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Ibogaine Block of the NMDA Receptor: *In Vitro* and *In Vivo* Studies

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Summary—Ibogaine is an hallucinogenic indole alkaloid claimed to have anti-addictive properties. Although its mechanism of action is unknown, binding studies have indicated that the drug may interact with *N*-methyl-D-aspartate (NMDA) receptors. We further investigated the nature of the interaction between ibogaine and NMDA receptors in voltage clamp and binding studies, and sought to confirm that the drug has NMDA receptor blocking activity *in vivo*. In whole-cell recordings from cultured rat hippocampal neurons, ibogaine caused a slow, concentration-dependent block of NMDA-induced currents (IC_{50}, 3.1 μM at −60 mV). In contrast, ibogaine failed to affect either kainate- or γ-aminobutyric acid-evoked currents. The blockade of NMDA currents was use- and voltage-dependent, and the long lasting ibogaine block could be occluded by co-application of Mg^{2+}. Ibogaine also inhibited equilibrium [^{3}H]dizocilpine binding to NMDA receptors in rat forebrain membranes (IC_{50}, 3.2 μM). We conclude that ibogaine is an open channel NMDA receptor antagonist. Administration of ibogaine to mice resulted in complete protection in the maximal electroshock test (ED_{50}, 31 mg/kg, i.p.) and partial protection against NMDA-induced lethality, confirming that ibogaine can block NMDA receptors *in vivo*. Published by Elsevier Science Ltd 1996.

Keywords—Ibogaine, voltage clamp recording, [^{3}H]dizocilpine binding, NMDA receptor, hippocampus, maximal electroshock (MES) test, seizure.

Ibogaine, an indole alkaloid found in the root bark of the African shrub *Tabernanthe iboga*, has central stimulant, hallucinogenic and tremorigenic effects (Goutarel *et al.*, 1993; Schultes, 1969; Zetler *et al.*, 1972). Interest in this psychoactive principal was heightened by claims that it is effective in the treatment of opiate, stimulant and alcohol abuse (Sheppard, 1994). Although ibogaine can affect various neurotransmitter systems (Maisonneuve *et al.*, 1992; Glick *et al.*, 1993; Sershen *et al.*, 1994; Harsing *et al.*, 1994), the mechanisms underlying its diverse pharmacological actions remain poorly understood. Recently, Popik *et al.* (1994) reported that ibogaine can displace binding of [^{3}H]dizocilpine ([^{3}H]MK-801) from NMDA receptors in rat forebrain membranes, suggesting that the alkaloid, like dizocilpine, is a channel blocking NMDA receptor antagonist (see also Mash *et al.*, 1995). In the present study, we further investigated this possibility using whole-cell voltage clamp recording techniques. Our results indicate that ibogaine blocks NMDA receptors by an open channel mechanism similar to dizocilpine, although with lower potency. To confirm that ibogaine blocks NMDA receptors *in vivo*, we determined whether ibogaine, like other centrally active NMDA receptor antagonists, is protective in the maximal electroshock (MES) seizure test (Rogawski, 1995). We also examined ibogaine for protective activity against NMDA-induced seizures and lethality which more directly evaluates a drug's ability to block NMDA receptors *in vivo* (Leander *et al.*, 1988). Our results indicate that ibogaine has NMDA receptor blocking activity both *in vitro* and *in vivo*.

METHODS

Voltage clamp studies in cultured hippocampal neurons

Cell culture. Hippocampal neurons from 19-day-old Sprague–Dawley rat embryos were grown in cell culture as previously described (Donevan *et al.*, 1992). In brief, hippocampi from 19-day-old rat embryos were triturated in modified minimal essential medium with Earle's salt (Advanced Biotechnologies, Columbia, MD) by repeated

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passage through a 10-ml pipette. The cell suspension was plated at a density corresponding to 1–1.5 hippocampi/dish on 35-mm polystyrene Petri dishes (Falcon 3001; Becton Dickinson Labware, Oxnard, CA) that were precoated with Matrigel (Collaborative Biomedical Products, Bedford, MA). The plating medium was supplemented with 10% horse serum (GIBCO, Grand Island, NY), 10% fetal calf serum, N3 (20 mg/ml transferrin, 200 μM putrescine, 60 nM sodium selenite, 20 ng/ml triiodothyronine, 10 mg/ml insulin, 40 nM progesterone, 40 ng/ml corticosterone), and 1% glutamine. The cultures were maintained at 37°C in a humidified atmosphere containing 10% CO_2. After 6 days, a few drops of fresh medium (without fetal calf serum and N3) were added to the cultures. Cells were used for electrophysiological recordings after 6–12 days *in vitro*.

Whole-cell recording. Before each experiment, the culture medium was removed, the cells were rinsed completely, and the culture dish was partially filled with bathing solution containing 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 0.1 mM $CaCl_2$, and 1 μM tetrodotoxin (to block voltage-activated Na^+ channels). The osmolality was adjusted to 315–325 mOsm/kg H_2O with sucrose and to a pH of 7.4 with NaOH.

Patch pipettes (3–6 MΩ) were prepared from filament-containing thin wall glass capillary tubes (1.5 mm outside diameter; World Precision Instruments, Sarasota, FL) using a four-stage horizontal pipette puller. The electrodes were filled with an intracellular solution containing 145 mM $CsCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, 0.1 mM $CaCl_2$, and 1 mM EGTA. The osmolality was adjusted to 310 mOsm/kg H_2O with sucrose and to a pH of 7.4 with CsOH.

Whole-cell recordings were performed with an Axopatch 1C patch-clamp amplifier (Axon Instruments, Burlingame, CA). Voltages corresponding to the membrane currents were displayed on a high-speed ink pen recorder (Gould Electronics, Cleveland, OH) and acquired on a microcomputer using the Axotape software (Axon). The holding potential was maintained at −60 mV, unless otherwise noted.

Drug application. Drug solutions were applied using a multibarrel, rapid perfusion system described previously (Donevan *et al.*, 1992). The solution exchange time constant at the tip of an open recording electrode was less than 2 msec. One barrel contained bathing solution while the other barrels contained agonists [NMDA, kainate or γ-aminobutyric acid (GABA)] and ibogaine either alone or in combination. Only one valve was opened at a time. NMDA perfusion solutions contained 10 μM glycine and 1 μM strychnine was added to block glycine-activated Cl^- channels. Drug solutions were made fresh on the day of use.

Data analysis. The fractional block (B) of an agonist-induced currents in the whole-cell recording experiments was calculated according to the formula $B = 1 - I_B/I$, where I is the steady-state current evoked by an agonist and I_B is current evoked by the agonist in the presence of ibogaine. Ibogaine concentration-block data were fit to the logistic equation

$$B = 1/\{1 + (IC_{50}/[D])^{n_H}\} \quad (1)$$

where $[D]$ is the ibogaine concentration, IC_{50} is the concentration resulting in 50% block, and n_H is an empirical parameter describing the steepness of fit and having the same meaning as the Hill coefficient. All quantitative data are expressed as mean ±SEM; n is the number of neurons tested.

[^{3}H]Dizocilpine binding assay

Ibogaine affinity for the dizocilpine binding site of the NMDA receptor complex was determined in rat brain homogenates using a displacement assay with [^{3}H]dizocilpine. Washed rat forebrain homogenates were prepared as described by Subramaniam and McGonigle (1993) and frozen for later use. Triplicate samples containing [^{3}H]dizocilpine (6 nM; specific activity 22 Ci/mM; Dupont NEN, Wilmington, DE) and thawed brain membranes (100–200 μg protein/tube) were incubated in 30 mM EPPS buffer (pH 7.45) containing 1 mM EDTA, 100 μM glutamate and 100 μM glycine in a final volume of 0.5 ml. Nonspecific binding was determined in the presence of 200 μM ketamine. The incubation was carried out at room temperature for 2.5 hr. At the end of the incubation period, 3 ml ice-cold buffer was added to each tube, and the incubation mixture was filtered through Whatman GF/B filter paper with a Brandel 24-well harvester. The filters were then washed three times with 3 ml of ice-cold buffer. The filters were soaked overnight in aqueous liquid scintillation cocktail (4–5 ml/vial) before counting.

In vivo *testing*

Male NIH Swiss mice (25–30 g) were obtained from the National Institutes of Health (NIH) animal program. Animals were allowed to acclimatize with free access to food and water for a 24-hr period before testing. All procedures were carried out under strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Use Committee.

Maximal electroshock (MES) seizure test. Fifteen minutes after intraperitoneal (i.p.) injection of ibogaine, mice were subjected to a 50-mA, 0.2 sec, 60 Hz electrical stimulus via corneal electrodes (5-mm diameter stainless steel balls) wetted with 0.9% saline. Animals failing to show tonic hindlimb extension were scored as protected.

NMDA-induced seizures and lethality. Injection of NMDA (257 mg/kg, s.c.) produced behavioral responses consisting of explosive running and jumping, followed by posturing (opisthotonos and Straub tail) and clonic movements of the forelimbs and hindlimbs, and ultimately tonic hindlimb extension and death. The ability of ibogaine to protect against NMDA-induced behavioral effects and lethality was evaluated by injecting it i.p. 15

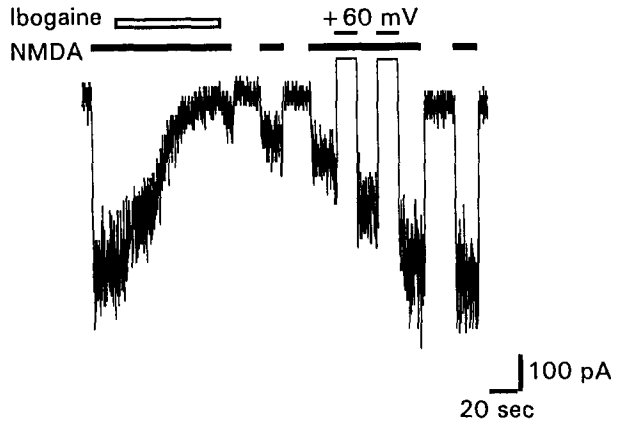

Fig. 1. Slow block of 10 μM NMDA (+10 μM glycine)-induced current by 30 μM ibogaine at a holding potential of −60 mV. Recovery was speeded by intermittently switching the holding potential to +60 mV in the presence of NMDA.

min before the s.c. administration of NMDA. Animals were observed for 60 min after the NMDA injection.

Motor toxicity test. Evaluation for motor toxicity was carried out using a modification of the horizontal screen test (Coughenour *et al.*, 1977), which determines an animal's ability to support its own body weight by grasping a grid. Mice were placed on a horizontally oriented grid (consisting of parallel 1.5 mm diameter rods situated 1 cm apart) and the grid was inverted. Animals which fell from the grid within 5 sec were scored as positive. Drug-free mice never fell from the grid. The horizontal screen test was carried out immediately before the imposition of the electrical stimulus in the MES test and the administration of NMDA in the NMDA lethality test.

Ibogaine lethality. Mice were injected with ibogaine (100–300 mg/kg, i.p.) and observed for 120 min. Lethality, when it occurred, was invariably seen within the first 20 min.

Data analysis. To construct dose–effect curves, ibogaine was tested at several doses spanning the ED_{50} or LD_{50} (i.e. the dose producing 50% effect, either protection or lethality, respectively). At least eight mice were tested at each dose. ED_{50} or LD_{50} values and their 95% confidence limits were determined by log-probit analysis using the computer programs accompanying Tallarida and Murray (1987).

Drugs

Ibogaine HCl was obtained from Sigma Chemical Co. (St Louis, MO), recrystallized, and verified to be pure by thin layer chromatography and reverse-phase high-performance liquid chromatography. NMDA was obtained from BACHEM Biochemical Inc. (King of Prussia, PA). Ketamine was from Research Biochemicals (Natick, MA). Other reagents were obtained from Sigma Chemical Co.

RESULTS

Ibogaine block of NMDA-induced currents

At a holding potential of −60 mV, application of 10 μM NMDA (in the presence of 10 μM glycine) evoked inward current responses that showed little rundown during NMDA applications lasting as long as 150 sec. Co-application of ibogaine caused a slow block of the

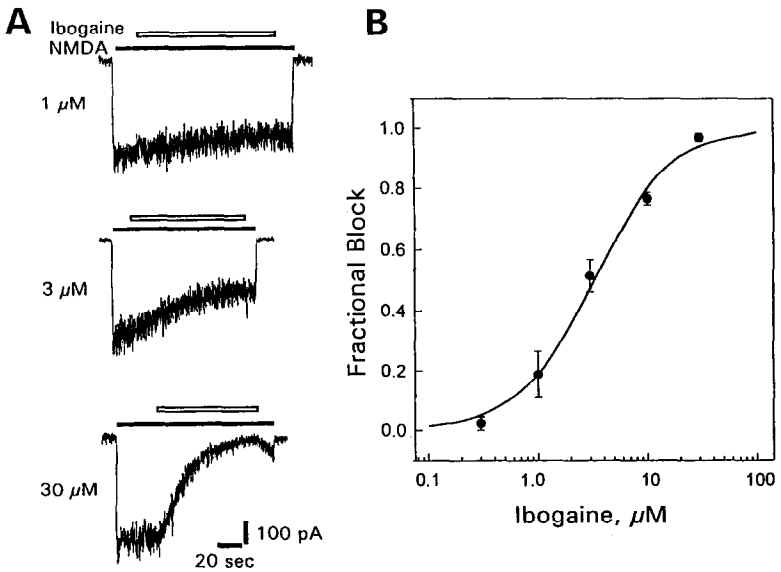

Fig. 2. Concentration-dependent inhibition of NMDA-induced current by ibogaine. (A) Effects of 1, 3 and 30 μM ibogaine. The traces are from three different neurons. The holding potential was −60 mV. (B) Concentration–response curve derived from experiments similar to those shown in (A). Each concentration point represents the mean ± SEM of fractional block values obtained in experiments with 5–8 neurons. The data were fit to the logistic equation given in the text (Eq. 1).

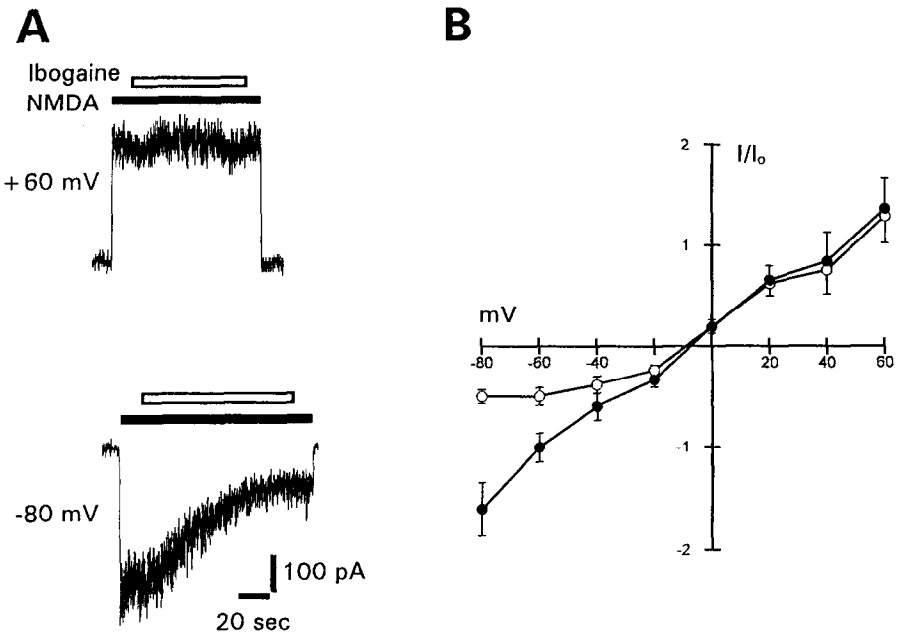

Fig. 3. Voltage-dependence of ibogaine block. (A) The effect of 3 μM ibogaine at holding potentials of +60 and −80 mV. (B) Current–voltage curves for NMDA-induced currents in the absence (●) and presence of (○) of 3 μM ibogaine. Data at each holding potential were derived from experiments with 4–6 cells. The currents were normalized to the control current at −60 mV (mean ± SEM: 302 ± 26 pA).

NMDA-induced currents. As illustrated in Fig. 1, the ibogaine block required more than 60 sec to reach a plateau. At the holding potential of −60 mV, it was difficult to demonstrate complete recovery of the NMDA currents from the ibogaine block due to slowness of recovery from block. However, full recovery could be obtained by switching the holding potential to +60 mV to speed unblocking (see below).

Concentration-dependence of ibogaine block

The concentration-dependence of ibogaine block was examined in a series of experiments where ibogaine was applied during the continuous application of NMDA. As illustrated in Fig. 2A, 1 μM ibogaine produced minimal block, whereas 3 and 30 μM ibogaine produced increasing degrees of block. In a series of similar experiments, mean fractional block values for concentrations of ibogaine from 0.3 to 30 μM were determined at or near steady-state. These values are plotted in Fig. 2B. The data points are fit to the sigmoidal curve defined by Eq. 1 with IC_{50} value of 3.1 μM (n_H, 1.2).

Selectivity of ibogaine block

The selectivity of ibogaine block was examined in experiments assessing the effects of the drug on kainate- and GABA-activated currents. Co-application of ibogaine at a concentration of 30 μM failed to affect currents evoked by 100 μM kainate (n = 3) or 2 μM GABA (n = 4).

Voltage-dependence of ibogaine block

In order to examine whether ibogaine block is voltage dependent, the steady-state current in the absence and presence of 3 μM ibogaine was determined at a series of holding potentials from −80 mV to +60 mV. These results are presented in Fig. 3A and B. Overall, the NMDA current–voltage relationship was roughly linear in the absence of ibogaine, but showed significant outward rectification in the presence of ibogaine. Thus, the block of NMDA-induced currents was greatest at the most negative holding potentials and diminished as the holding potential became more positive; no block was observed at potentials positive to −20 mV. Fractional current values from the data of Fig. 3B fit to a binding isotherm incorporating an exponential dependence of binding affinity on voltage (Eq. 3 in Subramaniam *et al.*, 1993). This analysis (after Woodhull, 1993) provided estimates of $K_D(0)$ (the binding affinity in the absence of applied transmembrane potential) and δ (the fraction of the transmembrane electric field sensed at the binding site). These values were 9.8 μM and 0.57, respectively. The $K_D(0)$ value was transformed to −60 mV according to the relationship of Woodhull (1973) describing the voltage-dependence of the binding affinity at a voltage-dependent blocking site (Eq. 2 in Subramaniam *et al.*, 1993). The derived value (2.6 μM), an independent measure of the binding affinity, agrees favorably with the value obtained from the concentration-block experiment of Fig. 2.

Use-dependence of ibogaine block

We next sought to determine whether agonist gating of the NMDA receptor is required for the blocking action of ibogaine. A comparison was made of the responses to test

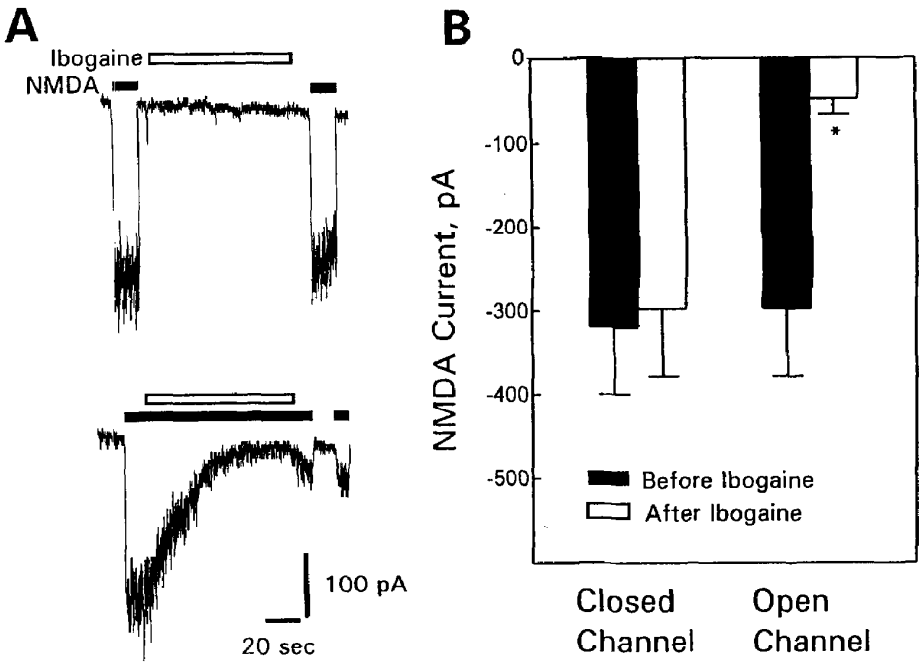

Fig. 4. Influence of agonist gating on ibogaine block of NMDA-induced current. (A) Ibogaine produces substantially greater block when applied together with NMDA. Top and bottom traces were from the same cell. The holding potential was −60 mV. (B) Summary of the results of four experiments similar to the one illustrated in (A). Filled and open bars indicate mean amplitudes of NMDA-induced currents before and after application of ibogaine, respectively. *Significant difference between mean amplitudes before and after ibogaine ($p < 0.05$, paired t-test).

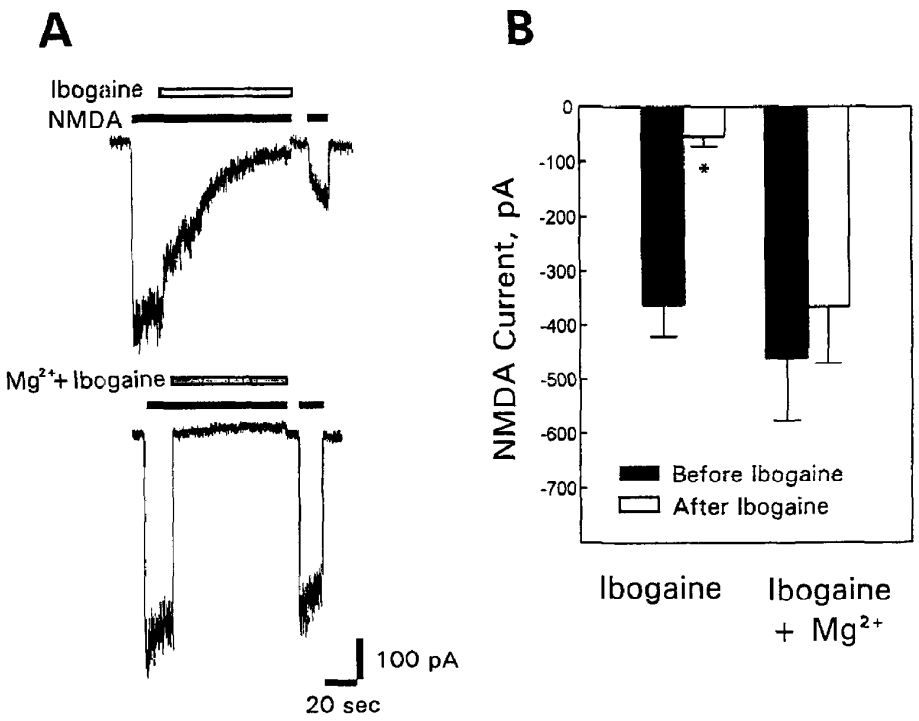

Fig. 5. Mg^{2+} occludes ibogaine block of NMDA-induced current. (A) Top, 30 μM ibogaine produces a slow, persistent block of NMDA-induced current. Bottom, application of 3 mM Mg^{2+} along with the ibogaine produces a more rapid block, but the persistent block characteristic of ibogaine alone is largely prevented. Top and bottom panels were from the same cell, but the record shown in the bottom panel was acquired first. The holding potential was −60 mV. (B) Summary of the results of four experiments similar to the one illustrated in (A). Filled and open bars indicate mean amplitudes of NMDA-induced currents before and after application of ibogaine, respectively. *Significant difference between mean amplitudes before and after ibogaine ($p < 0.05$).

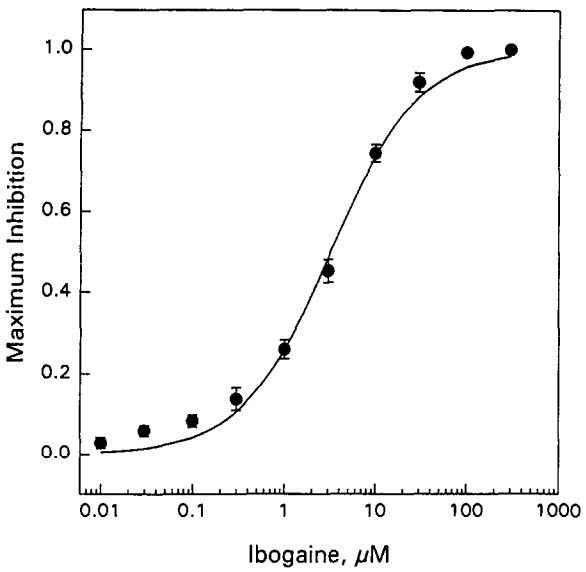

Fig. 6. Concentration-dependent inhibition of [^{3}H]dizocilpine binding by ibogaine. Each point represents the mean ± SEM of data from six separate experiments. The points were fit to text Eq. 1 with parameters as given in Results.

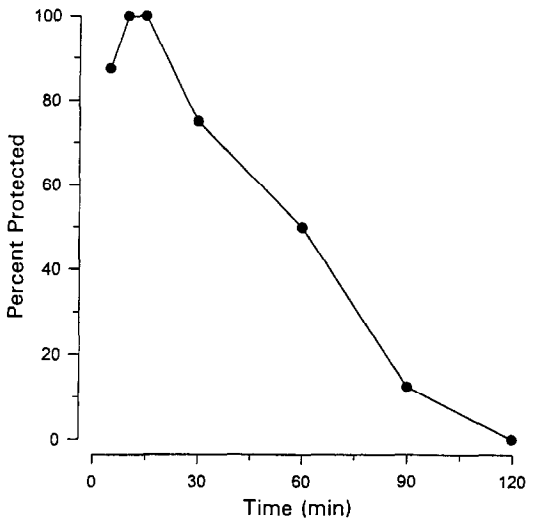

Fig. 8. Time course for protection in maximal electroshock (MES) test by ibogaine at a dose equivalent to twice its ED_{50} value (62 mg/kg). Each data point represents eight mice.

applications of NMDA applied before and after perfusion with ibogaine in the nominal absence of agonist. Since recovery from the ibogaine blocking effect is slow, substantial residual block would be expected after termination of ibogaine application if the drug were able to block closed channels. However, as illustrated in Fig. 4A (top), the NMDA response obtained 10 sec after a 1 min application of 30 μM ibogaine was similar in amplitude to the control response. By itself, ibogaine failed to affect the resting current of the cell. As illustrated in Fig. 4B which summarizes the results of four experiments similar to that of Fig. 4A (top), there was no statistically significant difference between the amplitudes of the NMDA-induced current response before and after ibogaine application. In contrast, when ibogaine was applied in conjunction with NMDA there was a slow block of the NMDA response and the current was significantly inhibited 20 sec later [Fig. 4A (bottom) and B]. These results indicate that ibogaine cannot bind and block NMDA receptors in the absence of NMDA, and that the drug requires agonist gating of the channel for block to occur.

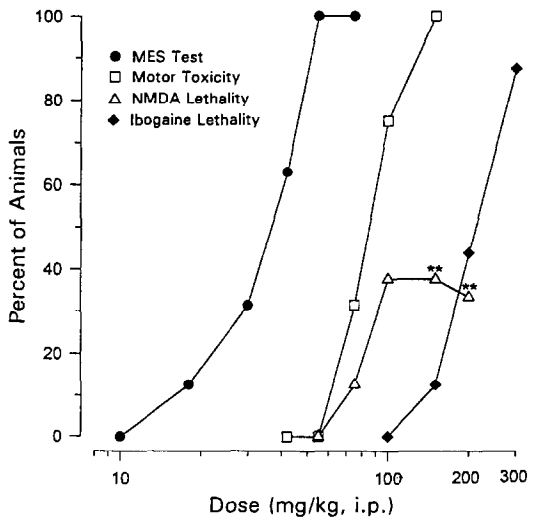

Fig. 7. Dose–effect relationships for protection by ibogaine against maximal electroshock (MES) seizures (●) and NMDA-induced lethality (△), and for ibogaine-induced motor toxicity (□) and lethality (◆). Each point represents data from eight to 16 mice. At high doses, ibogaine itself produced lethality (marked by asterisks) so that there was no dose-dependent protection against NMDA-induced lethality.

Mg^{2+} occlusion of ibogaine block

A possible mechanism to explain the use-dependent nature of ibogaine-induced block is that the compound is an open channel antagonist. In this case, ibogaine would directly occlude the ion conductance pathway of the NMDA receptor, but would gain access to its blocking site only when the receptor is in the ligand-gated open state. To investigate this potential mechanism, we examined whether Mg^{2+}, a well-known open channel blocker (Mayer *et al.*, 1984; Nowak *et al.*, 1984), would interfere with access of ibogaine to its binding site and thus occlude ibogaine block. It was feasible to test this possibility since recovery from Mg^{2+} block occurs much more rapidly than does recovery from ibogaine. Thus, as shown in Fig. 5A, co-application of 30 μM ibogaine with NMDA produced a persistent block of the NMDA-induced current. However, when 3 mM Mg^{2+} was applied together with the ibogaine, the long-lasting ibogaine block was largely prevented. Figure 5B summarizes the results of four similar experiments which confirm that ibogaine produces a persistent block of

NMDA receptor current, but this persistent block can be prevented by Mg^{2+}.

Inhibition of [^{3}H]dizocilpine binding

To confirm that ibogaine can interact with the channel blocking site of NMDA receptors, we investigated whether the drug could inhibit [^{3}H]dizocilpine binding to NMDA receptors in rat forebrain membrane. As shown in Fig. 6 (which summarizes the results of six experiments), ibogaine caused a concentration-dependent inhibition of [^{3}H]dizocilpine binding. The IC_{50} value derived from the logistic fit to these data was 3.2 $\pm$0.5 μM (n_H, 0.93 $\pm$0.06).

In vivo *studies*

As illustrated in Fig. 7, ibogaine protected mice in a dose-dependent manner against tonic hindlimb extension in the MES seizure test. The ED_{50} value for protection in the MES test was 31 mg/kg [95% confidence limits (C.L.): 23–41]. The time course for protection in the MES seizure test was determined following administration of ibogaine at a dose equivalent to twice its ED_{50} value (62 mg/kg). As shown in Fig. 8, the protective activity of ibogaine peaked at 10 to 15 min and was no longer apparent at 120 min. At doses higher than those that conferred seizure protection, ibogaine produced motor impairment in the horizontal screen test (Fig. 7). The ED_{50} value for motor impairment was 84 mg/kg (95% C.L.: 75–93). Additional signs of toxicity were observed when the dose of ibogaine was further increased, including severe gait ataxia and motionlessness, forelimb and hindlimb myoclonus, whole body tremors, depressed respiration (shallow and decreased respiratory rate) and lethality. The dose–response relationship for lethality is shown in Fig. 7. The LD_{50} value obtained from these data was 213 mg/kg (95% C.L.: 170–268). Ibogaine also protected mice against NMDA-induced lethality in a dose-dependent manner (Fig. 7). However, complete protection from NMDA lethality was not obtained. At higher doses ($\geq$150 mg/kg) ibogaine was severely toxic and, as noted above, by itself caused lethality.

DISCUSSION

Several lines of *in vitro* and *in vivo* evidence support the conclusion that ibogaine is an NMDA receptor antagonist. First, ibogaine produced a concentration-dependent block of NMDA-evoked currents in cultured hippocampal neurons (IC_{50}, 3.1 μM at –60 mV). Second, ibogaine inhibited [^{3}H]dizocilpine binding to NMDA receptors in rat forebrain membranes with similar potency to the effect on NMDA receptor currents. Finally, ibogaine was an effective anticonvulsant in the MES seizure test, and also was able to partially protect against NMDA-induced lethality (although the latter effect was limited by ibogaine's own lethality at higher doses).

Having demonstrated that ibogaine is a functional NMDA antagonist, we sought to determine its blocking mechanism. Prior studies with [^{3}H]dizocilpine binding raised the possibility that ibogaine is a channel blocking NMDA receptor antagonist (Popik *et al.*, 1994; Mash *et al.*, 1995). Indeed, the ability of ibogaine to inhibit equilibrium [^{3}H]dizocilpine binding, an effect consistent with this mechanism, was confirmed in the present study. Thus, ibogaine may bind to the same (or an adjacent) site as dizocilpine in the pore of the NMDA receptor channel (Huettner and Bean, 1988). (An alternative possibility compatible with the binding data is that ibogaine inhibits the NMDA receptor by another mechanism so that [^{3}H]dizocilpine association is slowed and equilibrium is not reached during the 2.5 hr incubation period.) Binding at such a site would be expected to block ion transit through the NMDA receptor channel resulting in functional NMDA receptor antagonism. Recently, Mash *et al.* (1995) have observed that ibogaine can inhibit NMDA-induced depolarizations of frog motoneurons, indicating that the alkaloid can, in fact, act as a functional NMDA receptor antagonist. This action was confirmed in the present voltage clamp studies where it was demonstrated that ibogaine blocks NMDA receptor currents. This blocking action occurred in a use-dependent fashion, as expected for an open channel blocker. In addition, the block was voltage-dependent, with relief of inhibition occurring at positive potentials. Ibogaine has a basic amino functionality with pK_A of 8.1. Consequently, at the physiological pH of the experiments, it would be largely in the (monovalent) cationic form. Thus, the reduction in block which occurred as the holding potential was brought to more positive potentials (Fig. 3) is likely to be the result of electrostatic field effects on the blocking particle. Indeed, similar voltage-dependent effects on NMDA receptors have been observed for a wide variety of cationic channel blocking NMDA receptor antagonists (see, e.g. Jones and Rogawski, 1992; Subramaniam *et al.*, 1993; McBain and Mayer, 1994). Analysis of the voltage dependence of block indicated that block occurs at a site that is located 57% into the transmembrane electric field (as measured from the outside). The depth of this blocking site is similar to that of the blocking site at which a variety of monovalent cationic open channel blockers bind to the NMDA receptor (see Subramaniam *et al.*, 1993). Thus, the NMDA receptor possess a deep, externally accessible blocking site to which many organic cations including ibogaine can bind. In addition to demonstrating use- and voltage-dependence, we have been able to establish that ibogaine block is occluded by Mg^{2+} (Fig. 5). Since it is well recognized that Mg^{2+} binds to a deep site within the NMDA receptor channel (Mayer *et al.*, 1984; Nowak *et al.*, 1984), this is perhaps the strongest piece of evidence supporting the concept that ibogaine acts by an open channel blocking mechanism and that it binds within the pore of the NMDA receptor. At a concentration 10-fold greater than the IC_{50} for block of NMDA receptor responses, ibogaine failed to

affect non-NMDA and $GABA_A$ receptor responses (evoked by kainate and GABA, respectively). The lack of effect of ibogaine on $GABA_A$ receptors is in agreement with a prior study which failed to observe an effect of ibogaine on $GABA_A$ receptors as assessed by radioligand binding and $^{36}Cl^-$ flux measurements (Deecher *et al.*, 1992).

Having established that ibogaine is an NMDA antagonist *in vitro*, we sought to determine whether the alkaloid could produce a functional blockade of NMDA receptors *in vivo* at doses comparable to those that have been reported to induce acute behavioral and anti-addictive effects. NMDA receptor antagonists are well recognized to have protective activity in the MES test (Rogawski, 1995), and ibogaine was highly effective in this model. The drug was protective at doses (ED_{50}, 31 mg/kg) within the range of those demonstrating behavioral, neurochemical and anti-addicative activity (Glick *et al.*, 1992; Cappendijk and Dzoljic, 1993; Dworkin *et al.*, 1995), but below those which have been associated with neuronal degeneration (O'Hearn and Molliver, 1993a, b; Glick *et al.*, 1994) or which cause severe toxicity and death (this study). While activity in the MES test is consistent with *in vivo* NMDA receptor blocking activity, other pharmacological actions of the drug could theoretically also produce such an anticonvulsant action. A more direct indicator of *in vivo* NMDA receptor blocking activity is protection against NMDA-induced lethality (Leander *et al.*, 1988). Ibogaine was partially protective in this model, but complete protection was not obtained due to the toxicity of ibogaine itself. The lethality of high doses of ibogaine is not likely to be due to the interaction with NMDA receptors, but rather could be related to effects of the drug on the cardiovascular system (Hamon *et al.*, 1985).

In conclusion, the present study has confirmed that ibogaine is a moderately potent channel blocking NMDA receptor antagonist. Doses of ibogaine that are commonly used in animal behavioral and neurochemical studies likely produce at least a partial functional blockade of brain NMDA receptors. Thus, the toxicity of ibogaine, including the ataxia seen in animals, and the hallucinations which have been reported by human subjects, could result to some extent from NMDA receptor blockade. It seems plausible that drug addiction—neuronal adaptation to repeated drug exposure—could result from the types neuronal plasticity that are sensitive to NMDA receptor antagonists. Indeed, Trujillo and Akil (1991) have observed that dizocilpine effectively attenuates the development of morphine dependence in rats. However, it remains to be determined whether ibogaine's anti-addictive properties are related to its NMDA receptor blocking activity.

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