Structurally modified ibogaine analogs exhibit differing affinities for NMDA receptors

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Abstract

Based on both preclinical findings and anecdotal evidence in man, the psychoactive indole alkaloid ibogaine has been suggested to have anti-addictive properties. Previous studies indicate that blockade of NMDA receptors may mediate at least some of the putative anti-addictive actions of ibogaine. The potencies of a series of ibogaine analogs to inhibit $(+)$-[3-^{3}H]5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine ([^{3}H]MK-801) binding to NMDA receptors were examined. This series of analogs included the putative ibogaine metabolite O-desmethylibogaine, its metabolism resistant analog O-t-butyl-O-desmethylibogaine, the iboga alkaloids $(\pm)$-ibogamine, $(\pm)$-coronaridine, tabernanthine, harmaline, and the indolotropanes endo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075194-190), exo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075237-190) and endo-3-(indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 025989-190). Among these compounds, ibogaine was the most potent inhibitor of [^{3}H]MK-801 binding ($K_i = \sim 1.2$ μM), whilst the compounds with the greatest structural similarity to ibogaine, O-desmethylibogaine and O-t-butyl-O-desmethylibogaine were less potent ($K_i = \sim 5.5$ and 179.0 μM, respectively). In morphine-dependent mice, ibogaine, but not O-desmethylibogaine or O-t-butyl-O-desmethylibogaine, attenuated naloxone precipitated withdrawal jumping. These findings are consistent with the hypothesis that inhibition of the expression of morphine dependence by ibogaine is related to its NMDA receptor antagonist properties.

Keywords: NMDA receptor; Ibogaine; O-Desmethylibogaine; Morphine; Naloxone

1. Introduction

Ibogaine is a psychoactive indole alkaloid originally isolated from the roots of the West African shrub *Tabernanthe iboga*. Ibogaine containing extracts were originally used in the religious rites of several West African secret societies. During the past decade, both preclinical studies (Aceto et al., 1990; Cappendjik and Dzoljic, 1993; Dzoljic et al., 1988; Glick et al., 1991, 1992, 1994; Sershen et al., 1993) and anecdotal reports (Lotsof, 1985, 1986, 1995) indicate that ibogaine can interfere with dependence and

withdrawal phenomena associated with a number of abused substances including cocaine and morphine (for review, see Popik et al., 1995b).

While the neurochemical bases for the these putative 'anti-addictive' actions of ibogaine remain unclear, several recent reports (Mash et al., 1995; Popik et al., 1994, 1995a; Sweetnam et al., 1995) have shown that at pharmacologically relevant concentrations, ibogaine acts as an NMDA receptor antagonist. Thus, ibogaine competitively inhibits $(+)$-[3-^{3}H]5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine ([^{3}H]MK-801) and [piperidyl-3,4-^{3}H(N)]-(N-(1-(2thienyl)cyclohexyl)-3,4-piperidine) ([^{3}H]TCP) binding to NMDA receptors (Mash et al., 1995; Popik et al., 1994, 1995a; Sweetnam et al., 1995), blocks glutamate-induced cell death (Popik et al., 1995a), and produces a voltage-dependent block of NMDA currents (Mash et al., 1995; Popik et al., 1995a). Moreover, ibo-

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gaine substitutes for MK-801 in a drug discrimination paradigm, and the ability of ibogaine to attenuate naloxone-precipitated jumping in morphine-dependent mice is abolished by glycine (Popik et al., 1995a). This latter observation is consistent with the hypothesis that ibogaine attenuates the expression of opiate dependence through a use-dependent block of NMDA receptors, and supports an emerging body of evidence indicating that NMDA receptor antagonists attenuate the tolerance and withdrawal associated with opioid dependence (Ben-Eliyahu et al., 1992; Higgins et al., 1992; Kolesnikov et al., 1994; Marek et al., 1991; Rasmussen et al., 1991; Tiseo et al., 1994; Tiseo and Inturrisi, 1993; Trujillo and Akil, 1991, 1995). Nonetheless, pharmacologically relevant concentrations of ibogaine have been reported to affect a number of other systems which may be relevant to drug abuse including κ opiate receptors (Deecher et al., 1992; Pearl et al., 1995; Sweetnam et al., 1995), σ receptors (Bowen et al., 1995; Mach et al., 1995; Popik et al., 1995b), and dopamine transporters (Sershen et al., 1992; Sweetnam et al., 1995).

A strategy which has been successfully employed to define loci of drug action is the synthesis and evaluation of structural derivatives of an active compound. Based on this strategy, it was hypothesized that if ibogaine attenuates the expression of opiate dependence through a blockade of NMDA receptors, then modification of this compound could lead to derivatives whose in vivo actions in this measure would parallel their in vitro affinities at NMDA receptors. To implement this strategy, we synthesized a number of ibogaine derivatives including O-desmethylibogaine (a putative ibogaine metabolite [Mash et al., 1995; Pearl et al., 1995]) and O-t-butyl-O-desmethylibogaine (an analog designed to resist O-dealkylation), and the potencies of these compounds to inhibit [^{3}H]MK-801 binding to NMDA receptor were compared to the parent alkaloid. Several of these compounds exhibiting a range of affinities at NMDA receptors were subsequently assessed for the ability to inhibit naloxone-induced withdrawal jumping in morphine-dependent mice. We report here that among structurally related derivatives of ibogaine, only the parent compound (which exhibited the highest affinity for NMDA receptors) was able to attenuate withdrawal-induced jumping in mice.

2. Materials and methods

2.1. Animals

Male NIH Swiss mice (20-25 g, HSD, Veterinary Resources Branch, NIH, Bethesda, MD, USA), Sprague-Dawley rats (200-250 g, Taconic Farms, Germantown, NY, USA), and Hartley guinea-pigs (300-500 g, Charles River Laboratories, Wilmington, MA, USA) were housed under standard laboratory conditions (lights on at 06:00 h,

lights off 18:00 h; room temperature 23 ± 1°C) with chow and tap water available ad libitum.

2.2. Drugs

Morphine, ibogaine HCl, and TCP were obtained from NIDA (Rockville, MD, USA). Naloxone was obtained from Dr. K. Rice (NIDDK, NIH, Bethesda, MD, USA). Memantine was the gift of Merz & Co. (Germany). Harmaline was obtained from Dr. J. Daly (NIDDK, NIH). Tabernanthine was obtained from Drs. P. Potier and C. Kan (Centre National de la Recherche Scientifique, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette Cédex, France). (±)Ibogamine HCl, (±)coronaridine HCl, (±)4-methyl-4-desethylcoronaridine HCl, and (±)4-desethylcoronaridine HCl were synthesized (M.E.K. and U.K.B.) as described (Glick et al., 1994). (+)U-50488, (−)U-50488 and nor-binaltorphamine were synthesized (C.M.B.) as described (De Costa et al., 1987; Lipkowski et al., 1986). O-Desmethylibogaine HCl and O-t-butyl-O-desmethylibogaine were synthesized (C.M.B.) as described below. Endo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075194-190), exo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075237-190) and endo-3-(indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 025989-190) were kindly donated by Dr. David Repke (Syntex Discovery Research, Palo Alto, CA, USA). Other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Morphine (free base) was converted to the HCl salt by addition of a small volume of 1 N HCl and subsequently dissolved in saline, the pH was adjusted to 7 with NaOH. Naloxone and memantine were dissolved in saline. Tabernanthine was dissolved in 10% ethanol in 0.1 N HCl, O-t-butyl-O-desmethylibogaine was dissolved in 0.1 N HCl, and all other compounds were dissolved in H_2O before dilution into appropriate buffers (see below). The doses of ibogaine used in this study were previously shown to be within the range that inhibited naloxone-precipitated jumping behavior in morphine-dependent mice (Popik et al., 1995a). All injections (0.2 ml) were administered i.p.

2.3. Morphine dependence and withdrawal

Mice were rendered morphine dependent and withdrawal precipitated with naloxone as previously described (Popik et al., 1995a; Popik and Skolnick, 1995). In brief, mice were administered morphine (30 mg/kg, twice daily, at 09:30 h and 17:30 h) for 3 days, and an additional last dose administered on the morning of the test (4th) day. 2 h and 15 min after the last dose of morphine, mice were injected with a test compound or vehicle. Mice were challenged with naloxone (4 mg/kg) 45 min after administration of the test compound and immediately placed in transparent plastic cylinders (42 cm high, 19 cm diameter).

The number of jumps was recorded during a 10-min test period. Animals were used only once. Immediately after the jumping session, some animals were placed on a rotorod (4.6 rpm) for 30 s. Data are presented as means $\pm$ S.E.M. of the number of jumps in 10 min. Data were evaluated by Kruskal-Wallis analysis of variance followed by Dunn's Multiple Comparisons test for comparison of individual groups, with $P < 0.05$ accepted as significant.

2.4. Radioligand binding

[³H]MK-801 (S.A. 22.5 Ci/mmol; Dupont-NEN, Boston, MA) binding was assayed in extensively washed membranes prepared from rat forebrain. The methods employed for tissue preparation and radioligand binding were identical to those described by Popik et al. (1994). To generate competition curves, membranes were incubated for 2 h (25°C) with 4–5 nM of [³H]MK-801 in the presence of 30 µM glycine and 30 µM glutamate. Non-specific binding was defined with 1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP) hydrochloride (100 µM).

[³H]U-69593 (S.A. 47.4 Ci/mmol; Dupont-NEN) binding was assayed in membranes prepared from rat, mouse, and guinea-pig forebrains. The methods employed for tissue preparation and radioligand binding were essentially as described (Lahti et al., 1985). In brief, forebrains were homogenized in ice-cold 50 mM Hepes-NH₄OH (pH 7.4) and centrifuged at 30000 × g for 10 min. The pellet was resuspended in Hepes-NH₄OH buffer, recentrifuged (20000 × g, 20 min) and resuspended in buffer to a volume of 1 : 50 w/v. Assays were performed in duplicate using polystyrene 96-well titre plates (Brandel, Gaithersburg, MD, USA). Membranes (500 µl representing ~ 100 µg protein/tube) were incubated with 3 nM [³H]U-69593 in 50 mM Hepes-NH₄OH buffer containing drugs and/or buffer in a total volume of 1 ml/assay. Non-specific binding was defined by 100 µM (−)U-50,488. Assays were initiated with tissue and terminated after 30 min (30°C) by filtration on Whatman GF/B filters using a Brandel MB-48R manifold. The filters were washed 3 times with 1 ml Hepes-NH₄OH buffer and the radioactivity retained on the filters measured in a Beckman LS-5801 liquid scintillation counter. Data were analyzed by iterative curve fitting using GraphPad-Inplot, Version 4.01.

2.5. Synthesis

2.5.1. instrumentation and methods

Proton NMR spectra were recorded in CDCl₃ on a Varian Gemini-300 spectrometer and the data are reported in the following format: chemical shift (all relative to Me₄Si), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, ap = apparent), integration, coupling constants, and exchangeability after D₂O addition. Electron impact (EI) mass spectra were recorded on a VG Micromass 7070F spectrometer. Thin-

layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25-mm plates. Preparative TLC was performed on Analtech silica gel GF 2.00-mm plates. Column chromatography was performed with Fluka silica gel 60 (mesh 220-440). Elemental microanalyses were performed by Atlantic Microlab. Melting points were recorded on a Thomas-Hoover capillary apparatus and are uncorrected.

2.5.2. Preparation of o-t-butyl-o-desmethylibogaine (CB-609.2)

A solution of O-desmethylibogaine (Bartlett et al., 1958) in freshly distilled N,N-dimethylformamide di-t-butyl acetal (1.2 ml, 5.0 mmol) was stirred at 110°C under an atmosphere of argon for 22.5 h and then at room temperature for 9.5 h. The reaction mixture was concentrated on a high vacuum line at room temperature and the residue was dissolved in N,N-dimethylformamide di-t-butyl acetal (1.0 ml, 4.2 mmol) and heated for 14 h at 110°C. TLC indicated incomplete reaction so the reagent was evaporated on a high vacuum line and the residue was taken up in chloroform (20 ml) and this solution was washed with half saturated brine (10 ml). The chloroform layer was dried (Na₂SO₄) and was concentrated to dryness. A mixture of the residue and benzene was concentrated to dryness and the resulting residue was dissolved in N,N-dimethylformamide di-t-butyl acetal (2.4 ml, 10 mmol) and stirred under argon at 110°C for 20 h. TLC indicated complete reaction. The excess reagent was evaporated utilizing a high vacuum line. The residue was taken up in chloroform and the solvent was evaporated on silica gel 60 (mesh 220–440, 2 g). The reaction product was purified on a silica gel column (50 g) by eluting with chloroform/methanol/28% NH₄OH (97 : 3 : 0.3). The fractions containing the major product ($R_f = 0.54$ in above solvent) were pooled and concentrated, yielding 106 mg of crude product. This was purified twice by preparative TLC with 2 × 2 mm Analtech silica gel plates with the eluting solvent chloroform/methanol/28% NH₄OH (98 : 2 : 0.2 first set of plates, 96 : 4 : 0.4 second). This yielded a yellow foam, 70 mg, that was crystallized from ethanol/water, yielding 51 mg of yellow crystalline solid. This was further purified on one preparative TLC plate with the eluting solvent chloroform/methanol/28% NH₄OH (98 : 2 : 0.2) yielding 46 mg of yellow crystalline residue. This was recrystallized from ethanol/water to yield 36 mg (17%), mp 200–202°C; ¹H NMR (CDCl₃): δ 7.54 (br s, 1H, ex. w/D₂O), 7.11 (d, 1H, $J = 8.7$ Hz), 7.06 (d, 1H, $J = 1.9$ Hz), 6.43 (dd, 1H, $J = 2.4$, 8.5 Hz), 3.33 (m, 2H), 3.12 (m, 2H), 2.97 (m, 1H), 2.90 (m, 1H), 2.60 (m, 1H), 2.03 (t, 1H, $J = 12.3$ Hz), 1.79 (m, 2H), 1.68 (qd, 1H, $J = 3.4$, 13.1 Hz), 1.51 (m, 4H), 1.34 (s, 9H), 1.21 (m, 1H), 0.89 (t, 3H, $J = 7.0$ Hz); MS (EI) m/z 352 (M⁺), 296 (M⁺ − C₄H₈); HRMS m/z 352.2513, experimental; 352.2515, calculated; Anal. calcd. for C₂₃H₃₂N₂O (C, H, N).

3. Results

The K_i values for ibogaine and structurally related derivatives to inhibit [^{3}H]MK-801 binding to NMDA receptors in rat forebrain are shown in Table 1. The K_i value obtained for ibogaine (1.1 ± 0.03 μM) is consistent with our previous results (Popik et al., 1994, 1995a) as well as the values reported by Mash et al. (1995) in human brain obtained at autopsy. The putative ibogaine metabolite O-desmethylibogaine (12-hydroxyibogamine) [Mash et al., 1995; Pearl et al., 1995], and the chemically related iboga alkaloids ($\pm$)ibogamine, ($\pm$)coronaridine and tabernanthine were ~ 5–10-fold less potent than the parent alkaloid (Table 1). The ~ 5-fold lower affinity of O-desmethylibogaine reported here is consistent with IC$_{50}$ values ~ 4–6-fold lower than ibogaine in human brain (Mash et al., 1995). Other compounds bearing a structural similarity to ibogaine, including harmaline, ($\pm$)4-methyl-4-desethylcoronaridine, ($\pm$)4-desethylcoronaridine and O-t-butyl-O-desmethylibogaine were > 45-fold less potent than ibogaine ($K_i = 47$–252 μM; Table 1). The potencies of a series of indolotropanes, a class of ibogaine congeners which retain the basic structural features of ibogaine (Repke et al., 1994), were also examined. The most potent indolotropane, endo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075194-190) was ~ 15-fold less potent than ibogaine ($K_i = ~ 16.8$ μM) while the other indolotropanes, exo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075237-190; $K_i = 48.6$ μM) and endo-3-(indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]oc-

tane (RS 025989-190; $K_i = 57$ μM), exhibited even lower affinities in this measure.

The potencies of ibogaine, O-desmethylibogaine, and O-t-butyl-O-desmethylibogaine to inhibit [^{3}H]U-69593 binding to brain κ-opioid receptors are shown in Table 2. Since radioligand binding to κ-opioid receptors varies among species (Lahti et al., 1985), these ligands were examined in membranes from rat, mouse and guinea-pig. Inclusion of the stereoisomers of U-50488 as positive controls demonstrated that the ($+$)-isomer was > 3 orders of magnitude less potent than the corresponding ($-$)-isomer. Moreover, the IC$_{50}$ value obtained for these isomers is consistent with literature values (Lahti et al., 1985; Nock et al., 1990). The IC$_{50}$ of ibogaine (13–29.8 μM) to inhibit radioligand binding to κ-opioid receptors is consistent with the values reported by Sweetnam et al. (1995) but slightly higher than reported by Repke et al. (1994) and Deecher et al. (1992). O-Desmethylibogaine was ~ 10-fold more potent than ibogaine (consistent with Pearl et al., 1995), and O-t-butyl-O-desmethylibogaine equipotent with the parent compound, respectively (Table 3).

Based on the affinities of O-desmethylibogaine and O-t-butyl-O-desmethylibogaine for NMDA and κ-opioid receptors, we evaluated the ability of these ibogaine derivatives to attenuate naloxone precipitated jumping in morphine-dependent mice (Table 3). Jumping was not observed in either saline-treated mice challenged with naloxone or morphine-dependent mice challenged with saline (data not shown). The NMDA receptor antagonist memantine (Borrmann, 1989) was used as a positive con-

Table 1
Inhibition of [^{3}H]MK-801 binding to NMDA receptors by ibogaine analogs

Compound	R1	R2	R3	R4	K_i (μM)
Ibogaine	CH$_2$CH$_3$	H	OCH$_3$	H	1.11 ± 0.03
O-Desmethylibogaine	CH$_2$CH$_3$	H	OH	H	5.48 ± 0.17
($\pm$)Ibogamine	CH$_2$CH$_3$	H	H	H	5.52 ± 0.12
($\pm$)Coronaridine	CH$_2$CH$_3$	CO$_2$CH$_3$	H	H	6.24 ± 0.32
Tabernanthine	CH$_2$CH$_3$	H	H	OCH$_3$	10.5 ± 1.1
($\pm$)4-Methyl-4-desethylcoronaridine	CH$_3$	CO$_2$CH$_3$H	H	H	67.9 ± 2.7
O-t-Butyl-O-desmethylibogaine	CH$_2$CH$_3$	H	OC(CH$_3$)$_3$	H	179 ± 13
($\pm$)4-Desethylcoronaridine	H	CO$_2$CH$_3$	H	H	252 ± 28

[^{3}H]MK-801 (4 nM) binding to rat forebrain membranes was assayed as described in Materials and methods. Non-specific binding was defined with 100 μM TCP. Basal [^{3}H]MK-801 binding was 2.07 ± 0.07 pmol/mg protein. Values represent mean ± S.E.M. of 3 experiments. The K_i of memantine (0.31 ± 0.02 μM), a voltage-dependent channel blocker included as a positive control to validate the assay, is consistent with previously reported values (Kornhuber et al., 1989). The K_i values of other compounds tested were: harmaline ($K_i = 46.6 \pm 5.2$ μM), endo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075194-190; $K_i = 16.8 \pm 2.5$ μM), exo-3-(1-methylindol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 075237-190; $K_i = 48.6 \pm 2.4$ μM) and endo-3-(indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane (RS 025989-190; $K_i = 57 \pm 4$ μM).

Table 2
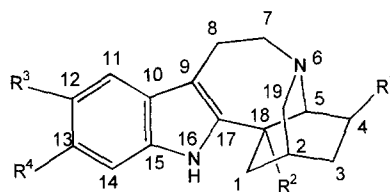Potencies (IC$_{50}$) of ibogaine analogs to inhibit [^{3}H]U-69,593 binding to κ-opioid opiate receptors

Compound	Rat	Mouse	Guinea-pig
Ibogaine	29.8 ± 8.3 μM	13.8 ± 0.6 μM	21.0 ± 1.1 μM
O-Desmethylibogaine	0.28 ± 0.11 μM	1.2 ± 0.1 μM	2.6 ± 0.5 μM
O-t-Butyl-O-desmethylibogaine	16.7 ± 2.95 μM	12.4 ± 0.3 μM	35.6 ± 10.5 μM
(+)U-50488	4.1 ± 1.3 μM	1.0 ± 0.1 μM	2.3 ± 1.7 μM
(−)U-50488	0.27 ± 0.11 nM	1.1 ± 0.1 nM	1.2 ± 0.1 nM
Nor-binaltorphamine	0.46 ± 0.15 nM	0.11 ± 0.04 nM	0.10 ± 0.01 nM

[^{3}H]U-69593 (3 nM) binding to forebrain membranes was assayed as described in Materials and methods. Non-specific binding was defined with 100 μM (−)U-50,488. Basal [^{3}H]U-69593 binding to rat, mouse and guinea-pig membranes was 5.1 ± 1.1, 18.5 ± 0.7 and 37.7 ± 2.7 fmol/mg protein. respectively. Values represent mean ± S.E.M. of 3 experiments and are expressed as IC$_{50}$ in μM or nM (boldface).

trol (Popik and Skolnick, 1995), and produced a significant, albeit incomplete attenuation of naloxone-induced jumping (Table 3). Consistent with previous reports (Dzoljic et al., 1988; Aceto et al., 1990; Glick et al., 1992; Popik et al., 1995a; but see also Sharpe and Jaffe, 1990; Francés et al., 1992) ibogaine reduced naloxone-precipitated jumping, with the first statistically significant effect observed at 80 mg/kg i.p. (Table 3). Neither O-desmethylibogaine (80 mg/kg) nor O-t-butyl-O-desmethylibogaine (40 and 80 mg/kg) inhibited naloxone-precipitated jumping. At higher doses (120 mg/kg), both O-desmethylibogaine and O-t-butyl-O-desmethylibogaine produced profound ataxia and convulsions which precluded studying the actions of these alkaloids on naloxone-induced jumping. Ataxia and convulsions were also produced by the abbreviated ibogaine congener RS 075194-190 (60–250 mg/kg). At doses capable of reducing naloxone-precipitated jumping in morphine-dependent mice, neither ibogaine (80 mg/kg) nor memantine (20 mg/kg) impaired rotorod (4.6 rpm, 30 s trial) performance. Similarly, at the highest dose tested (80 mg/kg), neither O-desmethylibogaine nor O-t-butyl-O-des-

Table 3
Effects of ibogaine derivatives on naloxone-precipitated withdrawal jumping in morphine-dependent mice

Compounds(mg/kg)	Control jumps/10 min (mean ± S.E.M.) [n]
Vehicle	32.5 ± 3.3 [59]
Memantine (20)	13.0 ± 4.2 * [20]
Ibogaine (40)	22.0 ± 5.4 [10]
Ibogaine (80)	19.7 ± 4.2 ** [19]
O-Desmethylibogaine (80)	28.2 ± 5.3 [14]
O-t-Butyl-O-desmethylibogaine (40)	37.3 ± 12.2 [10]
O-t-Butyl-O-desmethylibogaine (80)	26.3 ± 11.3 [10]

Effects of memantine, ibogaine and ibogaine analogs O-desmethylibogaine and O-t-butyl-O-desmethylibogaine on naloxone-precipitated withdrawal jumping in morphine-dependent mice. Ataxia and convulsions were produced by the abbreviated ibogaine congener RS 075194-190 (60–250 mg/kg). Withdrawal was precipitated by naloxone and vertical jumps were counted for 10 min. Values represent mean ± S.E.M. of the number of jumps. Kruskal-Wallis statistic $KW = 14.312$ (corrected for ties); $P = 0.0263$. * $P < 0.05$; ** $P < 0.001$ vs. placebo, Dunn's multiple comparisons test. The doses in mg/kg are given in parentheses. The number of mice tested are given in brackets.

methylibogaine impaired rotorod performance (data not shown).

4. Discussion

The present results demonstrate that modification of the ibogaine molecule has a dramatic impact on affinity for NMDA receptors. Ibogaine binds to NMDA receptors with moderate affinity (Table 1; Mash et al., 1995; Popik et al., 1994, 1995a; Sweetnam et al., 1995), and the methoxy function at C-12 appears to be an important determinant for the ligand-receptor interaction. Thus, both O-demethylation (O-desmethylibogaine) and phenolic deoxygenation (ibogamine) reduced the apparent affinity for NMDA receptors ∼ 5-fold compared to ibogaine. Moreover, replacement of the methoxy function at C-12 with the bulkier t-butoxy function (O-t-butyl-O-desmethylibogaine, designed to resist O-dealkylation in vivo) resulted in a ∼ 100-fold decrease in affinity relative to ibogaine. The importance of the 12-methoxy moiety is further underscored by the apparent affinity of tabernanthine ($K_i = $ ∼ 10.5 μM), a positional isomer of ibogaine with the methoxy group at C-13. These observations may be relevant to the hypothesis that the 'anti-addictive' properties of ibogaine are effected through a long-acting metabolite (Glick et al., 1991), since O-desmethylibogaine has been claimed to be the principal metabolite of ibogaine (Pearl et al., 1995; Mash et al., 1995). If the effects of ibogaine on morphine dependence (Aceto et al., 1990; Dzoljic et al., 1988; Glick et al., 1992; Popik et al., 1995a) are mediated via NMDA receptor blockade (Mash et al., 1995; Popik et al., 1995a), then the 5-fold lower affinity of O-desmethylibogaine indicates this compound would be a metabolite with diminished biological activity. Mash et al. (1995) have reached a similar conclusion, and reported that the potency of O-desmethylibogaine was ∼ 5-fold lower than ibogaine for NMDA receptors in human brain.

Coronaridine, an ibogamine analog with a carbomethoxy moiety at position C18 (R2 in Table 1) has an affinity similar to ibogamine (6.2 vs. 5.5 μM) which indicates that substitution at this position does not contribute substantially to the affinity of this alkaloid family at NMDA receptors. In contrast, substitution of the ethyl

group at position C4 with a methyl group (4-methyl-4-des-ethylcoronaridine) or removal of the ethyl group (4-des-ethyl coronaridine) resulted in affinities reduced by > 1 order of magnitude compared to the corresponding ethyl analogs. These findings indicate the ethyl moiety at position 4 (R1 in Table 1) is important for the interaction of ibogaine derivatives with NMDA receptors. Neither the structurally related alkaloid harmaline nor any of the indole tropanes tested exhibited a $K_i < 10$ μM for NMDA receptors. It should be noted that in some cases, racemic mixtures were used to determine affinity. Thus, the affinity of a potential active isomer would be underestimated.

Several reports have demonstrated that ibogaine also binds to κ-opioid receptors with moderate affinity (Deecher et al., 1992; Pearl et al., 1995; Sweetnam et al., 1995). While the affinity of ibogaine for κ-opioid receptors is slightly lower than for NMDA receptors, brain concentrations of ibogaine after pharmacologically relevant doses would be sufficient to occupy these receptors (Zetler et al., 1972). The recent report (Pearl et al., 1995) that the apparent affinity of O-desmethylibogaine in calf cortex is ~ 4 times higher than ibogaine prompted us to compare the affinities of ibogaine, O-desmethylibogaine, and O-t-butyl-O-desmethylibogaine for these receptors in several species. Interspecies comparisons were made since Lahti et al. (1985) have demonstrated differences in the percentage of total opioid binding sites which represent κ-opioid sites in rat, mouse and guinea-pig. Consistent with the report of Pearl et al. (1995), O-desmethylibogaine was ~ 10-fold more potent than ibogaine in inhibiting [^{3}H]U-69593 binding in membranes from the mouse and guinea-pig, and ~ 100-fold more potent in the rat, while the t-butyl derivative was equipotent (Table 2). These findings indicate that unlike ligand binding to NMDA receptors, a phenolic hydroxyl group at C-12 affords optimal affinity at κ-opioid receptors. This observation is consistent with previous studies (see Hite, 1989) demonstrating that among opiate alkaloids, a phenolic OH group confers a higher affinity for opiate receptors compared to methoxylated analogs (e.g. morphine vs. codeine).

Since our previous studies indicate that the ability of ibogaine to attenuate the expression of morphine dependence is effected through an action at NMDA receptors (Popik et al., 1995a), we compared the effects of O-desmethylibogaine and O-t-butyl-O-desmethylibogaine in order to test this hypothesis. Thus, if these effects of ibogaine are NMDA receptor mediated, then it would be predicted that O-desmethylibogaine would be somewhat less active, and O-t-butyl-O-desmethylibogaine inactive, respectively. In confirmation of previous findings (Popik et al., 1995a), ibogaine inhibited naloxone-precipitated jumping in morphine-dependent mice, whilst the other ibogaine derivatives were inactive at the highest doses tested. Since higher doses of O-desmethylibogaine and O-t-butyl-O-desmethylibogaine produced ataxia and convulsions, we were unable to use higher doses of these compounds.

Thus, these data support, but do not prove this hypothesis. Nonetheless, these data indicate that the interaction of ibogaine with κ-opioid receptors may not be responsible for this anti-addictive property of ibogaine since if κ-opioid receptors were involved, then O-desmethylibogaine should be significantly more potent than ibogaine in attenuating naloxone-precipitated jumping. Likewise, based on the ability of ibogaine to interact with σ receptors (Bowen et al., 1995; Mach et al., 1995; Popik et al., 1995b), it might be hypothesized that the ability of ibogaine to inhibit naloxone-induced jumping in morphine-dependent mice might be mediated through this system. However, since the affinity of O-t-butyl-O-desmethylibogaine for σ receptors (K_i for $\sigma_1 = 3.5 \pm 1.3$ μM; K_i for $\sigma_2 = 346 \pm 9$ nM [W.D. Bowen, personal communication]) is similar to that of ibogaine (K_i for $\sigma_1 = 8.5 \pm 1.1$ nM; K_i for $\sigma_2 = 201 \pm 23$ nM [Bowen et al., 1995]), it appears that σ receptors are not involved in this action of ibogaine. It must be noted that naloxone-precipitated withdrawal is a complex phenomenon, and only one measure (jumping) was examined in the present study. Whether other withdrawal-associated measures are differentially affected by ibogaine and its derivatives is unknown. Moreover, the effect of substitution of the 12-methoxy moiety on blood-brain barrier permeability is unknown. Nonetheless, based on structural considerations O-t-butyl-O-desmethylibogaine should be more and O-desmethylibogaine less lipophilic than ibogaine, respectively. The observation that both O-t-butyl-O-desmethylibogaine and O-desmethylibogaine produced convulsions suggests that these compounds do cross the blood-brain barrier.

Anecdotal reports indicate ibogaine interrupts addiction to a wide variety of abused substances, and most preclinical data are consistent with these reports (Glick et al., 1991, 1994; Lotsof, 1985, 1986, 1995; Cappendjik and Dzoljic, 1993; Rezvani et al., 1995; Sershen et al., 1993). This pharmacological profile is similar to that reported for NMDA receptor antagonists (Trujillo and Akil, 1995), and raises the possibility that the claimed anti-addictive properties of ibogaine are mediated by voltage-dependent block of NMDA receptors. However, unlike prototypical NMDA receptor antagonists (MK-801), pharmacologically relevant concentrations of ibogaine interact with a number of systems implicated in tolerance and dependence phenomena. The availability of ibogaine derivatives that exhibit differing affinities for NMDA receptors provides a potential means of localizing the molecular targets responsible for the putative anti-addictive properties of the parent alkaloid.

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