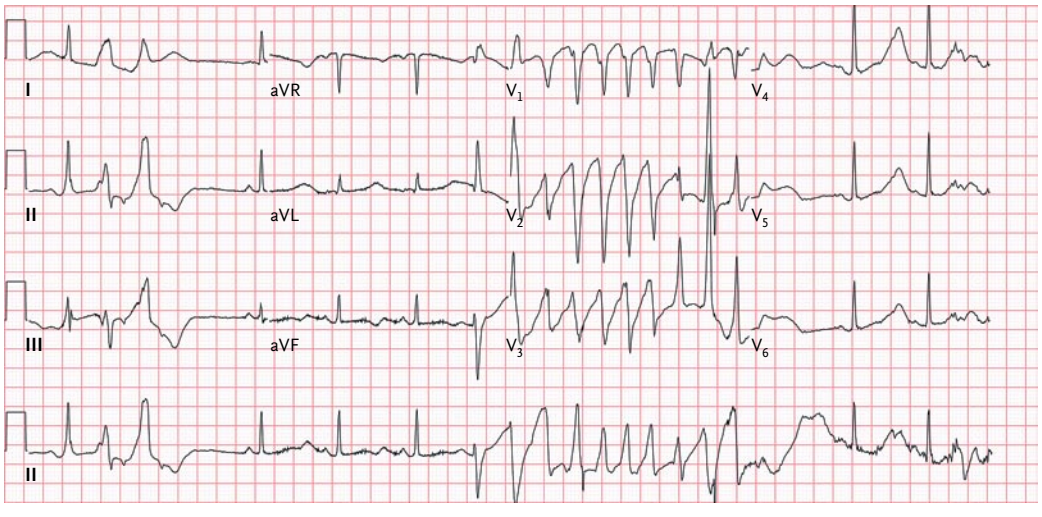

Figure 1. Electrocardiogram of the Patient, Showing Ventricular Tachyarrhythmias and a Prolonged QT Interval.

cardiac-rhythm abnormalities induced by QT-interval lengthening, ventricular tachyarrhythmias, or both.

At the doses currently used, ibogaine can lead to serious cardiac-rhythm abnormalities. The use and possible future trials of the drug should be permitted only under strict medical observation and continuous electrocardiographic monitoring.

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LETTER TO THE EDITOR

Ibogaine-associated ventricular tachyarrhythmias

To the Editor:

Ibogaine is an alkaloid with psychedelic effects derived from the Tabernanthe iboga plant and is used in alternative addiction treatment and connected to some sudden deaths.[1,2] We present ventricular tachyarrhythmias in an ibogaine-exposed patient.

A 33-year-old man ingested a single 600 mg dose of ibogaine after 2 days of cocaine, heroin and methadone abstinence. After 30 minutes, he lost consciousness after trying to urinate due to ventricular fibrillation (VF) which was defibrillated at 200 J DC. On admittance, his vital signs and laboratory results including electrolytes were normal. Electrocardiography showed a prolonged QTc-interval of 460 ms. During the first 10 hours, he had two more VFs, one after micturition, that were defibrillated with 200 J. Amiodarone infusion of 300 mg in 30 minutes followed by 1200 mg per day was started, and he developed sinus bradycardia, a QTc-interval prolongation (510 ms) and the 4th VF which was defibrillated. During transient amiodarone withdrawal he experienced the 5th VF and the QTc transiently shortened, but after amiodarone reintroduction, the QTc-interval prolonged up to 593 ms 42 hours post-ingestion, and he suffered three monomorphic VTs between 36 and 48 hours while trying to urinate or defecate, which were electroconverted with 100 J. Fourty- eight hours post-ingestion, the QTc-interval began to decrease and amiodarone was stopped on the 4th day, but the QTc-interval did not normalize until the 9th day. Ibogaine and noribogaine were detected by LC-MS/MS in his blood from day 1 to 9. The highest ibogaine level was 0.68 mg/L at the time of the last VF. No other drugs were revealed except methadone (0.04 mg/L) without EDDP on day 1. The genetic syndromes of the long QT-interval and structural heart disease were excluded.

In this patient, ibogaine was associated with VF/VT and QTc-interval lengthening, which could explain the sudden death syndrome after ibogaine. The mechanism of VF is not known, but later episodes of VT were probably due to QTc-interval prolongation. The influence of the autonomic nervous system in ventricular tachyarrhythmia is probable because 2/5 VFs and 3/3 VTs appeared at micturition and defecation, which are vagal maneuvers prolonging the QTc-interval.[2,3,4]

VF/VTs kept repeating for 2 days, and the QTc-interval was prolonged for 9 days, which coincided with an increased ibogaine level due to its half-time of 48 hours.[5] Amiodarone effect on the QTc-interval is probable regarding QTc-interval decrease during amiodarone discontinuation. Nevertheless, QTc-interval prolongation was primarily associated with ibogaine and its metabolite considering the observation that after 48 hours post-ingestion, the QTc-interval was decreasing despite ongoing amiodarone therapy, which could be related to progressive lowering of the ibogaine level. Furthermore, the QTc-interval normalized only 5 days after amiodarone withdrawal when ibogaine and noribogaine became undetectable, which is consistent with the role of ibogaine in QTc-interval prolongation. Methadone could have contributed only to a slightly prolonged QTc-interval on arrival.

In conclusion, ibogaine is associated with QTc-interval prolongation and VF/VT that might be provoked by vagal maneuvers. In ibogaine exposure, amiodarone did not appear effective in VF/VT therapy, and DC shock should be used first.

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Ibogaine-Associated Psychosis in Schizophrenia
A Case Report

To the Editors:

Ibogaine is the main psychoactive molecule of a plant (Iboga) used for religious ceremonies and traditional initiation purposes in Gabon. Ibogaine use is becoming increasingly widespread in Western countries and is being considered for opioid addiction therapy.[1] We describe here a patient whose schizophrenia was revealed by the ingestion of ibogaine.

A 26-year-old man of mixed origin (sub-Saharan Africa and West Indies), was admitted to our inpatient ward in 2006 for pathological wandering, rambling monologues, and carelessness. He presented persecutory and mystic delusions, with visual and acoustico-verbal hallucinations, along with major psychomotor agitation. The results of routine screening tests for narcotics were normal. Other laboratory examinations gave normal results. Hallucinations and delusions disappeared within 2 weeks of treatment with olanzapine (10 mg/d) and sedative medication.

Our patient explained that he was on a "mystic quest" and that he had been involved in Gabonese shamanism for around 5 years. As part of this quest, he took Iboga powder to fight his "internal demons." He consumed only small amounts of Iboga powder until 2 months before his hospitalization, when he began taking Iboga on a daily basis. He then had dream-like experiences in which he was hunted down by terrifying monsters.

Since the age of 22 years, the patient presented a progressive degradation of social, vocational, and familial interactions. He also experienced intermittent hallucinations and persecutory delusions. These may be acknowledged as attenuated psychotic symptoms.

The patient experienced 2 relapses: the first 2 months after his discharge from the hospital and the second in 2010. Both were caused by the interruption of his antipsychotic medication, in the absence of ibogaine consumption since his first hospitalization. The latter relapse occurred 2 weeks after the interruption of antipsychotics. A *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition,* diagnosis of schizophrenia was confirmed based on the persistent dissociative and persecutory delusions. The timeline of his 3 acute psychotic episodes strongly suggests that ibogaine was the cause of the first episode (although we cannot definitely prove it).

Neurotoxic and visual hallucinatory effects of ibogaine have been described in healthy controls, but this is the first description of the exacerbation of schizophrenia by ibogaine. Ibogaine is currently being considered for testing in clinical trials of opioid addiction treatment, but its adverse effects include long-QT syndrome.[2] Its consumption is becoming increasingly widespread in Western countries.[1] Our case report ibogaine associated psychosis in a patient with schizophrenia thus seems timely.

Ibogaine is an alkaloid that interacts with multiple binding sites; it inhibits the reuptake of serotonin and stimulates nicotinic cerebellum receptors.[1] It also decreases dopamine release in the nucleus accumbens, and it interacts with opioid receptors. Activities of ibogaine on these receptors potentially account for its antiaddictive properties.[3] *N*-methyl D-aspartic acid blockade may account for its hallucinogenic properties.[3]

Two conclusions can be drawn from our case. First, individuals presenting psychosis after engaging in mystic quests involving sub-Saharan African shamanic rituals should be asked about ibogaine consumption and/or screened for ibogaine use with tests recently developed.[3] Second, future trials involving ibogaine or its metabolites should include the careful assessment of psychotic side effects and consider excluding patients with schizophrenia.

AUTHOR DISCLOSURE INFORMATION

The authors declare no conflicts of interest.

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Effects of Add-On Cilostazol on Cognition in Patients With Schizophrenia
An Open-Label Pilot Trial

To the Editors:

Cognitive deficits in schizophrenia are core features of the illness that may be related to the daily functioning of patients.[1] The relationship between cognitive deficits and psychosocial functioning is complex and probably mediated by several factors, namely, positive, negative, and depressive symptoms.[2,3] Furthermore, the beneficial effect of atypical antipsychotic drugs on several domains of cognitive function is still a controversial issue.[4]

Cilostazol, a selective inhibitor type 3 phosphodiesterase, has been widely used as an antiplatelet agent for the treatment of chronic cerebral infarction and intermittent claudication.[5] Recently, Hashimoto et al[6] reported that cilostazol could attenuate hyperlocomotion and prepulse inhibition deficits in mice after administration of the *N*-methyl-D-aspartate receptor antagonist dizocilpine. Furthermore, the *N*-methyl-D-aspartate receptor antagonist phencyclidine-induced cognitive deficits in mice could be ameliorated by subsequent subchronic administration of cilostazol.[7] These preclinical findings suggest that cilostazol may have a potential antipsychotic activity in

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NEWS

Doctor is suspended for prescribing a drug for pornography "addiction" without giving the risks

Clare Dyer

BMJ

A doctor in private practice has been suspended for six months by the General Medical Council for giving the unlicensed hallucinogenic drug ibogaine to a man obsessed with internet pornography without fully explaining the drug's risks.

Peter Brackenridge, who saw private patients in London's Harley Street, was medical director of Ibogaine Therapy UK, which advertised the treatment on a website.

The drug, which has been used experimentally as a one-off "detox" treatment for heroin addicts because it can minimise or eliminate withdrawal symptoms, puts those who take it into a dreamlike state. Its initial effects usually last for 20-30 hours, during which someone must be with the patient.

The 25 year old pornography addict, named as Patient A, described it as a "horrendous treatment" and said that he went on to experience months of mental health problems, including insomnia and paranoia.

The drug has been linked with a number of deaths around the world and is banned in some countries. Sleeplessness, anxiety, and paranoia are well known side effects.

The GMC's fitness to practise panel found that the website, although it called ibogaine a "medicine," failed to mention that it was unlicensed in the United Kingdom and failed "to set out a single risk, let alone provide a summary of risks."

Along with a course of psychotherapy, Patient A was given ibogaine at Dr Brackenridge's home in August 2009. The panel found this was not an appropriate place because it lacked adequate facilities to treat an allergic reaction, give intravenous fluids, or provide pulmonary resuscitation or defibrillation.

The panel accepted that Dr Brackenridge had some medical equipment but lacked "the availability of other trained medical or nursing personnel for dealing with a medical or psychiatric emergency which could have arisen during the 30 hour period in which you were alone with the patient."

Dr Brackenridge, who qualified in Australia and worked in psychiatry in the NHS, was entitled to prescribe an unlicensed drug as long as no alternative existed that would meet the patient's needs. The panel found that there was no such alternative or any licensed drug that mirrored the effects and results of ibogaine.

But although he gave the patient a consent form that mentioned risks just before administering the treatment, the panel found that he did not adequately explain the risks. Those set out on the form were "almost exclusively physical," noted the panel's chairman, Jack Crane.

Dr Brackenridge also failed to carry out blood tests that the website said would be required and "inappropriately" refused to meet the patient's parents, who were outside his premises, in October 2009.

By then "there had been a clear deterioration in Patient A's mental health of which you were aware," Dr Crane told him. "The parents had serious concerns regarding their son's wellbeing to which you should have listened."

Dr Brackenridge, who now works as a psychotherapist, offered to give an undertaking never again to prescribe ibogaine. But Dr Crane said that the panel was concerned at his attitude and lack of insight and believed that six months' suspension would enable him to reflect on the panel's findings and deal with his deficiencies of judgment.

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PAPER

TOXICOLOGY

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Fatalities Temporally Associated with the Ingestion of Ibogaine

ABSTRACT: Ibogaine is a naturally occurring psychoactive plant alkaloid that is used globally in medical and nonmedical settings for opioid detoxification and other substance use indications. All available autopsy, toxicological, and investigative reports were systematically reviewed for the consecutive series of all known fatalities outside of West Central Africa temporally related to the use of ibogaine from 1990 through 2008. Nineteen individuals (15 men, four women between 24 and 54 years old) are known to have died within 1.5–76 h of taking ibogaine. The clinical and postmortem evidence did not suggest a characteristic syndrome of neurotoxicity. Advanced preexisting medical comorbidities, which were mainly cardiovascular, and/or one or more commonly abused substances explained or contributed to the death in 12 of the 14 cases for which adequate postmortem data were available. Other apparent risk factors include seizures associated with withdrawal from alcohol and benzodiazepines and the uninformed use of ethnopharmacological forms of ibogaine.

KEYWORDS: forensic science, toxicology, ibogaine, iboga alkaloid, substance abuse, human, fatality, opioid, opioid detoxification, ethnopharmacology

The iboga alkaloids are a group of monoterpene indole alkaloids, some of which reportedly reduce the self-administration of drugs of abuse and opiate withdrawal symptoms in animal models and humans (1,2). Ibogaine (Fig. 1), the most extensively studied iboga alkaloid, occurs in the root bark of the West African Apocynaceous shrub *Tabernanthe iboga* Baill. In Gabon, eboga, the scrapings of the root bark, has been used as a psychopharmacological sacrament in the Bwiti religion for several centuries (3,4). Elsewhere, including North America, Europe, and South Africa, ibogaine is used for the purpose of acute opioid detoxification, and to reduce craving and maintain abstinence from opioids and other abused substances including stimulants and alcohol, as well as for psychological or spiritual purposes (5).

Ibogaine is used most frequently as a single oral dose in the range of 10–25 mg/kg of body weight for the specific indication of detoxification from opioids (5,6). It is most commonly used in the form of the hydrochloride (HCl), which certificates of analysis typically indicate is 95–98% pure, with present retail prices in the range of *c.* $125–$250 USD per gram. Ibogaine is also used in the form of alkaloid extracts or dried root bark (Fig. 2).

Ibogaine is a schedule I substance in the United States, and similarly is illegal in France, Denmark, Sweden, Belgium, Switzerland, and Australia. However, it is unregulated in most countries, where it is neither illegal nor officially approved. Lay providers administer ibogaine in nonmedical settings and have accounted for the majority of treatments (5). Ibogaine is administered in medical settings in countries such as Mexico and South Africa, where physicians have the legal prerogative to prescribe unapproved medications.

Published case series and individual accounts regarding ibogaine for opioid detoxification tend to be consistent with regard to rapid remission of acute withdrawal symptoms following a single administration that is subsequently sustained without further ibogaine treatment or the use of opioids (1,6,7). This effect of ibogaine appears to be pharmacologically mediated and not accounted for by placebo, which has clinically negligible effects in opioid detoxification (8–10). In the naloxone-precipitated withdrawal model of opioid detoxification, iboga alkaloids have attenuated opioid withdrawal signs in 13 of 14 independent replications in two rodent and two primate species (11–24). Ibogaine administered to rats or mice as a single dose reduces the self-administration of morphine (25–28), cocaine (26,29,30), and alcohol (31,32), with sustained treatment effects for 48–72 h averaged for an entire sample, and an even longer duration in individual animals (25,26,28,30). The serum half-life of ibogaine in the rat is *c.* 1–2 h (33,34), indicating that the prolonged effect on self-administration outlasts the presence of ibogaine itself, without compelling evidence that it is mediated by a long-lived metabolite (35).

Ibogaine does not appear to be an abused substance. The National Institute on Drug Abuse (NIDA) did not identify potential abuse as an issue in the context of its research program on ibogaine, which included preclinical testing and the development of a clinical trial protocol (1). Animals do not self-administer 18-methoxycoronaridine (18-MC), a closely structurally related ibogaine congener with the same effects as ibogaine on self-administration and withdrawal in preclinical models (36). Aversive side effects such as nausea and ataxia limit ibogaine's potential for abuse.

Ibogaine potentiates the lethality of opioids (33,37–39). This is apparently because of an enhancement of opioid signaling (1,40), and not because of binding at opioid receptors as an agonist (such

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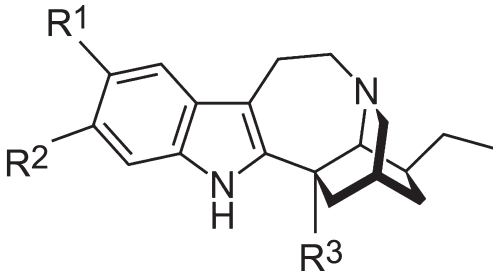

Iboga alkaloid	R^1	R^2	R^3
Ibogaine	OCH_3	H	H
Noribogaine	OH	H	H
Ibogamine	H	H	H
Ibogaline	OCH_3	OCH_3	H
Tabernanthine	H	OCH_3	H
Voacangine	OCH_3	H	CO_2CH_3

FIG. 1—*Chemical structures of ibogaine and its major metabolite noribogaine, and the alkaloids ibogamine, ibogaline, tabernanthine, and voacangine that co-occur with ibogaine in* T. iboga. *In the Chemical Abstracts system the positions of R^1, R^2, and R^3 on the ibogamine parent structural skeleton are respectively numbered 12, 13 and 18, whereas in the Le Men and Taylor system these same positions are numbered 10, 11 and 16.*

as methadone) or antagonist. Doses of ibogaine used in opioid detoxification do not produce signs of overdose in individuals who lack tolerance to opioids, such as African Bwiti adepts, or individuals in non-African contexts who take ibogaine for psychological or spiritual purposes or the treatment of addiction to substances other than opioids. If ibogaine was acting as an opioid agonist, it would not be tolerated by opioid-naïve individuals because the methadone dosage of 60–100 mg/day that is used to stabilize withdrawal symptoms in the maintenance treatment of opioid-dependent patients (41) substantially exceeds the estimated LD_{50} of 40–50 mg in humans who are not pharmacologically tolerant to opioids (42). Other evidence that ibogaine alters signaling through opioid receptors but is not itself an orthosteric agonist includes its potentiation of morphine analgesia in the absence of a direct analgesic effect (22,38,39,43–47). Ciba Pharmaceutical patented the use of ibogaine to reduce tolerance to opioid analgesics in 1957 (47).

Although ibogaine contains an indole ring and is designated as a "hallucinogen," it is pharmacologically distinct from the "classical" hallucinogens such as LSD, mescaline, or psilocybin, which are thought to act by binding as agonists to the serotonin type 2A ($5\text{-}HT_{2A}$) receptor (48). Serotonin agonist or releasing activity does not appear to explain ibogaine's effects in opioid withdrawal (2,49). There is no anecdotal or preclinical evidence for a significant effect of classical hallucinogens in acute opioid withdrawal, and in the animal model ablation of 90% of the raphe, the major serotonergic nucleus of the brain does not significantly affect the expression of opioid withdrawal (50). Descriptions of subjective experiences associated with ibogaine differ from those associated with the classical hallucinogens (5,48,51). The visual effects of classical hallucinogens are typically most strongly experienced with the eyes open and limited to alterations of colors, textures, and patterns. In contrast, the psychoactive state associated with ibogaine is experienced most intensely with the eyes closed and has been described as "oneiric" and likened to a "waking dream," with

FIG. 2—*Forms of availability of ibogaine: Ibogaine is available in form of the hydrochloride (HCl) dried root bark, or alkaloid extract. The upper left photo shows 96% pure ibogaine HCl in the form of powder in the upper left quadrant of the photo. In the lower left quadrant of the photo are five capsules. The four lighter colored capsules contain 96% pure ibogaine HCl; the smaller two contain 120 mg and the larger two contain 250 mg respectively. The largest capsule is darker and contains 330 mg of 85% ibogaine HCl. In the lower right quadrant of the photo is ground dried root bark. The upper right photo shows alkaloid extract with an estimated total iboga alkaloid content of about 40–50%. The lower photo shows a partially scraped dried* Tabernanthe iboga *root, with external bark layer, an inner bark layer, and wood. The alkaloid content is mainly concentrated in the inner root bark layer, which is exposed along the lower border of the bare wood in left middle portion of the photo (photos courtesy of Robert Bovenga Payne and Rocky Caravelli).*

interrogatory verbal exchanges involving ancestral and archetypal beings, and movement and navigation within visual landscapes. Another frequently described experience is panoramic memory, the recall of a rapid, dense succession of vivid autobiographical visual memories. Mechanistically, these subjective experiences associated with ibogaine might possibly suggest functional muscarinic cholinergic effects, which are prominent in the mechanisms of dreaming and memory (52). In animals, ibogaine is reported to enhance spatial memory retrieval (53,54), and to produce an atropine-sensitive EEG rhythm (55,56), commonly regarded as a model of REM sleep (57).

Ibogaine's highest affinity receptor interactions are as an agonist at the σ_2 receptor, and an antagonist at the *N*-methyl-D-aspartate-type (NMDA) glutamate and $\alpha 3\beta 4$ nicotinic acetylcholine receptors (1,2,58). Initially, ibogaine's mechanism of action in drug self-administration and withdrawal was hypothesized to involve NMDA receptor antagonism (59); however, this hypothesis is now viewed as unlikely because the synthetic ibogaine congener 18-MC has negligible NMDA receptor affinity but is equally effective as ibogaine in reducing withdrawal and self-administration in the animal model (2). Studies of iboga alkaloids and nicotinic agents (60–64) provide some support for antagonism of the $\alpha 3\beta 4$ nicotinic receptor as a possible mechanism of action with regard to drug craving and self-administration but do not appear to explain detoxification in the setting of extensive physical dependence on opioids. Likewise, the increased expression of glial cell-derived neurotrophic factor may mediate reduction in drug craving and self-administration (32) but does not explain ibogaine's effect in opioid detoxification.

Ibogaine was administered to human subjects in a clinical Phase I dose escalation study under a physician-initiated Investigational New Drug Application approved by the FDA in 1993 (65). The study was eventually discontinued because of disputes related to contractual and intellectual property issues (66); however, the available safety data indicated no adverse events (65). Most of the available preclinical pharmacological, toxicological, and pharmacokinetic data on ibogaine are derived from research supported by NIDA between 1991 and 1995. NIDA eventually ended its ibogaine project without having initiated a clinical trial apparently because of its high cost and complexity relative to NIDA's existing resources (1). Ibogaine's underlying structure cannot be patented because it is naturally occurring, which limits the financial incentive for its development. Ibogaine continues to be used in unregulated contexts with associated risks because of a lack of clinical and pharmaceutical standards (5).

Deaths have occurred temporally related to the use of ibogaine. This article presents a systematic review of all available autopsy, toxicological, and investigative reports on the consecutive series consisting of all known fatalities temporally related to the use of ibogaine that have occurred outside of West Central Africa from 1990 through 2008.

Materials and Methods

The Institutional Review Board of the New York University School of Medicine and the General Counsel of the New York City Office of Chief Medical Examiner (OCME) approved this research.

Identification of Cases

This series spans the time interval beginning with the first reported fatality in 1990 (1) until December 2008. Eighteen of the 19 fatalities in this series were found through contact with ibogaine treatment providers since the mid-1990s (5,6,67,68). One of these fatalities was also investigated by the OCME (69) as are all unexpected, violent, and suspicious deaths in New York City. One fatality was found by literature search (70). The ethnographic methodology and access to the network of the providers of ibogaine treatment and other participants in the ibogaine subculture are described in detail elsewhere (5,67).

All fatalities were followed up by contact with appropriate medico-legal death investigation agencies to obtain all available autopsy and toxicology reports, inquest testimony, and other investigative reports. In addition to documentary evidence, in most instances, treatment providers and other first-hand observers of the death scene were interviewed. Systematic evaluation of the literature included Medline searches from 1966 to June 2010 utilizing PubMed and ISI Web of Knowledge with the search terms "ibogaine" combined with "death" or "fatality" in addition to searches of periodical and nonindexed "grey" literature as described elsewhere (5,67).

Analytical Toxicology

Various methodologies for toxicological analysis of ibogaine (molecular weight 310.44) have been previously described, including liquid chromatography with flourimetric detection (71), gas chromatography/mass spectrometry (GC/MS) (72–76) liquid chromatography/mass spectrometry (LC-MS) (70,75,77–80), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (81–83). There is a potential for confusion because of the use of two different schemes for numbering the iboga alkaloid parent ibogamine skeleton (84), the *Chemical Abstracts* system, which is common in the biological and medical literature, and the Le Men and Taylor system, which tends to be favored by natural products and synthetic chemists and is also frequently encountered in the biological literature (see Fig. 1).

Ibogaine screening usually is not included in most routine forensic toxicological laboratories and a suspicion of use is required for analysis, which is typically performed by a referral laboratory. For two fatalities in this series (cases #3 and #10 in Table 1), the Forensic Toxicology Laboratory at the OCME performed the analysis. The presence of ibogaine was confirmed by GC/MS and the concentration determined using GC with a nitrogen phosphorus detector (69).

Cause of Death

The certified cause of death is included in Table 1, entitled "Official cause of death." The certified cause of death is that which is indicated by the official documentation, that is, autopsy report or death certificate, by the local authority that investigated and recorded the death. The available documentation varied greatly with regard to investigative rigor, level of detail, and geographic location of the official entity that issued the report. As an approach to controlling for this variance, a coauthor (JRG, a board-certified forensic pathologist) made a determination regarding the cause of each death on the basis of all available data, which in addition to the official documentation, included any information that was provided by treatment providers and other first-hand observers of the death scene, or friends and acquaintances of the decedent. Table 1 provides the conclusions of this systematic, critical evaluation of all available evidence in the far right-hand column entitled "Proximate cause of death."

The cause of death is defined as the original, etiologically specific, underlying medical condition that initiates the lethal sequence

TABLE 1—*Worldwide known fatalities outside of West Central Africa temporally associated with the ingestion of ibogaine, 1990–2008.*

	Age/Gender, Reason for Ibogaine Use	Country	Year	Circumstance	Time Interval from Most Recent Ingestion of Ibogaine Until Death	Ibogaine Form, Dose	Ibogaine (Blood, mg/L or mg/kg)	Other Toxicology (mg/L)	Other Autopsy or Historical Findings	Official Cause of Death	Proximate Cause of Death
1	44 F Psychological/ spiritual (1)	France	1990	Witnessed to become unresponsive during treatment	4 h	Ibogaine HCl 300 mg (*c.* 4.5 mg/kg)	0.24 Liver: 0.17 Kidney: 0.3	Negative	Hypertension; prior left ventricular myocardial infarct, marked 3-vessel coronary artery atherosclerosis, inverted T waves noted on EKG 3 months prior to death	Acute heart failure (autopsy)	Acute ibogaine intoxication. Contributing conditions: atherosclerotic and hypertensive cardiovascular disease
2	24 F Opioid detoxification (6)	Netherlands	1993	Died during ibogaine treatment; gurgling sounds	19 h	Ibogaine HCl 29 mg/kg	Cardiac: 0.74 Femoral vein: 0.75	Morphine: ''trace'' <0.01 Noribogaine: Cardiac: 11.28 Femoral vein: 3.96	Charred tin foil found in room	Undetermined; role of ibogaine unknown due to lack of information relating levels to toxic effects (autopsy)	Acute intoxication due to the combined effects of ibogaine and morphine
3	36 M Opioid detoxification, cocaine dependence (69)	USA	1999	Found dead at home. A syringe found near body	8–9 h	Ibogaine HCl; believed to be 16–20 mg/kg	Subclavian vein: 9.3 Brain: 18.6 Liver: 18.1	Benzoylecgonine: 0.6 Opiates: 0.1 (Morphine: <0.1)	Depression, adverse life events prior to treatment; decedent was aware of dangers of use of cocaine or heroin concurrently with ibogaine	Acute intoxication due to the combined effects of opiates, cocaine, and ibogaine (autopsy)	Acute intoxication due to the combined effects of opiates, cocaine, and ibogaine
4	40 M Opioid detoxification	United Kingdom	2000	Died in bathroom; vomited immediately prior to death	40 h	*Tabernanthe iboga* alkaloid extract 6 g administered over *c.* 6 h	0.36	Other toxicology: negative Noribogaine: detected Ibogamine: detected	Hepatitis C with liver fibrosis, pulmonary and cerebral edema	Fatal reaction to *Tabernanthe iboga* preparation. Contributing condition: Hepatitis C (autopsy)	Acute ibogaine intoxication
5.	35 F Psychological/ spiritual	Germany	2002	Found dead in bed (complained of not feeling well the day before)	1.5 h	Ibogaine HCl 500 mg (*c.* 8 mg/kg)	Unknown	Unknown	Childhood heart surgery congenital, moderate coronary artery atherosclerosis	Heart failure/ intoxication (autopsy)	Acute ibogaine intoxication (unknown if other drugs involved). Contributing conditions: atherosclerotic cardiovascular disease

Continued.

TABLE 1—*Continued.*

	Age/Gender, Reason for Ibogaine Use	Country	Year	Circumstance	Time Interval from Most Recent Ingestion of Ibogaine Until Death	Ibogaine Form, Dose	Ibogaine (Blood, mg/L or mg/kg)	Other Toxicology (mg/L)	Other Autopsy or Historical Findings	Official Cause of Death	Proximate Cause of Death
6.	32 M Opioid detoxification (self-administered by opiate abuser)	USA	2003	Found dead in bed at his residence	Unknown	Bag of brown powder at scene that tested positive for ibogaine (alkaloid extract vs powdered dried root bark)	Cardiac: 0.95 Femoral vein: 1.5 Liver: 8.0 Urine: 26 Vitreous: 0.54 Gastric: 2.9 Bile: 0.54	Benzoylecgonine 0.1 Methadone: <0.1 Nordiazepam: <0.1	Moderate coronary artery atherosclerotic stenosis. History of opiate abuse, and had been in methadone maintenance treatment at time of death	Ibogaine intoxication. Contributing conditions: atherosclerotic cardiovascular disease, cocaine use (autopsy)	Acute ibogaine intoxication. Contributing conditions: atherosclerotic cardiovascular disease, chronic cocaine abuse
7	54 M Opioid detoxification, alcohol dependence	Mexico	2003	Died at ibogaine treatment facility	60 h	Ibogaine HCl 13 mg/kg	Unknown	Unknown	Obesity, chronic alcoholism, smoker (unclear if autopsy was performed; report unavailable)	Pulmonary thromboembolism (death certificate)	Insufficient information
8	45 M Opioid detoxification, alcohol dependence	Mexico	2004	Died at ibogaine treatment facility	20 h	Ibogaine HCl 15 mg/kg	Unknown	Unknown	Chronic alcoholism, obesity, cardiac pacemaker	Acute hemorrhagic pancreatitis. Contributing conditions: Chronic alcoholism, obesity, opiate pain medication dependency (autopsy)	Acute hemorrhagic pancreatitis (during ibogaine treatment) complicating chronic alcoholism
9	48 F Opioid detoxification	Mexico (autopsied in the US)	2005	Died at ibogaine treatment facility	2 days	Ibogaine HCl 14 mg/kg	0.82 Liver: 0.72	Diazepam: 0.06 Oxazepam: 0.39 Temazepam (trace)	Prior gastric bypass surgery with 135 lb weight loss in 8 months preceding death. Fibromyalgia, benzodiazepine dependence that was not disclosed to treatment providers	Sudden cardiac death due to acute myocardial infarct due to acute coronary syndrome. Contributing conditions: Fibromyalgia, chronic pain medication dependency (autopsy)	Acute myocardial infarct due to coronary artery atherosclerosis during ibogaine therapy for opiate dependence complicating chronic fibromyalgia

Continued.

TABLE 1—*Continued.*

	Age/Gender, Reason for Ibogaine Use	Country	Year	Circumstance	Time Interval from Most Recent Ingestion of Ibogaine Until Death	Ibogaine Form, Dose	Ibogaine (Blood, mg/L or mg/kg)	Other Toxicology (mg/L)	Other Autopsy or Historical Findings	Official Cause of Death	Proximate Cause of Death
10	43 M Opioid detoxification, alcohol dependence	USA	2005	Witnessed cardiac arrest during self-administered ibogaine treatment. Witnessed apparent generalized tonic-clonic seizure 17 h after ibogaine ingestion	27 h	Ibogaine HCl, dose unknown	2.8	Diazepam: 0.03 Trimetho-benzamide: 0.85 Benzoylecgonine: detected Ibogamine, ibogaline: detected	Dilated cardiomyopathy, coronary artery atherosclerosis, pulmonary edema Hepatitis B	Valvular heart disease. Contributing conditions: Dilated cardiomyopathy (autopsy)	Acute ibogaine intoxication. Contributing conditions: Mitral insufficiency with dilated cardiomyopathy
11	51 M Opioid detoxification, methamphetamine and alcohol dependence	Mexico	2005	Died at ibogaine treatment facility	24 h	Ibogaine HCl 12 mg/kg	Unknown	Unknown	Autopsy not performed	Cardiorespiratory arrest due to acute myocardial infarction (death certificate, clinical diagnosis of attending physician)	Insufficient information
12	38 M Opioid detoxification	Mexico	2006	Died at ibogaine treatment facility. Found dead within 1 h of having last been seen alive	12 h	Ibogaine HCl 13 mg/kg	Unknown	Cocaine and morphine metabolites	Cutaneous abscesses, hepatitis. Autopsy was done, but inadequate for determination of a proximate cause of death	Pulmonary thromboembolism (death certificate)	Insufficient information
13	48 M Unknown (70)	France	2006	Ingested root bark of *Tabernanthe iboga* followed by vomiting and dyspnea	53 h	18 ''soup-spoons'' of a mixture of powdered *Tabernanthe iboga* root bark and sweetened condensed milk over 10 h	Vena cava: 6.6 Femoral vein: 5.4 Brain: 12.5 Liver: 40.5	Other toxicology: negative Noribogaine: Vena cava: 15.5 Femoral vein: 5.6 Brain: 18.7 Liver: 50.5 Ibogamine: detected	Pulmonary edema. Buprenorphine tablets and ''different objects and burned-out parts of plants found at the death scene suggested that some sort of esoteric ritual may have taken place.'' History of substance abuse	Acute ibogaine intoxication (autopsy)	Acute ibogaine intoxication (unknown if other drugs involved)

Continued.

TABLE 1—*Continued.*

	Age/Gender, Reason for Ibogaine Use	Country	Year	Circumstance	Time Interval from Most Recent Ingestion of Ibogaine Until Death	Ibogaine Form, Dose	Ibogaine (Blood, mg/L or mg/kg)	Other Toxicology (mg/L)	Other Autopsy or Historical Findings	Official Cause of Death	Proximate Cause of Death
14	28 M Opioid detoxification	The Netherlands	2006	Fluctuating level of consciousness following immersion in a warm bath for a 4-h period prior to death. Subject was observed and at no time was his head underwater, ruling out drowning	76 h	*Tabernanthe iboga* alkaloid extract, 7.5 grams over *c.* 18 h	Unknown	Quantitative toxicology results not available but ibogaine and cannabinoid concentrations reportedly "low." Negative for other drugs of abuse and ethanol	Choroid plexus papilloma involving hippocampus with hypoxic damage to hippocampus. Large duodenal ulcer with accumulation of blood in duodenum	Not conclusive regarding proximal cause of death. Possible causal and/or contributing factors were hemorrhagic complications of duodenal ulcer, increased intracranial pressure resulting from obstruction of third ventricle, and/or partial seizures originating from the temporal lobe "Toxicological cause not likely" (autopsy)	Hemorrhagic complications of duodenal ulcer
15	30 M Opioid detoxification	South Africa	2006	"Gurgling sounds" on expiration. Died en route to hospital after appearing to respond to resuscitative efforts	8 h	Ibogaine HCl 17 mg/kg (1.75 g) Single dose	Not tested	Not tested	Autopsy not performed	"Cardio-respiratory collapse secondary to drug related illness" (death certificate)	Insufficient information
16	27 M Unknown	France	2006	Discovered dead in meditation room at a center oriented toward psychological/spiritual use	≤20 h	Powdered root bark (7.2% ibogaine, 0.6% ibogamine). The actual amount ingested was not provided in the report. The medical examiner estimated 13 teaspoons at 1.5-g dried bark/teaspoon would have been required to achieve the measured ibogaine blood concentration	Peripheral blood immediately following death: 0.65 Peripheral blood at autopsy 8 days following death: 1.27	Peripheral blood immediately following death: Methadone: 0.077 Diazepam: 0.413 Oxazepam: 0.09 Temazepam: 0.04 Ibogamine: 0.05 Peripheral blood at autopsy 8 days following death: Ibogamine: 0.10	History of dependence on multiple substances including crack cocaine, benzodiazepines, and alcohol	Drug overdose due to ibogaine, methadone, diazepam, and temazepam (autopsy)	Acute intoxication due to the combined effects of ibogaine, methadone, and diazepam

Continued.

TABLE 1—*Continued.*

	Age/Gender, Reason for Ibogaine Use	Country	Year	Circumstance	Time Interval from Most Recent Ingestion of Ibogaine Until Death	Ibogaine Form, Dose	Ibogaine (Blood, mg/L or mg/kg)	Other Toxicology (mg/L)	Other Autopsy or Historical Findings	Official Cause of Death	Proximate Cause of Death
17	45 M Opioid detoxification	USA	2006	Found dead in bed following ibogaine treatment at a private residence	8–12 h	Ibogaine HCl 22 mg/kg	1.4	Diazepam: 77 ng/ml Fentanyl: 1.2 ng/ml Norfentanyl: 1.5 ng/ml Qualitative urine screen detected Oxycodone, Alpha-hydroxyalprazolam, Oxazepam, Temazepam, Ephedrine/ pseudo-ephedrine	Hepatic steatosis	Mixed drug intoxication (autopsy)	Acute intoxication due to the combined effects of ibogaine, fentanyl, and diazepam
18	33 M Opioid detoxification, crack cocaine dependence	Mexico	2007	Died at ibogaine treatment facility	6.5 h	Ibogaine HCl 11 mg/kg	Not tested	Not tested. History of having been caught using crack cocaine in the bathroom during a prior admission to the clinic	Family history of pulmonary thromboembolism in patient's father. Autopsy was done, but inadequate for determination of a proximate cause of death	Pulmonary thromboembolism (death certificate, clinical diagnosis of attending physician present at time of death)	Insufficient information
19	41 M Opioid detoxification, cocaine dependence	Mexico (autopsied in the US)	2007	Died at ibogaine treatment facility. Developed shortness of breath and became unresponsive during ibogaine treatment	6 h	Ibogaine HCl 13 mg/kg (1080 mg)	Not tested	Not tested	Cardiac hypertrophy Triglycerides: 397 mg/dL	Fatal arrhythmia during drug addiction treatment with cardiac hypertrophy (autopsy)	Acute ibogaine intoxication (unknown if other drugs involved). Contributing conditions: Cardiac hypertrophy

of events (85). A competent cause of death includes the proximate (underlying) cause, defined as that which in a natural and continuous sequence, unbroken by any efficient intervening cause, produces the fatality and without which the end result would not have occurred. Contributing conditions were additional disorders contributory to death but unrelated to the underlying cause of death.

The conclusion that death was caused by an acute intoxication requires that three conditions be met: the toxicological results are within the range typically encountered in such fatalities, the history and circumstances are consistent with a fatal intoxication, and the autopsy fails to disclose a disease or physical injury that has an extent or severity inconsistent with continued life (86). In deaths caused by drug intoxication with more than one drug in concentrations greater than trace amounts, it is customary to include all of the identified drugs in the cause of death.

Results

We report a summary of 19 ibogaine-associated deaths that have occurred worldwide between 1990 and 2008 including the probable causes of death based on the available clinical and pathologic information (see Table 1). There were 15 men and four women with a mean age of 39.1 ± 8.6 years ranging from 24 to 54 years. In 18 decedents, the estimated time intervals were available from the most recent ingestion of ibogaine in any form until death, and the mean interval was 24.6 ± 21.8 h and ranged from 1.5 to 76 h. In one other fatality (case #6) the time interval between death and the time when the decedent was last noted to be alive was 20 h, the decedent had been dead for at least several hours at the time the body was found. The time interval from the most recent ingestion of ibogaine until death in this instance was likely less than 76 h, but it was not included in the calculation of the mean interval.

Fifteen individuals took ibogaine for the indication of opioid detoxification, four of who were also dependent on alcohol, three on cocaine, and one on methamphetamine. Two individuals used it for a spiritual/psychological purpose and had no known substance abuse history, and two took it for unknown reasons but had a history of substance abuse. Ibogaine was given as the HCl form in 14 instances, as an alkaloid extract in two (cases #4 and #14), dried root bark in two (cases #13 and #16), and a brown powder that was probably either root bark or alkaloid extract in another (case #6). In the 12 fatalities where ibogaine was given as the HCl and a dose was reported, the mean dose was 14.3 ± 6.1 mg/kg (range 4.5–29 mg/kg). In the 10 fatalities in which ibogaine blood concentrations were determined, the mean was 2.38 ± 3.08 mg/L (range 0.24–9.3 mg/L), obtained at a mean of 25.5 ± 17.8 h following the ingestion of ibogaine (range 4–53 h). In addition, commonly abused drugs (including benzodiazepines, cocaine, opiates, and methadone) were detected in eight of 11 decedents on whom toxicological analysis for abused substances was performed.

Twelve of the decedents had medical comorbidities including liver disease, peptic ulcer disease, brain neoplasm, hypertensive and atherosclerotic cardiovascular disease, and obesity. Among the three decedents in which no other drugs of abuse were detected in postmortem toxicology analysis, one had advanced heart disease and another had liver fibrosis. Full toxicology and autopsy results were not available in eight and five decedents, respectively.

Discussion

In this series, 19 deaths occurred between 1990 and 2008, with an interval of 76 h or fewer between the most recent ingestion of ibogaine and death. In 14 instances, an autopsy was performed that allowed the determination of the proximate cause of death. The lack of clinical and pharmaceutical controls in settings in which ibogaine has been given, and the limited data regarding toxic concentrations of ibogaine in humans make the determination of the causes of these deaths difficult. Nonetheless, advanced comorbidities and contributing conditions appear to include preexisting medical, particularly cardiovascular disease, and drug use around the time of treatment.

This series of fatalities is consecutive in the sense that it represents a systematic application of an intensive methodology for identifying cases over the time interval spanned by this study. It is possible that additional fatalities may have occurred which were missed by death investigation agencies and this study. In the United States, this could relate to the surreptitiousness regarding the use of ibogaine because of its status as a schedule I substance, and individuals aware that ibogaine was used in temporal association with a fatal outcome might be reluctant to disclose that history. Without investigative information about the recent use of ibogaine, specialized analysis for ibogaine may not be performed. Under these circumstances, the cause of death of an individual treated with ibogaine for a substance use indication could be certified as a typical multidrug intoxication, particularly in view of the likelihood of detecting other drugs of abuse in these deaths. In most of the world, however, ibogaine is not illegal. In this series, outside of the United States, ibogaine was not illegal at the time of occurrence of the fatality in any country in which the fatality occurred.

In at least five instances, providers contacted the first author immediately regarding the death, and in a number of others, another individual close to the provider relayed the information, usually with the provider's consent. Their motivation to disclose this information included the wish to understand the causality of the death and prevent a future occurrence, abreaction regarding a traumatic event, and anxiety regarding legal liability. In a country in which ibogaine is not illegal, however, concealing its use is not necessarily perceived to be, or actually safer than disclosing it. Regardless of their distress regarding a death, experienced treatment providers such as those in Mexico or the Netherlands were aware that they did not face significant legal consequences. In a prior study by the first author of this article that surveyed the settings and extent of ibogaine use (5), it was estimated that 20–30% of the actual total number of ibogaine treatments had been missed by that study. Six of the series of 19 fatalities in this article occurred in settings and circumstances that are likely to have otherwise been hidden from the medical ethnographic study mentioned previously (5). While it is likely that some deaths temporally related to the use of ibogaine escaped inclusion in this series, it is also possible that treatments that are associated with a fatal outcome may come to attention relatively more frequently than those that are not.

For the purpose of this discussion, the terms "proximate cause" and "contributing condition" are used as they are defined previously in the methods section and appear in the extreme right-hand column of Table 1. A striking factor in this series of deaths is the identification of a comorbidity or intoxication (in addition to ibogaine) that could adequately explain or contribute to the death in 12 of 14 decedents that have adequate postmortem data. There are multiple possible pathways by which ibogaine may cause or contribute to death in these instances and include toxicological interactions with substances of abuse and direct cardiac effects.

Cardiac disease was a contributing condition or proximate cause in six deaths, suggesting cardiac mechanisms are an important mediator of fatal outcomes. Although preclinical toxicological testing by NIDA did not indicate prolongation of the QT interval (87),

it has been observed during ibogaine treatments with continuous EKG monitoring (88). Blockade of the potassium voltage-gated ion channel encoded by the human ether-a-go-go-related gene (hERG) is regarded as the most common cause of drug-related QT prolongation (89,90), which is associated with torsades de pointes (TdP), a morphologically distinctive polymorphic ventricular tachycardia. The effect of ibogaine differs from that of the hERG channel antagonist WAY-123.398 in studies of chromaffin cells (91–93); however, ibogaine is an hERG channel antagonist in the low micromolar range in human embryonic kidney tsA-201 cells (94). Ibogaine has low micromolar affinity for sodium channels (2,95,96), which might also possibly relate to cardiac risk in view of the possible association of sodium channel blockade with slowing of intraventricular conduction and the subsequent development of a re-entrant circuit resulting in ventricular tachyarrhythmia (89,97), and there is evidence for altered sodium channel functioning in some drug-induced forms of long QT syndrome (98–101).

QT prolongation is also regarded as a general correlate of cardiac instability that is associated with arrhythmias other than TdP (89,102,103), and with multiple risk factors relevant to the present study including bradycardia, coronary artery disease, dilated cardiomyopathy, recent myocardial infarction, ventricular hypertrophy, and liver disease (89,104). Bradycardia has been reported in humans in association with the ingestion of ibogaine in medical (88,105) and nonmedical (106) settings, and in some preclinical studies (33,36,107,108). The frequently altered nutritional status of substance abusers puts them at risk of hypomagnesemia and hypokalemia (90), which are associated with QT prolongation, as are bulimia and anorexia (109). Methadone is associated with QT prolongation, particularly in the presence of other drugs (110). Alcohol or cocaine use is associated with prolongation of the QT interval both acutely (111,112) and during withdrawal (113–115). In patients with alcohol dependence, QT prolongation has been observed to persist for 7 days after the last intake of alcohol (116), and withdrawal seizures contribute further independent and additive risk (114). Epileptic seizures, even in the absence of substance use or withdrawal, are an independent risk factor for QT prolongation (117).

A case report of QT prolongation and ventricular arrhythmia in association with the ingestion of *T. iboga* alkaloid extract (118) illustrates the variety of potential arrhythmogenic factors in the clinically uncontrolled settings in which ibogaine has been used. The patient survived in that case, which is not included in this present series. The patient had taken "Indra," an apocryphal brand of alkaloid extract that subsumes multiple sources of diverse origin, composition, and conditions of storage (67). Multiple confounding risk factors for QT prolongation and ventricular arrhythmia were present. The patient had presented with a witnessed generalized tonic-clonic seizure (GTCS) in the setting of acute alcohol withdrawal with hypomagnesemia and hypokalemia. Although the report made no mention of toxicological testing for illicit drugs, the patient had a prior history of cocaine abuse and a history of bulimia and had been purging prior to admission.

Bradycardia is a functional effect of potential medical significance that could possibly involve muscarinic cholinergic transmission. Ibogaine binds with reported affinities in the 10–30 μM range to M1 and M2 muscarinic cholinergic receptors and is generally assumed to act as an agonist (1,2); however, functional studies have not been performed. Although ibogaine is concentrated in brain tissue relative to serum in the animal model (119) and in the two cases reported here that reported on brain levels (cases #3 and #13), an older literature (120,121), as well as more recent data (122), indicates that the inhibition of acetylcholinesterase by ibogaine *in vitro* is negligible over the range of ibogaine concentrations observed in both blood and brain in this series. It is unclear whether the apparent association of ibogaine with bradycardia could possibly be related to orthosteric agonist actions at muscarinic cholinergic receptors, or to effects involving sodium channels (123) or other signal transduction pathways.

Pulmonary thromboembolism (PE) was the reported cause of death in three deaths (cases #7, #12, and #18) all of which occurred in Mexico. Two were not under direct observation at the time of the death. In all three of these cases, autopsy reports were inadequate as a basis for the determination of a proximate cause of death due the lack of evidence of systematic examination of the lungs and pulmonary vasculature. In Mexico, the death certificate provides the clinical conclusion reached by the physician who pronounced the death. In case #18, the attending physician patient observed the patient directly and based the clinical diagnostic impression of PE on acute dyspnea, tachypnea, and desaturation indicated by pulse oximetry. Although an adequate autopsy is lacking, the clinical picture mentioned previously is frequently seen with PE (124), and in instances where there is verification by a subsequent autopsy, the prospective clinical diagnosis of PE is less commonly falsely positive than falsely negative (125). The decedent had a family history of PE, and if he did indeed die from venous thrombotic disease, the family history suggests a possible etiological contribution because of genetic risk (126). Other possible risk factors for PE include travel to the treatment location (127) and/or inactivity and immobility during the treatment (128). Intravenous drug use is a risk factor for deep venous thrombosis (129–131), and hence for PE, and appears to be associated with injection per se, independent of the use of opioids versus other substances (132).

In this series, there appeared to be no clinical or postmortem evidence suggestive of a characteristic syndrome of neurotoxicity. Ibogaine's σ_2 agonist activity potentiates excitatory transmission in the olivocerebellar projection, where the redundancy of inputs to cerebellar Purkinje cells renders them vulnerable to excitotoxic injury (133,134). This is believed to be the mechanism of degeneration of cerebellar Purkinje cells observed in rats given substantially larger dosages of ibogaine than those used to study drug self-administration and withdrawal (135). Subsequent research found no evidence of neurotoxicity in the primate (65) or mouse (136) at dosages that produced cerebellar degeneration in the rat, or in the rat at dosages used in studies of drug self-administration and withdrawal (137). Neuropathological examination revealed no evidence of degenerative changes in a woman who had received four separate doses of ibogaine ranging between 10 and 30 mg/kg over a 15-month interval prior to her death due to a mesenteric artery thrombosis with small bowel infarction 25 days after her last ingestion of ibogaine (65).

In one fatality in this series, a GTCS occurred (case #10), which might have been due to alcohol or benzodiazepine withdrawal. In another death (case #14), a brain neoplasm might have explained the possibility of complex partial seizures mentioned in the autopsy report. The neurodegeneration observed in the rat following high dosages of ibogaine has mainly involved the cerebellum (134,135), which is an unlikely location for a seizure focus in humans. Seizures originating from the cerebellum in humans appear to be limited to rare instances in which a focus is located in a tumor mass distinct from normal cerebellar tissue, most commonly a gangliogliomа (138). Furthermore, cerebellar stimulation is viewed as a possible antiepileptic treatment (139), and ibogaine has been observed to protect against convulsions in animal models (140–142), which has been attributed to NMDA antagonist activity. Ibogaine causes

serotonin release in selected brain regions in the animal model (49), and seizures are sometimes seen in serotonin syndrome (143), but characteristic features of serotonin syndrome such as hyperthermia or rigidity were not present and a clinical picture suggestive of serotonin syndrome does not appear to have been evident in this series.

The apparent potentiation of both the analgesic (22,38,39,43–47) and toxic (33,37–39) effects of opioids by ibogaine may be mediated by enhanced transduction of signaling via opioid receptors (40), which might have been a factor in deaths involving the use of opioids in temporal proximity to the ingestion of ibogaine. In one fatality (case #2), it appeared that the decedent smoked heroin following ibogaine treatment and shortly before death (6). Toxicological analysis detected a low morphine concentration that nonetheless was in the range measured in human subjects within 30 min after inhalation of volatilized heroin (144), similar to the method of smoking heroin by heating tin foil known as ''chasing the dragon'' (145), and suggests possible potentiation of opioid toxicity by ibogaine in this death. Ibogaine increases cocaine-induced stereotypic motor behavior in the animal model (146), suggesting that ibogaine might also potentiate the toxicity of stimulants as well as opioids.

Postmortem toxicological analysis detected commonly abused drugs in eight of the 11 cases in which toxicological analysis was performed in this series. When considering a drug intoxication death because of multiple substances, it usually is not possible to differentiate the individual roles and complex interactions of these substances in causing the death. These deaths typically are certified as intoxications because of the combined effects of all substances detected. Therefore, it is not possible to determine whether the deaths in which drugs of abuse were detected were because of ibogaine alone, to one or more of the drugs of abuse, or a combination. There is also a general effect of the number of abused substances, with a larger number associated with a greater risk of death independent of the identity of specific substances involved (147). The unexplained variance of lethal outcome as a function of dose further adds to the difficulty of the determination of causality for ibogaine and drugs of abuse. For example, morphine concentrations associated with heroin overdose overlap substantially with concentrations obtained from living current heroin users (148), which may relate to the wide ranges of tolerance among opioid-dependent individuals, and within the same individual at different time points.

Systemic disease is a confounding factor that contributes to the mortality associated with substance use and further complicates the identification of the cause of death. The risk of death may represent a complex interaction involving a substance of abuse against a backdrop of systemic medical illness related to addiction. For example, the risk of death from opioid overdose is associated with cardiac hypertrophy and atherosclerotic disease (149), which were contributing conditions in this case series and which in turn are associated with a history of methamphetamine and cocaine use (150,151). The role of advanced preexisting medical comorbidities in this series of fatalities appears to be an instance of a more general association between systemic disease and risk of fatal overdose (149).

The reported elimination half-life of ibogaine in humans is on the order of 4–7 h (7,70), and that of noribogaine is apparently longer (7,35). Ibogaine is relatively lipophilic and accumulates preferentially in tissues containing a high density of lipids, such as brain or fat (119). Ibogaine undergoes demethylation to noribogaine via cytochrome P450 2D6 (CYP2D6) (152), which is expressed in the brain (153), where noribogaine may be ''trapped'' because it is more polar than ibogaine and may cross the blood–brain barrier more slowly. Postmortem redistribution of drugs and drug metabolites may occur due to passive drug release from drug reservoirs, cell autolysis, and putrefaction (154,155). In the three instances in which peripheral and cardiac concentrations of ibogaine were reported (cases #2, #6, and #13), the concentrations from the femoral and cardiac or vena cava sites were similar. However, the two that reported noribogaine concentrations (cases #2 and #13) demonstrated evidence for postmortem redistribution of noribogaine with ratios of *c*. 3:1 between cardiac and peripheral blood. The one instance that reported ibogaine concentrations at two time points (case #16) indicated 0.65 mg/L in blood at autopsy and 1.27 mg/L days following death.

The available data do not provide a basis for a reliable estimate of toxic concentrations of ibogaine. In humans administered fixed oral doses of ibogaine of 10 mg/kg, mean peak blood levels were 0.74 ± 0.08 and 0.90 ± 0.17 mg/L in extensive and poor CYP2D6 metabolizers, respectively (7). In series of cases reported here, the mean dosage was 14.3 ± 6.1 mg/kg (range 4.5–29 mg/kg), and the mean blood level was 2.38 ± 3.08 mg/L. The presence of cointoxicants and comorbidities, difference in dosages used, and the higher variance in dosages and blood levels in the present series does not provide for a meaningful comparison regarding a lethal dosage or level in humans.

In the rat, the animal model that is predominantly used in research on ibogaine, the dose that is usually used in models of drug self-administration and opioid withdrawal is 40 mg/kg administered intraperitoneally (i.p.) (1,2). This dose is approximately one-third of the LD_{50} of ibogaine administered i.p. (33), which in turn is approximately one-half to one-third of the LD_{50} by the intragastric route of administration (33,156). The animal data indicate a significant effect of abused substances on toxicity associated with ibogaine (33,37–39), and taken together with the clinical evidence for the effect systemic disease on fatal overdose (149) suggests that interactions involving cointoxicants and medical comorbidities preclude a reasonable estimate regarding a lethal dosage or level of ibogaine in humans.

Cointoxicants or contributing medical comorbidities were not reported in only two fatalities for which there were an adequate postmortem examination and toxicological analysis (cases #4 and #13). These two deaths involved the ingestion of crude alkaloid extract in one case, and root bark in the other. The overall composition, age, and origin of these sources of ibogaine are unknown. The iboga alkaloid content of *T. iboga* root bark extracts depends, among other factors, on the extraction method. The total alkaloid content of the root bark is *c*. 2–8% of the dry weight of the root bark, about half of which is iboga alkaloids, 80% of which is ibogaine (157,158). Utilization of water-soluble extractants yields an extract with an alkaloid fraction composed of *c*. 40% ibogaine, 10% related iboga alkaloids, and 50% other alkaloids, whereas utilization of an organic solvent such as acetone or methanol yields a total alkaloid fraction with relatively less non-iboga alkaloid content (157). Other iboga alkaloids that co-occur with ibogaine in *T. iboga* root bark include ibogamine, ibogaine, tabernanthine, and voacangine (157–159) (see Fig. 1). The overall iboga alkaloid composition of *T. iboga* alkaloid extracts may range from *c*. 15% to 50% (157) (C. Jenks, personal communication). Sources of ibogaine HCl are restricted and tend to be known to providers, and certificates of analysis have generally been available and corroborated when verified by independent laboratories, which up to the present time has distinguished ibogaine from the counterfeiting and adulteration seen with commonly abused ''street'' drugs (160).

Inexperience and lack of information regarding the use of ethnopharmacological forms of ibogaine may itself constitute a salient domain of risk, independent of the uncertain composition of alkaloid extracts and the undefined potential toxicity of the alkaloids that co-occur with ibogaine in *T. iboga* root bark. For example, one decedent (case #13) (70) may have ingested an amount of dried *T. iboga* root bark in excess of that which would typically be given in a full Bwiti initiation ceremony (5). The blood ibogaine concentration in this case was the second highest in the series, even though it was measured an estimated 53 h after ingestion, and does not take into account the likely presence of other alkaloids. This case additionally suggests that the bioavailability of the alkaloid content of dried root bark may be high.

The incidence of fatalities may have decreased in the recent past. As indicated in Table 1, in 2008, there were no known fatalities, and in 2007, there were 2. In contrast, there were a total of nine fatalities that occurred in 2005 and 2006. It is unlikely that this reflects a decline in the number of individuals treated, which appears to be continuing the trend of growth evident over the last decade (5). Greater recognition of medical risk on the part of treatment providers may have been a factor in the apparent reduction in the incidence of fatalities. Pretreatment screening including basic blood chemistries and EKG, the exclusion of patients with significant medical, particularly cardiac illness, and the recognition of the need to stabilize physical dependence on alcohol and benzodiazepines prior to ibogaine treatment has gradually become more widely accepted norms in the settings of ibogaine use (161). This might to a significant extent reflect the collective, cumulative experience of the fatal outcomes presented here.

In conclusion, in this series of 19 cases, advanced preexisting medical comorbidities, which were mainly cardiovascular, and/or one or more commonly abused substances explained or contributed to the death in 12 of the 14 cases for which adequate postmortem data were available. Significant factors in this series appear to include preexisting medical, particularly cardiovascular disease, possible PE, drug use during treatment, seizures associated with withdrawal from alcohol and benzodiazepines, and the uninformed use of ethnopharmacological forms of ibogaine.

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Treatment of Acute Opioid Withdrawal with Ibogaine

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Ibogaine is an alkaloid with putative effect in acute opioid withdrawal. Thirty-three cases of treatments for the indication of opioid detoxification performed in non-medical settings under open label conditions are summarized involving an average daily use of heroin of .64 ± .50 grams, primarily by the intravenous route. Resolution of the signs of opioid withdrawal without further drug seeking behavior was observed within 24 hours in 25 patients and was sustained throughout the 72-hour period of posttreatment observation. Other outcomes included drug seeking behavior without withdrawal signs (4 patients), drug abstinence with attenuated withdrawal signs (2 patients), drug seeking behavior with continued withdrawal signs (1 patient), and one fatality possibly involving surreptitious heroin use. The reported effectiveness of ibogaine in this series suggests the need for systematic investigation in a conventional clinical research setting. (Am J Addict 1999;8:234–242)

Detoxification is a necessary step in the treatment of opioid dependence. Ibogaine, an indole alkaloid found in the bark of the root of the African shrub *Tabernanthe iboga,* is alleged to have anti-addictive qualities, including efficacy in acute opioid withdrawal.[1] The Medications Development Division of the National Institute on Drug Abuse (MDD-NIDA) has given serious consideration to a clinical trial of ibogaine.[2] Currently, research on humans receiving ibogaine conducted in a conventional U.S. research setting has been limited to the administration of low subtherapeutic dosages in initial phase I dose escalation studies under the US Food and Drug Administration (FDA).[3] In contrast to the limited clinical experience with ibogaine in conventional U.S. research settings, an unofficial network has been providing treatment with ibogaine for over 30 years. Most of the clinical observations on ibogaine treatment of

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drug dependence in humans have been provided by this treatment network, which exists as a consequence of demand by addicts regardless of ibogaine's legal status in the U.S. While the existing informal treatment context is not optimally suited to conventionally rigorous clinical research, it appears warranted to direct some attention toward reports of efficacy of ibogaine for opioid withdrawal.

Evidence for ibogaine's effectiveness includes observations of reductions in morphine and cocaine self administration in animals and anecdotal reports in humans.[1] The reported efficacy of ibogaine in multiple substance dependence syndromes raises the possibility of implementing a pharmacologic strategy suggested by Leshner[4] of targeting "common effects that may underlie some common properties of all addictions."[4] If ibogaine is indeed effective, it is of great potential interest as representing a novel pharmacologic approach to treating addiction. Ibogaine does not appear to be a conventional dopamine or opioid agonist or antagonist or an amine re-uptake inhibitor.[1,5,6] Ibogaine has significant affinities for multiple binding sites within the central nervous system, including N-methyl-D-aspartate (NMDA), kappa opioid, and sigma and nicotinic receptors.[6] Ibogaine's mechanism of action is not known; however, interest has been focussed on NMDA antagonism as one possible mechanism of particular relevance to its putative effect on opioid withdrawal.[5–10] Because ibogaine apparently does not exert its effects by mechanisms of drugs currently in use for the treatment of drug dependence syndromes, it represents a potentially new strategic approach to understanding the neurobiology of addiction and the development of new treatments.

In this report, we present observations on patients treated with ibogaine for opioid detoxification over the time interval of 3 days after their last use of opiates. A specific focus on opioid withdrawal, in evaluating clinical reports from the existing informal ibogaine treatment network, is suggested for several reasons. One reason is that opioid dependence is the major reported indication for which addicts have sought out ibogaine treatment. Another reason to specifically focus on acute opioid withdrawal is to minimize the methodologic limitations of the informal treatment context by choosing to study a clinically robust phenomenon occurring within a relatively limited time frame. With respect to data that is currently available, the basic question of efficacy of ibogaine can possibly be most effectively addressed by studying opioid withdrawal due to the clinically obvious and unambiguous nature of the acute withdrawal syndrome. The distinctive, well recognized syndrome of opioid withdrawal contrasts, for example, with the lesser consensus regarding the clinical syndrome of cocaine withdrawal.[11,12] The current state of ibogaine research is such that the basic question of any human efficacy must be addressed, and opioid withdrawal provides a clearer and more readily available set of outcome measures than other drug withdrawal syndromes, such as cocaine or nicotine.

The support for efficacy of ibogaine in opioid withdrawal consists of animal studies and anecdotal reports in humans. In rats, ibogaine has been observed to attenuate the signs of morphine withdrawal[13–15] and to reduce heroin or morphine self administration.[16–18] Similar effects on morphine withdrawal have been reported in monkeys[19] and mice.[5] There are some case studies in humans in the literature[20–23] that describe ibogaine treatment in an aggregate total of 13 patients, as well as recent preliminary reports from a private clinic in the Caribbean.[3,24] Common features of these reports are reductions in drug craving and opiate withdrawal signs and symptoms within 1 to 2 hours and relatively complete resolution of the opioid withdrawal syndrome within 24 to 48 hours af-

ter the ingestion of ibogaine. These case studies appear consistent with general descriptions of ibogaine treatment.[25–27]

Patients treated with ibogaine describe the persistent elimination of withdrawal symptoms and craving beginning within hours of initiating treatment. Within 1 to 3 hours of ingestion, ibogaine produces its most intense subjective effects during a state lasting approximately 4 to 8 hours. The acute phase is characterized by the panoramic recall of a large amount of material relating to prior life events from long-term memory, primarily in the visual modality. Hallucinations have also been described but do not appear to be as prominent an aspect of the experience as the volume of images recalled from visual long-term memory. Following the acute phase is a state lasting approximately 8 to 20 hours in which the density of recall of visual images is greatly reduced and attention is directed toward evaluating the material recalled in the acute phase. The emotional tone of this second state appears to be generally characterized as neutral and reflective. Insomnia is often evident for 72 hours following administration of ibogaine to both opioid and non-opioid dependent patients, and it is responsive to sleep medication.[26] Patients have reported significant reductions or total cessation of substance use and craving for weeks to months or longer following treatment, although methodologically adequate follow-up observations are lacking. The purpose of this work is to systematically present a series of case reports of the possible efficacy of ibogaine in acute opioid withdrawal.

METHODS

The cases presented in this paper are a subset of 41 cases of patients treated with ibogaine between 1962 and 1993 that were presented at the Ibogaine Review Meeting held by MDD-NIDA in Rockville, MD, on March 8, 1995. Thirty-three of these cases were selected according to the following criteria:

(1) Heroin dependence, with or without other comorbid substance use disorders, as an indication for treatment with ibogaine (all 8 of the subjects who were receiving methadone at the time of their treatment also reported concurrent use of heroin). All patients in this study retrospectively met the DSM IV criteria[28] for Opioid Dependence with Physiological Dependence at the time of their treatment.

(2) Having been directly observed by either or both co-authors H.S.L. and/or G.M.N.F., continuously at the scene for at least 48 hours following treatment with ibogaine.

Eight of all 41 cases presented at the NIDA Ibogaine Review Meeting were excluded from this series. Five of those patients were not opioid dependent, and post treatment observation was lacking on 3 patients.

The demographic and drug use characteristics of the patients are summarized in Table 1. Treatments were provided in the setting of a hotel room or apartment under an open label condition with H.S.L. and/or G.M.N.F. continuously present on site to observe the patients for at least the first 48 hours following ibogaine administration. Observers well known to the above co-authors were additionally present when the co-authors slept and immediately notified the co-authors of withdrawal signs or symptoms or drug seeking behavior.

Patient behaviors between 48 and 72 hours were monitored by H.S.L., G.M.N.F., and/or their observers. In 1962 and 1963, a total of 7 treatments were carried out in the U.S., with the remaining 26 treatments taking place in the Netherlands between 1989 and 1993. Twenty-three of these treatments were observed by H.S.L., 9 by G.M.N.F., and 1 by both. Eighteen of the 33 patients in this study were under the care of Jan Bastiaans, M.D., Professor

TABLE 1. **Demographic and drug use characteristics of study sample**

Gender	22 (67%) male, 11 (33%) female
Mean Age	27.3 ± 4.7 years
Ethnicity	32 Caucasian, 1 Surinamese
Mean daily heroin use	.64 ± .50 grams/day
Predominant route of heroin self administration	26 intravenous, 4 intranasal, 3 smoking
Mean duration of heroin use	6.2 ± 5.8 years
Number of subjects with concurrent methadone maintenance	8 (24%)
Mean methadone dose (N = 8)	48 ± 30 milligrams
Number of subjects additional seeking treatment for concurrent cocaine use	8 (24%)
Mean daily cocaine use (N = 8)	1.4 ± 2.3 grams

Emeritus and former Chairman of the Department of Psychiatry at the State University of Leiden, whose areas of research emphasis within psychiatry included psychosomatic medicine and the medical uses of hallucinogens.[29,30] Dr. Bastiaans saw the patients before and after their treatments and was typically present for the first 4 to 8 hours, returning 24 hours post ibogaine administration. When present, Dr. Bastiaans provided corroboration regarding the observations made by H.S.L. and G.M.N.F. on the presence or absence of the clinical features of acute opioid withdrawal.

The subjects in this series of cases received an average dose of ibogaine of 19.3 ± 6.9 mg/kg (range of 6 to 29 mg/kg). Patients were instructed to ingest their last food, liquids, heroin, or other substances the night before treatment and received the ibogaine approximately 8 to 10 hours later the following morning. Patients on methadone took their last methadone dosage the morning before the next day's ibogaine treatment, approximately 24 hours prior to receiving ibogaine. During treatments, subjects were instructed to lie down in a dimly lit room whose location and ambient activity were made to be as quiet as possible.

Both H.S.L. and G.M.N.F. kept journals and recorded their observations of patient behaviors during treatment, which included assessment of signs of opioid withdrawal such as midriasis, sweating, elevated pulse rate, shivering, piloerection, or diarrhea. Vomiting is common during ibogaine treatment, but it typically occurs relatively suddenly as a single episode and is usually related to motion. Therefore, a single episode of vomiting was not regarded as an opioid withdrawal sign, although sustained vomiting and nausea persisting more than 2 hours was regarded as a sign of opioid withdrawal. Subjective symptomatic complaints such as chills, muscle pain, abdominal pain and nausea were also noted.

Both co-authors who assessed and recorded the presence or absence of withdrawal signs and symptoms were very experienced and familiar with the clinical syndrome of opioid withdrawal. H.S.L. has had clinical experience with withdrawing and detoxifying addicts over a 30 year period in the context of his involvement with the development of ibogaine as a treatment for acute opioid withdrawal.[31] G.M.N.F. has been involved with the Dutch and US addict self help and harm reduction movements since 1985, and is experienced in ethnographic field work with injection drug users.[32]

RESULTS

The outcomes with respect to opioid withdrawal signs and drug seeking behavior following ibogaine treatment are summarized in Table 2. Twenty-five (76%) of the patients had no signs or subjective complaints at 24 and 48 hours and did not seek to obtain or attempt to use opioids for at least 72 hours after the initial dose of ibogaine. The reported onset of relief of symptoms was rapid—within 1 to 3 hours for these patients, many of whom were already at least mildly symptomatic from having abstained from opioid use overnight prior to the morning of the ibogaine treatment.

An additional patient was noted to have sweating at 24 hours but not at 48 hours post treatment and did not seek, obtain, or attempt to use opioids within 72 hours post treatment. Another patient had chills that were present at 24 hours and 48 hours but nonetheless did not seek to obtain or use opioids for at least 72 hours post treatment. This particular patient was using 1 gram of heroin intravenously daily and received an ibogaine dose of 25 mg/kg. Four patients appeared to achieve resolution of opioid withdrawal, as judged by an absence of signs and subjective symptoms at 24 and 48 hours, but nonetheless returned immediately to opioid use within 72 hours. Two of these subjects, males aged 26 and 20, explicitly acknowledged a continued interest in pursuing a heroin-centered lifestyle despite the apparent elimination of the signs and symptoms of their opioid withdrawal. These two individuals received doses of only 8 mg/kg, and they were each using approximately only 0.1 grams per day of heroin. The two other individuals who relapsed immediately to continued heroin use, despite the apparent resolution of the opioid withdrawal syndrome, were both 27 year old males who were using approximately 0.4 grams and 0.75 grams of heroin a day, and received 23 and 25 mg/kg of ibogaine, respectively.

The only patient with clear objective signs and subjective complaints of opioid withdrawal following ibogaine treatment was a 27 year old female who used an average of 0.4 grams of heroin a day intravenously and received 10 mg/kg of ibogaine. This case is the only one in which ibogaine did not appear to provide significant relief from the opioid withdrawal syndrome, as this patient complained of nausea, chills, muscle aches, and was observed to be sweating with dilated pupils. This patient left the treatment environment and used heroin approximately 8 hours after the administration of ibogaine. The failure of ibogaine in this particular case was felt to be due to a dosage that was inadequate to the patient's level of opioid dependence.

Lastly (and importantly) is the case involving a fatal outcome in a 24-year-old female treated in the Netherlands in 1993.

TABLE 2. **Opioid detoxification with ibogaine: outcomes (N = 33)**

N	Signs of Opioid Withdrawal Post-Treatment	Drug Seeking During the 72 Hour Post-Treatment Interval
25	Fully resolved at 24 hours	−
4	Fully resolved at 24 hours	+
1	Partial resolution at 24 hours (sweating) fully resolved at 48 hours	−
1	Partial resolution at 24 and 48 hours (chills)	−
1	Multiple opioid abstinence signs	+
1	Fatality at 19 hours	?

This patient had a history of intravenous and smoking use of approximately 0.6 grams of heroin per day, and received an ibogaine dose of 29 mg/kg. The patient complained of muscle aches and nausea at 17 hours following the administration of ibogaine, without other evidence of signs of withdrawal. About an hour later, she suffered a respiratory arrest, possibly involving aspiration, and was pronounced dead at about 19 hours post treatment. Forensic pathological examination revealed no definitive conclusion regarding the probable cause of death[33] and cited the lack of information correlating ibogaine concentrations with possible toxic effects in humans. Another problem regarding the interpretability of postmortem levels of ibogaine, or its principal metabolite noribogaine, relates to potential for artifactual elevations of serum levels of drug or metabolites with a large volume of distribution due to postmortem release from tissue.[34–36] An additional source of uncertainty was the possibility of surreptitious opioid use, which was suggested by the finding of charred tin foil among the patient's effects, which is used to smoke heroin by the method of "chasing the dragon" (which is popular in the Dutch heroin scene).[37] There is evidence that suggests that the toxicity of opioids may be relatively greater following treatment with ibogaine.[5,38] Analysis of gastric contents for heroin or morphine, which might have confirmed recent heroin smoking, and analysis of blood for 6-monoacetyl morphine, a heroin metabolite whose presence indicates recent use,[39] were not performed.

DISCUSSION

Within the context of the methodological limitations imposed by the informal treatment setting, this series of open label case studies appears to provide some evidence for the efficacy of ibogaine in acute opioid withdrawal. Seventy-six percent of the patients in this series were reportedly free of opioid withdrawal signs and symptoms at 24 hours and did not seek drugs over the period of observation of 72 hours. Another 12 percent were without evidence of withdrawal but nonetheless chose to resume opioid use. While the lack of formal clinical research methodology such as a structured instrument rating withdrawal is unfortunate, the apparent validity of the findings rests largely on the ability of the two co-authors H.S.L. and G.M.N.F. to reliably recognize the features of the acute opiate withdrawal syndrome. Both of the above co-authors had extensive experience in observing the clinical features of opioid dependence including the acute withdrawal syndrome. The corroboration of Dr. Bastiaans on over half the cases provides some additional support for the accuracy of the above co-author's assessment regarding the presence or absence of acute opioid withdrawal.

The safety concern that has currently been most problematic for the development of ibogaine has been the one fatality in this series, following the administration of ibogaine in a female patient in the Netherlands in 1993. This incident was a significant factor in the decision not to pursue a clinical trial of ibogaine following the NIDA Review Meeting held in March of 1995 {F. Vocci, director, MDD-NIDA, personal communication, 1998}. This incident also underscores the need for the security procedures and medical supervision available in a conventional medical setting and for completion of the FDA dose escalation studies[3] to allow systematic collection of pharmacokinetic and safety data.

Another safety concern regarding potential neurotoxicity was raised by the observation of cerebellar damage in rats treated with ibogaine at a high dose of 100 mg/kg.[40] However, no evidence of toxicity was seen at the dose of 40 mg/kg demonstrated to reduce morphine or cocaine self-

administration in rats.[16,18,41,42] Helsley et al[43] treated rats with 10 mg/kg ibogaine every other day for 60 days and observed no evidence of neurotoxicity. Likewise, Mash et al[3] observed no evidence of neurotoxicity in monkeys treated for 5 days with repeated oral doses of ibogaine of 5 to 25 mg/kg or subcutaneously administered doses of 100 mg/kg. J. W. Olney has described the rationale for the use of ibogaine as an actual neuroprotective agent to minimize excitotoxic damage in stroke and anoxic brain injury.[44] The available evidence appears to suggest that the neurotoxic effects of ibogaine occur at levels higher than those observed to have effects on opioid withdrawal and self administration. In addition, the neurotoxic effects of ibogaine appear to be specifically mediated by activity at the sigma type 2 receptor and to be potentially dissociable from ibogaine's putative antiaddictive effect.[6] An ibogaine congener with relatively less sigma 2 activity, 18-methoxycoronaridine, reportedly produces effects similar to ibogaine on morphine and cocaine administration in rats, but has shown no evidence of neurotoxicity even at high dosages.[6]

The cases and literature reviewed here indicate significant clinical issues that will need to be addressed if ibogaine is to be considered as a clinical option for opioid detoxification. There are safety concerns (as discussed above) that must be addressed by careful investigation in clinical research settings. Ibogaine can presently be purchased at a wholesale price of approximately 200 US dollars per treatment, and that price could drop considerably if significant demand were to stimulate increased production. The time frame of treatment with ibogaine places it competitively within the time frame of rapid detoxification.[45] However, despite ibogaine treatments having taken place under conditions of relatively "low tech" improvisation, there is a question of whether ibogaine in a conventional medical setting, with its attendant evaluation and supervision, would still be economically competitive with other existing approaches to opioid detoxification. The need for supervisory personnel to serve the functions presently served by volunteer participants in the existing informal treatment network must be included in the overall cost of ibogaine treatment in a conventional medical setting. The significant subjective psychoactive state produced by ibogaine might not be widely desired or tolerated. Although anecdotal evidence suggests that ibogaine is well tolerated and that the material recalled in the psychoactive state might have potential psychotherapeutic significance,[21,25,27,46,47] a follow-up study of individuals treated with ibogaine assessing the tolerability of treatment would be useful. One limitation of such a study, however, would be the possible bias of self selection on a sample that has been sufficiently motivated to seek out ibogaine treatment in the existing unconventional network. It also remains to be seen whether a pharmacologic intervention or molecular modification of ibogaine can provide the option of resolving the psychoactive effects of the drug from its putative anti-addictive qualities.

The results reported here originate from an informal treatment context that presents methodologic disadvantages compared to a conventional clinical research setting. Nonetheless, the observations made in this setting do appear to provide some support for the efficacy of ibogaine in the treatment of acute opioid withdrawal. Whether or not ibogaine emerges as a viable conventional treatment option, the question of its pharmacologic effectiveness is interesting. If the results obtained under open label conditions summarized here are confirmed in controlled clinical studies, it would appear that ibogaine represents a novel pharmacologic mechanism that is not currently being utilized in the treatment of drug dependence. If it is in-

deed effective, ibogaine could eventually prove to be a productive paradigm for the study of the neurobiology and development of new approaches to addiction.

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——Chapter 8——

IBOGAINE IN THE TREATMENT OF HEROIN WITHDRAWAL

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I. Introduction

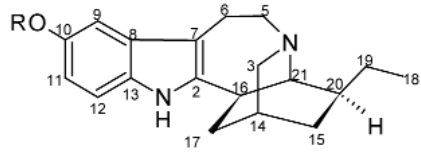
Ibogaine, is a naturally occurring, psychoactive indole alkaloid derived from the roots of the rain forest shrub *Tabernanthe iboga*. Indigenous peoples of Western Africa use ibogaine in low doses to combat fatigue, hunger, and thirst, and in higher doses as a sacrament in religious rituals (*1*). The use of ibogaine for the treatment of drug dependence has been based on anecdotal reports from groups of self-treating addicts that the drug blocked opiate withdrawal and reduced craving for opiates and other illicit drugs for extended time periods (*2-4*). Preclinical studies have supported these claims and provided proof-of-concept in morphine-dependent rats (*5,6*). While ibogaine has diverse CNS effects, the pharmacological targets underlying the physiological and psychological actions of ibogaine in general, or its effects on opiate withdrawal in particular, are not fully understood. Pharmacological treatments for heroin addiction currently employ two treatment strategies: detoxification followed by drug-free abstinence or maintenance treatment with an opioid agonist. Because agonist maintenance with methadone usually has the goal of eventual detoxification to a drug-free state, the use of medications to facilitate this transition is a clinically important treatment strategy. Anecdotal reports suggest that ibogaine has promise as an alternative medication approach for making this transition (*4*). Ibogaine has an added benefit to other detoxification strategies in that the treatment experience seems to bolster the patient's own motivational resources for change.

There have been few reports of the effects of ibogaine in humans. Anecdotal accounts of the acute and long-term effects of ibogaine have included only a small series of case reports from opiate and cocaine addicts with observations provided for only seven and four subjects, respectively (*2,3*). A retrospective case review of 33 ibogaine treatments for opioid detoxification in nonmedical settings under open label conditions has suggested further that the alkaloid has ameliorative effects in acute opioid withdrawal (*4*). However, objective investigations of ibogaine's effects on drug craving, and the signs and symptoms of opiate withdrawal, have not been done in either research or conventional treatment settings. Ibogaine is a drug with complex pharmacokinetics and an uncertain mechanism of action with regards to its alleged efficacy for the treatment of opiate dependence. Ibogaine is metabolized to noribogaine, which has a pharma-

cological profile that is different from that of the parent drug. We report here that ibogaine is effective in blocking opiate withdrawal, providing an alternative approach for opiate-dependent patients who have failed other conventional treatments. Identifying noribogaine's mechanism of action may explain how ibogaine promotes rapid detoxification from opiates after only a single dose.

II. Identification of a Primary Metabolite of Ibogaine

Our group developed an analytical method for quantifying ibogaine in blood samples from rats, primates, and humans (*7*,*8*). Using fullscan electron impact gas chromatography/mass spectrometry (GC/MS), a primary metabolite, 12-hydroxyibogamine (noribogaine) was detected for the first time in blood and urine samples. The analytical procedure involved a solvent extraction under basic conditions with D_3-ibogaine as an internal standard. Urines taken from dosed monkeys and humans were extracted under strongly basic conditions, extracts were evaporated, reconstituted, and analyzed by GC/MS in full scan electron impact ionization mode. Analysis of the resulting total ion chromatograms revealed a peak identified as ibogaine by comparison with an authentic standard. All samples were found to contain a second major component eluting after ibogaine. Similar spectral characteristics of this peak to ibogaine's spectrum defined it as an ibogaine metabolite, which is formed by the loss of a methyl group (Figure 1). The site for metabolic demethylation of ibogaine was the



Ibogaine, R = CH_3 (Le Men-Taylor numbering)
Noribogaine (10-Hydroxyibogamine)*, R = H

*Noribogaine has frequently been referred to as 12-hydroxyibogamine in the biological and medical literature, based on the Chemical Abstracts numbering system for this alkaloid skeleton.

FIGURE 1. MOLECULAR STRUCTURES OF IBOGAINE AND NORIBOGAINE. Ibogaine undergoes *O*-demethylation to form 12-hydroxyibogamine (noribogaine).

methoxy group, resulting in the compound 12-hydroxyibogamine (noribogaine). The identity of the desmethyl metabolite was confirmed using an authentic standard of noribogaine (Omnichem S.A., Belgium) and gave a single peak at the same retention time and with the same electron impact fragmentation pattern as the endogenous compound isolated from monkey and human urine (*7*).

III. Cytochrome P450 Metabolism and Genetic Polymorphisms

Ibogaine, like most CNS drugs, is highly lipophilic and is subject to extensive biotransformation. Ibogaine is metabolized to noribogaine in the gut wall and liver (Figure 2, schematic). Ibogaine is *O*-demethylated to noribogaine primarily by cytochrome P4502D6 (CYP2D6). An enzyme kinetic examination of ibogaine *O*-demethylase activity in pooled human liver microsomes suggested that two (or more) enzymes are involved in this reaction (*8*). In this study, ibogaine was incubated with a set of microsomes derived from cell lines selectively expressing only one human cytochrome P450 enzyme and with a series of human liver microsome preparations, characterized with respect to their activities toward cytochrome P450 enzyme selective substrates to estimate the relative contributions of the various P450 enzymes to the metabolism of ibogaine *in vitro*. The enzyme CYP2D6 showed the highest activity toward the formation of noribogaine, followed by CYP2C9 and CYP3A4 (*9*).

Depending on whether a particular isoenzyme is present or absent, individuals are classified as extensive or poor metabolizers. The influence of genetic polymorphisms on the biotransformation of ibogaine under *in vivo* clinical conditions has been examined in recent studies (*9*). The results demonstrate that there are statistically significant differences in the two populations with regard to Cmax and $t_{1/2}$ (elim) and area under the curve (AUC) of the parent drug and metabolite, indicating that the disposition of ibogaine is dependent on polymorphic CYP2D6 distribution (Table 1). Since some of the CNS activity may be the result of noribogaine, the CYP2D6 phenotype may prove to be an important determinant in the clinical pharmacology of ibogaine. Many CYP2D6 substrates are subject to drug interactions. In considering the potential patient population who might benefit from ibogaine, many of these patients may have taken other medications (prescription and/or illicit), increasing the potential for serious adverse drug interactions.

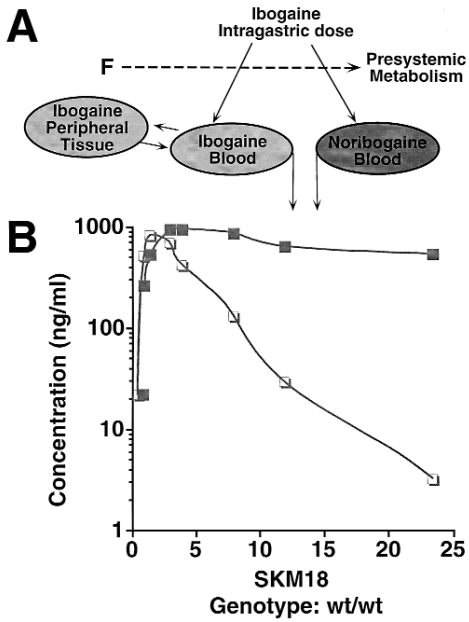


FIGURE 2. TIME COURSE OF WHOLE BLOOD CONCENTRATIONS OF IBOGAINE AND NORIBOGAINE AFTER ORAL ADMINISTRATION TO DRUG-DEPENDENT VOLUNTEER. Pharmacokinetics of ibogaine and noribogaine over the first 24 hours after oral dose in a human subject. Data shown are from a representative male subject (wt/wt, extensive metabolizer). Values for parent drug and desmethyl metabolite were measured in whole blood samples at the times indicated. Open squares indicate ibogaine concentrations and shaded squares indicate noribogaine concentrations. SK, St. Kitts, W.I., Subject Code.

TABLE 1.
PHARMACOKINETIC PARAMETERS OF IBOGAINE AND NORIBOGAINE IN HUMAN EXTENSIVE AND POOR METABOLIZERS (CYP2D6)

	*Extensive Metabolizers	**Poor Metabolizers
Ibogaine		
t_{max},hr	1.70 ± 0.15	2.50 ± 1.04
C_{max},ng/ml	737 ± 76	896 ± 166
$AUC_{0\text{-}24}$hr,ng • hr/ml	3936 ± 556	11471 ± 414
$t_{1/2}$,hr	7.45 ± 0.81	NQ
Noribogaine		
t_{max},hr	6.17 ± 0.85	3.17 ± 1.36
C_{max},ng/ml	949 ± 67	105 ± 30
$AUC_{0\text{-}24}$hr,ng • hr/ml	14705 ± 1024	3648 ± 435
$t_{1/2}$,hr	NQ	NQ

* N = 24 (10.0 mg/kg), 16 males and 8 females

** N = 3, 3 males (10.0 mg/kg)

IV. Ibogaine Pharmacokinetics

Pharmacokinetic measurements have been obtained from human drug-dependent patient volunteers who had received single oral doses of ibogaine (Table 1; Figure 2). Figure 2 illustrates the pharmacokinetic profile of ibogaine and the metabolite following oral doses of the drug in a representative male subject. Table 1 shows that CYP2D6 mediated metabolism of ibogaine resulted in high levels of noribogaine in blood, with Cmax values in the same range as the parent drug. The time required to eliminate the majority of absorbed ibogaine (>90%) was 24 hours post-dose (Figure 2). The pharmacokinetic profiles measured in whole blood demonstrate that the concentrations of noribogaine measured at 24 hours remained elevated, in agreement with previous findings (*10*). The still elevated concentrations of noribogaine in blood at 24 hours after drug administration limited the quantitation of the terminal half-life of the metabolite. Noribogaine was measured in CYP2D6 deficient subjects, but at concentrations that were markedly lower than for the extensive metabolizers. Conversion of the parent to noribogaine in CYP2D6 deficient subjects may reflect the metabolic contribution of other cytochromes (CYP2C9, CYP3A4). The concentration of noribogaine measured at 24 hours post-dose in the subject in Figure 2 was in the range of 800 ng/ml, similar to the peak concentration of ibogaine that was measured in this representative subject. Pharmacokinetic measurements in human volunteers administered oral doses of ibogaine showed that the area under the curve (AUC) for the parent compound was approximately three-fold less than for the active metabolite (Table 1). Thus, noribogaine reaches sustained high levels in blood after a single administration of the parent drug.

Since the metabolite has been shown in radioligand binding assays to have higher affinities for certain CNS targets, it can be estimated that the contribution of the metabolite to the total pharmacodynamic profile of ibogaine is significant. To display *in vivo* activity, it is necessary for CNS drugs to reach the brain. Since it is difficult to study these processes in humans, it is common to study the penetration of a CNS active drug into the brains of laboratory animals. The concentrations of ibogaine and noribogaine have been measured in rat brain following both oral and intraperitoneal (i.p.) administrations (*11,12*). The significance of micromolar interactions of ibogaine and noribogaine with various radioligand binding sites was related to the concentration of parent drug and metabolite in brain (Table 2). Regional brain levels of ibogaine and noribogaine were measured in rat cerebral cortex, striatum, brainstem, and cerebellum at 15 minutes, 1 and 2 hours postdrug administration. We have shown that ibogaine is rapidly detected in brain following oral administration. The metabolite was detected at the earliest time point (15 minutes), consistent with first pass metabolism of the parent drug (*11*). Administration of ibogaine (40 mg/kg i.p., 50

TABLE 2.
PHARMACOKINETIC PARAMETERS OF IBOGAINE AND NORIBOGAINE IN MALE RAT (SPRAGUE-DAWLEY)

	*Whole Blood 40 mg/kg i.p.	*Brain 40 mg/kg i.p.	**Brain 50 mg/kg p.o.
Ibogaine			
t_{max},hr	0.10 ± 0.03	1.00 ± 0.14	1.00 ± 0.21
C_{max},ng/ml or	3859 ± 789	3782 ± 418	5210 ± 480
ng/g [μM]	[11.2 ± 2.3]	[11.0 ± 1.2]	[15.1 ± 1.4]
AUC, ng • hr/ml or	10636 ± 341	22098 ± 922	NQ
ng/g [μM • hr]	[30.7 ± 1.0]	[63.9 ± 2.7]	
$t_{1/2}$,hr	2.38 ± 0.50	11.05 ± 1.15	NQ
Noribogaine			
t_{max},hr	2.40 ± 0.04	2.00 ± 0.16	2.00 ± 0.28
C_{max},ng/ml or	7265 ± 953	3236 ± 514	3741 ± 423
ng/g [μM]	[21.9 ± 2.9]	[9.8 ± 1.6]	[11.3 ± 1.3]
AUC, ng • hr/ml or	96920 ± 741	38797 ± 324	NQ
ng/g [μM • hr]	[292.0 ± 2.2]	[117.9 ± 1.0]	

NQ, not quantifiable
Noribogaine $t_{1/2}$ not quantifiable
* Noncompartmental pharmacokinetic analysis over a 24 hr. period
** Noncompartmental pharmacokinetic analysis over a 2 hr. period
Data represent the average values from individual animals (n = 4) assayed in duplicate.

mg/kg p.o.) in rodents resulted in levels of ibogaine and noribogaine that ranged from 10 to 15 μM and 10 to 12 μM, respectively. The results demonstrate that noribogaine reaches significant concentrations in brain following both routes of administration in rodents. Thus, the concentrations of noribogaine in brain may activate processes that cause the desired effects of suppressing opiate withdrawal signs and diminishing drug craving.

V. Setting and Study Design

We have had the opportunity to describe the clinical experience of a series of patients undergoing opiate detoxification with ibogaine. The study was conducted in a 12 bed freestanding facility in St. Kitts, West Indies. The treatment program had a planned duration of 12 to 14 days and stated goals of: (1) safe physical detoxification from opiates, (2) motivational counseling, and (3) referral to aftercare programs and community support groups (twelve-step programs). Subjects were self-referred for inpatient detoxification from opiates (heroin or

methadone) and met inclusion/exclusion criteria. All individuals were deemed fit and underwent treatment following a physician's review of the history and physical examination. Participants did not have histories of stroke, epilepsy, or axis I psychotic disorders. Results of the electrocardiogram and clinical laboratory testing were within predetermined limits. All subjects signed an informed consent for ibogaine treatment. Overall, the sample of 32 patients was predominately male (69%) and white (82%), with a mean age of 33.6 years and a mean length of addiction of 11.1 years.

All participants met DSM-IV criteria for opioid dependence and had positive urine screens at entry to the study. Participants were assigned to fixed-dose (800 mg; 10 mg/kg) of ibogaine HCl under open-label conditions. Subjects were genotypyed for the CYP2D6 alleles (*2, *4, *5 and wt alleles), as described previously (*13*). On admission, participants were administered the Addiction Severity Index (*14*) and received structured psychiatric evaluations before and after ibogaine treatment (SCID I and II). In cases where the participant's responses were deemed questionable due to intoxication or withdrawal signs, portions of all interviews were repeated later, as necessary. Additional information about substance use history and past/current medical condition(s) was gathered and later cross-referenced for accuracy through a separate comprehensive psychosocial assessment.

VI. Physician Ratings of Withdrawal

Two physicians rated as present or absent 13 physical signs typically associated with opiate withdrawal, based on a 10-minute period of observation (*14,15*). The Objective Opiate Withdrawal Scale (OOWS) data were analyzed from three assessments performed during the period spent in the clinic under medical monitoring, given that those points in relation to ibogaine administration were highly comparable among all patients. The attending physician performed the first assessment following clinic admission an average of 1 hour before ibogaine administration and 12 hours after the last dose of opiate. A psychiatrist without knowledge of the admitting OOWS score performed the second assessment an average of 10 to 12 hours after ibogaine administration and 24 hours after the last opiate dose. The attending physician performed the third assessment 24 hours following ibogaine administration and 36 hours after the last opiate dose. Physician's ratings were subjected to repeated measures analysis of variance (ANOVA) with treatment phase (pre-ibogaine, post-ibogaine, and program discharge) as the within-subjects factor.

VII. Subjects' Self-Report of Withdrawal Symptoms

The Opiate-Symptom Checklist (OP-SCL) was developed for the present study as a subtle assessment of withdrawal symptoms, given that many subjects' verbal reports about withdrawal experience were generally exaggerated, both in number and severity of symptoms. Each of the 13 items that comprises the OP-SCL scale were taken from the Hopkins Symptom Checklist-90, with the criteria for selection based on whether it appeared in two other self-report withdrawal questionnaires, the Addiction Research Center Inventory (*16*) and the Subjective Opiate Withdrawal (*17*) scales. Subjects also completed a series of standardized self-report instruments relating to mood and craving at three different time points during the study within 7 to 10 days after the last dose of opiate. Subjects were asked to provide ratings of their current level of craving for opiates using questions from the Heroin Craving Questionnaire (HCQN-29) (*18*). Self-reported depressive symptoms were determined by the Beck Depression Inventory (BDI) (*19*). Subjects' scores were subjected to repeated measures analyses of variance across treatment phase (pre-ibogaine, post-ibogaine, and discharge) as the within-subjects factor for the total score from the OP-SCL, BDI, and the HCQN-29.

VIII. Acute Detoxification and Behavioral Outcomes

Physical dependence on opiates is characterized by a distinctive pattern of signs and symptoms that make up the naturalistic withdrawal syndrome. The physical dependence produced by an opiate is assessed usually by discontinuation of opioid treatment (spontaneous withdrawal) or by antagonist-precipitated withdrawal. All of the subjects identified opiates as one of the primary reasons for seeking ibogaine treatment and demonstrated active dependence by clinical evaluation, objective observations, and positive urine screen. Physician ratings demonstrate that ibogaine administration brings about a rapid detoxification from heroin and methadone (Figure 3A). The post-ibogaine OOWS rating obtained 10 to 12 hours after ibogaine administration and 24 hours following the last opiate dose was significantly lower than the rating obtained 1 hour prior to ibogaine administration and 12 hours after the last opiate dose. At 24 hours after ibogaine administration and 36 hours after the last opiate dose, the OOWS rating was significantly lower than the pre-ibogaine rating. The blinded post-ibogaine ratings between doctors agreed well item for item and were not significantly different from one another in terms of the mean total OOWS score (mean ± 1 SD, N = 32). These objective measures demonstrate the effects of ibogaine on opiate

withdrawal assessed in this study. Objective signs of opiate withdrawal were rarely seen and none were exacerbated at later time points. The results suggest that ibogaine provided a safe and effective treatment for withdrawal from heroin and methadone. The acute withdrawal syndrome in addicts dependent on heroin begins approximately 8 hours after the last heroin dose, peaks in intensity at 1 to

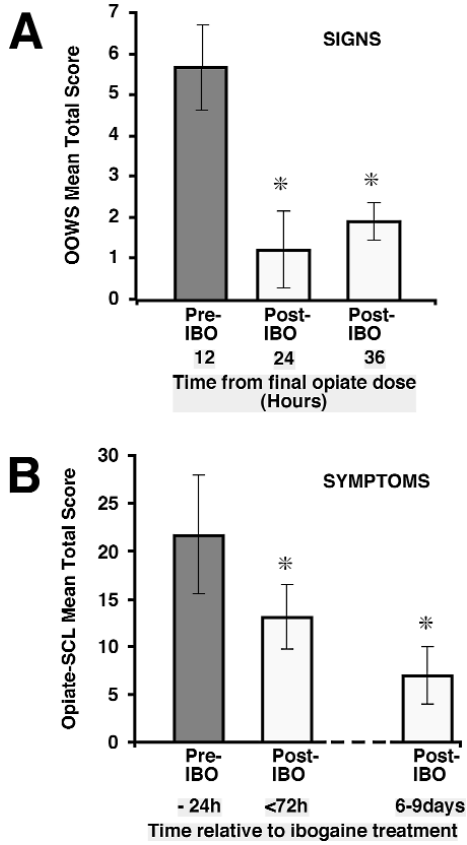


FIGURE 3. SCORES ON THE OBJECTIVE OPIATE WITHDRAWAL SCALE. (a) The effects of single-dose ibogaine treatment on opiate withdrawal signs at three physician-rated assessment times (12, 24, and 36 hours after the last dose of opiate). Average data are shown (mean ± 1 SD, N = 32). *P < .05. (b) The effects of single-dose ibogaine on patients self-report Opiate-Symptom Checklist (OP-SCL). The OP-SCL was developed for the present study as a subtle assessment of patients' subjective complaints based on 13 items selected from the Hopkins Symptom Checklist rated for intensity from 0 to 4. The maximum score attainable for the OP-SCL was 42.
* p < .05.

2 days, and subjective symptoms subside within 7 to 10 days. Self-reports of withdrawal symptoms shortly after recovery from ibogaine treatment (< 72 hours) were significantly decreased from the pre-ibogaine rating obtained 12 hours after the last use of opiates and were comparable to the level of discomfort reported at program discharge approximately one week later (Figure 3B). Thus, for subjects undergoing ibogaine detoxification, all of the subjects were successful during the detoxification process and many were able to maintain abstinence from illicit opiates and methadone over the months following detoxification (data not shown). Perhaps the most important observation was the ability of a single dose of ibogaine to promote a rapid detoxification from methadone without a gradual taper of the opiate. These preliminary observations of ibogaine treatment suggest that methadone withdrawal was not more difficult than heroin withdrawal following ibogaine detoxification. As discussed below, we suggest that the long-acting metabolite noribogaine may account for the efficacy of ibogaine treatment for both heroin and methadone withdrawal.

Craving is thought to be an important symptom contributing to continued drug use by addicts. Opiate-dependent subjects report increased drug craving during the early stages of withdrawal (*20*). We have previously reported that subjects undergoing opiate detoxification reported significantly decreased drug craving for opiates on five measures taken from the HCQN-29 scales at 36 hours posttreatment. These five measures inquired about specific aspects of drug craving, including urges, as well as thoughts about drug of choice or plans to use the drug. Questions are asked also about the positive reinforcing effects of the drug or the expectation of the outcome from using a drug of choice or the alleviation of withdrawal states. Perceived lack of control over drug use was included, since it is a common feature of substance-abuse disorders and is most operative under conditions of active use, relapse, or for subjects at high risk. The results demonstrated that across craving measures, the mean scores remained significantly decreased at program discharge (*10*). BDI scores were also significantly reduced both at program discharge and at 1-month follow-up assessments (*10*). Heroin craving is known to be dramatically reduced depending on the lack of availability of the abused drug in a controlled setting. Thus, more meaningful studies of ibogaine's ability to suppress heroin craving require further investigations done under naturalistic conditions.

IX. Cardiovascular Changes and Side Effects of Ibogaine

Ibogaine has a variety of dose-dependent pharmacological actions, which may not be relevant to its effectiveness for opiate detoxification and diminished drug

cravings, but may influence considerations for safety. However, toxicological studies in primates have demonstrated previously that ibogaine administration at doses recommended for opiate detoxification is safe (*21*). The FDA Phase I Pharmacokinetic and Safety investigations by our group have not advanced in the United States due to a lack of funds to support clinical investigations of ibogaine in patient volunteers. However, we have had the opportunity to obtain additional safety data in drug-dependent subjects under controlled conditions in human studies conducted in St. Kitts, West Indies. For these subjects, baseline screening included a medical evaluation, physical examination, electrocardiogram, blood chemistries, and hematological workup, as well as psychiatric and chemical dependency evaluations. In some cases, more extensive evaluations were done to rule out cardiac risk factors and to exclude subjects for entry to the study. The recognition of the cardiovascular actions of ibogaine date back to the 1950s, when the CIBA Pharmaceutical Company investigated ibogaine as an antihypertensive agent. Ibogaine at doses used for opiate detoxification may lower blood pressure and heart rate when the drug reaches peak concentrations in blood. In contrast, the opiate withdrawal syndrome is associated with increases in pulse, systolic and diastolic blood pressures, and respiratory rate.

Our observations of the safety of ibogaine have not been limited to opiate-dependent subjects. To date, we have evaluated ibogaine's safety in more than 150 drug-dependent subjects that were assigned to one of three fixed-dose treatments under open label conditions: 8, 10, or 12 mg/kg ibogaine. Adverse effects were assessed by clinician side-effect ratings and open-ended query. To date, no significant adverse events were seen under these study conditions. The most frequent side effects observed were nausea and mild tremor and ataxia at early time points after drug administration. Random regression of vital signs (respiration rate, systolic and diastolic blood pressures, and pulse) revealed no significant changes across time or by treatment condition for opiate-dependent subjects. However, a hypotensive response to ibogaine was observed in some cocaine-dependent subjects, which required close monitoring of blood pressure and which was responsive to volume repletion. Comparison of pre- and postdrug effects demonstrated that blood cell count, neurotrophil levels, and sodium and potassium levels were in the normal range. There were no significant changes from baseline seen on liver function tests. No episodes of psychosis or major affective disorder were detected at posttreatment evaluations. Intensive cardiac monitoring demonstrated that no electrocardiographic abnormalities were produced or exaggerated following ibogaine administration in subjects that were not comorbid for any cardiovascular risk factors. These preliminary results demonstrate that single doses of ibogaine were well tolerated in drug-dependent subjects. These preliminary observations are encouraging, but they do not diminish the possibility that ibogaine may have other medical risks not ordinarily associated with opiate withdrawal or with the use of tapering doses of methadone.

However, we anticipate, based on our clinical experience from offshore studies, that any potential adverse cardiovascular responses can be well managed within routine clinical practice.

X. Mechanism of Action

While the precise mechanism(s) underlying the expression of opiate withdrawal signs and symptoms are not fully understood, and may be different between humans and laboratory animals, the cellular and behavioral changes resulting from withdrawal and that have motivational relevance to drug-seeking behavior may involve the same neural circuits as those that participate in opiate dependence. Ibogaine and its active metabolite noribogaine act on a number of different neurotransmitter systems in the brain that may contribute to ibogaine's ability to suppress the autonomic changes, objective signs, and subjective distress associated with opiate withdrawal. However, we have speculated that the actions of noribogaine at mu-opioid receptors may account in part for ibogaine's ability to reduce withdrawal symptoms in opiate-dependent humans (*22*). For example, the desmethyl metabolite noribogaine has been shown to be a full agonist at the mu-opioid receptor (Table 3). This pharmacological activity, coupled with the

TABLE 3.
INHIBITORY POTENCY OF IBOGAINE AND NORIBOGAINE

	Ibogaine		Noribogaine		Pharmacodynamic
	IC_{50}(μM)	nh	IC_{50}(μM)	nh	Action
Serotonergic					
5-HT Transporter (RTI-55)	0.59 ± 0.09	0.8	0.04 ± 0.01	0.76	Reuptake Blocker
Opioidergic					
Mu (DAMGO)	11.0 ± 0. 9	1.0	0.16 ± 0.01	0.99	Agonist
Kappa 1 (U69593)	25.0 ± 0.6	1.1	4.2 ± 0.3	1.05	Partial Agonist (?)
Kappa 2 (IOXY)	23.8 ± 7.1	1.0	92.3 ± 9.2	1.03	Partial Agonist (?)
Glutaminergic					
NMDA (MK-801)	5.2 ± 0.2	0.9	31.4 ± 5.4	1.1	Channel Blocker

The values represent the mean ± SE of the IC_{50} value (μM) from 3-4 independent experiments, each performed in triplicate. nh, Hill slope

long duration of action may produce a self-taper effect in opiate-dependent patients.

The relative contributions of the parent and metabolite to the pharmacodynamic effects have yet to be established with precise certainty. Results from animal studies indicate that opiate withdrawal is associated with hyperactivity of the noradrenergic system and with changes in a variety of other neurotransmitter systems (*23*). Pharmacological agents may have differential effects on different components of opiate withdrawal. In addition to affecting mu-opioid receptors in the brain, noribogaine also has affinity at kappa-opioid receptors and the serotonin transporter (*8*). Indirect serotonergic agonists have been shown to attenuate neuronal opiate withdrawal (*24*). The 5-HT releaser d-fenfluramine and the 5-HT reuptake blockers fluoxetine and sertraline reduce the withdrawal-induced hyperactivity of locus ceruleus neurons. We have demonstrated previously that noribogaine elevates serotonin concentrations in brain by binding to the 5-HT transporter (Table 3) (*8*). Dysphoric mood states associated with opiate withdrawal may be a contributing factor for relapse, since addicts often experience drug craving in conjunction with dysphoric mood states (*20*). An action at the 5-HT transporter may explain the antidepressant effects seen following ibogaine administration in human opiate-dependent patients (*10*). Clinical studies have previously suggested that patients who abused opiates may have been self-medicating their mood disorders, indicating a possible role for endogenous opiates in major depression (*25*). Dysphoria and drug craving reportedly persist in opiate addicts even after detoxfication from opiates has been completed. Thus, noribogaine's effects at multiple opioid receptors and the 5-HT transporter may explain the easy transition following only a single dose of ibogaine in humans following abrupt discontinuation of opiates. These observations suggest that noribogaine may have potential efficacy for use as a rapid opiate detoxification treatment strategy. Recognition of the different components (autonomic changes and the objective signs versus subjective signs, dysphoric mood, and drug craving) may suggest the need for a medication strategy that targets multiple neurotransmitter systems for the treatment of opiate withdrawal and for relapse prevention. The identification of noribogaine's mix of neurotransmitter receptors and neurotransporter binding sites provides additional support for medications targeted to different aspects of the opiate withdrawal syndrome.

Opiate agonist pharmacotherapy with buprenorphine is a new alternative to methadone maintenance for the treatment of opiate dependence (*20*). Noribogaine has some pharmacologic similarities to the mixed agonist-antagonist analgesic buprenorphine. Buprenorphine and noribogaine both act as mu agonists. Compared to buprenorphine's high affinity partial agonist profile, noribogaine has lower receptor affinity, but increased intrinsic activity over buprenorphine as a mu agonist. Behavioral and physiological evidence suggest

that buprenorphine has kappa antagonist effects in addition to its action as a partial mu agonist. Noribogaine binds to kappa receptors, but acts as a partial agonist (Table 3). Both drugs have a long duration of action due to the slow rates of dissociation from opiate receptor sites. Thus, ibogaine's ability to inhibit opiate craving may be accounted for by the mixed mu- and kappa-opioid profile of the active metabolite noribogaine.

XI. Conclusion and Future Directions

Pharmacological treatments for opiate dependence include detoxification agents and maintenance agents. New experimental approaches have also been tried to reduce the time it takes to complete the process of detoxification or to further reduce persisting subjective reports of dysphoria and opiate craving. Ibogaine treatment is a novel approach that has similarities with other detoxification pharmacotherapies, including substitution with a longer-acting opiate (e.g., methadone or buprenorphine). However, ibogaine appears to be a prodrug with the beneficial effects residing in the active metabolite noribogaine. Thus, it would be useful to demonstrate that noribogaine alone is effective in detoxification of heroin-dependent and methadone-maintained patients. If noribogaine alone is safe and effective in open label studies, a randomized, double-blind study comparing noribogaine to clonidine-naltrexone detoxification would be justified. This clinical study would demonstrate whether noribogaine is more effective and has fewer adverse hemodynamic effects. Based on its spectrum of pharmacological activities, we suggest that noribogaine should also be considered as an alternative to methadone maintenance.

A pharmacological approach for the compliance problem has been the development of depot formulations that might be injected as infrequently as once a month. The long-acting pharmacokinetics of noribogaine suggests that the drug may, in fact, persist in the body for weeks to months. Thus, future development of depot noribogaine preparations may provide an optimal therapeutic approach for treating intractable opiate abusers. Another approach would be to combine a noribogaine taper with naltrexone. This approach may provide a means to shorten the time needed to initiate opiate antagonist therapy. Previous studies have also suggested the need for combination pharmacotherapies, such as antidepressants with buprenorphine (*20*). Interestingly, noribogaine has a pharmacological profile that includes actions on both serotonin and opiate systems in the brain. Although not discussed in this report, ibogaine provides an approach for the treatment of abuse of multiple substances including alcohol and cocaine. Many opiate-dependent patients abuse multiple drugs and alcohol. Thus, ibogaine and its

active metabolite noribogaine represent two additional pharmacological treatments for opiate dependence. However, clinical studies are needed to demonstrate whether they will become viable alternatives for treating opiate dependence in the future. It remains to be seen if the politics surrounding this controversial treatment approach will limit the promise for future development of either ibogaine or noribogaine.

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The ibogaine medical subculture

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Abstract

Aim of the study: Ibogaine is a naturally occurring psychoactive indole alkaloid that is used to treat substance-related disorders in a global medical subculture, and is of interest as an ethnopharmacological prototype for experimental investigation and possible rational pharmaceutical development. The subculture is also significant for risks due to the lack of clinical and pharmaceutical standards. This study describes the ibogaine medical subculture and presents quantitative data regarding treatment and the purpose for which individuals have taken ibogaine.
Materials and methods: All identified ibogaine "scenes" (defined as a provider in an associated setting) apart from the Bwiti religion in Africa were studied with intensive interviewing, review of the grey literature including the Internet, and the systematic collection of quantitative data.
Results: Analysis of ethnographic data yielded a typology of ibogaine scenes, "medical model", "lay provider/treatment guide", "activist/self-help", and "religious/spiritual". An estimated 3414 individuals had taken ibogaine as of February 2006, a fourfold increase relative to 5 years earlier, with 68% of the total having taken it for the treatment of a substance-related disorder, and 53% specifically for opioid withdrawal.
Conclusions: Opioid withdrawal is the most common reason for which individuals took ibogaine. The focus on opioid withdrawal in the ibogaine subculture distinguishes ibogaine from other agents commonly termed "psychedelics", and is consistent with experimental research and case series evidence indicating a significant pharmacologically mediated effect of ibogaine in opioid withdrawal.

Keywords: Ibogaine; *Iboga* alkaloid; Substance-related disorders; Opioid-related disorders; Substance withdrawal; Medical ethnography

1. Introduction

Ibogaine is the most studied of the *iboga* alkaloids (Bartlett et al., 1958), a group of naturally occurring and synthetic indole alkaloids, some of which reportedly reduce opioid withdrawal symptoms and drug self-administration in humans (Luciano, 1998; Alper et al., 1999; Mash et al., 2001) or preclinical models (Glick et al., 2001). Presently in the setting of homes, hotel rooms and private clinics in North America and Europe, individuals in increasing numbers are taking ibogaine in what has been termed "a vast uncontrolled experiment" (Vastag, 2005).

1.1. History

The ritual eating of iboga has been a psychopharmacological sacrament in the Bwiti religion for several centuries, and was likely practiced among Pygmies in much earlier times (Fernandez, 1982). In Gabon and elsewhere in West Central Africa, ibogaine is ingested in the form of scrapings of *Tabernanthe iboga* root bark. The ritual aim of eating iboga has been

Abbreviations: 18-MC, 18-methoxycoronaridine; AC, adenylyl cyclase; EKG, electrocardiogram; FDA, United States Food and Drug Administration; GDNF, glial cell line-derived neurotrophic factor; GPCRs, G protein-coupled receptors; Ibogaine HCl, ibogaine hydrochloride; LSD, lysergic acid diethylamide; mAChRs, muscarinic acetylcholine receptors; MDMA, 3,4-methylenedioxymethamphetamine; MI, myocardial infarction; NAc, nucleus accumbens; nAChR, nicotinic acetylcholine receptor; NIDA, United States National Institute on Drug Abuse; NMDA, *N*-methyl-D-aspartate.

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conceptualized as "binding"; the binding across time through ancestral contact, or binding participants socially on the basis of a common shared experience of a distinctive consciousness and system of belief (Fernandez, 1982; Fernandez and Fernandez, 2001). In the colonial era Bwiti became a context of collective psychological resistance to the anomie and demoralization related to the strain on indigenous community and family institutions. Bwiti offered a dignified realm of spiritual endeavor, "the work of the ancestors" and social cohesion. Following Gabonese independence in 1960, Bwiti has remained constellated with national identity and contemporarily retains significant social and political importance (Swiderski, 1988; Samorini, 1995).

Iboga has not commonly been used to treat addiction in the traditional African Bwiti context. Iboga has been sought as a treatment for some somatic conditions, in particular for infertility (Fernandez, 1982). In the colonial era the indigenous community experienced a crisis due to a sharp decline in fertility caused by venereal disease stemming from prostitution and the separation of men from their families by the large-scale physical relocation of indigenous workers. The possibility of an objective basis for the use of iboga in this setting is suggested by evidence associating *iboga* alkaloids with antimicrobial activity or effects on cell-mediated immunity. *Iboga* alkaloids are reportedly active against *Candida albicans* in the intact animal (Yordanov et al., 2005). In vitro studies indicate reversal of multidrug resistance in human cancer cells (Kam et al., 2004) and activity against *Mycobacterium tuberculosis* (Rastogi et al., 1998), human immunodeficiency type 1 virus (Silva et al., 2004), and the tropical parasite *Leishmania amazonensis* (Delorenzi et al., 2002).

The first observation of ibogaine as treatment for substance-related disorders in 1962 involved a network of lay drug experimenters who ingested a variety of hallucinogens and systematically recorded their experiences (Lotsof and Alexander, 2001). Withdrawal symptoms were unexpectedly absent in heroin-dependent individuals who had taken ibogaine. Common to various sociological definitions of the term "subculture" is a system of beliefs, norms and values apart from a superordinate culture (Clarke, 1974; Dowd and Dowd, 2003). The ibogaine subculture has elicited wariness from the "superordinate culture" of conventional clinical medicine (Kleber, 2001), and has been invoked regarding the null hypothesis that ibogaine's reported effect in opioid withdrawal is not pharmacologically mediated, but is instead accounted for by suggestion and ritual (Sharpe and Jaffe, 1990). The ibogaine subculture is also significant as the setting of case report evidence that influenced the decision of the National Institute on Drug Abuse (NIDA) to pursue its ibogaine project (Alper, 2001), and the Food and Drug Administration (FDA) to approve a clinical trial (Mash et al., 1998).

Ibogaine is unscheduled in most of the world, with the exception of the US, Belgium, Denmark, France, Sweden, Switzerland, and Australia where it is illegal. Ibogaine has not been popular as a recreational drug regardless of its legal status (Kleber, 2001), and apparently only two arrests involving ibogaine are known to have occurred in the US (Ranzal, 1967; Lane, 2005). *Iboga* alkaloids reportedly are not self-administered, and do not produce withdrawal signs following chronic administration in animals (Aceto et al., 1992). As of late 2006, ibogaine hydrochloride (HCl) was available for $400–$500 USD per gram (ethnogarden.com, 2006), and the dosage typically used for opioid withdrawal is in the range of 1–2 g. Purity on the order of 97–98% has been reported on certificates of analysis for supplies of ibogaine HCl used in the subculture. Ibogaine is also available as *Tabernanthe iboga* extract or dried root bark.

1.2. Clinical use

Ibogaine, either as *Tabernanthe iboga* root bark or ibogaine HCl is the only *iboga* alkaloid that has reportedly been administered to humans, with apparently only one exception, a study in which 12 normal volunteers were evaluated with some brief neuropsychological tests after receiving the naturally occurring *iboga* alkaloid ibogaline (Schmid, 1967). Ibogaine HCl has been typically administered as a single oral dose in the range of 10–25 mg/kg of body weight. Patients physically dependent on opioids have described significant attenuation of withdrawal symptoms within several hours of ingesting ibogaine, with subsequently sustained resolution of the opioid withdrawal syndrome (Alper et al., 1999; Mash et al., 2001). The advantages attributed to ibogaine are higher tolerability relative to other standard treatments for acute opioid withdrawal, and an interval of diminished drug craving that may last days to months following a treatment. Individuals also take ibogaine in search of psychological or religious insight, typically at dosages lower than those used in the treatment of opioid withdrawal.

There are no randomized controlled clinical trials of ibogaine, and the available clinical data is limited mainly to two open label case series. One series from the US and the Netherlands included self-reported outcomes of a consecutive series of 52 treatments involving 41 different individuals, some of who were treated on multiple occasions mainly for the indication of dependence on opioids or stimulants (Alper, 2001). Thirty-six percent of the treatments were associated with self-reported intervals of 6 months or longer of abstinence from the primary drugs of dependence for which treatment had been sought. A subset of 33 individuals were treated for the indication of opioid withdrawal with a single dose of ibogaine averaging 19.3 mg/kg (Alper et al., 1999). Twenty-five of these patients had full resolution of opioid withdrawal without drug seeking behavior that was sustained throughout a 72-h period of post-treatment observation, and another four individuals denied withdrawal symptoms but expressed their preference to continue to use heroin. The other series, from a clinic in St. Kitts consists of 32 patients treated with a fixed dose of 800 mg of ibogaine HCl for the indication of withdrawal from heroin (Mash et al., 2001). Physician-rated structured instruments indicated resolution of withdrawal signs and symptoms at 24 h after the last use of opioids (an interval of abstinence commonly associated with significant withdrawal symptoms) that was sustained during subsequent observation for 1 week following ibogaine administration.

An unpublished Dutch doctorandus thesis (Bastiaans, 2004) presents data obtained from 21 subjects who responded to a

Web-based questionnaire adapted from the European Addiction Severity Index a mean of 21.8 months after they had taken ibogaine for treatment of a substance-related disorder. Seventeen of the 21 patients (81%) identified opioids as the primary drug of dependence for which they had sought treatment. Five individuals reported stopping the use of all substances following treatment with ibogaine, and another nine reported stopping the use of their primary drug while continuing to use alcohol or cannabis. Nineteen patients reported stopping their use of their primary drug for at least a week following treatment, suggesting frequent resolution of acute opioid withdrawal.

1.3. Preclinical research

Research utilizing animal models has involved the *iboga* alkaloids ibogaine (Alper, 2001) and its desmethylated metabolite noribogaine (Baumann et al., 2001), and a synthetic congener, 18-methoxycoronaridine (18-MC) (Maisonneuve and Glick, 2003). Eleven of the 13 published preclinical studies of *iboga* alkaloids in opioid withdrawal indicate a significant attenuation of opioid withdrawal signs in the rat (Dzoljic et al., 1988; Sharpe and Jaffe, 1990; Maisonneuve et al., 1991; Glick et al., 1992; Cappendijk et al., 1994; Rho and Glick, 1998; Parker et al., 2002; Panchal et al., 2005), mouse (Frances et al., 1992; Popik et al., 1995; Layer et al., 1996; Leal et al., 2003), and primate (Aceto et al., 1992). *Iboga* alkaloids are also reported to reduce the self-administration of morphine (Glick et al., 1991; Glick et al., 1994; Glick et al., 1996; Maisonneuve and Glick, 1999; Pace et al., 2004), cocaine (Cappendijk and Dzoljic, 1993; Glick et al., 1994), amphetamine (Maisonneuve et al., 1992), methamphetamine (Glick et al., 2000; Pace et al., 2004), alcohol (Rezvani et al., 1995; Rezvani et al., 1997; He et al., 2005) and nicotine (Glick et al., 1998; Glick et al., 2000), and to diminish dopamine efflux in the nucleus accumbens (NAc), which is regarded as a correlate of drug salience (Berridge, 2007), in response to opioids (Maisonneuve et al., 1991; Glick et al., 1994; Glick et al., 2000; Taraschenko et al., 2007b) or nicotine (Benwell et al., 1996; Maisonneuve et al., 1997; Glick et al., 1998).

1.4. Mechanisms of action

Initially, ibogaine's mechanism of action was hypothesized to involve antagonism at the *N*-methyl-D-aspartate-type glutamate (NMDA) receptor (Skolnick, 2001). However, 18-MC, which has negligible NMDA receptor affinity, also reduces opiate withdrawal and drug self-administration in the animal model (Glick et al., 2001). Antagonism of the α3β4 nicotinic acetylcholine receptor (nAChR) is a possible mechanism of action, as indicated by a series of studies of *iboga* alkaloids and nicotinic agents (Fryer and Lukas, 1999; Glick et al., 2002a,b; Pace et al., 2004; Taraschenko et al., 2005). The α3β4 nAChR is relatively concentrated in the medial habenula and interpeduncular nucleus, where 18-MC's antagonism of α3β4 nAChRs diminishes sensitized dopamine efflux in the NAc (Taraschenko et al., 2007a,b).

Ibogaine's mechanism of action has frequently been suggested to involve the modification of neuroadaptations related to prior drug exposure (Rabin and Winter, 1996b; Popik and Skolnick, 1998; Alper, 2001; Glick et al., 2001; Sershen et al., 2001; Levant and Pazdernik, 2004). Ibogaine may modulate intracellular signaling linked to opioid receptors, and potentiates the morphine-induced inhibition of adenylyl cyclase (AC) (Rabin and Winter, 1996b), an effect that is opposite to the activation of AC that is classically associated with opioid withdrawal (Sharma et al., 1975). In animals, ibogaine enhances the antinociceptive effect of morphine or other μ opioids without by itself having an effect on nociception (Schneider and McArthur, 1956; Schneider, 1957; Frances et al., 1992; Bagal et al., 1996), and inhibits the development of tolerance to morphine antinociception (Cao and Bhargava, 1997). Prior exposure to morphine potentiates ibogaine's diminution of sensitized dopamine efflux in the NAc in response to morphine (Pearl et al., 1996) or ibogaine's enhancement of morphine antinociception (Sunder Sharma and Bhargava, 1998), suggesting an effect on neuroadaptations related to opioid tolerance or dependence.

Increased glial cell line-derived neurotrophic factor (GDNF) in the ventral tegmental area has been suggested to mediate decreased ethanol consumption following the administration of ibogaine to rats (He et al., 2005; He and Ron, 2006). GDNF enhances the regeneration of dopaminergic function (Ron and Janak, 2005) and is increased by antidepressant treatment (Hisaoka et al., 2007). The hypothesis that GDNF may mediate improvement in hedonic functioning and mood in chronic withdrawal from addictive substances is appealing, but does not appear likely to explain efficacy in acute opioid withdrawal.

Although designated as a hallucinogen, ibogaine's use in opioid withdrawal distinguishes it from other compounds that are commonly termed "psychedelics", namely the serotonin type 2A receptor agonist classical hallucinogens such as lysergic acid diethylamide (LSD), psilocybin and mescaline, or the serotonin releasing substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA). In contrast with ibogaine, there is no preclinical or case report evidence that suggests a significant therapeutic effect of classical hallucinogens or MDMA in acute opioid withdrawal. Ibogaine's effects in opioid withdrawal do not appear to involve serotonin agonist or releasing activity (Wei et al., 1998; Glick et al., 2001). Serotonergic neurotransmission does not appear to play a significant role in mediating the expression of the opioid withdrawal syndrome, which remains unchanged even after extensive lesioning of the raphe (Caille et al., 2002).

The phenomenology of the subjective state produced by ibogaine has been attributed with the quality of a "waking dream" and distinguished from the state associated with classical hallucinogens (Goutarel et al., 1993; Lotsof and Alexander, 2001). The visual phenomena associated with ibogaine tend to occur with greatest intensity with the eyes closed, and to be suppressed with the eyes open, and often involve a sense of location within an internally represented visual or dream landscape, in contrast to an alteration of the visual environment experienced with the eyes open while awake which is often reported with classical hallucinogens. The occurrence of an atropine-sensitive

electroencephalogram (EEG) rhythm in animals treated with ibogaine (Schneider and Sigg, 1957; Depoortere, 1987) suggests a waking neurophysiological state with an analogy to rapid eye movement sleep (Goutarel et al., 1993; Alper, 2001).

1.5. Research objectives of this study

A previous publication provides a history and description of the ibogaine subculture in the U.S. and Europe from its origin in 1962 until early 2001 (Alper et al., 2001). The major objectives of this study are the qualitative analysis of observational and textual data (Bailey, 1994; Malterud, 2001) to provide an updated description as well as a typology of the ibogaine medical subculture, and the systematic collection of quantitative data regarding treatment and the purpose for which individuals took ibogaine.

2. Methods

The Institutional Review Board of the New York University School of Medicine approved this research.

2.1. Observational methods

The providers of ibogaine treatment were conceptualized as participants in a global medical subculture and studied from an "observing participant" research perspective (Gold, 1958). The qualitative and quantitative information was obtained from face to face discussions, phone conversations and e-mail correspondence with treatment providers and other participants.

2.2. Study sample

The study included only treatment providers who had already publicly identified their activities by maintaining Web sites, publishing in the lay or scientific press, presenting at public meetings, or posting to ibogaine list servers. Because ibogaine is not regulated in most of the world, providers are very often open about their activity.

A "scene" is defined in this study as a provider of ibogaine in an associated setting. The term "ibogaine subculture" refers to all ibogaine scenes collectively outside of Africa. The sample in this study that represented the ibogaine subculture consisted of all known presently or previously existing ibogaine scenes outside of Africa involving publicly identified providers, with the exception of a scene in Gabon which was included that involved European and US participants and African Bwiti adept providers. Otherwise no systematic attempt was made to study the Bwiti religious context in Africa. No data was encountered regarding the use of *Lambarene*, a tablet that was marketed in France between 1939 and 1970 that contained an estimated 8 mg of ibogaine (Goutarel et al., 1993).

2.3. Excluded scenes

A large ibogaine scene was alleged to have existed in the Christiana squatter community in Copenhagen but was concluded to lack corroborative evidence on the basis of communication with the Danish Drug Users Union and former Christiana residents (Alper et al., 2001). Reported ibogaine scenes in Pakistan and Thailand were not included due to lack of independent verification. Due to the inability to obtain quantitative data, the study did not include a sample of probably about 20 individuals who were provided ibogaine by Dan Lieberman, a South African ethnobotanist who died in a motor vehicle accident in August 2000. Psychologist Leo Zeff and others administered ibogaine (typically as a single doses in the range of 150–300 mg) and other hallucinogens as an adjunct to psychotherapy beginning in the 1950s in a scene that existed on the West Coast of the US (Stolaroff, 2004) that was excluded due to a lack of quantitative data.

2.4. Data collection

The data collection for this study began with a previously published description and history of the ibogaine subculture as of early 2001 (Alper et al., 2001). The authors subsequently continued their contact with the ibogaine subculture by email, phone, and in person. Quantitative information that was assessed systematically from the providers included cumulative numbers of people treated, percentage seeking treatment for addiction and specifically acute opioid withdrawal, as well as ibogaine form and dosage and the cost of treatment. The approach to pretreatment medical screening and laboratory evaluation, and monitoring during the treatment was also discussed. The estimates of cumulative numbers of subjects treated obtained from providers are current as of February 2006 except for the figure for the St. Kitts Clinic, which is taken from an abstract published in June 2005 (Mash et al., 2005).

To determine if any further scenes existed in addition to those of which the authors were aware, in May of 2005 a series of messages was posted to Mindvox (Kroupa, 2006), the most frequently used ibogaine list server. The Internet, which is an important aspect of the ibogaine subculture and comprises an extensive unpublished "grey literature" (Boukacem-Zeghmouri and Schöpfel, 2006) was searched monthly from May 2005 to February 2006 using the terms "ibogaine" or "iboga" alone, and combined with the term "treatment". The list server postings and Internet searches yielded no usable information regarding new scenes that had not already been previously obtained by longstanding, ongoing contact with subculture participants. In May of 2005, and again near the conclusion of data gathering in February 2006, all known treatment providers were systematically contacted to update the quantitative information. The typology of scenes was created between the first and second data collections.

The study also reviewed the academic literature, and the "white literature" (Boukacem-Zeghmouri and Schöpfel, 2006) including public media and officially published government or industrial documents. Databases with white literature content including ProQuest, LexisNexis, and the New York Times, and academic literature databases including PubMed, PsycInfo, JSTOR, UMI Dissertation Abstracts, WorldCat, and the SAGE Sociology Full-Text Collection were searched utilizing the terms

"ibogaine" and "iboga". The references cited by the articles retrieved utilizing the above searches were reviewed until they no longer yielded new references containing the search terms. The above searches yielded relatively little material that was new to the authors because of their extensive prior use of the *iboga* alkaloid conventional and nonconventional literature, as well as substantial access to ibogaine-related material that is not indexed in any searchable database (Lotsof, 1985; Alper, 2001; Alper et al., 2001; Lotsof and Wachtel, 2003; Lotsof, 2007).

2.5. Data validation

Triangulation of the data, i.e., viewing the data from multiple observational perspectives (Malterud, 2001; Denzin and Lincoln, 2005), was possible for all of the currently operating scenes listed in Table 1 on the basis of independent corroboration from provider and patient participants in the same scenes, providers regarding other providers, and other informants. Most treatment providers interviewed in the present study had previously supplied data that was published 5 years earlier (Alper et al., 2001) and were known to at least two of the authors. For three medical model scenes that no longer exist (Lexington 1955–1956, Santiago 1966–1967, and Zürich 1980–1989) textual evidence was used for validation. This study omitted providers who had not publicly disclosed their activity, which would tend to lead toward underestimation of the total numbers of individuals who have taken ibogaine. This is particularly likely for scenes involving small numbers of patient participants, such as individuals obtaining ibogaine from the Internet. In order to account for this effect, estimates of hidden populations were obtained from individuals with extensive contact with the subculture as described below in Section 3.2.

2.6. Data analysis

A typology of scenes was constructed (Bailey, 1994), based on the classificatory dimensions of setting and the provider's set and credentials. The *setting* is the physical and ecological location in which the treatment takes place: a clinic or hospital, a private residence or hotel, or a religious shrine. A *provider* is an individual or group that administers ibogaine to the patient participant, and specifies the form and dose to be given. The provider determines the parameters of the treatment such as setting, inclusion and exclusion criteria, and medical monitoring. Providers may or may not have a credential as a licensed physician. The provider's set consists of the beliefs, expectations, attitudes and motivation that determine the intention to provide ibogaine. Provider set subsumes beliefs and expectations regarding ibogaine as a treatment for substance-related disorders, a psychotherapeutic adjunct, or religious sacrament. Motivational aspects of set may include the giving of care, activism, or ritual.

Quantitative data included the number of individuals who took ibogaine, and the number who took it for the treatment of a substance-related disorder, and specifically for acute opioid withdrawal. This data for each scene was included in Table 1, and the totals for each of the four types of scenes are indicated in Table 2.

3. Results

3.1. Typology

As indicated in Table 1, four types of scenes were identified and classified on the basis of the features of treatment setting, provider credentials and provider set; "medical model", "lay provider/guide", "activist/self-help" and "religious/ceremonial".

3.1.1. Medical model

In this type of scene the provider is a licensed physician. In the variation of the medical model type involving clinical research, some roles of the provider are distributed among authors of the study protocol and the physician investigator who prescribes ibogaine. Settings of the medical model are medical hospitals or clinics, or clinical research facilities, which are officially credentialed according to national and local requirements in a given country, as well as offices or residential settings in the case of treatment intended as psychotherapy. The set of the treatment provider includes the aim of emulating existing conventional medical standards in the treatment of addiction, clinical research, and/or psychotherapy.

Historically, the use of ibogaine in the medical model began in the 1950s, when clinicians and researchers viewed ibogaine much as they did other compounds classified as hallucinogens. Some, such as Jan Bastiaans, M.D. (Snelders and Kaplan, 2002), Leo Zeff, Ph.D. (Stolaroff, 2004), and Claudio Naranjo, M.D. (Naranjo, 1973), were interested in ibogaine as an adjunct to psychotherapy. Ibogaine, like other hallucinogens, was of interest as an experimental model of psychosis (Turner et al., 1955; Fabing, 1956; Salmoiraghi and Page, 1957; Schneider and Sigg, 1957). As with other hallucinogens, ibogaine may have also been investigated for military or intelligence purposes as a "truth serum", or a means of "brainwashing" or incapacitating an adversary which was the focus of MKULTRA (1977), a US Central Intelligence Agency project acknowledged to have existed from 1953 until 1964. Harris Isbell, M.D., an apparent participant in the MKULTRA project, directed the Addiction Research Center in Lexington, Kentucky where he reported administering ibogaine to human subjects in a letter to the Ciba Pharmaceutical, the manufacturer at that time (Isbell, 1955).

The medical treatment model presently exists mainly in countries adjacent to the US, such as Mexico, where ibogaine is subsumed within a physician's legal prerogative to prescribe experimental treatment, or Saint Kitts, where the government includes ibogaine in its national formulary and provides specific approval to the clinic there to administer it. The most common setting is a private clinic with less frequent use of hospitals. The clinics' Web sites tend to emphasize images that suggest comfort, safety, and the experience and expertise of the clinical team.

The standard of care varies among scenes in the medical model, but typically at a minimum involves pretreatment laboratory and electrocardiogram (EKG), vital signs and evaluation

Table 1
Ibogaine scenes: quantitative and descriptive features, grouped by scene type

Scene, year began- (year ended, if applicable)	Reason for taking ibogaine: *n*[a], *n* (%)[b] [*n* (%)][c]	Other non-substance-related reason for taking ibogaine	Dose/form	Setting/provider, medical evaluation and monitoring	Cost
Medical model type					
US, 1955 Lexington, KY (Isbell, 1955)	8 (research, subjects not seeking treatment)	Research, determination of psychoactive threshold	50–300 mg ibogaine HCl	Clinical research, US Public Health Service Hospital, Lexington, KY; Harris Isbell, M.D. Subjects were prisoners with prior histories of opioid dependence who had been abstinent for periods of months	Volunteers/prisoners
Chile, 1966–1967 Santiago (Naranjo, 1973)	30 None	Adjunct to psychotherapy	3–5 mg/kg ibogaine HCl	Claudio Naranjo, M.D., psychiatrist. Ibogaine administered in office setting in context of ongoing psychotherapy	N/A
Switzerland, 1980–1989 Zürich (Prins, 1988)	34 None	Adjunct to psychotherapy	4–10 mg/kg ibogaine HCl	Peter Baumann M.D., psychiatrist. Ibogaine administered in office or residential setting, given in context of ongoing psychotherapy	N/A
US, 1994–1995 Miami FL (Mash et al., 1998)	15 (Phase I clinical trial subjects not seeking treatment)	FDA approved clinical research	1–4 mg/kg ibogaine HCl	Phase I/II dose-ranging study. Juan Sanchez-Ramos, Ph.D., M.D., Principal Investigator, Deborah Mash, Ph.D., Co-Investigator. Jackson Memorial Hospital in Miami	Volunteers
Panama, 1994–1995 Panama City (Luciano, 1998)	11, 11 (100%) [9 (82%)]	Substance dependence only	10–25 mg/kg ibogaine HCl	Hospital Centro Medico Paitilla, full medical staff. Pretreatment evaluation included EKG, blood chemistry, medical and psychiatric history	No cost to $35,000 USD
Brazil, 1994–Sao Paulo (Sandberg, 2006)	9, 9 (100%) [1 (11%)]	Substance dependence only	10–20 mg/kg ibogaine HCl	Hospital Maternidade Maria Perpetua Piedade Goncalves, full medical staff. Pretreatment evaluation includes EKG, blood chemistry, medical and psychiatric history	$3000 USD
St. Kitts, 1996- (Mash et al., 2001)	400, 400 (100%) [316 (79%)][d]	Substance dependence only	600–1200 mg ibogaine HCl	See text for description of the approach to medical evaluation and monitoring developed by Jeffrey Kamlet, M.D	$10,000–$12,500 USD
Mexico, 2001– Playas de Tijuana, Baja California (Ibogaine Association, 2006)	283, 252 (89%) [186 (74%)]	psychotherapeutic, spiritual	12–18 mg/kg ibogaine HCl	Clinic or hospital. Pretreatment evaluation includes EKG, blood chemistry, medical and psychiatric history. Continuous EKG monitoring and presence of a nurse in the room with the patient during the treatment	$4000 USD
Mexico, 2005– Cancun (villaserena.org, 2006)	34, 34 (100%) [6 (18%)]	Substance dependence only	8–18 mg/kg ibogaine HCl	Clinic, private rooms. See text regarding medical evaluation and monitoring	$6000 USD

Lay provider/guide type					
US, 1962–1963 New York City (Lotsof and Alexander, 2001)	20; none sought treatment, 7 were opioid dependent	Lay experimentation and research, Psychotherapeutic	0.14–19.0 mg/kg ibogaine HCl	Apartments, private homes. No medical support. Self-administration and systematic self-observation	$15 USD for 500 mg
Central America, Caribbean, 1993– (Taub, 2006)	607, 455 (75%) [309 (68%)]	Psychotherapeutic, spiritual	9–36 mg/kg ibogaine HCl	Rented cottages in resort settings, private residences. Pretreatment medical and psychiatric history, EKG and blood chemistry	No cost to $4000 USD
Italy/France, 1994–(Naeher, 2006)	101, 44 (44%) [34 (77%)]	Psychotherapeutic, spiritual	10–23 mg/kg ibogaine HCl	Apartment. Physician available. Pretreatment medical and psychiatric history, EKG and blood chemistry	$1500 USD
Netherlands, 1999– Breukelen (Glatt, 2006)	200, 160 (80%) [144 (90%)]	Psychotherapeutic, spiritual	2–6 g *Tabernanthe iboga* extract (estimated 15% ibogaine)	Private home. Use of other "plant medicine or fungi" in combination with ibogaine. Pretreatment medical and psychiatric history, no medical testing	No cost to $2000 USD
Czech Republic, 2000– (Mariano, 2006)	102, 94 (92%) [73 (71%)]	Psychotherapeutic, spiritual	900–1600 mg ibogaine HCl	Apartments and private homes. Medical assessment by local consulting clinic, including medical and psychiatric history, EKG and blood chemistry	£600 GBP
UK, 2000– London, West Sussex (Conn, 2006; Wells, 2006)	83, 54 (65%) [46 (85%)]	Psychotherapeutic, spiritual	14–20 mg/kg, or 250–2000 mg ibogaine HCl	Multiple Providers; apartment or private home. Pretreatment medical and psychiatric history, EKG and blood chemistry	£400–£850 GBP
Canada, 2002–Vancouver, Toronto (ibogatherapyhouse.net, 2007)	64, 52 (81%) [36 (69%)]	Psychotherapeutic, spiritual	16–23 mg/kg ibogaine HCl	Multiple Providers; dedicated clinic, private residences. Pretreatment medical and psychiatric history, EKG and blood chemistry. Emergency medical technician on premises during treatment at clinic	$1000–$3500 CAD
South Africa, 2004–Eldoraigne (Rossouw, 2006)	36, 36 (100%) [23 (64%)]	Substance dependence only	15–19 mg/kg ibogaine HCl	Treatments conducted in private residences. Pretreatment medical and psychiatric history, EKG and blood chemistry. Arrangement for very rapid response emergency medical support	$3000 USD
Activist/Self-Help type					
Netherlands, 1989–1993 Rotterdam, other Dutch cities (Alper et al., 2001)	40, 40 (100%) [37 (93%)]	Substance dependence only	10–29 mg/kg ibogaine HCl	Multiple treatment providers; private residences and hotels. Pre and post-treatment medical evaluation. Strong involvement of activist drug user network	No cost to $18,000 USD
US, 2003– New York, San Francisco, other U.S. cities (Freedomroot.com, 2007)	160, 160 (100%) [152 (95%)]	Substance dependence only	21–24 mg/kg ibogaine HCl	The "ibogaine underground"; multiple treatment providers. Private residences and hotels. Pretreatment medical and psychiatric history, EKG and blood chemistry	No cost to $1500 USD
Religious/Ceremonial type					
Slovenia/Croatia, 1995– Ljubljana (Sacrament of Transition, 2006)	433, 424 98% [403 (95%)]	Psychotherapeutic, spiritual	20 mg/kg ibogaine HCl	Religious Ritual, treatment guide/priest. The Republic of Slovenia officially recognizes the Church of the Sacrament of Transition as a religion. Private homes. Initiates are interviewed and sign a statement attesting to their good health without clinical evaluation	€750 EUR

Table 1 (*Continued*)

Scene, year began- (year ended, if applicable)	Reason for taking ibogaine: *n*[a], *n* (%)[b] [*n* (%)][c]	Other non-substance-related reason for taking ibogaine	Dose/form	Setting/provider, medical evaluation and monitoring	Cost
France, 2000- (Meyaya, 2006)	378 45 (12%) [36 (80%)][d]	Bwiti initiation, psychotherapeutic, spiritual, general health issues	Dried root bark, 6–10 teaspoons[e]	Bwiti Religious ritual. Chateau, private residences. Nganga (ritual leader or priest) with African and European assistants. Requires only a doctor's or the prospective patient's statement assuring good health without clinical evaluation.	€ 650 EUR
France/UK, 2003–(myeboga.com, 2006)	316, 32 (10%) [6 (19%)]	Bwiti initiation, psychotherapeutic, spiritual, general health issues	Dried root bark 3–4 teaspoons[e]	Bwiti religious ritual, retreat. Resort settings, private residences. European initiates of the African Bwiti tradition with African and European assistants. Pretreatment medical and psychiatric history, blood chemistry and EKG. Medical doctor in attendance throughout the treatment	€500 EUR
Gabon, 1999– (Ebando, 2006)	50, 8 (16%) [5 (63%)]	Bwiti initiation, psychotherapeutic, spiritual, general health issues, fertility	Infusion, 20–25 teaspoons of fresh root bark scrapings	Bwiti religious ritual. Bwiti chapels in which Europeans and non-Africans are accepted. Prospective interview by a Nganga, without clinical evaluation	$4000 USD (for 1 month stay)

[a] *n* = number of individuals within each scene who took ibogaine.

[b] *n* (%) = subset of *n* who took ibogaine for the treatment of any substance-related disorder, expressed as absolute number, and as (%) of *n*.

[c] [*n* (%)] = subset of *n* who took ibogaine for the treatment of opioid withdrawal expressed as absolute number, and as (%) of the number of individuals who took ibogaine for the treatment of any substance-related disorder.

[d] Data regarding the number of individuals taking ibogaine for opioid withdrawal among all those who took ibogaine for the treatment of any substance-related disorder were missing for the St. Kitts and France 2000 scenes. For these scenes, the proportion seeking treatment specifically for opioid withdrawal relative to all those seeking treatment for any substance-related disorder was assumed to be the same as the rest of the sample. This yielded estimates of ($0.79 \times 400 = 316$) for St. Kitts, and ($0.79 \times 45 = 36$) for France 2000.

[e] One teaspoon of dried *Tabernanthe iboga* root bark weighs approximately 2–3 g. Also often referenced as a "coffee spoon" by French speaking providers.

Table 2
Numbers of individuals and reason for having taken ibogaine in each the four types of scenes

Type of scene	n[a]	Non-substance-related (%)[b]	Substance-related (%)[b]	Opioid[c] (%)[b]	%Opioid[d] of substance-related (%)
Medical model	824	118 (14%)	706 (86%)	518 (63%)	74
Lay provider/guide	1213	318 (26%)	895 (74%)	665 (55%)	74
Activist/self-help	200	0	200 (100%)	189 (95%)	95
Religious/ceremonial	1177	668 (57%)	509 (43%)	450 (38%)	89
Total	3414 (=N)[e]	1104 (32%)[f]	2310 (68%)[f]	1822 (53%)[f]	79

Summary data regarding numbers of individuals who took ibogaine and their reason for taking it, from the second column of Table 1 entitled "Reason for taking ibogaine. . .". The rows in this table summarize the data from the individual scenes for each type.

[a] n = total number of individuals who took ibogaine within each of the four types of scenes indicated in the left hand column.

[b] Percentage of n.

[c] Opioid = number of individuals who took ibogaine for the treatment of opioid withdrawal in the given type of scene.

[d] %Opioid of substance-related = percentage of individuals who took ibogaine for the treatment of opioid withdrawal relative to the number of individuals who took ibogaine for the treatment of any substance-related disorder.

[e] N = the total number of individuals who took ibogaine across all scenes.

[f] Percentage of N.

of the medical and psychiatric history, and some participation of nurses and physicians. The most intensive approach appears to have been that developed at the St. Kitts clinic (see Table 1). A recently started clinic in Mexico reported a similar clinical standard (villaserena.org, 2006). Prior to treatment with ibogaine, opioid dependent patients are converted to equivalent doses of orally administered short acting opioids. All centrally acting medications are tapered and discontinued for at least three serum half lives, although no specific drug interactions with ibogaine have yet been identified. Evaluation includes pretreatment Holter monitor and 12 lead EKG, and the following continuously during the treatment: EKG, vital sign and pulse oximetry monitoring, intravenous access, and the presence on site of an emergency physician with advanced cardiac life support certification and a registered nurse in the room with the patient continuously throughout the treatment (Mash et al., 2000, 2001).

3.1.2. Lay provider/guide

The term lay provider designates a provider without an official medical credential. The set and purpose of the treatment may be the medical treatment of addiction, psychotherapy, and/or spiritual growth. The dosage utilized for "psychospiritual" goals is typically on the order of half that required for opioid withdrawal. The treatment setting is a private residence or hotel, and the provider typically functions in the role of a "guide", or in the UK, a "sitter", and manages the treatment setting and the interaction with the patient with the goal of facilitating the therapeutic process. The treatment is conducted in a quiet, darkened room over a time interval of 12–18 h. Interaction with the patient is typically minimized during the treatment unless the patient initiates verbal communication because of the importance attributed to the patient's focus on the content of the experience. Some guides view ibogaine as unlinking mental representations from the pathological salience and obsessive motivational states with which they have become associated, allowing an opportunity for insight and positive change (Stolaroff, 2004). Goutarel et al. (1993) described the use of ibogaine in dosages of 10–50 mg as an antidepressant, and some contemporary lay providers presently use similar dosages given daily over periods of several days or weeks, to which they attribute an antidepressant effect or the diminution of craving (Kroupa and Wells, 2005). Interestingly, the low dose regimen is also reportedly used to limit or reduce opioid tolerance, which is an effect attributed to ibogaine in a patent obtained by Ciba Pharmaceutical 50 years ago (Schneider, 1957), and has been observed in subsequent preclinical research (Cao and Bhargava, 1997).

Regardless of their beliefs concerning ibogaine's psychotherapeutic benefits, lay treatment providers are aware of medical risk, which they make some attempt to minimize. A downloadable manual for ibogaine treatment (Lotsof and Wachtel, 2003) reflects collective views among lay providers regarding clinical issues such as the use of exclusion criteria and pretreatment laboratory tests, assuring adequate hydration during a treatment, or contingencies for accessing emergency medical intervention. A significant consensus exists among lay providers regarding the use of EKG and liver function tests in pretreatment screening, and with respect to a set of medical and psychiatric conditions are commonly designated as exclusionary such as cardiac disease, acute hepatitis and psychotic disorders.

3.1.3. Activist/self-help

This type of scene involves a lay provider with an activist or evangelical set that prominently includes the explicit objective of gaining acceptance of the use of ibogaine. Activist self-help providers often view their activities as a form of civil disobedience affirming the right to better treatment for a stigmatized group. A nexus exists involving the harm reduction movement and the ibogaine subculture. The ibogaine scene that existed in the Netherlands from 1989 to 1993 featured strong participation of European and U.S. addict self-help including the Dutch Junkiebond, which was a model for subsequent European drug user unions and a vanguard of the harm reduction movement (Grund, 1995; De Rienzo and Beal, 1997; Alper et al., 2001; Frenken, 2001; Lotsof and Alexander, 2001). The "ibogaine underground", or "Freedomroot" (Freedomroot.com, 2007) is a scene that recently emerged in the U.S. that recalls the earlier

aggressive advocacy in the Netherlands. It is a network of individual providers, many of whom themselves are former patient participants in the subculture, who actively reach out to heroin users in New York and some other US cities.

The following quote from a post to an ibogaine list server captures some important attitudes and beliefs of the subculture associated with the activist/self-help type. These include the identification of individuals with severe opioid dependence as a marginalized population abandoned by the institution of conventional medicine, the theme of self-help, and the attribution of aspects of the medical model to "underground providers", who are referenced sympathetically as doing "most of the research".

> ". . .No one with the money and clout to do so wants to touch ibogaine. . . The reasons are numerous, from its illegal status in some places, to the stigma attached to drug addiction to begin with . . . with the result that most of the research is being done by underground providers who only have lists like this and the internet to help share information with each other. I can tell you from personal experience with an 8+ year opiate addiction . . . if it wasn't for ibogaine I doubt I would be clean today, two and a half years later. There are many more people on this list who can also tell you the same thing from their own personal experience. It's a risk to be sure. The risk of death, and the risk that it might not work . . . But for me it came down to the fact that absolutely nothing else had worked for me . . . in the end it was through ibogaine that I finally got clean."

3.1.4. Religious/ceremonial

Scenes of this type involve a lay provider and the setting of Bwiti religious shrine in Africa, or any residential, or hotel or resort setting intended to provide a religious or ceremonial context. A provider set of identification with traditional Bwiti ritual culture may exist in scenes in either Gabon or Europe. The scenes listed here are those involving participants from Europe or the US. Individuals who take ibogaine in these scenes tend to be seeking a spiritual experience, although even in the religious scene type about a third of participants primarily seek treatment for substance dependence. As in Africa, there are also those who seek to use ibogaine in a traditional context as treatment for medical illness or infertility.

In their comparative analysis of the African Bwiti religious context and an addict self-help scene, Fernandez and Fernandez (2001) identify the construct of personal transformation, guided by insight or new knowledge mediated by iboga/ibogaine, as a common feature of central importance, and reference ibogaine as a "transitional alkaloid". The similarly named Sacrament of Transition (2006) is a ritual context of Western creation that is officially recognized as a religion in Slovenia with a large proportion of participants who took ibogaine for heroin withdrawal. The Bwiti theme of personal transformation is shared among diverse religious cultures, regardless of the ritual use of hallucinogens, and in their own narratives patients in conventional treatment settings frequently characterize recovery from substance dependence as a spiritual transformation (Galanter, 2006).

3.2. Quantitative data

Table 2 summarizes the quantitative data regarding numbers of individuals taking ibogaine and their reason for taking it, totaled across each scene type from Table 1. The total number of individuals across all scenes who have taken ibogaine is 3414. This is approximately a fourfold increase relative to the estimate of 857 of 5 years before based on previously published quantitative data from early 2001 (Alper et al., 2001). As indicated in table, 68% of the total number of individuals across all scenes took ibogaine for the treatment of substance-related disorders, and 53% specifically for opioid withdrawal.

The effect of hidden populations would lead to underestimation of the true number of participants in the ibogaine subculture. In order to estimate this effect, the editors of the most frequently utilized ibogaine list server (Kroupa, 2006), and a popular ibogaine Web site (Sandberg, 2006) were asked to blindly and independently estimate the "hidden proportion", i.e. the proportion of participants in the ibogaine subculture in scenes that would have been overlooked by the criteria used in this study. Both estimates of the hidden proportion fell within a range of 20–30% (personal communication, Patrick Kroupa, December 3, 2006 and Nick Sandberg, December 4, 2006), as did the hidden proportion of an unpublished sample (Bastiaans, 2004), suggesting that most treatments involve experienced providers who are open about their activity. These sources agreed regarding the view that opioid withdrawal was the most common reason for taking ibogaine, and that the subculture had expanded greatly over the prior 5 years. Taking the hidden proportion estimates into account yields an estimated range of approximately 4300–4900 individuals who took ibogaine outside of Africa as of February 2006.

4. Discussion

4.1. A medical subculture, distinct from other drug subcultures

The clinical focus on the treatment of opioid withdrawal distinguishes the ibogaine subculture from subcultures associated with psychedelic or other illegal drugs. The reason for taking ibogaine was more frequently to alleviate the symptoms of opioid withdrawal than to pursue spiritual or psychological goals. In the US, the expansion of the ibogaine subculture coincides temporally with a substantial increase in the public health impact of opioid use disorders (Compton and Volkow, 2006). The incidence of opioid-related deaths in the US doubled between 1999 and 2004 (Fingerhut, 2007), with methadone and oxycodone accounting for most of this increase. In contrast to trends regarding opioids, there was no increase in use of hallucinogen and MDMA among young adults in the US between 2002 and 2005 (Substance Abuse and Mental Health Services Administration, 2006), suggesting that the recent expansion of the ibogaine subculture is not an epiphenomenon of popular interest in psychedelic drugs and the availability of psychoactive substances on the Internet (Schifano et al., 2006).

The ibogaine subculture is not a counterculture (Yinger, 1960) because its identity is not defined on the basis of opposition to conventional medicine. The subculture is to a significant extent an innovation by its participants in response to a demand for a treatment that is unavailable in the conventional medical setting. Although it involves alternative means, the ibogaine subculture shares with the conventional medical culture the common goal of providing treatment, which it emulates in the medical model type, or the utilization by lay treatment providers of medical tests for pretreatment evaluation. Criminality per se is not a significant focus of the subculture, which exists because of ibogaine's lack of availability within the institution of clinical medicine, and not its illegality. Ibogaine is not illegal and available by Internet in most of the world. It is illegal in the US, Australia and five EU countries, but it is available throughout Europe and the Americas including Canada and Mexico.

4.2. Study limitations and methodological concerns

The issue of observer subjectivity is an important concern regarding the participant–observer approach. The need to establish rapport and elicit collaboration and disclosure may conflict with the imperative of scientific objectivity (Jackson, 1983; Malterud, 2001), and beliefs and attitudes that motivated interest in ibogaine and provided contacts and access within the subculture are also a potential cause of bias. Qualitative methodology acknowledges that the problem of observer subjectivity always exists, and addresses it by and attempting to account for its effect by the use of multiple validating approaches (Malterud, 2002). Validating approaches utilized in this study include triangulation of the data across providers, patient participants and other informants, as well as longitudinal observation involving multiple contacts over time.

The authors' collective access and intensive observation of the ibogaine subculture suggests that this study approaches an exhaustive, and not merely representative sampling of publicly identified ibogaine scenes. The intensiveness of the sampling in this study indicates that most of the use of ibogaine outside of Africa has taken place in the scenes that are included in Table 1, even taking into account the problem of hidden populations.

4.3. Implications of the "vast uncontrolled experiment"

Frank Vocci, who oversaw NIDA's ibogaine project as the head of Medications Development (Vastag, 2005), characterized the ibogaine subculture as a "vast uncontrolled experiment". The term has significant literal merit. The data on *iboga* alkaloids collectively subsumes significant elements of a drug development process in various stages of completion, including substantial preclinical and open label study evidence, preclinical toxicological studies, and some initial Phase I safety and pharmacokinetic data.

Reports of efficacy of ibogaine in opioid withdrawal may be valid irrespective of the methodological limitations associated with the clinical settings in which ibogaine is presently used. Unlike other outcomes such as post-treatment drug abstinence or craving, the clinical expression of acute opioid withdrawal occurs within a limited time frame, is easily operationalized, tends to be robust, and can be assessed accurately by typically experienced lay providers. It appears unlikely that suggestion or placebo could solely mediate the effect attributed to ibogaine in acute opioid withdrawal. Recent Cochrane reviews on the management of acute opioid withdrawal with α_2-agonists (Gowing et al., 2004), buprenorphine (Gowing et al., 2006) or methadone taper (Amato et al., 2005) evaluated a combined total of 56 studies. Overall rates of treatment completion among studies of α_2-agonists, buprenorphine, or methadone, respectively ranged from 48 to 64%. Only 3 of the 56 studies included a placebo comparison, and all indicated a strong distinction of placebo from any active drug treatment at a level of significance of $p < .001$ on the basis of more frequent failure to complete detoxification (Benos, 1985; San et al., 1992) or higher ratings of withdrawal symptoms (Gerra et al., 1995) in the placebo group. The relatively negligible effect of placebo in acute opioid withdrawal is likely further reduced with the greater severity of physical dependence that is generally characteristic of individuals who take ibogaine, such as a series of 33 patients whose average daily heroin use was 0.64 g, mainly by the intravenous route (Alper et al., 1999; Frenken, 2001).

The authors are aware of a total of 11 individuals that are reported to have died within 72 h of taking ibogaine from the time of the first such fatality in 1990 (Alper, 2001) until February 2006. Collectively, the cases suggest that cardiac rhythm may be a particularly significant domain of medical risk. Deaths were most commonly attributed to a cardiac cause in association with significant risk factors such as a prior myocardial infarction, cardiomyopathy or valvular disease, or to pulmonary embolus. Other deaths were regarded as mixed drug overdoses involving opioids with or without the additional ingestion of cocaine (Alper et al., 1999; Marker and Stajic, 2002). Deaths not involving the above factors have been associated with the use of *Tabernanthe iboga* alkaloid extract (Alper, 2001) or dried root bark (Kontrimaviciute et al., 2006b). This subset of fatalities may reflect a general hazard associated with the use of indigenous ethnopharmaceutical forms outside of their traditional context by the uninformed or inexperienced (Callaway and Grob, 1998; Maas and Strubelt, 2006). For example, one death (Kontrimaviciute et al., 2006b) involved ingestion of an amount of powdered dried root bark that is at least twice the maximum upper limit used by the providers in this study who are traditional African Bwiti adepts. Other potential hazards may be associated with the lack of pharmaceutical standards for ibogaine, including unregulated procedures for manufacturing and storage or the possibility of naturally co-occurring toxic alkaloids (Singbartl et al., 1973; Jenks, 2002; Kontrimaviciute et al., 2006a).

Cerebellar Purkinje cell degeneration reported in rats administered ibogaine at high dosages of 100 mg/kg (O'Hearn and Molliver, 1993, 1997) prompted concern regarding potential neurotoxicity. The FDA was aware of this research at the time it approved the Phase I study; other work indicated no evidence of neurotoxicity rats at the dosage of 40 mg/kg typically used to

study drug self-administration and withdrawal (Molinari et al., 1996). Evidence of neurotoxicity due to ibogaine is reportedly absent in mice (Scallet et al., 1996), primates (Mash et al., 1998) and a postmortem neuropathological examination of a woman who had taken ibogaine four times in the prior 15 months at doses up to 30 mg/kg. In the Phase I study, quantitative dynamic measures of cerebellar motor function were unremarkable in human subjects that received low doses of ibogaine of 1 and 2 mg/kg (Mash et al., 1998). The study was never finished due to contractual disputes with eventual litigation among the study sponsors, unrelated to clinical issues. The σ_2 receptor mediates neurotoxic injury (O'Hearn and Molliver, 1997; Bowen, 2001), and is apparently not involved in effects of ibogaine on drug self-administration and withdrawal (Glick et al., 2001). This suggests that the therapeutic and neurotoxic effects of *iboga* alkaloids can be resolved from one another by rational drug design, as indicated by the example of 18-MC, which has lower affinity for the σ_2 receptor and is not associated with evidence of neurotoxicity even at very high dosages (Maisonneuve and Glick, 2003).

4.4. Suggestions for future research

Experimental pharmacologists are increasingly interested in the development of approaches to addiction that extend beyond the present repertoire of agonist or antagonist actions, and instead are targeted at effects on intracellular signaling downstream from the receptor (Bonci and Carlezon, 2005). Ibogaine may provide a prototypic example of an agent with such novel mechanisms of action. Future work should replicate and extend on prior research indicating that *iboga* alkaloids modulate signal transduction in second messenger pathways linked to G protein-coupled receptors (GPCRs) (Rabin and Winter, 1996a,b).

Constitutive spontaneous activity without the binding of an agonist (Costa and Cotecchia, 2005) occurs in GPCRs such as opioid receptors (Shoblock and Maidment, 2006). Constitutive signaling mediated by conformational states of receptor-associated proteins may be modulated relatively rapidly and span a wide signal range, consistent with a possible role in the highly dynamic neuroadaptations associated with opioid tolerance and withdrawal. It may be worthwhile to investigate the possibility that *iboga* alkaloids interact allosterically or orthosterically with GPCRs to affect constitutive signaling. A possible role of orphan receptors should be also considered (Civelli et al., 2006).

Functional and clinical evidence of muscarinic cholinergic actions of *iboga* alkaloids includes the occurrence of a state with some neurophysiological and behavioral features common to REM sleep (Schneider and Sigg, 1957; Depoortere, 1987; Goutarel et al., 1993; Alper, 2001), and recent work suggesting that muscarinic acetylcholine receptors (mAChRs) as well as nAChRs in the habenulopeduncular pathway mediate the effects of 18-MC on dopamine efflux in the NAc (Taraschenko et al., 2007a,b). Ibogaine interacts with cholinergic neurotransmission in multiple ways; as a strong antagonist at nAChRs (Daly, 2005), binding to mAChRs with affinities on the order of approximately 10 μM with actions that are not well characterized with regard to antagonist versus agonist effects, and according to an older literature, inhibition of acetylcholinesterase (Vincent and Sero, 1942). The knockout mouse, which exists for each of the five basic subtypes of mAChRs (Wess et al., 2003), provides an in vivo approach that makes it possible to study functional correlates of activity at mAChRs such as the EEG or cardiac electrophysiology, as well as the role of mAChRs in ibogaine's effects on models of substance-related disorders.

Structure–function relationships mediating toxic and therapeutic effects of *iboga* alkaloids have been identified and utilized to guide rational synthesis (Glick et al., 1994; Kuehne et al., 2003; Maisonneuve and Glick, 2003). Preclinical toxicological testing, and if appropriate, clinical research on *iboga* alkaloids will require the development of pharmaceutical synthetic and chemical manufacturing technology in order to produce adequate quantities of investigational drug in conformance with international Good Manufacturing Practice standards. The chemical, manufacturing and control stage of pharmaceutical development generally is accomplished in the private sector, but the pharmaceutical industry historically has shown less interest in developing drugs for substance-related disorders relative to other indications (Gorodetzky and Grudzinskas, 2005), indicating an important need for involvement of the public sector.

5. Conclusions

The estimated number of participants in the ibogaine subculture increased fourfold relative to the prior estimate of 5 years earlier, an average yearly rate of growth of approximately 30%. The existence and expansion of the subculture indicates a demand for new treatment, which is sought regardless of medical risk, inconvenience, expense, and in some cases legal prohibition. Across a diversity of settings, most individuals who took ibogaine did so for the treatment of a substance-related disorder, specifically for opioid withdrawal. Ibogaine's effect in opioid withdrawal is consistent with case series and preclinical evidence, and is unlikely to be mediated by placebo. The mechanism of ibogaine's action in opioid withdrawal merits further investigation as a paradigm for neurobiological research and rational pharmaceutical development.

Conflict of interest statement

We declare that we have no conflict of interest. Howard Lotsof was awarded multiple patents on the use of ibogaine in substance-related disorders, which he divested in 1998.

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Ibogaine, an anti-addictive drug: pharmacology and time to go further in development. A narrative review

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Ibogaine is an indole alkaloid derived from the bark of the root of the African shrub *Tabernanthe iboga*. Psychoactive properties of ibogaine have been known for decades. More recently, based on experimental data from animals and anectodal reports in human, it has been found that this drug has anti-addictive effects. Several patents were published between 1969 and 1995. The pharmacology of ibogaine is quite complex, affecting many different neurotransmitter systems simultaneously. However, the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood. Ibogaine is rapidly metabolized in the body in noribogaine. The purpose of this article was to review data from the literature concerning physicochemical properties, bio-analytical methods, and pharmacology of ibogaine; this article will be focused on the use of this drug as anti-addictive agent.

Key words: bioanalytical methods; ibogaine; noribogaine; pharmacodynamic studies; pharmacokinetics; safety

Introduction

The development process of the medicinal product is a system consisting of many operational aspects designed to solve certain organizational, scientific, and regulatory questions.[1,2] Ideally one can have a clear view about this system, meet the needs, and have a product on the market. The real problems are the practicalities that hinder implementation of ideal principles and make product failed. Bibliographical review of ibogaine development might be one of the learning case studies that we can learn from others. Ibogaine is one of the psychoactive indole alkaloids naturally occurring in the West African shrub *Tabernanthe iboga*. The major components of *T. iboga* root bark extracts are ibogaine (approximately 80%), ibogaline (15%), and ibogamine (up to 5%), which confirms the complexity of the extract.[3] From the results of the preclinical studies and anecdotal reports from American and European addict self-help groups, ibogaine could be a promising drug in addiction therapy. Unfortunately, lost opportunities to confirm a positive benefit risk balance during both preclinical and clinical developments as well as losses of financial supports have lead to the stopping of the ibogaine development in the treatment of drug dependence. Future will show the strategy one will obtain and outcome thereof – will we forget about this novelty in addictive therapy or will we have a finalized development.

The mechanism of action of ibogaine in the treatment of drug addiction appears to be distinct from other existing pharmacotherapeutic approaches. The purpose of this article was to review data from the literature concerning physicochemical properties, bio-analytical methods, and pharmacology of ibogaine; this article will be focused on the use of this drug as anti-addictive agent.

To identify articles for this review, we use Internet-based Grateful Med to access electronic databases: MEDLINE and Currents Contents 1957–2007. We searched, without language limitations, for the subject terms "ibogaine", "noribogaine", "mechanism of action", "quantification", "pharmacokinetics", and "pharmacodynamics". We further narrowed the search by using the terms "anti-addictive properties", "animals", "healthy volunteers", and "dependent patients". We then improved the search using the terms "withdrawal signs" and "drug craving". We identified additional citations from the reference sections of articles retrieved and consulted these articles. We completed this search using the website "google.com" and the engine "Copernic".

History

Ibogaine is a naturally occurring plant indole alkaloid. The root bark of the Apocynaceous shrub *T. iboga* is the most frequently cited source of

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ibogaine. The Iboga tree is the central pillar of the *Bwiti* religion practiced in West-Central Africa, mainly Gabon, Cameroon, and the Republic of the Congo, which uses the alkaloid-containing roots of the plant for its psychoactive properties in a number of ceremonies. Ibogaine is also used by indigenous peoples in low doses to combat fatigue, hunger, and thirst.[4] Other sources of ibogaine are *Voacanga thouarsii* var. *Ortusa.*,[5] *Tabernaemontana australis*[6], and *Tabernaemontana orientalis*.[7] Although known for many centuries for tribes in West Africa, research of ibogaine started in late 19th century. The first description of *T. iboga* is published in 1985 from specimens of the plant brought to France from Gabon.[8] A published description of the ceremonial use of *T. iboga* in Gabon appears in 1885.[9] Ibogaine was first extracted and crystallized from the *T. iboga* root in 1901.[10–12] Ibogaine structure has been established in 1957 through chemical studies,[13] and X-ray crystallographic investigations have fixed the configuration of the ethyl group.[14] Moreover, ^{13}C nuclear magnetic resonance data[15] were reported in comparison with several iboga similar structures. The total synthesis of ibogaine and its availability in the form of the racemate was reported in 1966.[16]

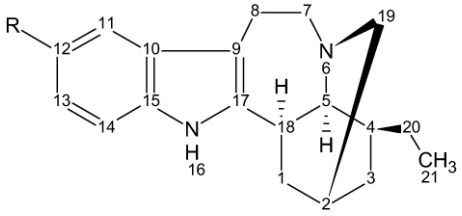
The interest of ibogaine to contemporary pharmacology is that this drug possesses anti-addictive properties. Between 1969 and 1995, the anti-addictive properties of ibogaine and its use in the treatment of heroin, cocaine, amphetamine abuse, alcohol, and nicotine dependence, and even some drug abuse have been patented in the United States and in France. A French patent for the psychotherapeutic use of ibogaine at a dosage of 4–5 mg/kg was published in 1969.[17] US patents have been published by Lotsof for the use of ibogaine in opioid withdrawal, dependence on cocaine and other stimulants, alcohol, nicotine, and polysubstance abuse.[18–22] These patents claim that an oral or rectal 4–25 mg/kg dose of ibogaine interrupts addictive drug behavior for a period of 6–36 months.

Chemistry

Ibogaine (12-methoxyibogamine, (6R, 6aS, 7S, 9R)-7-ethyl-2-methoxy-6, 6a, 7, 8, 9, 10, 12, 13-octahydro-5*H*-6, 9-methanopyrido[1',2':1,2]azepino[4,5-*b*]indole) has a molecular weight of 310.44 (Figure 1). Extraction of ibogaine from *T. iboga* shrub requires professional training. Mainly haloalkanes or alcohols were used for extraction. Chromatography was the method of choice for its purification. Extraction of ibogaine from *T. iboga* root bark using diluted vinegar and ammonia was described.[3] This drug can also be obtained semisynthetically from voacangine[3] or synthetically from nicotinamide by way of a 13 or 14 step process,[23] although extraction from the iboga root is a simpler method for obtaining the compound. Ibogaine has a melting point of 153 °C and a pK_a of 8.1 in 80% methylcellosolve; its heptane/water partition coefficient of 28 confirms the lipophilicity of the compound. Recently, a structural analysis of ibogaine and of its main active metabolite, noribogaine (or 12-hydroxyibogamine, (6R, 6aS, 7S, 9R)-7-ethyl-6, 6a, 7, 8, 9, 10, 12, 13-octahydro-5*H*-6, 9-methanopyrido [1',2':1,2]azepino[4,5-*b*]indol-2-ol, Figure 1), using Fourrier transform-infrared spectroscopy, 1D and 2D nuclear magnetic resonance spectroscopy, and liquid chromatography-electrospray mass spectrometry (LC/ESI-MS) has been published.[24] In accordance with the article of Taylor,[25] a fragmentation pattern in LC/ESI-MS is proposed (Figure 2). Ibogaine and noribogaine in solution suffer facile autoxidation under light- and heat-exposure giving iboluteine and ibochine, and desmethoxyiboluteine and desmethoxyibochine, respectively.[24–26] Recently, it has been shown that at 20 °C with daylight exposure, ibogaine (22.4 ng/mL) and noribogaine (25 ng/mL) showed a monoexponential decrease in drug concentrations; the corresponding half-lives were 81.5 min for ibogaine and 11 min for noribogaine.[27]

Analytical methods

Ibogaine was determined in complex mixtures of *T. iboga* and in biological matrices (brain homogenate, urine, and plasma) by spectrophotometry,[28] thin-layer chromatography,[29] or gas chromatography with flame ionization,[29–31] nitrogen-specific[32] or mass-spectrometric (electron impact or chemical ionization)[32–35] detection. Most of these methods involved a derivatization procedure. A method for determining opiate agonists including ibogaine by liquid chromatography-atmospheric-pressure chemical-ionization mass spectrometry procedure has been also described (LC/APCI-MS).[36] Recently, a high performance liquid chromatography method with fluo-



R = OH Noribogaïne
R = OCH3 Ibogaine

Figure 1 Molecular structures of ibogaine and noribogaine.

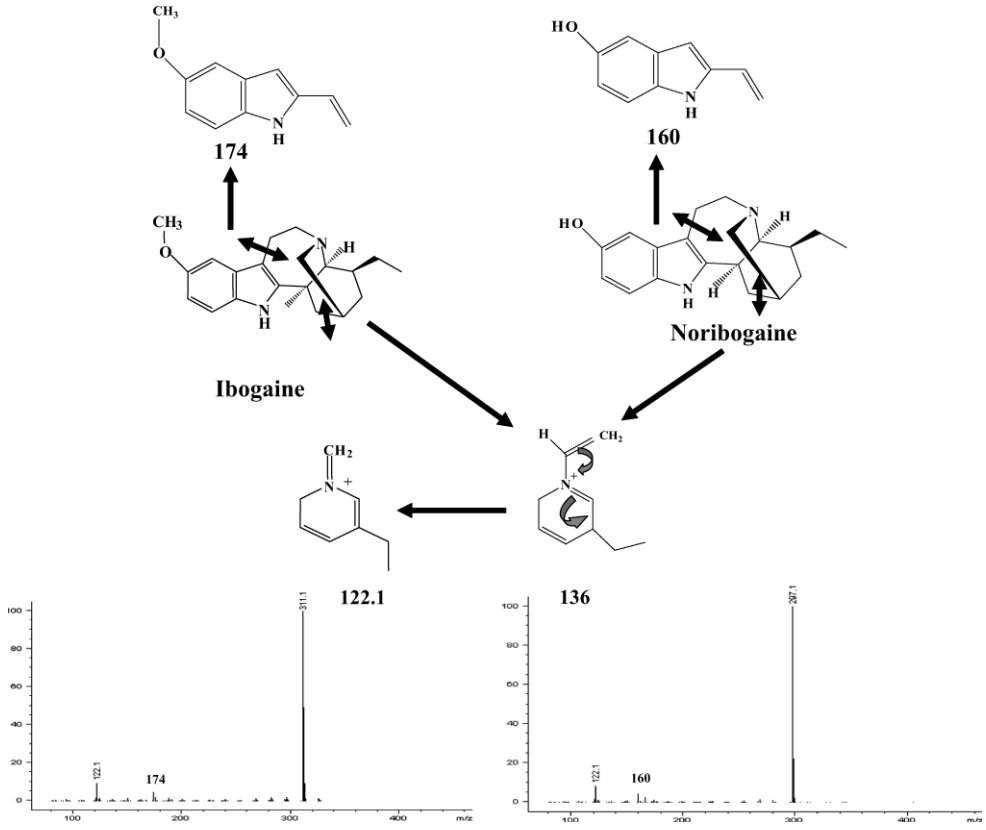


Figure 2 Fragmentation pattern of ibogaine and noribogaine.

rescence detection[27] and LC-MS methods with electrospray ionization[37,38] have been published to quantify ibogaine in plasma, blood, and urine. These methods involved liquid–liquid or solid-phase extraction of the biological samples. Some of them reported simultaneous quantitation of ibogaine and its 12-hydroxy metabolite[27,34,35,37,38] The main characteristics of these methods are summarized in Table 1.

Formulations

In traditional use, ibogaine was consumed by chewing the root bark of *T. iboga*. Commercially available formulations include plant extracts and crystalline ibogaine hydrochloride salt. From 1901 to 1905, ibogaine was recommended as a treatment for "asthenia" at a dosage range of 10–30 mg/day. Tablets from extracts of the roots of *Tabernanthe manii*, containing about 200 mg of extract or 8 mg of ibogaine per tablet, were sold in France as a neuromuscular stimulant between 1939 and 1970 under the trade name of Lambarene®. This marketed formulation was recommended in the treatment of fatigue, depression, and recovery from infectious disease.[9] Another ibogaine containing preparation was Iperton®, used as a tonic or stimulant, delivering 40 mg of the total *T. iboga* extract.[39] The ibogaine hydrochloride salt (98% purity) was favored for research. Capsules containing 100 or 200 mg of ibogaine were available.[40]

Most of preclinical experimentations have been reported by laboratories preparing the administered dose from the ibogaine hydrochloride acquired from Sigma Chemical Co. (compound No. I-7003, St. Louis, Mo, USA). Future research will permit the acquisition of ibogaine salt from the National Institute of Drug Abuse (NIDA). The use of ibogaine in the form of Endabuse®, the trademarked procedure to synthesize ibogaine for use in human drug abusers, provided another source for the compound. Three formulations containing ibogaine (the Sigma compound, the NIDA compound, and Endabuse®) were tested in rat for their discriminative dose–response effects. Results indicated that these drugs were equipotent.[41]

The main metabolite of ibogaine was noribogaine (Figure 1). Noribogaine may selectively mediate putative anti-addictive effects that persist for prolonged periods of time. Evaluation of pharmacokinetics and metabolism peculiarities of ibogaine supports the possibility of development of a slow

Table 1 Analytical methods used for the determination of ibogaine and noribogaine in biological fluids

References	*Analytes*	*Methods*		*Analytical column*	*Extraction*	*LLOQ*	*Extraction recovery, %*
		Chromatography	*Matrix*				
Dhahir, *et al.*[28]	Ibogaine	Identification TLC Silica G and UV spectra λmax = 278 nm λmin = 248 nm	Urine Blood Tissue	—	Liquid–liquid Petroleum ether pH 10–11; back-extraction with 0.5 N HCL	—	—
Bertol, *et al.*[29]	Ibogaine	Identification TLC Silica 60 GC-FID	Urine	1% SE–30 silanized on Chromosorb W; 80–100 mesh (2 m)	Liquid–liquid Ether pH 12 after purification with 0.1 N HCL (pH 1)	—	—
Cartoni and Giarusso[31]	Ibogaine	Quantitation TLC-cellulose GC-FID	Urine	1% SE–30 silanized on Chromosorb W 0.1% SE–30 on silanized glass beads; 80–100 mesh (1.80 m, 3 mm I.D.)	Liquid–liquid Ether pH 12–14 after purification with HCL pH 1	LLOD TLC: 1 µg LLOD GC: 0.05 µg	
Gallagher, *et al.*[32]	Ibogaine	Quantitation GC-EI-MS	Tissue	DB–5 (30 m × 0.25 mm I.D.); film thickness, 0.1 µm	Liquid–liquid n-Hexane 10 N KOH; back-extraction with 0.01 N HCL Derivatization	LLOD: 180 ng/g	98%
Hearn, *et al.*[34]	Ibogaine Noribogaine	Quantitation GC-MS	Plasma Urine Blood Tissue	DB–5 (15 m × 0.25 mm I.D.), film thickness 0.1 µm	Liquid–liquid Ethyl acetate pH > 10 Derivatization	5–10 ng/g	80–90%
Alburges, *et al.*[35]	Ibogaine Noribogaine	Quantitation GC-CI-MS	Plasma	DB–1 (15 m × 0.32 mm I.D.); film thickness 0.25 µm	Liquid–liquid n-butyl chloride/acetonitrile pH 13 Derivatization	10 ng/mL	Ibogaine: 55% Noribogaine: 14%
Ley, *et al.*[33]	Ibogaine	Quantitation GC-CI-MS	Plasma	DB–1 (30 m × 0.25 mm I.D.); film thickness 0.25 µm	Solid–liquid C18 Bond – Elut cartridge; elution with methanol	1–3 ng/mL	ND
Bogusz, *et al.*[36]	Ibogaine	Quantitation LC-APCI-MS	Serum Urine Blood Tissue Bile	Superspher RP 18 (125 mm × 3 mm I.D.); particles size 4 µm	Solid–liquid C18 Bond – Elut cartridge; elution with methanol/ acetic acid	1 ng/mL	96%
Kontrimavičiūtė, *et al.*[27]	Ibogaine Noribogaine	Quantitation HPLC, fluorimetric detection	Plasma	Supelcosil C18 (75 mm × 4.6 mm I.D.); particle size, 3 µm	Solid–liquid Oasis-HLB cartridge after protein precipitation; elution with methanol	Ibogaine: 0.89 ng/mL Noribogaine: 1 ng/mL	Ibogaine: 94.2% Noribogaine: 96.2%
Kontrimavičiūtė, *et al.*[37]	Ibogaine Noribogaine	Quantitation LC/ESI-MS	Plasma (P) Blood (B)	Zorbax eclipse XD8 C8 (150 mm × 4.6 mm I.D.) Particle size, 5 µm	Solid–liquid Oasis-HLB cartridge after protein precipitation; elution with methanol	Ibogaine: 0.89 ng/mL (P) 1.78 ng/g (B) Noribogaine: 1 ng/mL (P) 2 ng/g (B)	Ibogaine: 94.2% (P) 57% (B) Noribogaine: 96.9% (P) 62% (B)
Kontrimavičiūtė, *et al.*[38]	Ibogaine Noribogaine	Quantitation LC/ESI-MS	Urine	Zorbax eclipse XD8 C8 (150 mm × 4.6 mm I.D.) Particle size, 5 µm	Solid–liquid Oasis-HLB cartridge; elution with methanol	Ibogaine: 1.78 ng/mL Noribogaine: 2 ng/mL	Ibogaine: 70.0% Noribogaine: 81.7%

TLC, thin-layer chromatography; GC-FID, gas chromatography–flame ionization detection; GC-MS, gas chromatography–mass spectrometry; GC-EI-MS, gas chromatography–electron impact–mass spectrometry; GC-CI-MS, gas chromatography–chemical ionization–mass spectrometry; HPLC, high performance liquid chromatography; LC-APCI-MS, liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry; LC-ESI-MS, liquid chromatography–electrospray ionization–mass spectrometry; LLOD, lower limit of detection; LLOQ, lower limit of quantitation.

release formulation of noribogaine as an anticraving medication for opiates and psychostimulants.[4]

Preclinical pharmacokinetics

Absorption

Studies in rats showed dose-dependent and gender-dependent bioavailability after oral route suggesting that ibogaine absorption, and/or first pass elimination, is non-linear.[8] After oral administration of Iperton® capsules, containing 40 mg of natural extract of *T. iboga*, a substantial sex difference in rat brain and plasma concentrations of ibogaine was observed.[31] These data were consistent with those of Pearl, *et al.*,[42] reporting that plasma levels of ibogaine after oral administration were approximately threefold higher in female than in male rats, and the bioavailability of ibogaine was approximately twofold higher in female than in male.[42] After oral administration of 5 and 50 mg/kg to rat, the bioavailability was 16% and 71% in female and 7% and 43% in male, respectively.[43]

Distribution

An intriguing property of ibogaine is its prolonged duration of action when behavioral and neurochemical effects are identified after one or more days following oral, intraperitoneal (i.p.), or subcutaneous administration. Pharmacokinetics of ibogaine in rat were consistent with a two-compartment model, but the extremely high concentrations found in adipose tissues suggests the possibility of more complex pharmacokinetics.[44] One hundred times greater concentrations in fat and 30 times greater concentrations in brain, than in plasma found 1 h after administration were consistent with the highly lipophilic nature of ibogaine.[45] It was proposed that prolonged actions of ibogaine could be explained by adipose tissue reservoir with release and metabolism to active metabolite noribogaine over an extended period of time.[45,46]

Another depot might be the platelets or other blood components, as concentrations of ibogaine were higher in the whole blood than in plasma.[8] The concentrations of ibogaine and noribogaine have been measured in rat brain following both oral and i.p. administrations (40 mg/kg i.p., 50 mg/kg per os.).[47–49] The significance of micromolar interactions of ibogaine and noribogaine with various radioligand binding sites was related to the concentration of the parent drug and its metabolite in brain. Concentrations of these two drugs in rat cerebral cortex, striatum, brainstem, and cerebellum were measured 15 min, 1 and 2 h following drug administration. It was shown that ibogaine was rapidly detected in brain following oral administration. Noribogaine was detected at the earliest time point (15 min) consistent with a first pass metabolism of the parent drug.[48] After i.p. and oral administrations of ibogaine in rat, maximum concentrations were 11–15 μM in the whole blood and the brain for ibogaine, and 21.9 μM in the whole blood and 9.8–11.3 μM in the brain for noribogaine. In the whole blood and in the brain, area under concentration-time curve (AUC) values were 9- and 1.8-times higher for noribogaine than for ibogaine, respectively. The AUC ratios (brain/whole blood) were equal to 2 for ibogaine and 0.4 for noribogaine.[49] These results report that noribogaine reaches significant concentrations in brain following both routes of administration in rat. Thus, the concentrations of noribogaine in brain may activate processes that cause the desired effects of suppressing opiate withdrawal signs and diminishing drug craving.

Metabolism

Ibogaine is metabolized by cytochrome P4502D6 (CYP2D6) into a major (active) metabolite – noribogaine.[50] An important aspect is that this isoform is subjected to polymorphic expression particularly in Caucasians. From in-vitro study using human liver microsomes, Obach, *et al.*[50] reported that two (or more) enzymes were involved in this reaction. These authors identified two kinetically distinguishable ibogaine *O*-demethylases involved in this reaction corresponding, to high (K_M, > 200 μM) and low (K_M, 0.1 μM) values of the apparent Michaelis constant. The importance of the route of administration has been underlined, indicating that the noribogaine/ibogaine concentration ratio in the bloodstream was higher when ibogaine is injected by the i.p. route rather than by the intravenous route.[51] As well as, higher concentrations of ibogaine in plasma, brain, kidney, liver, and fat were observed following subcutaneous versus i.p. administration suggesting a substantial "first pass" effect after i.p. administration involving hepatic extraction.[45]

It seems that noribogaine could be a safer and possibly more efficacious alternative to ibogaine as a medication for the treatment of various types of addiction.[51]

Elimination

The half-life of ibogaine in rat was 1–3 h.[28,45,51] This elimination half-life could be underestimated since 3-h post-dose ibogaine concentrations remained very high in fat.[45] Ibogaine and noribogaine are excreted via the renal and the gastrointestinal tract (60–70% of the administered dose in 24 h in rat[43]).

Pharmacodynamic studies in animals and mechanism of action

Primary and secondary pharmacology

Several reviews have reported the pharmacological profile of ibogaine.[8,26,49,52] It has been shown that ibogaine and noribogaine interact with multiple binding sites within the central nervous system (CNS), including *N*-methyl-D-aspartate (NMDA) receptor-coupled ion channels, κ-opioid (κ_1 and κ_2), μ-opioid and σ_2, serotonin (5-HT_2 and 5-HT_3), muscarinic (M_1 and M_2) receptors and monoamine uptake sites, and nicotinic acetylcholine receptors. The pharmacological profile of noribogaine is different from that of ibogaine.[49,51–58] Ibogaine is more potent than noribogaine (i) in binding to the NMDA receptor in brain tissue[49,54] and (ii) as a stimulator of the hypothalamic-pituitary-adrenal axis.[54,55] Although ibogaine and 5-HT display chemical similarities, because both molecules contain an indole as part of their structure, noribogaine is much more potent than ibogaine in its ability to elevate extracellular 5-HT in the brain.[51] Thus, this drug is 10-times more potent in binding to serotonin transporter and inhibiting reuptake of serotonin.[49,51,53–55] Noribogaine is also (i) much more potent than ibogaine for binding to μ-opioid receptor and is a full μ-opioid agonist[49,54,56–58] and (ii) more potent binding to κ_1 and less potent binding to κ_2 opioid receptors. Affinities of ibogaine and noribogaine to some of these receptors are reported in Table 2.[49] Although not apparent in binding studies, functional studies indicate significant activity of ibogaine as a non-competitive antagonist at the nicotinic acetylcholine receptor.[4]

Conflicting results were reported about the alteration of extracellular dopamine levels in the nucleus accumbens. According to Baumann, *et al.*,[51] neither ibogaine nor its metabolite significantly altered dopamine levels, whereas Glick, *et al.*[46,59] reported that these two drugs cause significant decreases in dopamine levels.

Signal transduction pathways were examined during several preclinical trials. The examination of discriminative stimulus effects of ibogaine and noribogaine in rats in relation to their concentrations in blood plasma and brain regions and to receptor systems suggested that noribogaine may be the major entity that produces the discriminative effect of ibogaine.[47] It has been reported that the observed increase in phosphoinositide hydrolysis by noribogaine should be accompanied by an activation of protein kinase C which mediated a variety of long-term changes and might be involved in the behavioral effects of ibogaine.[60] The selective increase in receptor-mediated inhibition of adenyl cyclase activity caused by ibogaine and noribogaine might also be involved in the pharmacological activity of these compounds.[61] Recently, it has been shown that the remedial effect of ibogaine as anti-addictive drug is mediated, at least partially, through an influence on energy metabolism.[62] Among the recent proposals for ibogaine mechanisms of action is the activation of the glial cell line–derived neurotrophic factor (GDNF) pathway in the ventral tegmental area of the brain.[63] This work has principally been accomplished in preclinical ethanol research, where 40 mg/kg of ibogaine caused increase of RNA expression of GDNF in keeping with reduction of ethanol intake in the rat and absence of neurotoxicity or cell death. Short-term ibogaine exposure results in a sustained increase in GDNF expression, resulting in an increase in GDNF mRNA leading to protein expression and to the corresponding activation of the GDNF signaling pathway.[64]

It was also shown that ibogaine and noribogaine may stimulate the secretion of corticosterone from the adrenal cortex and prolactine from the anterior pituitary.[51] These two drugs also caused similar increase in plasma prolactine. Relevance of these physiological changes for primary or secondary pharmacology should be elucidated more in the future.

Table 2 Affinities of ibogaine and noribogaine to some receptors (according to Mash, *et al.*[49])

	Ibogaine	*Noribogaine*	*Pharmacodynamic activity*
	IC50, μM		
Serotonergic			
5-HT transporter (RTI-55 DAT sites)	0.59	0.04	Reuptake blocker
Opioidergic			
Mu (DAMGO)	11.0	0.16	Agonist
Kappa 1 (U69593)	25.0	4.2	Partial agonist (?)
Kappa 2 (IOXY)	23.8	92.3	Partial agonist (?)
Glutaminergic			
NMDA (MK-801)	5.2	31.4	Channel blocker

DAT, dopamine transporters; NMDA, *N*-methyl-D-aspartate.

Anti-addictive activity in animals

NMDA, opioid, and serotonin receptors have been targeted successfully for many years as anti-addictive treatment of opioid and/or cocaine addiction.[65,66] Activities of both ibogaine and noribogaine on these receptors provided a biological plausibility to expect anti-addictive efficacy for ibogaine in human also. Initial findings, suggestive of the efficacy of ibogaine in animal models of addiction, including diminished opioid self-administration and withdrawal[67–69] and diminished cocaine self-administration,[70] were published in late 1980s and in early 1990s.

Animal models of addiction were used to study the activity of ibogaine in the treatment of drug dependence. The administration of ibogaine reduced self-administration of cocaine, morphine, heroin, alcohol, and reduced nicotine preference.[8] The decrease in cocaine consumption has been described in mice after i.p. administration of two 40 mg/kg dose at 6 h interval[71] and in rat after i.p. administration of 2.5–80 mg/kg given as single or repeated doses (daily or weekly, $n = 3$).[67,70,72] According to Cappendijk and Dzoljic, the maximum effects were observed when ibogaine was given weekly for 3 weeks.[70] The decrease in opiate consumption has been described after administration of 2.5–80 mg/kg ibogaine in morphine- and heroin-dependent rat.[67,72,73]

Ibogaine also eliminates some of the signs of opiate withdrawal precipitated by naloxone or naltrexone in morphine-dependent rats given 20, 40, or 80 mg/kg ibogaine i.p.[68,74] and monkeys given 2 or 8 mg/kg ibogaine subcutaneously.[75,76] However, Sharpe and Jaffe[77] failed to report that ibogaine administered subcutaneously attenuated naloxone-precipitated withdrawal in rat receiving 5, 10, 20, and 40 mg/kg of ibogaine. Conflicting results were observed in mice. At doses ranging from 40 to 80 mg/kg i.p., a reduced naloxone-precipitated jumping in morphine-dependent mice was observed[77,78]; however, opposite effects were found after a 30 mg/kg i.p. dose.[79] These discrepancies might be related to ibogaine administration: before[77,78] or after[79] naloxone administration.

Although ibogaine has diverse effects on the CNS, the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood.

Preclinical safety studies

Several safety studies were performed during preclinical development. These studies raised several safety concerns, mainly neurotoxicity and possible cardiotoxicity.

Multiple laboratories have reported on the degeneration of cerebellar Purkinje cells in rat receiving i.p. administration of ibogaine at a dose of 40–100 mg/kg.[51,74,80] These include abnormal motor behavior (such as tremors, ataxia) related to histologically proven neurotoxicity.[80] Single-dose investigations showed that a 25 mg/kg i.p. dose was found to correspond to a no-observed-adverse-effect-level (NOAEL).[81] Helsley, *et al.* observed no evidence of neurotoxicity in a study where rats received 10 mg/kg of ibogaine per day for 60 days.[82] However, the neurotoxic effects of ibogaine may occur at levels higher than those observed to have effects on opioid withdrawal and self-administration. The monkey appears to be less sensitive to potential ibogaine neurotoxicity than the rat.[4] Mash, *et al.* observed no evidence of neurotoxicity in monkeys treated for 5 days with repeated oral doses of ibogaine of 5–25 mg/kg or subcutaneously administered doses of 100 mg/kg.[4] Another species difference in sensitivity is the mouse, which unlike the rat showed no evidence of cerebellar degeneration at a 100 mg/kg i.p. dose of ibogaine.[83]

Animal studies showed certain cardiotoxicities. Observed cardiotoxicity could be dose dependent. No changes in resting heart rate or blood pressure were found at a dose of ibogaine of 40 mg/kg i.p., which has been used in drug withdrawal or self-administration studies. Higher doses of ibogaine (100 and 200 mg/kg) decreased the heart rate without an effect on blood pressure.[84] However, Binienda, *et al.*[85] found a significantly decreased heart rate in rats given ibogaine 50 mg/kg i.p. The lethal dose 50% of ibogaine was 145 mg/kg i.p. and 327 mg/kg intragastrically in the rat, and 175 mg/kg i.p. in the mouse.[28]

In conclusion, preclinical development showed that ibogaine is acting on several mediators in CNS that have been targeted in treatment of drug-dependence. Neurotoxiciy and cardiotoxicity are safety concerns to be investigated further. No sufficient long-term safety non-clinical studies are available. All these data make further investigation of ibogaine's activity as potential therapeutic agent biologically plausible.

Clinical pharmacokinetic and pharmacodynamic studies

Clinical studies

Clinical development of ibogaine has continued for some decades. Development was governed by

several sponsors handed over management form one hand to other. Development was carried by several separate academicians and companies.

The first pharmacodynamic studies of ibogaine have been performed during 1901–1905. First anti-addictive attempts were done by Harris Isbell in 1955, administering doses of ibogaine of up to 300 mg to eight already detoxified morphine addicts at the United States. Addiction Research Centre in Lexington, Kentucky.[8] In 1962–1963, Lotsof administered ibogaine at the dose of 6–19 mg/kg, to 19 individuals including seven subjects with opioid dependence who noted an apparent effect on acute withdrawal symptomatology.[18,19] In 1967–1970, the World Health Assembly classified ibogaine with hallucinogens and stimulants as a "substance likely to cause dependency or endanger human health". In 1970, the US Food and Drug Administration (FDA) classified ibogaine as a Schedule I Controlled Substance, along with other psychedelics such as LSD and mescaline. The International Olympic Committee banned ibogaine as a potential doping agent. Thus, sales of Lambarène® were stopped in France.[9] Since that time, several countries, including Sweden, Denmark, Belgium, Switzerland, and recently (since March 2007) France, have banned the sale and possession of ibogaine.

The available data from private clinics described in scientific reports, where ibogaine has been used for informal addiction treatment, stated that ibogaine has been taken orally at an average dose of 19.3 ± 6.9 mg/kg.[86,87] Another study reported six heroin-addicted individuals and one subject who were addicted to codeine treated with ibogaine at doses ranging from 700 to 1800 mg.[40]

From 1989 to 1993, treatments were conducted outside of conventional medical settings in the Netherlands involving the International Coalition of Addict Self-Help, Dutch Addict Self Help, and NDA International.[8,88] In 1991, NIDA Medication Development Division began its ibogaine project. This initiative was based on case reports and preclinical evidence suggesting possible efficacy. The major objectives of the ibogaine project were preclinical toxicological evaluation and development of a human protocol. In August 1993, FDA Advisory Panel meeting formally considered Investigational New Drug Application filed by Dr Deborah Mash, Professor of Neurology at the University of Miami. Approval for human trials was given with 1, 2, and 5 mg/kg of ibogaine dosage levels. The Phase I dose escalation study began in December 1993, but activity was eventually suspended.[4,8] From October 1993 to December 1994, phase I/II protocols were discussed by the NIDA and fixed doses of ibogaine of 150 and 300 mg versus placebo for the indication of cocaine dependence were proposed.[8,89] The next year, a NIDA ibogaine review meeting decided to end the ibogaine project but to continue to support some preclinical research on iboga alkaloids. A fatality occurred during a heroin detoxification treatment of a 24-year-old women in the Netherlands in June 1993. This incident was a significant factor in the NIDA decision not to fund a clinical trial of ibogaine in 1995.[8] But the drug-addicted persons continue taking purified ibogaine hydrochloride powders or a whole plant extract that contains an unidentified number of other biologically active compounds. Some practically applied recommendations instruct taking between 2 and 6 g of powdered iboga.[90]

Some clinical experiences were gained during mid 1990s to 2001. At that time, ibogaine was available in alternative settings and studies based on a conventional medical model were carried out in Panama and in St Kitts. Informal protocols were developed in the United States, Slovenia, Britain, the Netherlands, and the Czech Republic. The ibogaine mailing list began in 1997 and heralded an increasing utilization of the Internet within the ibogaine medical subculture.

In early 2006, the creation of a non-profit foundation addressing the issue of providing ibogaine for the purpose addiction interruption within establishment drug treatment care was formed in Sweden (Stiftelsen Iboga's web site, accessed march 2007).

Pharmacokinetics

Pharmacokinetic data relative to ibogaine in human are limited.[8,49,56,91] Most of these studies have been carried out in drug-dependent patients. Following single oral doses of ibogaine (500–800 mg) to individual subjects, maximum ibogaine and noribogaine blood concentrations of 30–1250 ng/mL and 700–1200 ng/mL were obtained approximately 2 and 5 h after drug administration, respectively.[49,56] Thereafter, ibogaine was cleared rapidly from the blood, whereas noribogaine concentrations remained high. Indeed, concentrations of noribogaine measured at 24 h post-dose were in the range of 300–800 ng/mL whereas those of ibogaine were about 100 times lower. From blood concentration-time profiles of ibogaine published by Mash, *et al.*,[49,56] after an oral dose of 800 mg, the steady-state volume of distribution uncorrected for bioavailability was about 13 l/kg and the half-life of the terminal part of the curves was 4–7 h. Ibogaine being metabolized by the CYP2D6 into noribogaine, the pharmacokinetic profile of this drug was different in extensive and poor metabolizers. After single oral doses of ibogaine (10 mg/kg), maximum concentrations of noribogaine

were nine times lower in poor metabolizers ($n = 3$) than in extensive metabolizers ($n = 24$), whereas maximum concentrations of ibogaine were about 18% higher comparing to extensive metabolizers. This gap reflects speculation that conversion rate of the parent compound to noribogaine in CYP2D6 deficient subjects may reflect the metabolic contribution of other cytochromes (CYP2C9, CYP3A4). The blood AUC values, poor metabolizers versus extensive metabolizers, were almost three times higher for ibogaine and four times lower for noribogaine. These AUC levels were more representative for understanding systemic exposures in extensive and poor metabolizers. In extensive metabolizers, the blood AUC ratio, noribobaine/ibogaine, was approximately 3. Thus, the contribution of noribogaine to the total pharmacodynamic effect of the parent drug was significant. The calculated terminal half-life of ibogaine in this study was 7.45 h in extensive metabolizers.

In a recent study, Kontrimavičiūtė, *et al.*[91] reported for the first time the tissue distribution of ibogaine and noribogaine, in a subject dead after a poisoning involving ingestion of root bark of the shrub *T. iboga*. The highest concentrations of ibogaine and noribogaine were found in spleen, liver, brain, and lung. The tissue/sub-clavian blood concentration ratios averaged 1.78, 3.75, 1.16, and 4.64 for ibogaine and 0.83, 2.43, 0.90, and 2.69 for noribogaine, for spleen, liver, brain, and lung, respectively. Very low concentrations of the two drugs were found in the prostatic tissue. No compounds were detected in the cardiac tissue. Both ibogaine and noribogaine are secreted in the bile and cross the blood–brain barrier.

Results are summarized in Table 3.

Pharmacodynamic effects

Some case-report studies showed that ibogaine is active as psychotropic agent with possible anti-addictive activity in acute opioid withdrawal.[4,41,86,87] After administration of ibogaine, individuals can experience (i) certain subjective new experience and (ii) reductions of drug craving and withdrawal signs and symptoms.

Subjective new experiences described by patients treated with ibogaine Preliminary data shows that patients experience several different phases that may be categorized into acute, evaluative, and residual stimulation stages (Figure 3).[8]

The first phase (acute phase) is experienced within first 1–3 h after exposure and lasts 4–8 h. During this phase, patients report panoramic delivery of long-term memory, mainly visual; "visions" or "waking dream" states experiencing contact with transcendent beings, passage along a lengthy path, floating, etc. Although visual experiences are not reported by all patients and seem depend of drug exposure, it is also noticed that they were associated and enhanced with eye closure. Unfortunately, difference between these dreams and hallucinations are not clear enough.

Table 3 Pharmacokinetic parameters from clinical studies

		Extensive metabolizers (n = 24)[49]	*Poor metabolizers (n = 3)*[49]
Ibogaine doses	500–800 mg	10 mg/kg	10 mg/kg
Ibogaine			
t_{max}, h	2	1.7	2.5
C_{max}, ng/mL	30–1250	737	896
V_{ss}/F, l/kg	13	—	—
$t_{1/2}$ (last phase), h	4–7	7.5	—
AUC_{0-24h}, ngxh/mL	—	3936	11471
Tissular distribution (tissue/blood concentration ratios)	Spleen: 1.78 Liver: 3.75 Brain: 1.16 Lung: 4.64 Bile: 1.97	—	—
Noribogaine			
t_{max}, h	5	6.2	3.2
C_{max}, ng/mL	700–1200	949	105
C_{24h}, ng/mL	300–800	—	—
AUC_{0-24h}, ngxh/mL	—	14705	3648
Tissular distribution (tissue/blood concentration ratios)	Spleen: 0.83 Liver: 2.43 Brain: 0.90 Lung: 2.69 Bile: 0.54	—	—

n, number of subjects; C_{max}, maximum concentration; t_{max}, time of C_{max}; $C_{24\ h}$, concentration 24 h post-dose; $AUC_{0-24\ h}$, area under concentration-time curve; V_{ss}, steady-state volume of distribution; $t_{½}$, half-life.

Opiate withdrawal	**Reduction** of drug craving	**Reduction** of drug withdrawal	**Reduction** of depression and drug craving (up to one month)	**Reported cessation** of drug use (up to more than 1 year)*
Personal new experience	***Acute*** phase with "oneiric" experiences (onset: 1-3 h, duration: 4-8 h)	***Evaluative*** phase with "neutral" and "reflective" emotional tone (onset: 4-8 h, duration: 8-20 h)	***Residual Stimulation*** phase with return of normal allocation of attention (onset: 12-24 h, duration: 24-72 h)	

* Reported cessation from the sample of 41 individuals: nine individuals were treated twice and one was treated three times for a total of 52 treatments. Fifteen (29%) of the treatments were reportedly followed by cessation drug use for less than 2 months, 15 (29%) for at least 2 months and less than 6 months, 7 (13%) for at least 6 months and less than one year, 10 (19%) for a period of greater than one year (8, 89, 93).

Figure 3 Clinical pharmacodynamic effects after ibogaine administration.

The second phase (evaluative phase) starts approximately 4–8 h after ingestion and lasts 8–20 h. During this phase, dreams decreased slowly and the emotional tone is generally described as neutral and reflective. Patients reflect that their attention is focused on inner subjective experiences (i.e., by evaluating the experiences of the acute phase).

During these two first phases, patients tend to stay focused on their experiences and avoid any external distraction.

The third phase (residual stimulation phase) starts 12–24 h after exposure and lasts 24–72 h. Patients regain normal attention to the external environment. Subjective psychoactive experience lessens, remaining with mild residual subjective arousal or alertness. Decrease in the need to sleep for several days to weeks can be observed.

Reductions of withdrawal signs and symptoms, drug craving and depression After administration of 6–29 mg/kg dose of ibogaine, acute reduction in drug craving and opiate withdrawal signs and symptoms are observed in 1–2 h. Resolution of withdrawal was observed during 1 week, reduction of craving and depression – 1 month after exposure.

Opiate physical dependence is assessed usually by discontinuation of opiate treatment (spontaneous withdrawal) or by antagonist-precipitated withdrawal.[49] Usually, acute withdrawal syndrome in case of heroin addiction may begin approximately 8 h after the last heroin dose, peaks in intensity at 24–28 h, and subsides within 7–10 days. In one of the opiate (heroin or methadone) addiction studies including 32 patients, rapid detoxifications of these patients was assessed after single-dose ibogaine treatment (10 mg/kg). Results are summarized in Table 4. The post-ibogaine Objective Opiate Withdrawal Scale (OOWS) blinded rating obtained 10–12 h and 24 h after ibogaine administration (i.e., 24 and 36 h after the last dose of opiate, respectively) was statistically lower than the rating obtained 1 h before ibogaine administration. The OOWS mean total score decreases from approximately 5.6 to 1.1 and 1.9, 12, and 24 h following ibogaine administration, respectively. Authors noticed that objective signs of opiate withdrawal were rarely seen and none were exacerbated at later time points.

A second measurement used was "self-reports of withdrawal symptoms according to Opiate-Symptom Checklist" (OP-SCL). This "discomfort" measurement showed statistically significant decreases in mean scores: from approximately 21 (score observed 24 h before ibogaine treatment) to 12 shortly after recovery from ibogaine treatment (<72 h) and down to 7 (at program discharge, approximately 6–9 days later).

Impressive success of single dose of ibogaine detoxification process was noticed as well as the fact that many of the patients were able to maintain abstinence over the months following detoxification.[49] This relatively small study suggests that methadone withdrawal was not more difficult to detoxify than heroin withdrawal. Speculation that long-acting metabolite noribogaine may account for the efficacy of ibogaine can be done.

Craving is an important symptom reported by opiate-dependent subjects during the early stages of withdrawal contributing to continued drug use.[49,92] Craving symptoms for opiates could be evaluated by using the "Heroin Craving Questionnaire scales (HCQN-29)". Thirty-six hour post-ibogaine treatment, the mean scores on five measures about specific aspects of drug craving (including urges, thoughts about drug of choice, or plans to use the drug) show significant decreases and lasted at program discharge.

Evaluation of patients using Beck Depression Inventory scores showed also significant reduction of scores both at program discharge and at 1-month follow-up assessments.[49,56]

Subjects undergoing cocaine detoxification also reported significant decrease in drug-craving 36 h post-ibogaine treatment and at discharge for three of the five category scales of the Cocaine Craving Questionnaire (CCQN)-45 (anticipation of positive outcomes, relief of negative states, and lack of control).

There is only limited retrospective experience in long-term outcomes (Figure 3).[8,88,93] No clear negative or positive conclusions could be drawn. It seems that relapses could be attempted with ibogaine re-challenge, as this was done several times in anecdotal cases.

It seems that pharmacodynamic effects experienced by patients varied and may be related to dose, bioavailability, and interindividual variabilities.[8] Sequential pattern of clinical pharmacodynamic effects could be summarized as presented in Figure 3.

Clinical safety

Neurotoxicity observed in animal studies was also noticed in human studies (e.g., effects on postural stability, body tremor, and appendicular tremor). In 1994, a fatal case of woman treated with ibogaine was reported. Fifteen months before her death, this woman had undergone four separate treatments with ibogaine in rather high dosages (ranging from 10 to 30 mg/kg). Death was not attributed to ibogaine but was related to mesenteric arterial thrombosis related to chronic cellulitis.[4,8]

Similar to the findings in animals, some cardiac side-effects were also observed during clinical investigations.[8,94] Thirty-nine patients dependent on cocaine and/or heroin, who received fixed oral doses of ibogaine of 500, 600, 800, or 1000 mg, were monitored. Six subjects exhibited some significant decrease of resting pulse rate; one of them evidenced a significant decrease in blood pressure (attributed to a transient vasovagal response). No evidence of electrocardiogram abnormalities was showed. There were hypotensive episodes (responsive to volume repletion) noticed during ibogaine therapy in some cocaine-dependent subjects.[49]

The safety of ibogaine was also evaluated in more than 150 drug-dependent subjects receiving 8, 10, or 12 mg/kg ibogaine.[49] The most frequent side-effects encountered were nausea and mild tremor, and ataxia earliest after drug administration. A hypotension was observed in some cocaine-dependent subjects, who required close monitoring of blood pressure. No other significant adverse events were seen under the study conditions; and, therefore, there are no clear evidences till now that there are big issues in tolerance after single dose of ibogaine.

In conclusion, clinical development could be considered as started only and needs essential exploratory program first.

Main unanswered questions

Experience available in public domain suggests that ibogaine might have some activity in anti-addiction treatment. Although data seems quite promising, several issues remain to be elucidated first before moving further towards controlled pivotal clinical trials. Four major product development issues to be solved relates to the areas of pharmaceutical formulation development and starting clinical exploratory studies:

1) Is ibogaine pharmaceutical formulation developed enough to ensure proper constant composition form certain active ingredients?

Table 4 Effects of single-dose ibogaine (10 mg/kg) on opiate withdrawal signs

	Time after the last opiate dose, h	*Time after the ibogaine dose, h*	*OOWS*	*OP-SCL*	*HCQN-29*[a]	*BDI*
Before ibogaine treatment			5.6 (1 h)	21 (24 h)	3.26–4.88	16.9
	24	10–12	1.1*			
	36	24	1.9*			
		36			1.57–3.67	10.4
		<72 h		12*		
		6–9 days		7*		
Discharge					1.22–2.85***	3.0**
1-month follow-up						2.29**

OOWS, Objective Opiate Withdrawal Scale; OP-SCL, Opiate-Symptom Checklist; HCQN-29, Heroin Craving Questionnaire scales (subscales: desire to use, intention to use, anticipation of positive outcomes, relief of negative states and lack of control); BDI, Beck Depression Inventory scores.
[a]According to the subscale.
*$P < 0.05$; **$P < 0.0005$; ***$P < 0.0001$.

2) What is ibogaines' pharmacodynamic activity in controlled exploratory trial in drug-dependent stabilized subjects?
3) What is ibogaines' potential for abuse by drug-dependent subjects (in both pharmacodynamic and economic measurements)?
4) What is the most rational dosage range to be studied in dose–response studies in treatment of some drug dependences (first, in opiate and/or cocaine dependencies?

Conclusion

Experience available in public domain suggests that ibogaine might have some activity in anti-addiction treatment, but current data are not sufficient to do further in development. Data gathered during more than 100 years of pharmaceutical, non-clinical, and clinical developments need to be validated. Good laboratory and clinical practice environment is essential for future investigations. Several major objectives of preclinical and clinical investigations should focus on core-identified questions first.

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Regulation

composed by
The International Center for Ethnobotanical Education, Research & Service
Advisors: José Carlos Bouso & Kenneth R. Alper, MD

CLASSIFICATION OF MEDICINES

Minutes of the November 2009 Medicines Classification Committee Meeting

Classification of codeine

Following the consultation period, Medsafe is considering submissions asking to delay the gazettal date for the reclassification recommendations regarding codeine.

MINUTES OF THE 42ND MEETING
OF THE MEDICINES CLASSIFICATION COMMITTEE
HELD IN THE MEDSAFE BOARDROOM, LEVEL 6, DELOITTE HOUSE
10 BRANDON STREET, WELLINGTON
ON TUESDAY 3 NOVEMBER 2009 COMMENCING AT 9:30am

Present:
Dr Stewart Jessamine (Chair)
Ms Natalie Gauld
Dr Timothy Healy
Dr Jill Peckham
Ms Andrea Kerridge (Secretary)

In Attendance:
Ms Carole Firth (Advisor - Science, Medsafe)
Dr Ruth Lopert (Principal Medical Advisor, Therapeutic Goods Administration)
Mrs Mary Miller (Senior Advisor - Science, Medsafe)
Mr Andrew Orange (Advisory Pharmacist, Medicines Management Limited)
Dr Enver Yousuf (Principal Medical Advisor, Medsafe)

Apologies:
Dr Melissa Copland
Dr David Galler

1 WELCOME

The Chair opened the 42nd meeting at 9:30am and welcomed members and guests. The Chair explained that Mr Orange, who was observing the meeting, would be replacing Ms Gauld on the Committee early next year.

2 APOLOGIES

Apologies were received from Dr Copland, whose apology was a result of illness in transit to the meeting, and Dr Galler.

Dr Lopert was only present, via telephone, for Agenda Item 5.2. Mrs Miller joined the meeting for Agenda Items 5.2 and 5.8. Ms Firth joined the meeting for Agenda Item 5.8 only.

3 CONFIRMATION OF THE MINUTES OF THE 41ST MEETING HELD ON THURSDAY 14 MAY 2009

Items from this meeting that may be considered at the next meeting were:

- diclofenac 12.5 mg film coated tablet (agenda item 6.3)
- gingko biloba (agenda item 7.3)
- HMG-COA reductase inhibitors ('statins') (agenda item 8.2.2a).

10 GENERAL BUSINESS

10.1 Ibogaine and its metabolite noribogaine

Medsafe had received several enquiries about importing ibogaine into New Zealand. In response to these requests, Medsafe decided to clarify the scheduling status.

The Committee was therefore asked to consider the scheduling of ibogaine.

Ibogaine is a naturally occurring indole alkaloid derived from the roots of the rain forest shrub *Tabernanthe iboga*. It is used in low doses by the indigenous peoples of western Africa to combat fatigue, hunger and thirst, and in higher doses as a sacrament in religious rituals. The use of ibogaine for the treatment of drug dependence had been based on anecdotal reports from American and European addict self-help groups that it decreased the signs of opiate withdrawal and reduced drug craving for cocaine and heroin for extended time periods. Although ibogaine has diverse effects on the central nervous system (CNS), the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood.

The purported efficacy of ibogaine following single-dose administrations may be due to the formation of an active metabolite. Ibogaine is O-demethylated to 12-hydroxyibogamine (noribogaine) by the activity of liver enzymes. Noribogaine appears to have a slow clearance rate in humans, suggesting that some of the after effects of ibogaine may be due to the actions of the metabolite.

The Committee was provided with a table of data which attempted to state what is known regarding ibogaine related fatalities in the public domain. The figures suggest that the number of deaths due to methadone, the most controlled substance, were a little higher that those associated with ibogaine, which is unregulated.

The most frequently reported use for ibogaine is for the reduction or elimination of addiction to opiates. Ibogaine is reported to alleviate the symptoms of opiate withdrawal. It has also been suggested that ibogaine may be useful in treating dependence to other substances such as alcohol, methamphetamine and nicotine.

Given its use for the therapeutic purpose of managing/treating addiction and the need for this treatment to be under supervision, Medsafe believed that there was a case for classifying ibogaine and its metabolite noribogaine as prescription medicines. This would not necessarily restrict the medical use in a therapeutic environment but would limit attempts at self treatment and prevent its development for recreational use as a "party pill", even though the documented experience of using it is usually not that pleasant.

Medsafe had also sought opinion from a psychiatrist who is proposing to conduct a clinical study utilising ibogaine. He was of the opinion that although its appeal as a recreational drug is low, he shared concerns that use in an *ad hoc* fashion as a self medication for drug addiction could occur following the media interest in the product and that this could be dangerous. The psychiatrist was supportive of classification of ibogaine as a prescription medicine.

Due to the potential for therapeutic use of the product, the safety profile and the potential for misuse, Medsafe suggested that the substance ibogaine and its metabolite noribogaine met the criteria for classification under the Medicines Act 1981. This would provide ability to control the import and supply of ibogaine, its metabolite or any products containing each or both of the substances.

The Committee agreed with the recommendation to classify ibogaine as a prescription medicine.

Recommendation

That ibogaine and its metabolite noribogaine should be classified as prescription medicines.

10.2 Committee members

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THE NEED FOR IBOGAINE IN DRUG AND ALCOHOL ADDICTION TREATMENT

Jennifer R. Donnelly*

> Clearly, in a world devastated by addictions to alcohol, cocaine, heroin, amphetamines, methadone, and nicotine, with all the accompanying death, disease and crime, in a society where dysfunctional behavior is the rule rather than the exception, in a humanity hungering to reconnect with God, ibogaine has profound implications.[1]

INTRODUCTION

Samantha Jones graduated valedictorian of her high school class. She had big dreams of becoming a surgeon and had even been offered a full scholarship at an Ivy League university. Something, however, went terribly wrong. At 20 years old, Samantha has been disowned by her family, fired by her employer, evicted by her landlord, and has lost her daughter to foster care. She is living on the streets, selling her body for sex to get enough money to feed her $200-a-day addiction to heroin. On a good day, Samantha wakes up and immediately shoots heroin to "get well." On a bad day, she is forced to go out on the street and beg for money or sell her body just to come up with enough money to shoot up.

Samantha has been in and out of rehab facilities and has spent time in jail for prostitution and possession of a controlled substance. Each time, she relapses within days of her release. She is currently on methadone maintenance in an effort to ward off heroin withdrawal symptoms, but instead of taking the methadone, Samantha is selling it on the streets for money to buy the heroin

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[1] Eric Taub, *Ibogaine: I Begin Again*, *in* I BEGIN AGAIN TREATMENT CENTERS (2003), http://www.ibeginagain.org/articles/eric_taub.shtml.

she desperately craves. She knows that she has lost all control and realizes that her addiction may soon take her life, but up against a wall with nowhere to turn, Samantha needs a miracle.

What Samantha does not know is that there is a drug that could interrupt her dependency on heroin for long enough that she could get the therapy that she needs. Unfortunately, this miracle drug, ibogaine, is illegal in the United States and while it is legally available in other countries, travel and treatment are expensive. Samantha, like thousands of others in her position, simply cannot afford the treatment, as every penny she manages to scrounge goes to feed her addiction.[2]

Section I of this commentary discusses the economic and societal costs of drug abuse in the United States, as well as the current treatment methods available for drug addiction. Section II examines the history of ibogaine, including the available studies on its use for addiction and the importance of aftercare. Section III analyzes the common arguments against legalizing ibogaine. Section IV concludes with suggestions for the future of ibogaine in the United States.

I. DRUG ADDICTION IN THE UNITED STATES

John Belushi, Chris Farley, Janis Joplin, Jimmy Hendrix, Judy Garland, Billie Holiday, River Phoenix, Elvis Presley, Tim Hardin, Sid Vicious, and Jim Morrison: these are only a few in the long list of celebrities who died in the prime of their lives as a result of their drug and alcohol addictions.[3] Drug abuse and addiction have no boundaries; they affect people of all races, cultures, and socio-economic backgrounds. The results from the 2009 National Survey on Drug Use and Health, conducted by the Department of Health and Human Services, indicates the rate of substance abuse or dependence by class/ethnicity as "15.5 percent for American Indians or Alaska Natives, 13.2 percent for persons reporting two or more races, 10.1 percent for Hispanics, 9.0 percent for whites, . . . 8.8 percent for blacks" and 3.5% for Asians.[4] Additionally, college graduates typically had lower abuse and dependence

[2] Although Ms. Jones is a fictional character, her plight is not unique, as the 2004 National Survey on Drug Use and Health reported that "an estimated 3.7 million people had used heroin at some time in their lives" and "from 1995 through 2002, the annual number of new heroin users ranged from 121,000 to 164,000." *See* NAT'L INST. DRUG ABUSE RESEARCH REPORT SERIES, HEROIN ABUSE AND ADDICTION 1-2 (Rev. May 2005), http://www.drugabuse.gov/PDF/RRHeroin.pdf.

[3] *See*, *e.g.*, Casa Palmera, *Top 20 Drug Induced Celebrity Deaths*, http://www.casapalmera.com/articles/how-20-prominent-people-died-as-a-result-of-drug-use/ (last visited Nov. 25, 2010).

[4] SUBSTANCE ABUSE & MENTAL HEALTH SERVICES ADMIN., U.S. DEP'T HEALTH & HUMAN SERVICES, PUB. NO. SMA 10-4586 FINDINGS, RESULTS FROM THE 2009 NATIONAL SURVEY ON DRUG USE AND HEALTH 78 (2010), http://oas.samhsa.gov/NSDUH/2k9NSDUH/2k9ResultsP.pdf.

rates (7.5%) than college dropouts (9.9%), high school graduates (8.9%), and high school dropouts (11.6%).[5]

In the United States, however, drug addiction is treated as a crime, rather than the disease that it is.[6] The United States spends billions of dollars each year to imprison addicts or force them into treatment programs that have low success rates.[7]

A. Costs of Drug Addiction on Society

Americans spend approximately $64.8 billion every year to purchase illicit drugs, including $36 billion for cocaine, $11 billion for marijuana, $10 billion for heroin, $5.4 billion on methamphetamine, and $2.4 billion on other illegal drugs.[8] Roughly 22.2 million Americans experience a substance abuse problem each year, while only 3.9 million of those receive any kind of treatment for their addiction.[9]

The Office of National Drug Control Policy estimates that drug abuse in the United States costs society approximately $181 billion a year, $107 billion of which is directly attributable to drug-related crime.[10] Moreover, this cost is rising sharply, despite the "War on Drugs,"[11] evidenced by the fact that the societal cost of illicit drug addiction has risen $64 billion between 1997 and 2002.[12] Alcohol abuse, meanwhile, costs society an additional $185 billion per year.[13] These costs are felt by all of society through higher insurance premiums and health care expenses, as well as higher incidence of crime throughout the nation.[14]

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[5] *Id.*

[6] NAT'L INST. DRUG ABUSE, U.S. DEP'T OF HEALTH & HUMAN SERVICES, UNDERSTANDING DRUG ABUSE AND ADDICTION 1 (JUNE 2008), http://www.drugabuse.gov/PDF/InfoFacts/Understanding08.pdf.

[7] *See* Dave Bewley-Taylor et al., The Beckley Foundation Drug Policy Programme, *The Incarceration of Drug Offenders: An Overview* 12 (Mar. 2009), http://www.idpc.net/php-bin/documents/Beckley_Report_16_2_FINAL_EN.pdf (estimating that the United States spends $12.3 billion to keep state and federal drug offenders incarcerated); *see also* SUBSTANCE ABUSE POLICY RESEARCH PROGRAM, POLICY BRIEF: SUBSTANCE ABUSE TREATMENT BENEFITS AND COSTS, http://www.saprp.org/knowledgeassets/knowledge_brief.cfm?KAID=1 (last visited Nov. 18, 2010) (finding that nearly $18 million is spent annually on drug and alcohol treatment, mostly with public funds).

[8] OFFICE OF NAT'L DRUG CONTROL POL'Y: DRUG POLICY INFORMATION CLEARINGHOUSE FACT SHEET, DRUG DATA SUMMARY 2 (Mar. 2003), http://www.whitehousedrugpolicy.gov/pdf/drug_datasum.pdf (last visited Mar. 1, 2010).

[9] KATHARINE R. LEVIT ET AL., PROJECTIONS OF NATIONAL EXPENDITURES FOR MENTAL HEALTH SERVICES AND SUBSTANCE ABUSE TREATMENT 2004-2014, U.S. DEP'T HEALTH & MENTAL SERVICES, SAMHSA PUB. NO. SMA 08-4326, at 27 (2008).

[10] *Id*.

[11] *See* Claire Suddath, *The War on Drugs*, TIME MAG., Mar. 25, 2009, at 74.

[12] Brian Vastag, *Addiction Treatment Strives for Legitimacy*, 288 J.A.M.A. 3096, 3100 (2002).

[13] LEVIT, *supra* note 9.

[14] *Id.*

Addiction's effects on crime in the United States are profound. While possessing and consuming an illicit drug are crimes themselves, substance abuse contributes substantially to property damage and violent crime. According to a 1997 National Household Survey on Drug Abuse (NHSDA) conducted by the United States Department of Health and Human Services (HHS), individuals who reported using an illicit drug during the previous year were: 16 times more likely to be arrested for property crimes, such as larceny and theft; 14 times more likely to be arrested for driving under the influence; and nine times more likely to be arrested for assault.[15] In 1991, 21% of state inmates and 59% of federal inmates were incarcerated specifically for drug-related crimes.[16] By 1997, 22.4% of federal prison inmates and 32.6% of state prison inmates reported that they had been under the influence of drugs at the time of the crime for which they were incarcerated.[17] Drug use continues to be a major contributor in violent offenses, with 29.4% of federal and 26.8% of state inmates convicted of murder having been under the influence of drugs at the time of their crime.[18] Additionally, 7.9% of federal and 21.5% of state inmates reported being under the influence of drugs when they committed a sexual assault; 27.8% and 39.9% for robbery; and 13.8% and 24.2% for assault.[19] Moreover, 19% of state inmates and 15% of federal inmates reported that they had committed the offense they were incarcerated for in an effort to obtain money for drugs.[20]

B. Current Treatment Methods Available

Currently, pharmaceutical treatment for substance abuse addiction in the United States is limited to two basic types: (1) replacement therapy; and (2) aversion therapy.[21] Replacement therapy is characterized by substituting or replacing the drug that the person is addicted to with a "safer drug" under the theory that the individual can be weaned off the replacement drug over time.[22] The most prominent examples of this are methadone maintenance for heroin addiction and nicotine replacement drugs for smokers.[23] Unfortunately,

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[15] OFFICE OF NAT'L DRUG CONTROL POL'Y: DRUG POL'Y INFO. CLEARINGHOUSE FACT SHEET, DRUG-RELATED CRIME 1-2 (2000), http://www.whitehousedrugpolicy.gov/publications/pdf/ncj181056.pdf.

[16] OFFICE OF NAT'L DRUG CONTROL POL'Y: DRUG POL'Y INFO. CLEARINGHOUSE FACT SHEET, DRUG TREATMENT IN THE CRIMINAL JUSTICE SYSTEM 1 (2001), http://www.whitehousedrugpolicy.gov/publications/pdf/94406.pdf.

[17] OFFICE OF NAT'L DRUG CONTROL POL'Y, *supra* note 8, at 3.

[18] *Id.*

[19] *Id.*

[20] *Id.*

[21] Jonathon Freedlander, *Ibogaine: A Novel Anti-Addictive Compound, A Comprehensive Literature Review*, 1 J. DRUG EDUC. & AWARENESS 79, 88-90 (2003).

[22] *Id.*

[23] *Id.*

there are no "safer drugs" available for individuals with addictions to cocaine, crack, or methamphetamine.[24]

Aversion therapy, on the other hand, involves the use of drugs that interact negatively with the drug of addiction, such as disulfiram, which is used to treat alcoholism.[25] This treatment choice posits that the individual will be deterred from using the drug to which they are addicted because, when combined with aversion drugs, it induces nausea, vomiting, and physical pain.[26]

The problems associated with these treatment methods, however, are numerous. Both require long-term treatment, which greatly increases the chance that an addict will quit treatment and return to using.[27] Replacement therapy simply replaces one drug with another, and, as is the case with methadone, the "safer drug" is itself addictive. In fact, both the illegal use of methadone and the deaths caused by the drug are on the rise.[28] The Centers for Disease Control and Prevention (CDC) reported at least 786 deaths caused by methadone in 1999.[29] Five years later, the number of deaths had jumped to 3,839.[30] The unpleasant side effects associated with aversion therapy, however, result in many patients stopping treatment and relapsing.[31]

Fortunately, there is a natural plant whose roots may be the answer to many substance addictions, including addictions to heroin, cocaine, crack, methamphetamines, alcohol, and even nicotine. Unfortunately, this vehicle for treatment is unavailable in the United States because it has hallucinogenic qualities and is, therefore, illegal.[32]

II. IBOGAINE—AN ADDICTION INTERRUPTER

A. Ibogaine's History

Ibogaine is a naturally occurring psychoactive substance derived from the roots of the Tabernanthe iboga shrub and other West African plant species generally found in rain forests.[33] It has been used for centuries by the

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[24] NAT'L INST. DRUG ABUSE, U.S. DEP'T HEALTH & HUMAN SERVICES, TREATMENT APPROACHES FOR DRUG ADDICTION 3 (Sept. 2009), http://www.nida.nih.gov/PDF/InfoFacts/IF_Treatment_Approaches_2009_to_NIDA_92209.pdf.

[25] Freedlander, *supra* note 21.

[26] *Id*.

[27] *Id*.

[28] Rich Daly, *Government Undertakes Effort to Cut Methadone-Related Deaths*, 42 PSYCH. NEWS 13, 13 (2007).

[29] *Id*.

[30] *Id.*

[31] Freedlander, *supra* note 21.

[32] 21 U.S.C. § 812 (2009).

[33] *See, e.g.,* MAPS, *Ibogaine: Treatment Outcomes and* Observations, 13 MAPS 16, 16 (2003), http://www.maps.org/news-letters/v13n2/13216two.pdf; Howard S. Lotsof et al., *Ibogaine in the Treatment*

indigenous peoples of Western Africa, mainly for use as a sacrament in spiritual initiation ceremonies.[34] It has also been used in traditional medicine, however, to combat such things as fatigue, hunger, and thirst.[35] Additionally, ibogaine was used as a mental and physical stimulant in France, under the name Lambarene, until 1970 and was popular with post-World War II athletes before being banned by the Olympic Committee as a potential doping agent.[36]

In the 1950s and early 1960s, American psychologist Leo Zeff and Chilean psychiatrist Claudio Naranjo used ibogaine in their psychotherapy practices.[37] Naranjo conducted several studies to determine the psychotherapeutic effects ibogaine had on patients seeking closure for unresolved emotional issues.[38] These studies illustrated that ibogaine allowed patients to revisit their past experiences objectively and without the negative emotions experienced during the actual incident, which, in turn, enabled them to confront and resolve deep personal conflicts.[39] During the same time, the CIBA Corporation was also evaluating ibogaine's usefulness as an anti-anxiety drug.[40]

When taken at low doses, ibogaine causes a stimulant effect eliciting increased alertness, and reducing fatigue, hunger, and thirst.[41] Within three hours after ingesting a higher dose of ibogaine, the user will enter into the "acute phase," typically lasting four to eight hours.[42] It is during this phase that the user experiences ibogaine's most intense effects, characterized as the "panoramic recall of a large amount of material relating to prior life events from long-term memory, primarily in the visual modality," or the "waking dream" state.[43] If the user is an addict, he or she will usually be taken back to the place and time where the underlying issue leading to the addiction arose,

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of Chemical Dependence Disorders: Clinical Perspectives, 3 MAPS BULL. 5, 5 (1995), http://www.ibogaine.org/ibogaine_trauma.html; Freedlander, *supra* note 21, at 79; Piotr Popik & Phil Skolnick, *Pharmacology of Ibogaine and Ibogaine-Related Alkaloids*, *in* THE ALKALOIDS 197 (Geoffrey A. Cordell ed., 1998).

[34] Freedlander, *supra* note 21, at 79.

[35] *See, e.g.,* MAPS, *supra* note 33, at 16; Lotsof, *supra* note 33, at 5; Freedlander, *supra* note 21; *see also* Popik & Skolnick, *supra* note 33.

[36] Vince Beiser, *One Pill Makes You Better*, NEWS REVIEW, Jan. 6, 2005, http://www.newsreview.com/sacramento/content?oid=33181; Freedlander, *supra* note 21, at 2; Romaldas Mačiulaitis et al., *Ibogaine, An Anti-Addictive Drug: Pharmacology and Time to Go Further in Development, A Narrative Review*, 27 HUMAN & EXPERIMENTAL TECHN. 181, 188 (2008); MAPS, *supra* note 33, at 16.

[37] Simon Witter, *Ibogaine*, *in* I BEGIN AGAIN TREATMENT CENTERS (2003), http://www.ibeginagain.org/articles/times.shtml.

[38] Freedlander, *supra* note 21, at 79-82.

[39] *Id*. at 3.

[40] Witter, *supra* note 37.

[41] Freedlander, *supra* note 21, at 79.

[42] Kenneth R. Alper et al., *Treatment of Acute Opioid Withdrawal with Ibogaine*, 8 AM. J. ADDICTIONS 234, 236 (1999).

[43] *Id*.; *see also* Freedlander, *supra* note 21.

allowing the addict to gain critical insight into the reasons why he or she abuses.[44]

The user, however, remains in control during the entire ordeal and can terminate negative visions by simply opening his or her eyes.[45] After the acute phase has ended, the user then enters a reflective and neutral state that lasts 8 to 20 hours.[46] The experiences during this stage may vary individual to individual, with some using this time to reflect on what they learned in the first stage.[47] In the majority of cases, the user will be unable to sleep, unless assisted by sleep medication, as ibogaine can cause insomnia for up to 72 hours.[48] In the third and final stage, which can last for an additional 24 hours, the person will be functional, albeit slower and more open or vulnerable.[49]

Following ibogaine treatment, "former addicts" will have two months to one year, free of drug cravings, giving them a "window of opportunity" to seek therapy and support for their addictions.[50] Follow-up therapy is extremely important, as addicts who do not receive the assistance needed to sustain drug-free lives during the window of opportunity often relapse when the psychological issues originally turning them on to drugs return.[51] The larger window of opportunity after taking ibogaine, however, allows patients to "get a head start in their recovery," while patients treated in traditional clinics must "learn very basic and concrete ways to stay clean[,] as taught in self-help meetings, and emphasized in psychotherapy."[52]

Ibogaine's physical effects include ataxia,[53] dystonia,[54] nausea and vomiting, tremors, and light sensitivity.[55] The many physical side effects, the duration of these side effects, and the fact that ibogaine is not necessarily hallucinogenic in the same sense as LSD, make ibogaine an unpopular choice for recreational drug use.[56] Moreover, it is highly improbable that ibogaine is addictive, evidenced by the many people who have experienced its effects and related it to a "rough trip," an unpleasant experience many do not wish to repeat.[57]

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[44] Taub, *supra* note 1.
[45] IBOGAINE: RITE OF PASSAGE (Lunart Productions 2003) (Dutch documentary film about Ibogaine; DVD copies are available at www.ibogainefilm.com).
[46] Alper, *supra* note 42.
[47] Taub, *supra* note 1.
[48] Alper, *supra* note 42, at 236; Taub, *supra* note 1.
[49] Taub, *supra* note 1.
[50] *Id*.; Witter, *supra* note 37.
[51] Taub, *supra* note 1; Witter, *supra* note 37.
[52] Witter, *supra* note 37.
[53] WEBSTER'S NEW WORLD MEDICAL DICTIONARY 34 (3d ed. 2008) (defining ataxia as the loss of muscle coordination).
[54] *Id.* at 130 (defining dystonia as "involuntary movements and prolonged muscle contraction").
[55] Freedlander, *supra* note 21, at 79-82.
[56] *Id.*
[57] *Id.*

In 1962, the anti-addictive property of ibogaine was inadvertently discovered by 19-year-old heroin addict Howard Lotsof.[58] Lotsof was part of an experimental group of mostly 20-something Caucasians attending college.[59] The group, which included seven heroin addicts, shared a common interest in experimenting and subjectively evaluating their experiences with various psychoactive drugs, including Mescaline, LSD, DMT, and psilocybin,[60] in an effort to determine the psychotherapeutic value of hallucinogenic drugs.[61] As psychedelic drugs were not illegal at the time, Lotsof had access to many of these drugs through his company, S & L Laboratories.[62] Rather than simply providing the hallucinogenic, euphoriant high the group anticipated, the heroin addicts noticed that ibogaine actually alleviated their craving for heroin.[63] Lotsof ceased using heroin, cocaine, and all other drugs during the six months following his initial dose of ibogaine, an effect most members of the group shared.[64]

In 1966, many of the psychedelic drugs which Lotsof possessed were classified as Schedule I narcotics: drugs that have no acceptable medical value and a high potential for abuse.[65] As Lotsof had already drawn the attention of the Food and Drug Administration (FDA) several years earlier, the government was highly aware of Lotsof's activities and had him arrested and charged with drug conspiracy.[66] During his trial, Lotsof tried to speak out about ibogaine's anti-addictive property, but his testimony was stricken from the record, and he was sentenced to 14 months in jail.[67]

Ibogaine enjoyed a relatively brief stint in the drug market during the 1960s, first appearing in a small number of cases in New York and California.[68] Shortly after these reported cases, however, ibogaine disappeared from the drug market completely.[69] This short visit is likely explained by the fact that there was no profit for drug dealers in selling a product that, because of its anti-addictive effect, actually decreased their clientele.[70]

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[58] *Id.*; *see also* Nick Sandberg, *Ibogaine*, http://www.ibogaine.co.uk/index.htm (noting that Lotsoff passed away on Jan. 31, 2010).

[59] Howard S. Lotsof & Norma E. Alexander, *Case Studies of Ibogaine Treatment: Implications for Patient Management Strategies, in* THE ALKALOIDS, *supra* note 33, at 293-94.

[60] *See* NAT'L INST. DRUG ABUSE, U.S. DEP'T HEALTH & HUMAN SERVICES, PUB. NO. 01-4209, HALLUCINOGENS AND DISSOCIATIVE DRUGS 1-2 (Mar. 2001) (noting that mescaline, lysergic acid diethylamide, dimethyltryptamine, and psilocybin are drugs which have hallucinogenic qualities).

[61] *Id.*; Witter, *supra* note 37.

[62] Freedlander, *supra* note 21, at 82-84.

[63] Witter, *supra* note 37.

[64] *Id*.

[65] Freedlander, *supra* note 21, at 82-84.

[66] *Id.*

[67] *Id.*

[68] *Id.* at 2.

[69] *Id.*

[70] *Id*.

Despite ibogaine's known anti-addictive properties, in 1967, the federal government classified the drug as a Schedule I controlled substance, where it remains today.[71] Ironically, ibogaine is classified in the same category as the very drugs it can counteract.[72] Throughout most of the world, ibogaine remains legal,[73] albeit somewhat restricted in Australia, Belgium, Sweden, and Switzerland.[74]

B. Formal and Informal Studies

In 1986, more than two decades after his initial discovery, Lotsof: founded NDA International (NDA); obtained patents for the use of ibogaine in treating opiate, cocaine, amphetamines, and alcohol addictions under the name Endabuse; and started unofficially distributing ibogaine to addicts in Holland.[75] In 1991, impressed with the positive results ibogaine had on substance addiction in animals, the United States National Institute for Drug Abuse (NIDA) began studying the anti-addictive drug to determine its relative safety and to develop treatment protocols for human use.[76] In 1993, the FDA approved the first clinical trial, which was to be conducted by Dr. Deborah Mash of the University of Miami on behalf of NDA.[77] The future looked bright for ibogaine and the drug addicts who would benefit from its use, but the death of a young woman in Holland taking ibogaine for her heroin addiction abruptly halted promising ibogaine research.[78] This death ended the Holland trials, though it was believed that the woman had died as the result of a heroin overdose while under the effects of ibogaine.[79]

Then, in 1993, a 24-year-old woman was pronounced dead 19 hours after her initial ibogaine dose was administered.[80] The autopsy did not produce a definite cause of death, but the forensic pathologist suspected the cause was related to the lack of information in administering the proper dose of ibogaine.[81] A piece of charred tin foil, however, was found among her effects, and it is likely that she smoked heroin shortly after ingesting ibogaine without realizing that its toxicity would be increased, which may have caused a heroin overdose.[82]

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[71] Mačiulaitis, *supra* note 36.
[72] Witter, *supra* note 37.
[73] Sandberg, *supra* note 58.
[74] *See* I BEGIN AGAIN, http://www.ibeginagain.org/faqs.shtml (last visited Nov. 26, 2010) (noting that in Australia, it is illegal to import ibogaine without a license and in Belgium, Switzerland, and Sweden, the purified alkaloid (salt) ibogaine-hydrochloride, is a restricted substance).
[75] Ed Platt, *The Dreaming*, THE INDEPENDENT ON SUNDAY, Mar. 29, 1999, at 1.
[76] Sandberg, *supra* note 58.
[77] *Id.*
[78] *Id.*
[79] *Id.*
[80] *Id.*
[81] *Id.*
[82] *Id.*

Dr. Mash's clinical trials technically commenced, despite the Holland death, but disputes between the NIDA and the University of Miami over funding issues brought the trial to a close before it really even began.[83] Shortly thereafter, the NIDA suspended all further activity involving ibogaine, citing the death of the Holland patient as a concern, but political factors and criticism from the pharmaceutical industry likely played a role.[84]

In 1995, the results of 33 patients treated with ibogaine between 1962 and 1993 were presented at the Ibogaine Review Meeting in Rockville, Maryland.[85] Seven of these treatments were conducted in the United States prior to 1963, and the remaining 26 occurred in the Netherlands between 1989 and 1993.[86] The 33 subjects were selected for inclusion because they were heroin dependent and had been directly observed by Lotsof or Frenken for at least 48 continuous hours after treatment began.[87] The patients were also observed between 48 and 72 hours by Lotsof, Frenken, or other trusted observers.[88] Ibogaine treatment was provided to these patients in either a hotel or an apartment setting.[89]

The patients did not eat, drink, ingest, or consume any drugs for eight hours prior to the administration of ibogaine, and patients on methadone received their last methadone dose approximately 24 hours before treatment.[90] Lotsof and Frenken recorded their observations of patient behaviors, including any physical signs of opioid withdrawal.[91] Both researchers had extensive knowledge with opioid withdrawal symptoms and detailed signs, such as "midriasis, sweating, elevated pulse rate, shivering, piloerection, and diarrhea," as well as subjective symptoms, including "chills, muscle pain, abdominal pain, and nausea" in their journals.[92] Because vomiting is a common side effect of taking ibogaine, the researchers did not record it as a withdrawal symptom unless the vomiting and nausea lasted for more than two hours.[93]

Most of the patients included in this study had begun experiencing opioid withdrawal symptoms because they had not used heroin the night before, yet they experienced relief of opioid withdrawal symptoms within one to three hours of ingesting ibogaine.[94] Additionally, 76% of the patients had no outward

[83] *Id.*
[84] *Id.*
[85] Alper, *supra* 42, at 236.
[86] *Id.*
[87] *Id.*
[88] *Id.*
[89] *Id.*
[90] *Id*. at 237.
[91] *Id.*
[92] *Id.*
[93] *Id.*
[94] *Id.*

signs of withdrawal, nor any subjective complaints of withdrawal, within the 48 hours after taking ibogaine, and none of these 25 patients attempted to obtain heroin or another opioid for at least 72 hours following the initial dose of ibogaine.[95] One patient had documented sweating at 24 hours, but this had been alleviated by 48 hours, and he did not use drugs within 72 hours of treatment.[96] Another experienced chills through the first 48 hours but did not use drugs within 72 hours of treatment.[97] Four other patients who did not experience any subjective or objective withdrawal symptoms within 48 hours returned to heroin use within 72 hours, with two patients explicitly acknowledging that they wished to continue using heroin despite the loss of physical craving.[98] Only one patient, a 27-year-old woman who used approximately 0.4 grams of heroin[99] a day and received a low dose of ibogaine did not experience relief from opioid withdrawal and returned to heroin use within eight hours of her initial ibogaine dose.[100] The researchers believed that treatment failed in this case because an inadequate dose of ibogaine was given to treat her level of opioid dependence.[101]

In 1996, Dr. Mash opened a state-of-the-art ibogaine treatment clinic in the Caribbean.[102] There, drug addicts are properly screened to ensure that they are healthy enough to be treated with ibogaine, are hooked up to heart monitors and continually monitored by a staff of nurses during treatment, and are given an individualized rehabilitation plan drawn up by a Harvard Professor.[103]

Between 1996 and 2000, Dr. Mash treated approximately 70 addicts at her clinic.[104] At an ibogaine conference in New York in November of 1999, she announced that 83% of the drug addicts she treated with ibogaine at her clinic experienced no withdrawal symptoms or cravings after 24 hours.[105] While she admits that ibogaine does not work for every addict, she believes that "it is a hell of a lot better than anything else we've got."[106] Although still in operation, the cost of treatment at this facility is approximately $10,000.[107] This high

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[95] *Id.*
[96] *Id.*
[97] *Id.*
[98] *Id.*
[99] *See* NAT'L HIGHWAY TRAFFIC SAFETY ADMIN., DRUGS & HUMAN PERFORMANCE FACT SHEETS: MORPHINE & HEROIN, http://www.nhtsa.gov/people/injury/research/job185drugs/morphine.htm (noting that the average daily dosage for heroin users is between 0.3 and 0.5 grams).
[100] Alper, *supra* note 42, at 238.
[101] *Id*.
[102] Freedlander, *supra* note 21, at 82-85.
[103] Jerome Burne, *One-Step Cure for Addiction?*, *originally in* FOCUS MAG., July 2000, http://www.ibogaine-therapy.net/index408b.html?Page=42.
[104] *Id.*
[105] *Id.*
[106] *Id.*
[107] *Id.*

price effectively precludes treatment for a large portion of drug addicts, as most cannot afford such expensive therapy.

Another study, conducted in 2001 and 2002, treated a number of individuals for substance abuse addiction in the United Kingdom.[108] With the exception of two patients treated in their own homes, these individuals were treated in a facility in West Sussex.[109] To be included in the study, the individual was required to meet a number of criteria.

1. Subject participation must be voluntary and informed.
2. Subject must sign an informed consent indicating their understanding of the possible risks and potential benefits of ibogaine.
3. Subject must have done some research and investigation into ibogaine and given some thought to the process.
4. Subject must obtain an ECG and report.
5. Subject must provide reports from a liver function test and blood work.
6. Subject must sign a form stating that they have not taken any narcotic analgesics, cocaine, amphetamines or alcohol for the last 12 hours before arriving and that they have none of these substances in their possession.
7. Subject must provide a next of kin in case of emergency.[110]

Additionally, the individual was denied treatment, although there were some negotiations and compromises, if they had any of the following.

1. Significantly impaired liver function.
2. Any signs of abnormalities on the ECG or any previous heart problems.
3. Severe mental health problems such as schizophrenia or bipolar disorder.
4. HIV positive or HEP C symptomatic.
5. Presently taking antipsychotic medication.
6. On long-term medication for which there is no prior data available regarding possible interactions with ibogaine or psychoactive compounds.[111]

Patients taking ibogaine for substance addiction received a dose ranging from 15 to 20 mg/kg of body weight, with the most effective dose appearing to be between 17 mg/kg and 19 mg/kg.[112] Of the 18 individuals who received ibogaine to interrupt their addiction, six remained clean and sober during the entire two year follow-up.[113] Two remained clean for approximately one year and went back to using drugs to self-medicate for pain, while another two

[108] MAPS, *Ibogaine: Treatment Outcomes and* Observations, 13 MAPS 16, 17 (2003), http://www.maps.org/news-letters/v13n2/13216two.pdf.
[109] *Id.*
[110] *Id.*
[111] *Id.* at 18.
[112] *Id.*
[113] *Id.*

remained clean for three to six months following their initial dose of ibogaine, but ultimately returned to intermittent drug use.[114] One individual died of a heroin overdose six months after treatment, and five patients returned to drugs within one month of treatment.[115] The last two patients did not remain in contact with the researchers, but remained sober for at least one week following treatment.[116] In contrast, approximately 70% to 90% of addicts who receive traditional substance abuse treatment relapse within the first year.[117]

Between 2000 and 2005, researchers Patrick Kroupa and Hattie Wells treated 45 drug addicts with ibogaine.[118] While they reported that only four of these individuals were so impacted by their first dose of ibogaine that they were immediately cured of their addiction, Kroupa and Wells found that retreatment with ibogaine greatly increased one's chances of success.[119] For example, they found that booster doses of ibogaine, given shortly after the initial dose, greatly benefited individuals with a long history of drug dependence and may be the difference between success and relapse.[120] Additionally, they found that tune-ups given several weeks and months later to those who have maintained sobriety, but may be slipping, or to those that have already returned to drug use, are beneficial.[121]

C. Aftercare—The Difference Between Success and Relapse

As these researchers point out, and those most familiar with the treatment will testify, ibogaine is not, in general, a "cure" for drug addiction.[122] In the majority of cases, however, it does eliminate or substantially decrease signs and symptoms of withdrawal and the individual typically emerges some 36 hours later without physical dependence on the drug.[123] "Ibogaine doesn't eradicate the underlying causes of addiction, which for many people may take years to understand and come to terms with. Ibogaine is more than a detox, but it's a catalyst, not a 'cure.'"[124] Ibogaine creates a "window of opportunity" where the individual can cognitively choose to take back control of his or her life.[125] It is during this window of opportunity that a drug addict can be

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[114] *Id.*
[115] *Id.*
[116] *Id.*
[117] Addiction Solutions, *Relapse—Is It Possible to Stay Clean?*, http://www.alcohol-drug-treatment.net/relapse.html.
[118] Patrick K. Kroupa & Hattie Wells, *Ibogaine in the 21st Century: Boosters, Tune-ups and Maintenance* 15 MAPS 21, 22 (2005), http://www.maps.org/news-letters/v15n1/iboga21.pdf.
[119] *Id.*
[120] *Id.*
[121] *Id.*
[122] MAPS, *supra* note 108, at 18; Kroupa & Wells, *supra* note 118, at 22.
[123] MAPS, *supra* note 108, at 18.
[124] Kroupa & Wells, *supra* note 118, at 24.
[125] MAPS, *supra* note 108, at 19.

reached, and that is why aftercare and therapy are so important to ibogaine's success.[126]

Because ibogaine is illegal in the United States, individuals who have or are able to secure the resources to travel to Mexico, Canada, or the Caribbean for treatment often return home with no support waiting for them.[127] Although they are no longer physically dependent, the memories elicited during treatment may begin weighing them down and many turn back to drugs in the absence of any real therapy to help them deal with these underlying issues positively.[128] While many clinics offer short after-care programs, even those that can afford treatment likely cannot afford an extended stay in a foreign country.

III. ARGUMENTS AGAINST THE LEGALIZATION OF IBOGAINE

There are a number of reasons typically given in opposition to the use of ibogaine as a treatment for drug and alcohol addiction. Some argue that ibogaine is a hallucinogenic drug with no medicinal value and serious safety concerns.[129] Others explain that pharmaceutical companies are unwilling to invest in ibogaine because it potentially increases their liability, given the greater fatality rate among drug users in general.[130] Finally, some explain that there is simply no profit to be made on a drug that need be taken only once or twice, which eliminates the incentive to study, develop, and mass-produce the drug.[131]

A. Ibogaine's Status as a Schedule I Controlled Substance

One of the main arguments against the use of ibogaine for drug and alcohol treatment is that it has hallucinogenic qualities and, therefore, is addictive.[132] Having been discovered by a drug addict, rather than by scientists in a laboratory, ibogaine has been condemned from the very beginning.[133] Classified as a Schedule I controlled substance, ibogaine is listed in the same category with the very drugs it counteracts because its hallucinogenic properties arguably outweigh its medicinal value.

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[126] *Id*.
[127] Taub, *supra* note 1.
[128] *Id*.
[129] Vince Beiser, *One Pill Makes You Better*, NEWSREVIEW.COM, Jan. 6, 2005, http://www.newsreview.com/sacramento/content?oid=33181.
[130] Derek Williams, *Drug Addiction Treatments: Is There Hope for the Future*, DISABLED WORLD, June 28, 2010, http://www.disabled-world.com/medical/pharmaceutical/addiction/addict-Ibogaine.php.
[131] Vastag, *supra* note 12, at 3096-101.
[132] Beiser, *supra* note 129.
[133] *Id*.

Although ibogaine is a psychoactive drug, it does not share the same hallucinogenic qualities as other psychoactive drugs.[134] Those who have taken ibogaine relate its effects to more of a "waking dream," as such visions may be avoided by opening one's eyes.[135] While in this "altered state of consciousness," users typically "relive [their] childhood experiences," so as to "get to the root of [their] addictions."[136] Furthermore, ibogaine users find it "too emotionally unsettling, mentally exhausting, and physically stressful to be any kind of party drug."[137]

Many researchers have lost all hope that ibogaine will ever be legalized in the United States. As a result, some researchers have begun work on developing ibogaine derivatives with the same anti-addictive effect as ibogaine without the negative side effects and hallucinations.[138] Dr. Mash, for example, has isolated the molecule noribogaine, which she believes is the key agent that blocks an addicts' cravings, and Dr. Stanley Glick has synthesized a chemical form of ibogaine, which he calls 18-MC.[139] Both derivatives may be the answer to effectively treating drug addiction in jurisdictions where ibogaine may never be legalized because these derivatives do not cause hallucinations, which both Dr. Mash and Dr. Glick believe to be a necessary factor for FDA approval.[140] Mash points out, however, that eliminating the psychedelic effects may diminish the effectiveness for treating addiction.[141] Many believe that this state of waking dreams allows the addict to gain insight into the reasons for taking drugs in the first place, which ultimately helps address those issues in therapy, thus increasing the chance for continued sobriety.

B. Safety Concerns

Some cite safety concerns for opposing ibogaine's use to treat addiction, as several deaths have been linked to ibogaine use.[142] The first documented ibogaine-related death occurred in France in 1990, when a 44-year-old woman died four hours after ingesting ibogaine.[143] Her cause of death was acute heart failure, and the autopsy showed that she had a prior myocardial infarction of the left ventricle, severe atherosclerotic changes, 70% to 80% stenosis of all three major coronary artery branches, a history of hypertension, and may have

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[134] Nina L. Diamond, *Does One Trip Equal 30 Years on a Therapist's Couch?*, 16 OMNI 16 (1994).
[135] *See, e.g.,* Mara Shalhoup, *Ibogaine's Long Strange Trip*, CREATIVE LOAFING, http://atlanta.creativeloafing.com/gyrobase/Content?oid=oid%3A5355.
[136] Diamond, *supra* note 134.
[137] Beiser, *supra* note 129.
[138] *Id*.
[139] *Id*.
[140] *Id*.
[141] *Id*.
[142] *Id.*; *see also* Myeboga Essentials, *Ibogaine Related Fatalities*, http://www.myeboga.com/fatalities.html.
[143] Myeboga Essentials, *supra* note 142.

taken other drugs while on ibogaine.[144] This was followed by the death of the 24-year-old Holland woman mentioned above, who died of respiratory arrest 19 hours after taking ibogaine.[145] No definite cause of death was found in the autopsy, but it was noted that she may have taken opiates while on ibogaine.[146] In 1994, a woman treated in Panama died shortly after a follow-up visit at the University of Miami.[147] Her cause of death was later revealed to be unrelated to ibogaine use, caused by "mesenteric artery thrombosis associated with small bowel infarction (blockage), complicated by a general 'hypercoagulable state' of the blood due to chronic infection in the thigh."[148]

In 2000, a 40-year-old man died 40 hours after treatment, after asphyxiating on his own vomit.[149] The coroner reported that his death "need not necessarily be laid at the feet of ibogaine," as he also had hepatitis C.[150] In 2002, a 35-year-old woman died of unknown causes a half hour after taking a small dose of ibogaine for psychospiritual reasons.[151] She had taken ibogaine before with no problem, despite health issues with her heart, breasts, and uterus, including a history of congenital heart defects.[152] In 2005, a patient died of natural causes during a period of low-dose ibogaine treatment.[153] The autopsy found that he had acute myocardial infarction, acute coronary syndrome, fibromyalgia, and chronic opiate dependency.[154] Later that year, a 43-year-old heroin addict died in Las Vegas three days after taking ibogaine.[155] His cause of death was determined to be vascular heart disease.[156] In 2006, a 38-year-old Santa Barbara man died at an ibogaine clinic in Tijuana.[157] His cause of death was pulmonary thrombosis.[158] Later that year, a 48-year-old man died of massive pulmonary edema with hemorrhagic alveotis and vascular congestion, consistent with a drug overdose, after receiving ibogaine treatment.[159]

Although these nine deaths occurred after the patients used ibogaine, none have been directly attributable to ibogaine use. Most of these patients would have been denied ibogaine treatment had their medical histories been

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144 *Id.*
145 *Id.*
146 *Id.*
147 *Id.*
148 *Id.*
149 *Id.*
150 *Id.*
151 *Id.*
152 *Id.*
153 *Id.*
154 *Id.*
155 *Id.*
156 *Id.*
157 *Id.*
158 *Id.*
159 *Id.*

known. Even if the assumption were to be made that ibogaine was to blame for these deaths, every drug, legal or not, has negative side effects, many of which can be serious, if not fatal. Methadone, for example, a legal, synthetic opiate primarily thought to be a "safer drug" used in replacement therapy for heroin and opiate addicts, was associated with nearly 10,000 deaths spanning a two-year period, not including vehicle fatalities caused by those under the influence of methadone.[160] The federal Drug Abuse Warning Network (DAWN) reported in 2001 that an estimated 10,725 people were seen in hospital emergencies rooms for methadone abuse.[161] James McDonough, director of the Florida Office of Drug Control, believes that methadone is "the fastest rising killer drug."[162] Prescription drugs alone kill an estimated 106,000 people every year in the United States,[163] and adverse drug reactions to prescription drugs is the fourth leading cause of death in the United States.[164]

Although ibogaine has potential side effects, including ataxia, tremors, photosensitivity, nausea, vomiting, slight changes in blood pressure, and hallucinations, all of these side effects dissipate within 48 hours of treatment.[165] Degeneration of cerebellar Purkinje cells[166] has also been reported after receiving high doses of ibogaine.[167] While these safety concerns are valid, they also show how desperately the United States needs ibogaine clinics and physicians who are able to legally and safely administer ibogaine to drug addicts, ensuring that it is administered under medical supervision and only to those individuals who do not have prior medical histories that may react negatively to the treatment. Every drug has risks, but those risks must always be weighed against potential and actual benefits. In the case of ibogaine, the benefits far outweigh the risks for most (otherwise healthy) addicts.

C. Pharmaceutical Hesistance in Funding Ibogaine Studies

Perhaps the most prominent reason ibogaine has not been legalized for medical use in the United States is the lack of financial incentives to produce the drug. Pharmaceutical companies feign resistance to the drug's potential

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[160] *See* Helping America Reduce Methadone Deaths, *Methadone History and Facts,* http://www.harmd.org (reporting nearly 4,000 methadone-related deaths in 2004 and over 4,700 in 2005).

[161] Pam Belluck, *Methadone, Once the Way Out, Suddenly Grows as a Killer Drug*, N.Y. TIMES, Feb. 9, 2003, at 11.

[162] *Id.*

[163] Jessica Fraser, *Statistics Prove Prescription Drugs Are 16,400% More Deadly than Terrorists*, NATURALNEWS.COM (July 5, 2005), http://www.naturalnews.com/009278.html.

[164] *See, e.g.,* Jason Lazarou et al., *Incidence of Adverse Drug Reactions in Hospitalized Patients*, 279 J.A.M.A. 1200, 1205 (1998).

[165] Iboga Therapy House, *Frequently Asked Questions*, http://www.ibogatherapyhouse.net/cms/content/view/19/32/.

[166] *See* DALE PURVES ET AL., NEUROSCIENCE 432-34 (4th ed. 2007) (explaining that Purkinje cells are some of the largest neutrons in the cerebellum of the brain and control motor movement).

[167] Dao-Yao He et al*., Glial Cell Line-Derived Neurotrophic Factor Mediates the Desirable Actions of the Anti-Addiction Drug Ibogaine Against Alcohol Consumption*, 25 J. NEUROSCI. 619, 619 & 624 (2005).

increases in pharmaceutical liability caused by larger fatality rates of drug and alcohol addicts.[168] While drug and alcohol users may have a higher risk of death, the more likely reason for such resistance is the lack of profit to be made from an anti-addictive drug.

In 1999, the Pharmaceutical Research and Manufacturers of America (PhRMA) reported that there were only 10 anti-addiction agents in clinical trials, while more than 400 cancer drugs were in clinical development.[169] While a spokesperson for PhRMA could not explain the disparity, the most likely reason is that pharmaceutical companies cannot profit from a drug that could treat an addict with only one or two doses.[170] Pharmaceutical companies, after all, are in the business to study, develop, and market drugs that are going to be profitable. Many prescription drugs are taken over long periods of time, while ibogaine treatment can succeed with very few administrations. This is one reason methadone is so popular: once a heroin addict stops using methadone, he or she experiences withdrawal symptoms.[171] Methadone is addictive, and the continued need for methadone keeps the addict going back and spending money on the drug.[172] Even over-the-counter drugs like aspirin have a larger repeat-customer base than ibogaine ever will.

IV. SUGGESTIONS FOR THE FUTURE OF IBOGAINE

Ibogaine can play an important role in United States society—to aid in treating the costly, deadly, and overly abundant problems associated with drug and alcohol addiction. The best way to enable ibogaine to provide the relief that it is capable of is for the legislature to remove the drug from the list of Schedule I controlled substances. By doing so, formal studies and treatments under medically supervised conditions may be conducted. As pharmaceutical companies are unlikely to fund these clinical studies, trillions of dollars could be saved in the long run if these studies were funded by taxpayers. Another option available is for states to decriminalize the use and possession of ibogaine, such as many have done for medical marijuana.

A. Removing Ibogaine from Schedule I

Ibogaine is wrongly classified as a Schedule I controlled substance under the guise that it has a high potential for abuse, it has no accepted medical

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[168] Williams, *supra* note 130.
[169] Vastag, *supra* note 12, at 3101.
[170] *Id.*
[171] NARCONON ARROWHEAD, METHADONE & METHADONE ADDICTION, http://www.heroinaddiction.com/heroin_methadone.html#ht=498
[172] *Id.*

use, and it has serious safety concerns that cannot be addressed by medical supervision of treatment.[173] In truth, ibogaine is not a "fun" drug to use: it is 36 hours of one's life that most wish to never experience again.[174] As a result of ibogaine's anti-addictive qualities and the physical effects it elicits, it is unlikely that anyone would choose to take ibogaine as a recreational drug, and, therefore, the potential for abuse is extremely low. Moreover, the fact that there are no legal ibogaine clinics in the United States does not establish that there is no accepted medical use for the drug. Several researchers have conducted clinical ibogaine studies and have opened treatment clinics outside of the United States. Ibogaine has an accepted medical use, and the only obstacle keeping ibogaine clinics from resurrecting in the United States is politics. Further, while there are safety concerns associated with ibogaine, as there are with any drug, these concerns can be addressed by making ibogaine available under the direct supervision of trained medical professionals.

Ibogaine should be redesignated a Schedule II controlled substance, at a minimum, so that formal clinical trials can be conducted in the United States.[175] Removal to Schedule II would allow physicians to prescribe ibogaine to their patients to be used under medical supervision. This would greatly reduce, if not eliminate, its adverse effects, as the patients would be thoroughly screened prior to treatment to ensure that they are good candidates for ibogaine. Moreover, by allowing physicians to prescribe and supervise ibogaine treatment, more formal research on ibogaine can be conducted.

B. Taxpayer Funding of Clinical Studies

If the pharmaceutical companies are unwilling to fund controlled clinical studies of ibogaine, perhaps taxpayers should. As discussed above, Americans spend $181 billion on drug abuse and drug related crime and $185 billion on alcohol abuse each year.[176] The amount of money necessary to fund a controlled clinical study on ibogaine would be much less costly than the current costs of alcohol and drug abuse. By funding these studies and actually treating drug and alcohol addicts, billions of dollars spent on the incarceration of drug and alcohol addicts could be directed elsewhere and, likely, reduced. These studies should ultimately lead to the broad legalization of ibogaine for

[173] 21 U.S.C. § 812(b)(1) (2009).

[174] Chip Carter, *Ibogaine—The Magic Plant that Could Cure Addiction, Still Banned in the U.S.*, http://www.asylum.com/2010/04/27/we-investigate-ibogaine-iboga-howard-lotsof-addiction-cure.

[175] *See* 21 U.S.C. § 812(b)(2) (2010) (classifying a drug as a Schedule II Controlled Substance if it has a high potential for abuse, has a currently accepted medical use, and abuse of the drug may lead to severe psychological or physical dependence).

[176] LEVIT, *supra* note 11.

medical purposes, lowering the overall crime rate and making the nation a safer place.

C. Decriminalizing Use, Possession, and Distribution

In December 2005, a Wyoming couple was arrested for conspiracy to import ibogaine, after Drug Enforcement Agency (DEA) agents raided their home and learned of their plans to smuggle ibogaine from Switzerland to Wyoming.[177] The couple was attempting to import ibogaine to treat their drug addictions; they faced up to 20 years in prison for trying to cure their ailments.[178] If the federal government is unwilling to act, perhaps it is time for the states to do for ibogaine what many have done for medical marijuana. Twelve states, including Alaska,[179] California,[180] Colorado,[181] Maine,[182] Massachusetts,[183] Minnesota,[184] Mississippi,[185] Nebraska,[186] Nevada,[187] New York,[188] Ohio,[189] and Oregon,[190] have decriminalized the use of marijuana for recreational use, making the possession of small amounts of marijuana punishable only by fine. Moreover,

[177] Aina Hunter, Health Watch Editorial, *Busted for Iboga: Ibogaine Enthusiasts Are Getting Bad Vibes from Conspiracy Arrests*, THE VILLAGE VOICE, Dec. 21, 2005, at 14.

[178] *Id*.

[179] Raven v. Alaska, 537 P.2d 494, 511-12 (Alaska 1975) (holding possession of marijuana at one's home for personal use is protected by the privacy guaranty in the Alaska Constitution); Noy v. Alaska, 83 P.3d 538, 545 (Alaska App. 2003) (limiting prohibitions on marijuana possession to possession of four or more ounces).

[180] CAL. HEALTH & SAFETY CODE § 11357 (b) (2007) (limiting the penalty for possession of 28.5 grams or less of marijuana to a fine of not more than $100).

[181] COLO. REV. STAT. § 18-18-406 (2004) (limiting the penalty for possession of two ounces or less of marijuana to a fine of not more than $100).

[182] 22 ME. REV. STAT. § 2383 (2004) (limiting the penalty for possession of 1.25 ounces or less of marijuana to a fine of not more than $600 and limiting the penalty for possession of 2.5 ounces or less of marijuana to a fine of not more than $1,000).

[183] MASS. GEN. LAWS 94C § 32L (2010) (limiting the penalty for possession of one ounce or less of marijuana by an adult to a fine of $100 and forfeiture of the marijuana).

[184] MINN. STAT. § 152.027(a) (2005) (limiting the penalty for possession of a "small amount of marijuana" to a petty misdemeanor conviction and participation in a drug education program).

[185] MISS. CODE § 41-29-139 (2010) (limiting the penalty for possession of 30 grams or less of marijuana to a fine not less than $100 and not more than $250).

[186] NEB. REV. STAT. § 28-416(13) (2009) (limiting the penalty for possession of one ounce or less of marijuana to a fine of $300 and attendance in a drug education course).

[187] NEV. REV. STAT. § 453.336(4) (2009) (limiting the penalty for possession of one ounce or less of marijuana to a misdemeanor, a fine of not more than $600, and an examination by a drug abuse treatment facility for determination of drug dependency status).

[188] N.Y. PENAL LAW § 221.05 (2008) (limiting the penalty for possession of marijuana to a fine of not more than $100).

[189] OHIO REV. CODE § 2925.11 (2008) (limiting the penalty for possession of less than 100 grams of marijuana to a minor misdemeanor, not to constitute a criminal record).

[190] OR. REV. STAT. § 475.864(3) (2005) (limiting the penalty for possession of less than one ounce of marijuana to a fine of not more than $1,000).

16 states, including Alaska,[191] Arizona,[192] California,[193] Colorado,[194] Hawaii,[195] Maine,[196] Maryland,[197] Michigan,[198] Montana,[199] New Jersey,[200] Nevada,[201] New Mexico,[202] Oregon,[203] Rhode Island,[204] Vermont,[205] and Washington,[206] as well as the District of Columbia,[207] permit the use of marijuana for medicinal purposes. By decriminalizing ibogaine, physicians and psychiatrists would be more likely to administer ibogaine to treat drug addition, because the penalties, if any, would be small enough to justify the risk of prosecution.

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[191] ALASKA STAT. § 17.37.030(a) (2009) (creating an affirmative defense against criminal prosecution related to marijuana for registered patients, primary caregivers, and alternate caregivers); *Id*. § 17.37.030(c) (immunizing physicians for advising patients about medicinal marijuana use).

[192] ARIZ. REV. STAT. § 36-2812 (2010) (creating an affirmative defense against criminal prosecution related to marijuana for qualifying patients and caregivers).

[193] CAL. HEALTH & SAFETY CODE § 11362.5(d) (2007) (immunizing patients and caregivers from prosecution for possession and cultivation of marijuana); *Id.* § 11362.5(c) (immunizing physicians for recommending medicinal marijuana use).

[194] COLO. CONST., art. 18, § 14(2)(a) (2010) (creating an affirmative defense against criminal prosecution related to marijuana for qualifying patients and caregivers); *Id.* § 14(2)(b) (immunizing physicians for advising patients about medicinal marijuana use).

[195] HAW. REV. STAT. § 329-125 (2000) (creating an affirmative defense against criminal prosecution related to marijuana for qualifying patients and caregivers); *Id.* § 329-125 (2000) (immunizing physicians for certifying the medical use of marijuana for qualifying patients).

[196] 22 ME. REV. STAT. § 2427(1) (2010) (creating an affirmative defense against criminal prosecution related to marijuana for qualified patients and caregivers).

[197] MD. CODE ANN., CRIM. LAW § 5-601 (c)(3) (2003) (authorizing "medical necessity" as a mitigating factor in prosecution for marijuana use or possession and limiting the penalty for such use or possession to a fine of not more than $100).

[198] MICH. COMP. LAWS § 333.26428 (2008) (creating an affirmative defense against criminal prosecution related to marijuana for qualified patients and caregivers).

[199] MONT. CODE ANN. § 50-46-206 (2009) (creating an affirmative defense against criminal prosecution related to marijuana for qualified patients and caregivers).

[200] N.J. REV. STAT. § 24:6I-6 (2010) (creating an exemption from criminal liability for the acquisition, possession, transport, or use of marijuana by qualified patients, caregivers, alternative treatment centers, and physicians).

[201] NEV. REV. STAT. § 435A.310(1)(a) (2009) (creating an affirmative defense against criminal prosecution for possession, delivery, or production of marijuana for patients and those assisting patients); *Id.* § 435A.500 (prohibiting disciplinary action against physicians for advising patients about medicinal marijuana use).

[202] N.M. STAT. § 26-2B-1 (2007) (exempting qualified patients and caregivers from criminal liability for possession and use of medical marijuana).

[203] OR. REV. STAT. §§ 475.324 (2003) (creating an affirmative defense against criminal prosecution for possession or production of marijuana for qualified patients and caregivers).

[204] R.I. GEN. LAWS § 21-28.6-8 (2007) (creating an affirmative defense against criminal prosecution related to marijuana for qualified patients and caregivers).

[205] 18 VT. STAT. § 4474b(a) (2003) (exempting registered patients and caregivers from criminal liability for possession and use of medical marijuana); *Id.* § 4474b(b) (immunizing physicians for assisting patients with the registration process for medicinal marijuana use).

[206] WASH. REV. CODE § 69.51A.040 (2007) (creating an affirmative defense against criminal prosecution related to marijuana for qualified patients); *Id.* § 69.51A.030 (immunizing health care professionals for advising patients about medicinal marijuana use).

[207] D.C. CODE § 7-1671.02 (2010) (permitting qualifying patients and caregivers to possess and administer medical marijuana).

CONCLUSION

The rate of drug and alcohol addiction in the United States is alarming, and it costs society billions of dollars every year with no end in sight. Treating drug and alcohol addicts with ibogaine promises the real possibility of substantially lowering the costs shifted to society by drug and alcohol abuse. In a world where drug addiction is treated as a crime, addicts like Samantha, who have been in and out of rehab and prison, have no chance of overcoming their addictions or living a functional life. If society is ever to make progress in addressing the substance abuse issues that currently run rampant, it must begin treating drug addiction as the illness it is.

Punishing drug addicts has not worked, as evidenced by the high recidivism rate of drug offenders, and the current treatment models available for substance abusers, which either replace one drug with another or demand abstinence from drug use altogether, are rarely effective, as again proven by the high rate of relapse. Ibogaine may well be a viable answer to substance abuse problems in the United States, but, as the law currently stands, it is barred from being a viable option. Society must be willing to at least seriously explore a treatment that could solve these issues.

Ibogaine has great potential and could be one of the answers to winning the War on Drugs. As ibogaine is not addictive and has great medical value, it should be removed from the list of Schedule I controlled substances and reclassified as a Schedule II controlled substance. If, after the controlled substance schedule classification issue is addressed, pharmaceutical companies are unwilling to fund clinical studies on ibogaine, society should demand public funding of these much needed studies. These clinical studies could inevitably lead to the legalization of ibogaine for medical use, which could, in turn, substantially decrease the cost of drug treatments, as well the costs of drug-related crime and incarceration costs associated with substance abuse. If the federal government shirks this responsibility, the states should decriminalize ibogaine and allow physicians and psychiatrists to treat addicts without fear of incarceration or loss of their medical license.

Although ibogaine is not a cure for drug and alcohol addiction, the available studies and patient accounts establish that it opens a window of opportunity that would not otherwise exist with regard to treating addiction. This window of opportunity allows an addict to begin psychotherapy without physical dependence, greatly increasing the chances of continued sobriety. For many addicts, ibogaine may be the only hope of recovery. If the law does not change, these addicts may never have the opportunity to regain control of their lives, custody of their children, or even a warm place to call home.

Ibogaine—be informed before you promote or prescribe

Many primary health care providers working with addiction patients ask, 'what is ibogaine and is it safe'? Recent interest in this compound, largely generated by websites (e.g. http://ibogaine.org.nz/) and the media (e.g. *Law and Order: SVU* Season 11 Episode 7), has highlighted its apparent efficacy in the treatment of opioid dependence. Consequently some patients are requesting it as part of their treatment regimen.

Ibogaine is the major active alkaloid extracted from the root bark of the Central West African rain forest shrub *Tabernanthe iboga*,[1,2] traditionally used by indigenous people for medicinal purposes.[2] It appears to have dose-dependent effects, with low doses acting as a stimulant,[3] and high doses as a hallucinogen. In the early 1960s the Western world began to use it for its putative 'anti-addictive properties'.[3,4] Anecdotal human reports and case studies indicate that ibogaine decreases the severity of opioid withdrawal and reduces craving for heroin and cocaine for extended periods of time.[3,4]

Ibogaine's pharmacology is complex, with much still unknown about its mode of action. It is rapidly metabolised in the liver to the active metabolite, noribogaine.[1,3,5] Both drugs have been shown to mediate their effects on multiple neurotransmitter systems in the central nervous system (CNS), including N-methyl-D-aspartate (NMDA) receptor coupled ion channels, opioid, muscarinic, serotonin and nicotinic acetylcholine receptors and monoamine uptake sites.[1,3,5,6] It is thought that their anti-addictive effects are mediated via the effect on dopamine signalling pathways.[1] Furthermore, there are reports that it alters the user's state of consciousness, causing an initial 'dream state' soon after ingestion (within one to three hours) which lasts for four to eight hours, progressing to an evaluative and personal reflective stage for the following eight to 20 hours, and ending in a residual stimulation stage (starting after 12–24 hours) that may last up to 72 hours following ingestion.[1,5,7]

Despite the anecdotal evidence of its subjective effects, evidence of its efficacy in humans is restricted to open case studies.[1,5,7] A case series conducted in the United States (US) and the Netherlands with heroin-dependent patients in withdrawal showed that 76% had no withdrawal symptoms at 24 and 48 hours post-treatment and no opioid use or drug seeking was reported for at least 72 hours.[1,4] As yet no randomised controlled clinical trials in humans have been published. There are no long-term effectiveness studies of ibogaine treatment published, but anecdotal reports of long-term abstinence following treatment have been noted.[1,2]

A number of adverse effects have been reported after use of ibogaine in humans. The main concerns are neurotoxicity (body tremors, postural stability, cerebellar damage and ataxia), cardiac effects (reduced heart rate and blood pressure, and QT-interval prolongation), cardiotoxicity, death, nausea and vomiting.[1,2,4,5,7] Hence, insufficient long-term safety data are available to inform prescribing.

We were unable to find any official New Zealand (NZ) or international data with regards to ibogaine's prevalence of use, availability, cost and treatment utility. In most countries, including the US and many European countries, ibogaine is an unlicensed medicine and is illegal to possess, sell or distribute.[1,4,8] Indeed due to ibogaine's hallucinogenic properties, the US has classified ibogaine in the same category as LSD (a Schedule 1 hallucinogenic drug).

On 11 February 2010 Medsafe gazetted ibogaine and its metabolite, noribogaine as Prescription Medicines under the Medicines Act

1981.[9,10] Thus the use of ibogaine or its metabolite for the therapeutic purpose of managing or treating addiction needs to be under medical supervision. It is important to be cognisant of the fact that although ibogaine (or noribogaine) is classified as a prescription medicine, the medicine and its use has not been approved, nor has its safety, quality and efficacy been evaluated by Medsafe. Nevertheless, Section 25 of the Medicines Act permits a NZ-registered medical practitioner to prescribe, procure and administer unapproved medicines for the treatment of a patient in his or her care.[11] The Code of Health and Disability Services Consumers' Rights then places obligations on the medical practitioner i.e. 'The consumer has the right to treatment of an appropriate ethical and professional standard...' and '...requires written consent for experimental use of a medicine'.[12]

We believe that prescribers and medical professionals should be cautious before promoting ibogaine as a 'treatment option' until there is a robust body of knowledge about its efficacy and safety in humans. This caution is endorsed by the New Zealand Drug Foundation (personal communication, Ross Bell).[13]

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