Psychopharmacology (1994) 114:672–674

**Psychopharmacology**


*Rapid communication*

# The putative anti-addictive drug ibogaine is a competitive inhibitor of [$^3$H]MK-801 binding to the NMDA receptor complex

**Piotr Popik, Richard T. Layer, Phil Skolnick**
Laboratory of Neuroscience, NIDDK, National Institutes of Health, Building 8, Room 111, Bethesda, MD 20892–0008, USA

Received: 3 January 1994 / Accepted: 10 February 1994

**Abstract.** Ibogaine is a putative anti-addictive drug with potential efficacy for the treatment of opiate, stimulant, and alcohol abuse. We now report ibogaine is a competitive inhibitor ($K_i$, 1.01 ± 0.1 μM) of [$^3$H]MK-801 binding to *N*-methyl-D-aspartate (NMDA) receptor coupled cation channels. Since MK-801 can attenuate the development of tolerance to morphine and alcohol as well as sensitization to stimulants in preclinical studies, the reported ability of ibogaine to modify drug-seeking behavior in man may be attributable to a blockade of NMDA receptor coupled cation channels.

**Key words:** MK-801 (dizocilpine) – Ibogaine – NMDA receptors – Drug abuse

Ibogaine (NIH 10567, Endabuse) is an indole alkaloid originally isolated from *Tabernanthe iboga.* The use of ibogaine in the treatment of drug addiction has been proposed based on claims that it may decrease dependence and symptoms of withdrawal to opiates (US Patent 4,499,096), stimulants (US Patent 4,587,243), and ethanol (US Patent 4,857,523). Preclinical findings that ibogaine reduces morphine self-administration, ameliorates symptoms associated with morphine withdrawal (Glick et al. 1991 and references therein) and decreases preference for cocaine consumption (Sershen et al. 1994) are generally consistent with these claims.

Converging lines of evidence have implicated the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor in tolerance and dependence phenomena. Thus, administration of the NMDA antagonist MK-801 (dizocilpine) can attenuate both the development of tolerance to the analgesic effect of morphine and morphine dependence (Trujillo and Akil 1991). Other studies have shown that MK-801 and another non-competitive NMDA antagonist, ketamine, can also block the development of tolerance to the motor incoordinating actions of ethanol (Khanna et al. 1993; Wu et al. 1993). MK-801 has also been reported to block sensitization ("reverse tolerance") to the behavioral activating effects of amphetamine and cocaine (Karler et al. 1989; Pudiak and Bozarth 1993). The similarity between these preclinical effects of MK-801 and the therapeutic claims which have been made for ibogaine prompted us to determine if MK-801 and ibogaine share a common neurochemical action at NMDA receptors.

## Materials and methods

[$^3$H]MK-801 (specific activity 22.5 Ci/mmol) binding was assayed in extensively washed membranes prepared from rat forebrain. The methods employed for tissue preparation and radioligand binding were essentially as described by Marvizon et al. (1989). Membranes were incubated for 2 h (25°C) with either 0.1–25 nM (to generate saturation isotherms) or 4 nM [$^3$H]MK-801 (to generate competition curves) in the presence of 30 μM glycine and 30 μM glutamate. Nonspecific binding was defined with 100 μM 1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP) hydrochloride.

Ligand binding to glutamate receptors was assayed in rat forebrain membranes prepared as described by Honore et al. (1988). [$^3$H]Amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([$^3$H]AMPA, specific activity 49.3 Ci/mmol) binding (5 nM) was assayed in 30 mM TRIS-HCl buffer with 2.5 mM $CaCl_2$ and 100 mM potassium thiocyanate (KSCN); [$^3$H]kainate (specific activity 58.0 Ci/mmol) binding (5 nM) was assayed in 50 mM TRIS-citrate buffer. Nonspecific binding of both was defined with 100 μM L-glutamate. [$^3$H]L-Glutamate (specific activity 56.59 Ci/mmol) was used to measure binding (10 nM) to metabotropic glutamate and *N*-methyl-D-aspartate (NMDA) receptors. Assays were performed in 30 mM TRIS-HCl buffer in the presence of NMDA, AMPA, kainate and 4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid (SITS) (each 100 μM); non-specific was defined by 100 μM trans-(1*S*,3*R*)-1-amino-1,3-cyclopentanedicarboxylic acid [(1*S*,3*R*)-ACPD] (Schoepp and True 1992). For [$^3$H]L-glutamate binding to glutamate receptors including the NMDA subtype, assays were performed in 30 mM TRIS-HCl buffer; L-glutamate (100 μM) was used to define nonspecifc binding.

Radioligands were purchased from DuPont-NEN (Boston, Mass.). Ibogaine and TCP were donated by the National Institute on Drug Abuse. Other chemicals were obtained from Sigma Chemical Company (St Louis, Mo.).

P. Popik is a Fogarty Visiting Fellow on leave from Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.
*Correspondence to*: P. Popik

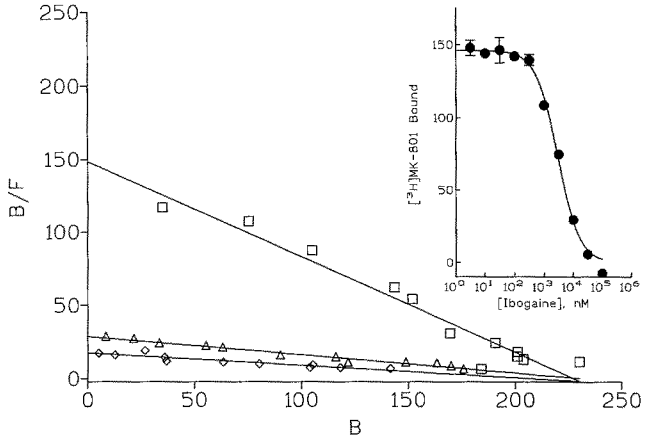


**Fig. 1.** Ibogaine competitively inhibits [$^3$H]MK-801 binding to the NMDA receptor complex. [$^3$H]MK-801 binding was assayed in extensively washed rat forebrain membranes (~70 μg protein/tube) in the presence of 30 μM glutamate and 30 μM glycine. In this representative experiment, addition of 0 (□), 5 μM (△) and 10 μM (◇) ibogaine increased the $K_d$ of [$^3$H]MK-801 from 1.3 nM to 8.6 and 16.1 nM, respectively. The corresponding $B_{max}$ values were: 3.6, 3.9, and 4 pmol/mg protein, respectively. Symbols: *B*, [$^3$H]MK-801 bound (fmol/assay); *F*, free radioligand (nM). *Inset*: competition curve. In this representative experiment, [$^3$H]MK-801 (4 nM) binding was inhibited by ibogaine with a $K_i$ of 0.90 nM ($n_H$ 1.17). Both types of experiments were repeated three times (see text for details). Data were analyzed using Inplot 4.0

## Results

Ibogaine inhibited [$^3$H]MK-801 binding (Fig. 1) with a $K_i$ of 1.01 ± 0.1 μM ($n_H$ 1.2 ± 0.04). This inhibition was effected through a concentration dependent reduction in the apparent affinity of [$^3$H]MK-801 with no concomitant change in the maximum number of binding sites ($B_{max}$). The $K_D$ of [$^3$H]MK-801 increased from 1.7 ± 0.3 nM in control membranes to 7.8 ± 0.5 and 14.3 ± 1.7 nM in the presence of 5 and 10 μM ibogaine, respectively. No statistically significant effects of ibogaine were observed on the corresponding $B_{max}$ values: 3.5 ± 0.1, 3.4 ± 0.2 and 3.3 ± 0.3 pmol/mg protein, respectively (Fig. 1). No inhibition of radioligand binding to kainate, AMPA, NMDA or metabotropic glutamate receptors was observed at ibogaine concentrations up to 100 μM (data not shown).

## Discussion

Ibogaine has been claimed to decrease dependence and withdrawal symptoms to opiates (US Patent 4,499,096), stimulants (US Patent 4,587,243), and ethanol (US Patent 4,857,523). The molecular mechanisms responsible for these putative anti-addictive properties are unknown. Nonetheless, ibogaine has recently been shown to inhibit [$^3$H]WIN 35,248 binding to the dopamine transporter ($IC_{50}$, 1.5 μM) (Sershen et al. 1992), [$^3$H]U-69593 binding to κ opioid receptors ($K_i$, ~ 2 μM) and [$^3$H]batrachotoxinin A-20-α-benzoate binding to voltage-dependent sodium channels ($K_i$, 8.1 μM) (Deecher et al. 1992). These are all potential molecular targets for ibogaine, since peak brain concentrations have been estimated at 100 μM following pharmacologically effective doses in rodents (Glick et al. 1993). However, the therapeutic claims which have been made for ibogaine bear a striking similarity to preclinical findings demonstrating that use of dependent channel blockers such as MK-801 (Huettner and Bean 1988) attenuates addiction-related phenomena such as the tolerance and dependence to opiates (Trujillo and Akil 1991) as well as psychomotor stimulants (Karler et al. 1989) and alcohol (Khanna et al. 1993; Wu et al. 1993). Ibogaine has a complex pharmacological profile, producing euphoria, increase in libido and a sense of inebriation in man (Dybowsky and Landrin 1901; Schneider and Sigg 1957). It is unknown whether the hallucinogenic actions of ibogaine are related to its effects on the NMDA receptor complex. In animals ibogaine produces generalized tremor, akinesia and fear (Glick et al. 1992). These observations indicate ibogaine may act at several sites in the CNS (Deecher et al. 1992). Nonetheless, in view of the present finding that ibogaine is a relatively potent ($K_i$, 1.01 μM), competitive inhibitor of [$^3$H]MK-801 binding, the claimed anti-addictive properties of this alkaloid may be mediated through the NMDA receptor complex.

## References


Deecher DC, Teitler M, Soderlund DM, Bornmann WG, Kuehne ME, Glick SD (1992) Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies. Brain Res 571:242–247

Dybowsky J, Landrin E (1901) Sur l'Iboga, ses propriétés excitantes, sa composition et sur l'alcaloïde noveau qu'il renferme, l'ibogaïne. C R Acad Sci (Paris) 133:748–750

Glick SD, Rossman K, Steindorf S, Maisonneuve IM, Carlson JN (1991) Effects and aftereffects of ibogaine on morphine self-administration in rats. Eur J Pharmacol 195:341–345

Glick SD, Rossman K, Rao NC, Maisonneuve IM, Carlson JN (1992) Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor. Neuropharmacology 31:497–500

Glick SD, Rossman K, Wang S, Dong N, Keller RW Jr (1993) Local effects of ibogaine on extracellular levels of dopamine and its metabolites in nucleus accumbens and striatum: Interactions with D-amphetamine. Brain Res 628:201–208

Honore T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE (1988) Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241:701–703

Huettner J, Bean B (1988) Block of *N*-methyl-D-aspartate – activated current by the anticonvulsant MK-801: selective binding to open channels. Proc Natl Acad Sci USA 85:1307–1311

Karler R, Calder LD, Chaudhry IA, Turkanis SA (1989) Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 45:599–606

Khanna JM, Kalant H, Shah G, Chau A (1993) Effect of D-cycloserine on rapid tolerance to ethanol. Pharmacol Biochem Behav 45[4]:983–986

Marvizon JC, Lewin AH, Skolnick P (1989) 1-Aminocyclopropane carboxylic acid: a potent and selective ligand for the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. J Neurochem 52:992-994

Pudiak CM, Bozarth MA (1993) L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. Life Sci 53[20]:1517–1524

Schneider JA, Sigg EB (1957) Neuropharmacological studies on ibogaine, an indole alkaloid with central stimulant properties. Ann NY Acad Sci 66:765–766

Schoepp DD, True RA (1992) 1S,3R-ACPD – sensitive (metabotropic) [$^3$H]glutamate receptor binding in membranes. Neurosci Lett 145:100-104

Sershen H, Hashim A, Harsing L, Lajtha A (1992) Ibogaine antagonizes cocaine-induced locomotor stimulation in mice. Life Sci 50:1079–1086

Sershen H, Hashim A, Lajtha A (1994) Ibogaine reduces preference for cocaine consumption in C57BL/6By mice. Pharmacol Biochem Behav 47[1]:13–19

Trujillo KA, Akil H (1991) Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251:85–87

Wu PH, Michic SJ, Liu J-F, Dzung Le A, Kalant H (1993) Blockade of chronic tolerance to ethanol by the NMDA antagonist, (+)-MK 801. Eur J Pharmacol 231:157–164