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Pharmacological screen for activities of 12-hydroxyibogamine: a primary metabolite of the indole alkaloid ibogaine

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Abstract The purported efficacy of ibogaine for the treatment of drug dependence may be due in part to an active metabolite. Ibogaine undergoes first pass metabolism and is *O*-demethylated to 12-hydroxyibogamine (12-OH ibogamine). Radioligand binding assays were conducted to identify the potency and selectivity profiles for ibogaine and 12-OH ibogamine. A comparison of 12-OH ibogamine to the primary molecular targets identified previously for ibogaine demonstrates that the metabolite has a binding profile that is similar, but not identical to the parent drug. Both ibogaine and 12-OH ibogamine demonstrated the highest potency values at the cocaine recognition site on the 5-HT transporter. The same rank order (12-OH ibogamine > ibogaine), but lower potencies were observed for the [^{3}H]paroxetine binding sites on the 5-HT transporter. Ibogaine and 12-OH ibogamine were equipotent at vesicular monoamine and dopamine transporters. The metabolite demonstrated higher affinity at the kappa-1 receptor and lower affinity at the NMDA receptor complex compared to the parent drug. Quantitation of the regional brain levels of ibogaine and 12-OH ibogamine demonstrated micromolar concentrations of both the parent drug and metabolite in rat brain. Drug dependence results from distinct, but inter-related neurochemical adaptations, which underlie tolerance, sensitization and withdrawal. Ibogaine's ability to alter drug-seeking behavior may be due to combined actions of the parent drug and metabolite at key pharmacological targets that modulate the activity of drug reward circuits.

Key words Ibogaine · 12-Hydroxyibogamine · Ligand binding · Neuroreceptors · Neurotransporter · Drug dependence

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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 a rain forest alkaloid found in the root of *Tabernanthe Iboga* (Apocynaceae family), a shrub that grows in West Central Africa. Ibogaine is used by native peoples in low doses to combat fatigue, hunger and thirst, and at high doses for its hallucinogenic properties in religious rituals. Ibogaine has been claimed by members of American and European addict self-help groups to promote long-term drug abstinence from addictive substances, including psychostimulants, opiates and alcohol (for review, Popik et al. 1995). The purported efficacy is based on anecdotal reports that after a single dose of ibogaine, symptoms associated with cocaine and opioid withdrawal are eliminated and drug "craving" is inhibited for extended periods of time (Sisko 1993; Lotsof 1995). These findings are supported by preclinical animal studies which demonstrate

that ibogaine decreased morphine and cocaine self-administration (Glick et al. 1991, 1994; Cappendijk et al. 1994; Sershen et al. 1994) and blocked some of the symptoms of naloxone-precipitated withdrawal (Dzoljic et al. 1988; Glick et al. 1992; Cappendijk et al. 1993). Ibogaine is centrally active and at high doses produces adverse effects, including tremors and hallucinations (Popik et al. 1995). The development of ibogaine as an anti-addiction drug has been hindered due to uncertainties over potential neurotoxicity (O'Hearn and Molliver 1993; Touchette 1993). While ibogaine has a variety of CNS effects that are dose-related, the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood.

Ibogaine interacts with multiple targets within the CNS, including dopaminergic, cholinergic, glutamatergic, opioidergic and serotonergic systems (Popik et al. 1995). Radioligand binding surveys of ibogaine in rat brain demonstrated micromolar potencies for the dopamine (DA) transporter (Sershen et al. 1992) kappa-1 and mu opioid receptors, alpha-1 adrenergic receptors and voltage-dependent sodium channels (Deecher et al. 1992; Sweetnam et al. 1995). Ibogaine has low affinity for muscarinic receptor subtypes (Sweetnam et al. 1995) and competitively inhibits [^{3}H]MK-801 binding to the NMDA receptor complex (Popik et al. 1994; Mash et al. 1995a). Ibogaine possesses moderate affinity for putative sigma$_2$ receptors (Bowen et al. 1995; Mach et al. 1995). Ibogaine administrations cause a 5-HT behavioral syndrome in rats, suggesting an interaction with serotonergic targets (Popik et al. 1995). These multi-site interactions with a diverse grouping of neuropharmacological targets contribute to the wide spectrum of CNS activities. Recent studies have suggested that some of the neurobehavioral and physiological aftereffects of ibogaine may be mediated by a long-lasting metabolite. The principal metabolite of ibogaine has been identified in humans and primates as 12-hydroxyibogamine (12-OH ibogamine; Hearn et al. 1995a; Mash et al. 1995b). In the present study, we used radioligand binding assays to compare the potencies of ibogaine and 12-OH ibogamine at neuroreceptors and neurotransporters to help define the molecular mechanisms of action that may account for ibogaine's anti-addictive properties.

Materials and methods

Neurological tissue specimens

Human neuropathological tissue specimens were obtained at routine autopsy from accidental death victims (males; age range 20–46 years; autolysis times 13–21 h). Brain tissue specimens were frozen and stored at −70 °C until the day of assay. For binding assays, the region of interest (Table 1) was dissected and membrane homogenates were prepared by homogenizing tissue in ice-cold ligand-specific buffer with a Brinkman polytron (15 s, setting 3). Membrane homogenates were centrifuged and washed to remove endogenous ligand.

Ligand binding assays

Radioligands were commercially available from NEN/Dupont (Boston, Mass.) or Amersham Corp. (Arlington Heights, Ill.) with the exceptions of [^{125}I]iodovinyltetrabenazine and [^{125}I]IOXY which were custom synthesized and radiolabeled by Dr. Hank Kung (University of Pennsylvania) and Dr. Richard Rothman (Addiction Research Center/NIDA), respectively. Ibogaine and 12-OH ibogamine were obtained from s.a. Omnichem, Belgium. BIT (2-(*p*-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidiazole-HCl) and FIT (*N*-phenyl-*N*-[1-[2-(4-isothiocyanatophenyl) ethyl-4-piperidinyl] propanamide) were synsthesized in the Laboratory of Medicinal Chemistry, NIDDK, Bethesda, Md.). All other unlabeled drugs were purchased from Research Biochemicals (Natick, Ma.).

A summary of the radioligands used in competition assays with ibogaine and 12-OH ibogamine is shown in Table 1. All binding assays were conducted as described previously (see Table 1 for references). CHO cells stably transfected with genes for each muscarinic receptor subtype were prepared for ligand binding screens at m1–m5 receptor subtypes as described previously (Ferrari-Dileo et al. 1994). The ability of ibogaine and 12-OH ibogamine to inhibit binding to neuroreceptors or transporters was first assessed at doses of 100 nM and 10 μM. Positive controls were routinely assayed in parallel using specific drugs with known affinities (Table 1). Assay tubes were incubated under the specified conditions and filtered through Whatman 934AH filters on Millipore manifolds. Nonspecific binding was defined as the cpm bound in the presence of a saturating concentration of an established competing ligand. All binding assays demonstrated a total signal of at least 2000 dpm with at least 60–90% specific binding for each radioligand. Ibogaine and 12-OH ibogamine were considered active at the defined receptor site if there was 50% or higher inhibition at a concentration of 10 μM. To determine potency values accurately, full competition curves were obtained at relevant binding sites using 10–15 concentrations of ibogaine or 12-OH ibogamine. Ligand competition data were analyzed using the DRUG program of EBDA/LIGAND (Biosoft, Elsevier).

Quantitative gas chromatography/mass spectrometry (GC/MS)

Using GC/MS, the concentrations of ibogaine and 12-OH ibogamine were determined in brain specimens of rats treated with a single dose of ibogaine (50 mg/kg PO) and killed at the times indicated. Briefly, brain tissue was diluted with 1% NaCl (1:1), homogenized (30 s) and sonicated (5 min). The alkaloids were extracted from the tissue homogenate using a solvent extraction under basic conditions with D$_3$-ibogaine as an internal standard and derivatization of the metabolite to an ethyl ether as described previously (Hearn et al. 1995a, b). The GC/MS was operated in the full scan electron ionization mode scanning from M/Z 45 to 450 at 1 s/scan. Ibogaine and its principal metabolite were identified by subjecting brain extracts to full scan electron impact GC/MS on Finnegan 4521 (quadrupole) and Finnegan ITS-40 (ion trap) mass spectrometers. Compound identification was based upon comparison of retention times and fragmentation patterns obtained from authentic standards (s.a. Omnichem, Belgium). Ion ratios for the molecular ion of ibogaine (m/z – 310), and 12-OH ibogamine ethyl ether (m/z – 324) to that of the internal standard *O*-D$_3$-methyl-ibogaine (m/z – 313) were subjected to least squares linear regression versus concentration. The resulting standard curves were linear ($r^2 = 0.999$) and reproducible. Intraassay coefficients of variation (CV) in brain were 1.1% for ibogaine and 4.0% for 12-OH ibogamine. Limits of detection and

Table 1 Survey of ibogaine and 12-OH ibogamine targets in radioligand binding assays

Binding site	Radioligand	Nonspecific	Tissue	Reference
Adenosinergic				
A_1 receptor	[^{3}H]NECA	R-PIA	Caudate	(Bruns et al. 1986)
A_2 receptor	[^{3}H]CGS-21680	CGS21680	Caudate	(Jarvis et al. 1989)
Cholinergic				
M_1 receptor	[^{3}H]NMS	QNB	CHO-ml	(Ferrari-DiLeo et al. 1994)
M_2 receptor	[^{3}H]NMS	QNB	CHO-m2	(Ferrari-DiLeo et al. 1994)
M_3 receptor	[^{3}H]NMS	QNB	CHO-m3	(Ferrari-DiLeo et al. 1994)
M_4 receptor	[^{3}H]NMS	QNB	CHO-m4	(Ferrari-DiLeo et al. 1994)
M_5 receptor	[^{3}H]NMS	QNB	CHO-m5	(Ferrari-DiLeo et al. 1994)
Vesicular transporter	[^{3}H]Vesamicol	(−) ABV	Caudate	(Kish et al. 1990)
Dopaminergic				
D_1 receptor	[^{3}H]SCH23390	(+) Butaclamol	Caudate	(DeKeyser et al. 1989)
D_2 receptor	[^{3}H]YM-09151-2	(+) Butaclamol	Caudate	(Jarvie et al. 1987)
	[^{3}H]Haloperidol	(+) Butaclamol	Caudate	(Whitaker and Seeman 1977)
D_3 receptor	[^{3}H]-(+)-7-OH-DPAT	(+) Butaclamol	Nuc. Acc.	(Burris et al. 1994)
DA transporter	[^{125}I]RTI-121	(−) Cocaine	Caudate	(Staley et al. 1995)
	[^{3}H]Mazindol	(−) Cocaine	Caudate	(Staley et al. 1995)
Vesicular transporter	[^{125}I]-TBZ	Tetrabenazine	Caudate	(Kung et al. 1994)
Glutamatergic				
NMDA receptor complex	[^{3}H]-(+)MK801	(+) MK801	Caudate	(Popik et al. 1994)
Noradrenergic				
NE transporter	[^{3}H]Nisoxetine	Desipramine	Dentate Gyrus	(Tejani-Butt et al. 1990)
Opioidergic				
$Kappa_1$	[^{3}H]U69593	Naloxone	Insular Ctx	(Nock et al. 1988)
$Kappa_2$	[^{125}I]-IOXY	Naloxone	Caudate	(Ni et al. 1993)
$Sigma_1$	[^{3}H]-(+)-Pentazocine	(±) Pentazocine	Cerebellum	(Zabetian et al. 1994)
Serotonergic				
5-HT_{1A} receptor	[^{3}H]-8-OH-DPAT	Serotonin	Hippocampus	(Gozlan et al. 1983)
5-HT_2 receptor	[^{3}H]RP 62203	Ketanserin	Frontal Ctx	(Malgouris et al. 1993)
5-HT transporter	[^{125}I]RTI-55	(−) Cocaine	Occipital Ctx	(Staley et al. 1994)
	[^{3}H]Paroxetine	Paroxetine	Occipital Ctx	(Backstrom et al. 1989)
Vesicular transporter	[^{125}I]-TBZ	Tetrabenazine	Occipital Ctx	(Kung et al. 1994)

quantitation were 5 ng/g for both ibogaine and derivatized 12-OH ibogamine in brain, respectively.

Results

The results of the radioligand binding survey of the potencies of ibogaine and 12-OH ibogamine at various neuroreceptors and neurotransporters are shown in Table 2. At a 10 μM dose, both ibogaine and 12-OH ibogamine competed for at least 50% of the total binding to the DA transporter, 5-HT transporter and vesicular monoamine transporter. In addition, ibogaine was active in displacement assays at the cholinergic vesicular transporter and NMDA receptor complex, while 12-OH ibogamine was more potent than ibogaine at the kappa-1 receptor. Both ibogaine and its primary metabolite were nonselective and weak inhibitors of binding to m1–m5 muscarinic receptor subtypes. The results of the present study confirm and extend previous reports that ibogaine and its primary metabolite do not interact with biogenic amine receptors, including dopaminergic (Deecher et al. 1992; Sweetnam et al. 1995) and serotonergic receptor subtypes (Deecher et al. 1992). Neither ibogaine nor 12-OH ibogamine demonstrated significant binding affinity at the norepinephrine transporter, sigma-1 binding sites or adenosine (A1 and A2) receptor subtypes assayed in human brain.

The significance of micromolar interactions of ibogaine and 12-OH ibogamine with various radioligand binding sites was related to the concentration of parent drug and metabolite in brain. Regional brain levels of ibogaine and 12-OH ibogamine were measured in rat cerebral cortex, striatum, brainstem and cerebellum at 15 min, 1 and 2 h post-drug administration. The results demonstrate that ibogaine is rapidly detected in brain following oral administration (Fig. 1). The metabolite was detected at the earliest time point (15 min), consistent with first pass metabolism of the parent drug (Hearn et al. 1995a). Administration of ibogaine (50 mg/kg PO) in rodents resulted in levels of ibogaine and 12-OH ibogamine ranging from 4 to 17 μM and 1 to 17 μM, respectively. It is interesting to note that over the 2-h time period the concentration of 12-OH ibogamine increased 10-fold across all regions examined, while the levels of the parent drug decreased (cortex, brainstem) or stayed the same (striatum, cerebellum). These data provide

Table 2 Empirical screen of the inhibitory potency of ibogaine and 12-OH ibogamine

Inactive (10 μM) test dose[a]	Active[b]
Ibogaine	
[^{3}H]SCH 23390	[^{3}H]MK-801
[^{3}H]YM01915-2	[^{3}H]Mazindol
[^{3}H]-(+)-7-OH-DPAT	[^{125}I]RTI-121
[^{3}H]Haloperidol	[^{125}I]-TBZ
[^{3}H]NECA	[^{125}I]RTI-55/Benztropine
[^{3}H]CGS 21680	[^{3}H]Paroxetine
[^{3}H]Nisoxetine	[^{3}H]Vesamicol
[^{3}H]-8-OH-DPAT	[^{3}H]NMS-CHO-m1
[^{3}H]RP62203	[^{3}H]NMS-CHO-m2
[^{3}H]NMS-CHOm5	[^{3}H]NMS-CHO-m3
[^{3}H]-(+)-Pentazocine	[^{3}H]NMS-CHO-m4
[^{3}H]U69593	
[^{125}I]IOXY	
12-OH Ibogamine	
[^{3}H]SCH 23390	
[^{3}H]YM 01915-2	[^{3}H]Mazindol
[^{3}H]-(+)-7-OH-DPAT	[^{125}I]RTI-121
[^{3}H]-8-OH-DPAT	[^{125}I]-TBZ
[^{3}H]RP62203	[^{125}I]RTI-55/Benztropine
[^{3}H]Vesamicol	[^{3}H]Paroxetine
[^{3}H]NECA	[^{3}H]U69593
[^{3}H]Haloperidol	[^{3}H]NMS-CHO-m1
[^{3}H]-(+)-Pentazocine	[^{3}H]NMS-CHO-m2
[^{3}H]CGS21680	[^{3}H]NMS-CHO-m3
[^{3}H]Nisoxetine	[^{3}H]NMS-CHO-m4
[^{3}H]MK-801	[^{3}H]NMS-CHO-m5
[^{125}I]IOXY	

[a]Inactive – Ibogaine or 12-OH ibogamine competed for binding with an IC_{50} value greater than 10 μM
[b]Active – Ibogaine or 12-OH ibogamine inhibited binding with an IC_{50} value equal to or less than 10 μM

further evidence that micromolar activities of ibogaine and the *O*-demethylated metabolite are relevant for defining binding site activities.

Ibogaine and 12-OH ibogamine exhibited distinct neurotransporter and neuroreceptor binding profiles (Fig. 2). The highest potency value observed in this activity screen was for the inhibition of [^{125}I]RTI-55 binding by 12-OH ibogamine at cocaine recognition sites on the 5-HT transporter (IC_{50} = 40.7 ± 11.6 nM). Interestingly, 12-OH ibogamine competed for [^{3}H]paroxetine binding to the 5-HT transporter with 20-fold lower potency (IC_{50} = 0.9 ± 0.06 μM) than at RTI-55 binding sites. In keeping with the demonstrated rank order of potency, 12-OH ibogamine was 10-fold more potent than ibogaine at displacing both [^{125}I]RTI-55 and [^{3}H]paroxetine binding (Table 3). The metabolite also was more potent than the parent drug at the kappa-1 receptor and displaced binding in the low micromolar range. Ibogaine displayed 4- and 6-fold higher potencies than the metabolite at kappa-2 receptors and at MK-801 sites on the NMDA receptor complex, respectively. Ibogaine and 12-OH ibogamine were equipotent at the DA transporter and vesicular monoamine transporter and displaced binding in the low micromolar range (Fig. 2, Table 3).

Discussion

The present study provides additional insights into the ligand binding profiles for ibogaine and its primary metabolite that may be relevant for understanding the putative anti-addictive properties. Comparison of the in vitro binding profiles for ibogaine and 12-OH ibogamine demonstrates that they interact with neurotransporters and subclasses of neurotransmitter receptors, which are associated with the reinforcing actions of cocaine and opiates. Ibogaine and 12-OH ibogamine are active at specific pharmacological targets that have been linked to the phenomena of sensitization and tolerance. While micromolar potencies of ibogaine and its desmethyl metabolite may be considered ancillary for defining primary sites and mechanisms of action (Sweetnam et al. 1995), our pharmacokinetic studies demonstrate that both ibogaine and 12-OH ibogamine are found in rat brain following oral administrations (50 mg/kg) at levels ranging from 1 to 17 μM. The pharmacological relevance of micromolar brain

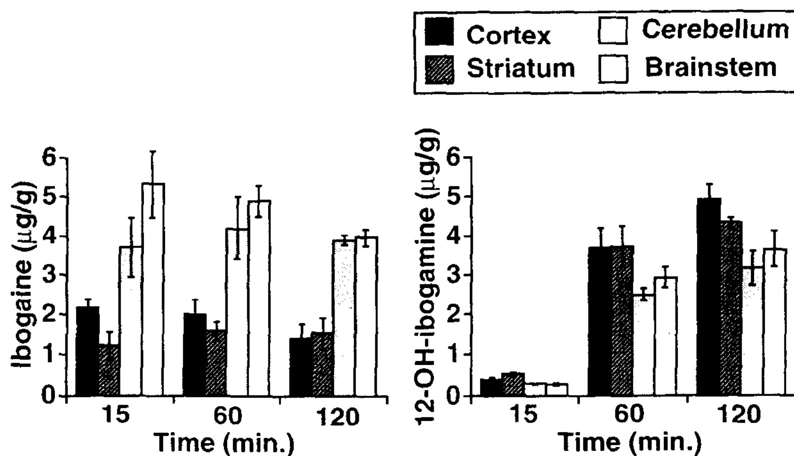

Fig. 1 Regional brain levels of ibogaine (*left*) and 12-OH ibogamine (*right*). Regional samples of rat cerebral cortex, striatum, cerebellum and brainstem were assayed at the indicated times post-adminstration of ibogaine (50 mg/kg PO). Data represent the average values from individual animals (n = 4) assayed in duplicate

Fig. 2 Potency of ibogaine and 12-OH ibogamine for binding to CNS targets. Various concentrations of ibogaine (*upper panel*) and 12-OH ibogamine (*bottom panel*) were incubated in the presence of [^{125}I]RTI-55 (10 pM)/1 μM benztropine. [^{125}I]RTI-121 (50 pM), [^{3}H]MK-801 (2 nM), [^{125}I]TBZ (1 nM) and [^{3}H]U69593 (1 nM) with membrane homogenates from the human brain. The data shown are the mean ± SE of at least three independent experiments. Potency values were obtained using DRUG (EBDA/LIGAND)

concentrations of ibogaine and 12-OH ibogamine is supported by previous studies which have demonstrated that doses of ibogaine within the range of 30–80 mg/kg are active in behavioral studies designed to assess reinforcing effects, tolerance and withdrawal symptoms associated with psychomotor stimulants and opiates. Ibogaine has been shown to reduce signs of morphine withdrawal (Glick et al. 1992), antagonize cocaine-induced locomotor stimulation (Sershen et al. 1992) and inhibit cocaine (Cappendijk and Dzoljic 1993; Glick et al. 1994) and morphine self-administration (Glick et al. 1994).

At present, the metabolism and intracerebral disposition of ibogaine in the brain is not completely known. Both the parent drug and metabolite have high heptane/phosphate buffer partition coefficients (Zetler et al. 1972), indicating their ability to penetrate the blood-brain barrier and consistent with the rapid entry of ibogaine into the brain shown here. Partitioning of the parent drug into brain lipid may serve as a slow release storage "depot". Sequestration of iboga alkaloids into lipophilic compartments in brain, may result in lower concentrations of the parent drug and metabolite in the extracellular fluid. The more polar nature of 12-OH ibogamine suggests that there may actually be higher extracellular fluid concentrations of the metabolite as compared to ibogaine. If ibogaine is *O*-demethylated to 12-OH ibogamine in brain, then it is reasonable to conclude that the slow elimination of a CNS trapped polar metabolite may contribute to some of the reported aftereffects of single dose administrations of ibogaine in humans (Mash et al. 1995b). However, further studies are needed to estimate precisely the intracerebral distribution of ibogaine and 12-OH ibogamine and their relative concentrations in extracellular fluid as compared to brain extracts.

The metabolite 12-OH ibogamine inhibited binding of the cocaine congener [^{125}I]RTI-55 to the 5-HT transporter with the highest potency reported to date. Interestingly, ibogaine and 12-OH ibogamine exhibited 15- to 20-fold lower potency for inhibition of paroxetine binding to the 5-HT transporter. The disparity in the potency values for binding to the 5-HT transporter suggests that 12-OH ibogamine and ibogaine may recognize a binding domain on the transporter that is preferred by cocaine-like, but not structurally dissimilar

Table 3 Inhibitory potency of ibogaine and 12-OH ibogamine. The values shown represent the mean ± SE of the IC_{50} value (μM) from 3–4 independent experiments each performed in triplicate

Binding target (Radioligand)	Ibogaine		12-OH Ibogamine	
	IC_{50} (μM)	nH	IC_{50} (μM)	nH
Dopaminergic				
DA transporter (RTI-55)	4.11 ± 0.45	1.10	3.35 ± 0.50	1.01
Vesicular transporter (TBZ)	14.63 ± 4.47	0.98	29.45 ± 9.13	1.05
Glutamatergic				
NMDA receptor (MK-801)	5.20 ± 0.24	0.90	31.41 ± 5.43	1.05
Opioidergic				
$Kappa_1$ (U69593)	25.00 ± 0.57	1.09	4.24 ± 0.28	1.05
$Kappa_2$ (IOXY)	23.80 ± 7.10	0.99	92.30 ± 9.21	1.03
Serotonergic				
5-HT transporter (RTI-55)	0.59 ± 0.09	0.80	0.04 ± 0.01	0.76
(paroxetine)	9.30 ± 1.70	1.15	0.90 ± 0.06	1.10
Vesicular transporter (TBZ)	2.23 ± 0.22	0.75	4.99 ± 0.48	0.98

transport inhibitors. Previous studies have shown that there are multiple cocaine-like recognition sites associated with the 5-HT transporter (Rothman et al. 1994; Staley et al. 1994). Interestingly, paroxetine has been shown to label two RTI-55 recognition sites on the 5-HT transporter with distinct affinities (Rothman et al. 1994). These observations provide additional evidence for the non-identity of binding sites associated with the 5-HT transporter and suggest that ibogaine and its desmethyl metabolite may be useful probes for dissecting their functional relevance.

In keeping with its potency at the 5-HT transporter, previous studies have shown that 12-OH ibogamine elevates extracellular levels of 5-HT in microdialysis assays following intravenous injections in rats (Mash et al. 1995b). Preliminary studies have suggested that drugs (fluoxetine, sertraline) that enhance 5-HT neurotransmission are indicated for the treatment of heroin and cocaine dependence (Batki et al. 1994; Kleber 1995; Walsh et al. 1994). Chronic drug use is associated with a 5-HT deficit form of 5-HT dysregulation, causing depressed mood and alterations in affect and cognition (Baumann et al. 1993, 1995; Levy et al. 1994; Parsons et al. 1995). Improvements in mood and cognition have been associated with the alleviation of drug "craving", which is a predictor of drug relapse. Mood measures are also important indicators of the effect of treatment for cocaine abuse, and some aspects of cravings intersect with depression and anxiety (Covi et al. 1995). These observations suggest that the interaction of a longer acting metabolite with the 5-HT transporter may be relevant for explaining the reported ability of ibogaine to rapidly alleviate dysphoria and improve mood and cognition in abstinent-drug abusers.

While ibogaine and 12-OH ibogamine displayed high affinity for the 5-HT transporter, both the parent and metabolite failed to demonstrate significant potency for binding to the 5-HT_{1A} and 5-HT_2 receptors assayed in human brain. These results conflict with a previous report, which demonstrated potencies in the low micromolar range for ibogaine binding to the 5-HT_2 receptor subtype (Sweetnam et al. 1995). In this study, [^{3}H] ketanserin was used to label the 5-HT_2 receptor subtype. It is known that this ligand binds with nanomolar affinity to the vesicular monoamine transporter (Darchen et al. 1988). This inconsistency between studies may reflect species differences or the overlapping affinities of [^{3}H] ketanserin for the 5-HT_2 receptor and vesicular monoamine transporter. The lack of affinity of ibogaine for 5-HT_2 receptors shown here may indicate that the hallucinogenic activities of the drug may be mediated by either a different population of 5-HT receptor or that ibogaine does not fit the binding site profile of classical hallucinogens (Glennon 1990).

The rewarding effects of abused drugs are mediated by activation of the mesolimbic DA system (reviewed by Di Chiara 1995). In particular, the DA transporter is a primary substrate for the reinforcing potential of psychostimulants. Ibogaine recognizes the cocaine binding site on the DA transporter and inhibits DA uptake in rat brain with a potency value corresponding to its affinity for the cocaine binding site (M. Baumann, personal communication). These findings suggest that ibogaine and 12-OH ibogamine may interact with the DA transporter in a similar manner to cocaine, but with 10-fold lower potency (Staley et al. 1994). Ibogaine and its primary metabolite may function as "cocaine partial agonists" at the DA transporter by blocking the access of cocaine and decreasing the rapid elevation of DA mediating the rewarding effects of cocaine. This explanation may account for ibogaine's ability to decrease cocaine place preference and inhibit cocaine self-administration (Cappendijk et al. 1994; Glick et al. 1994; Sershen et al. 1994). Ibogaine may affect DA transmission also by promoting a redistribution of DA synaptosomal pools in addition to its blockade of DA transporter function. Ibogaine has been shown to increase DA efflux from the cytoplasmic pool by reversal of the DA transporter (Harsing et al. 1994). Since ibogaine displays moderate affinity at the vesicular monoamine transporter, it may regulate the distribution of DA between vesicular and cytoplasmic pools. If ibogaine blocks vesicular storage of DA, the higher concentrations of cytoplasmic DA would lead to reversal of the DA transporter. In keeping with this "redistribution" hypothesis, ibogaine pretreatment significantly reduced the cocaine-induced rise in DA levels in the nucleus accumbens measured by in vivo voltametry (Broderick et al. 1994).

Mesolimbic DA neurotransmission is known to be modulated by tonic activation of the mu and kappa opioid receptors within the limbic reward sectors of the striatum. According to the model by Spanagel and Shippenberg (1992), opioids tonically regulate mesolimbic DAergic transmission through two opposing mechanisms that regulate DA release via distinct actions at cell body and terminal fields. Activation of mu receptors increase, whereas kappa agonists decrease DA release in the nucleus accumbens. Ibogaine and 12-OH ibogamine have demonstrated micromolar affinities for mu and kappa opioid receptors (Pearl et al. 1995), suggesting an additional mechanism for regulating the DAergic reward pathway. We have confirmed that ibogaine and 12-OH ibogamine have affinities in the low micromolar range at kappa receptor subtypes in agreement with previous reports (Deecher et al. 1992; Reid et al. 1994; Pearl et al. 1995; Sweetnam et al. 1995). In the present study, the *O*-demethylated metabolite displayed the highest potency at kappa-1 receptor sites assayed in human brain membranes. The role of specific kappa receptor subtypes within limbic reward pathways of the human brain are at present unknown. The selective kappa-1

agonist U-69593 attenuates cocaine-induced behavioral sensitization (Heidbreder et al. 1993), while the non-selective kappa antagonist nor-binaltorphimine enhances morphine-induced sensitization (Spanagel et al. 1992; Spanagel and Shippenberg 1993). Ibogaine and 12-OH ibogamine may regulate DA release via their interactions with kappa receptors (Reid et al. 1994). The actions of the parent drug and metabolite at kappa receptors may be important for understanding the anti-craving aftereffects of ibogaine. While kappa agonists are markedly dysphoric, the additional and potent actions of 12-OH ibogamine at the 5-HT transporter may significantly elevate mood, and thereby limit the potential adverse effects of kappa receptor activation.

The ability of ibogaine to inhibit [^{3}H]-MK801 binding to the NMDA receptor complex (Popik et al. 1994; Mash et al. 1995b; Sweetnam et al. 1995) may be of relevance for understanding the anti-addictive actions of ibogaine. MK-801 has been reported to block sensitization (reverse tolerance) to the behavioral activating effects of cocaine and amphetamine (Karler et al. 1989; Pudiak and Bozarth 1993). The administration of MK-801 attenuates both the development of tolerance to the analgesic effect of morphine and morphine dependence (Trujillo and Akil 1991, 1995). On the basis of these converging lines of evidence, Skolnick and coworkers suggest that ibogaine's ability to modify drug-seeking behavior results primarily from their blockade of NMDA receptor-coupled cation channels (Popik et al. 1994, 1995). Previously, we have shown that 12-OH ibogamine is less potent than ibogaine at inhibiting [^{3}H] MK-801 binding in assays of human brain striatum and cerebellum (Mash et al. 1995b). The effects of MK-801 on sensitization and withdrawal described above are compatible with the idea that some of the anti-addictive properties of ibogaine may result from an interaction with NMDA receptor-coupled cation channels. Since 12-OH ibogamine inhibits [^{3}H]MK-801 binding with a lower potency as compared to ibogaine, it is unlikely that the channel activity of 12-OH ibogamine is additive to ibogaine. This observation may indicate that some of the acute effects of ibogaine administration, but not the extended aftereffects, may be linked to its activities at MK-801 binding sites. It has been suggested also that ibogaine's interaction with NMDA receptor-coupled cation channels may contribute to the adverse effects of the drug, including the psychotropic (PCP-like actions) and high dose neurotoxic changes in cerebellar Purkinje cells (Sweetnam et al. 1995). Further studies are needed to address the functional significance of NMDA-receptor coupled channel blockade to ibogaine's purported anti-addiction properties.

In the present study, radioligand binding assays were conducted with 12-OH ibogamine to identify the molecular mechanism(s) accounting for ibogaine's putative long-lasting anti-addictive properties. The results demonstrate that ibogaine and its primary metabolite have pharmacologic sites of action associated with biogenic amine systems, opioidergic and glutamatergic synapses. Dependence on psychostimulants and opiates results from distinct, but inter-related processes which include tolerance, sensitization and withdrawal (Nestler and Hyman 1993, Nestler 1994). The neurochemical substrates underlying drug tolerance and reverse tolerance (sensitization), as well as the expression of withdrawal symptoms are likely to be unique and separate processes occurring within discrete cellular locations and affecting distinct molecular targets. The multi-target CNS actions of ibogaine and its primary metabolite suggest that simultaneous modulation of two or more neural mechanisms may be a more effective approach for designing anti-addiction agents.

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