Properties of ibogaine and its principal metabolite (12-hydroxyibogamine) at the MK-801 binding site of the NMDA receptor complex


Keywords: Ibogaine; 12-hydroxyibogamine; MK-801; N-Methyl-D-aspartate receptors; Spinal cord; Caudate; Cerebellum; Drug abuse

Ibogaine (NIH 10567, Endabuse™) is the principal indole alkaloid obtained from the root bark of the African shrub Tabernanthe iboga. Anecdotal reports from addict self-help groups suggest that ibogaine may be therapeutically useful in the treatment of both the physiological and psychological symptoms associated with withdrawal from opiates (US Patent 4,499,096), stimulants (amphetamine, cocaine; US Patent 4,587,243), and ethanol (US Patent 4,857,523). In support of these findings, animal studies have indicated that ibogaine attenuates morphine self-administration, cocaine preference, and amphetamine-, cocaine-, and morphine-induced increases in motor activity, reduces symptoms associated with morphine withdrawal, and decreases preference for cocaine [1,3–5,10,14,15].

Neither the site nor the mechanism of action of ibogaine is known. The alkaloid may, however, be active at excitatory amino acid receptor sites. Skolnick et al. [12] have shown that ibogaine competitively displaced the binding of the potent, selective, non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate ([3H]MK-801) in rat forebrain. NMDA receptors have been implicated in the development of tolerance to, and dependence on, addictive substances. In particular, low doses of MK-801 are reported to affect the development of behavioral sensitization and tolerance to morphine, ethanol, and stimulants [7,13,17,18].

The present pharmacological studies were undertaken to further define the actions of ibogaine and its principal metabolite 12-hydroxyibogamine at the MK-801 binding site of the NMDA receptor complex [6]. In parallel investigations, we compared the binding and physiological actions of ibogaine and 12-hydroxyibogamine. Radioligand binding studies were conducted on neuropathological tissue specimens obtained from the University of Miami Brain Endowment Bank (two accident victims; males, aged 26 and 37; no history of neurologic disease; drug-free at the time of death; postmortem intervals of 9.5 and 13 h respectively) and on frog (Rana pipiens).
spinal cords. Briefly, well-washed membranes (5 mg/ml, caudate; 10 mg/ml, cerebellum, spinal cord) were incubated with 4 nM [3H]MK-801 (NEN/Dupont; S.A., 22 Ci/ mmol), 30 μM glycine, and 30 μM glutamic acid in the presence of increasing concentrations of (+)MK-801, ibogaine, or 12-hydroxyibogamine for 4 h at 25°C in 5 mM Tris–HCl, pH 7.4. Samples were filtered through Whatman 934-AH filters presoaked in 0.1% polyethyleneimine on Millipore manifolds packed in dry ice. Filters were washed 3 times with 4 ml of ice-cold buffer. Competition curves were analyzed using the DRUG program from EBDA/Ligand (Biosoft). The data are presented as IC50 values and were not converted to Kd values because of the complex nature of MK-801 binding. At a concentration of 4 nM, [3H]MK-801 occupied 70–80% of the high affinity binding sites and 20–30% of the low affinity binding sites.

Differential sucrose gap recording from the IXth ventral root (VR) was used to determine NMDA-induced changes in the membrane potential of frog motoneurons. The methods have been described in detail elsewhere [2]. The cord was continuously superfused with oxygenated HCO3−-buffered Ringer’s solution maintained at 18°C and flowing at a rate of 10 ml/min. Mg2+ was omitted from the medium to facilitate study of responses mediated by NMDA receptors. Cords were exposed to normal superfusion medium or to medium containing chemicals which were dissolved in Ringer’s solution just prior to application. Solution changes were accomplished rapidly by means of a solenoid valve system. NMDA (100 μM) and (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 10 μM) were applied for 5 s periods (which were shown by preliminary experiments to be sufficient for potential changes to reach a plateau) and at rates that minimize desensitization.

The results of competition assays to determine the effects of ibogaine and 12-hydroxyibogamine on [3H]MK-801 binding are shown in Fig. 1 and Table 1. Both compounds inhibited [3H]MK-801 binding, but ibogaine was 4–6-fold more potent than its metabolite. Both ibogaine and 12-hydroxyibogamine were 50–1000-fold less potent than MK-801 with regard to binding to the NMDA receptor. As shown in Table 1, the displacement profiles for both ibogaine and 12-hydroxyibogamine at MK-801 sites were comparable in human caudate and cerebellum and frog spinal cord.

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Competitor</th>
<th>IC50 (μM)</th>
<th>nH</th>
</tr>
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<tbody>
<tr>
<td>Human Caudate</td>
<td>(+)MK-801</td>
<td>0.002 ± 0.1</td>
<td>0.36 ± 0.03</td>
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<tr>
<td></td>
<td>Ibogaine</td>
<td>5.2 ± 0.2</td>
<td>0.90 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>12-hydroxyibogamine</td>
<td>31.4 ± 5.4</td>
<td>1.05 ± 0.19</td>
</tr>
<tr>
<td>Human Cerebellum</td>
<td>(+)MK-801</td>
<td>0.07 ± 0.03</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Ibogaine</td>
<td>9.8 ± 2.3</td>
<td>1.10 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>12-hydroxyibogamine</td>
<td>38.2 ± 8.3</td>
<td>1.15 ± 0.06</td>
</tr>
<tr>
<td>Frog Spinal Cord</td>
<td>(+)MK-801</td>
<td>0.07 ± 0.04</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Ibogaine</td>
<td>3.6 ± 1.7</td>
<td>0.90 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>12-hydroxyibogamine</td>
<td>21.3 ± 4.8</td>
<td>0.96 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 3 independent determinations each assayed in triplicate. Increasing concentrations of competitor were incubated in the presence of 4 nM [3H]MK-801. Nonspecific binding was determined with 10 μM (+)MK-801. The values were determined using the DRUG program of EBDA/Ligand. nH, Hill slope.
Fig. 2. Antagonism of NMDA- and AMPA-induced motoneuron responses by ibogaine and MK-801. Applications of NMDA indicated by (△) and AMPA by (▲). (A) Effects of ibogaine on NMDA- and AMPA-depolarizations. Negativity is indicated by an upward pen deflection and signifies depolarization of the motoneuron membrane. The cord was continuously bathed in medium containing TTX (tetrodotoxin, 0.625 μM); (1) control applications of NMDA (100 μM, 5 s application) and AMPA (10 μM, 5 s); (2) first application of NMDA and AMPA after 10 min exposure to ibogaine (100 μM); (3) third application of NMDA and AMPA. Clear antagonism of the response to NMDA by ibogaine is shown, but little change in the AMPA-depolarization is seen; (4) recovery of the NMDA-response following removal of ibogaine (45 min washout). Ibogaine was present in the Ringer’s solution for the time indicated by the solid bar below the traces. (B) Effects of MK-801 on NMDA- and AMPA-responses. (1) control applications of NMDA and AMPA; (2) first application of NMDA and AMPA after 10 min exposure to MK-801 (10 μM); (3) second application of NMDA and AMPA; (4) third application of NMDA and AMPA. Distinct antagonism of the response to NMDA by MK-801 is demonstrated with little effect on the AMPA-response. MK-801 was present in the Ringer’s solution for the time indicated by the solid bar below the traces. Vertical calibration, 2.5 mV; horizontal calibration, 2.0 min.

Similar findings were induced by application of MK-801 (10 μM; 6.0 ± 2.8%, n = 3; Fig. 2B2,3,4) and 12-hydroxyibogamine (1.0 mM; 10 ± 2%, n = 2; not illustrated). Ibogaine, 12-hydroxyibogamine, and MK-801 did not produce changes in motoneuron membrane potential. That the ibogaine-, 12-hydroxyibogamine-, and MK-801-induced reductions of NMDA-responses were dependent upon activation of NMDA receptors in the presence of the blockers was demonstrated by the finding that the depolarization evoked by the first application of NMDA after exposure to the 3 compounds was only slightly reduced, but the 2nd and 3rd applications were substantially blocked. The effects of ibogaine (Fig. 2A) and 12-hydroxyibogamine were readily reversed upon washout. The effects of MK-801 on NMDA-depolarizations were persistent and were not reversed after 2 h of exposure to normal medium. In contrast to the effects on NMDA-depolarizations, AMPA-induced motoneuron depolarizations were not decreased by ibogaine, 12-hydroxyibogamine, and MK-801.

The present results demonstrate that both ibogaine and 12-hydroxyibogamine displace specific [3H]MK-801 binding to the NMDA receptor complex [12,16] may be of relevance to the understanding of the anti-addictive actions of ibogaine. MK-801 and the non-competitive NMDA antagonist, ketamine, block the development of tolerance to motor disturbances that result from ethanol administration [8,18]. In addition, MK-801 has been reported to block sensitization (reverse tolerance) to the behavioral effects of cocaine and amphetamine [7,13] and has been shown to attenuate the development of tolerance to the analgesic effect of morphine [17]. On the basis of these converging lines of evidence, Skolnick et al. [12] have suggested that ibogaine’s ability to modify drug-seeking behavior is attributable to its ability to blockade NMDA receptor-coupled ion channels. The 4–6-fold lower potency of 12-hydroxyibogaine to inhibit [3H]MK-801 binding indicates that it is likely that the dwell time for the metabolite in the ion channel is brief and implies that it is not contributing additional activity to the efficacy of ibogaine. Alternatively, Sweetnam et al. [16] have suggested that ibogaine’s interaction with the NMDA receptor-coupled cation channels may contribute to the psychotropic and high dose neurotoxic reactions of ibogaine [11]. If degeneration does occur through an interaction with the NMDA receptor complex, then 12-hydroxyibogamine may potentially have fewer neurotoxic properties than the parent drug.
Our electrophysiological observations indicate that MK-801 blocked NMDA-induced motoneuron depolarizations in the frog cord. Ibogaine and 12-hydroxyibogamine also blocked NMDA-depolarizations and, in agreement with binding data, ibogaine was much more effective than 12-hydroxyibogamine. The blocking effect of both alkaloids was similar to the inhibitory effect of MK-801 in that the onset of the block of NMDA-responses was a function of repeated presentations of the agonist, i.e., blockade of NMDA-depolarizations showed use-dependency. Biophysical data indicate that MK-801 impedes passage of permeant cations by binding selectively to the open, activated channel operated by activation of the NMDA receptor [9]. Use-dependent block of NMDA-responses by ibogaine and 12-hydroxyibogamine would be in keeping with an open channel-blocking mechanism.

Taken together our binding and electrophysiological data are compatible with the idea that both ibogaine and 12-hydroxyibogamine act at the MK-801 binding site located in the NMDA receptor channel. The findings have important implications for understanding ibogaine’s reported anti-addictive properties.

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