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Short communication

Evidence for roles of κ-opioid and NMDA receptors in the mechanism of action of ibogaine

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Abstract

Ibogaine, a putatively anti-addictive alkaloid, binds to κ-opioid and NMDA receptors. In the present study we investigated the roles of κ-opioid and NMDA actions in mediating ibogaine's (40 mg/kg, i.p.) behavioral and neurochemical effects in rats. A combination of a κ-opioid antagonist (norbinaltorphimine, 10 mg/kg, s.c.) and a NMDA agonist (NMDA, 20 mg/kg, i.p.) partially prevented ibogaine-induced inhibition of intravenous morphine self-administration and ibogaine-induced antagonism of morphine-induced locomotor stimulation. The combination, as well as norbinaltorphimine and NMDA alone, blocked the acute effects of ibogaine on dopamine release and metabolism in the striatum. The data suggest that both κ-opioid agonist and NMDA antagonist actions of ibogaine contribute to its putative anti-addictive effects.

Keywords: Ibogaine; Noribogaine; Morphine; κ-Opioid receptor; *N*-Methyl-D-aspartate receptor; Addiction; Drug self-administration; In vivo microdialysis

Ibogaine, an alkaloid extracted from *Tabernanthe iboga*, is being studied as a potential long-acting treatment for both opioid and stimulant abuse. While there have been only anecdotal reports of long-term efficacy in humans, e.g., [17], studies in this [4,6] and other [1] laboratories have shown that ibogaine can decrease both morphine and cocaine self-administration for several days in some rats; similar effects are produced by noribogaine, a metabolite of ibogaine [5]. Acutely, ibogaine and noribogaine decrease extracellular levels of dopamine in the nucleus accumbens and striatum while ibogaine pretreatment (19 h beforehand) blocks morphine-induced dopamine release [8] and morphine-induced hyperactivity [9,14]. Because both ibogaine and its metabolite noribogaine bind to κ-opioid, e.g., [3,13,16] and NMDA receptors, e.g., [11,15], we have been investigating the roles of κ-opioid and NMDA mechanisms in mediating ibogaine's behavioral and neurochemical effects. The interactions of a κ-opioid antagonist (nor-binaltorphimine, norBNI) and/or a NMDA agonist (*N*-methyl-D-aspartic acid, NMDA) with ibogaine were assessed in terms of three of ibogaine's reported effects: inhibition of morphine self-administration [6], inhibition of morphine-induced motor stimulation [9,14], and inhibition of dopamine (DA) release in the striatum [8]. The result of these studies suggest that both κ-agonist and NMDA antagonist actions of ibogaine and noribogaine contribute to, but may not entirely account for, the resultant effects.

All subjects were naive female Sprague–Dawley (Taconic, Germantown, NY) rats, approximately 3 months old and weighing 230–250 g at the beginning of an experiment. Rats were maintained on a normal light/dark cycle (lights on/off at 07.00 h/19.00 h).

The intravenous self-administration procedure has been described previously, e.g., [4,6]. Briefly, responses on either of two levers (mounted 15 cm apart on the front wall of each operant test cage) were recorded on an IBM compatible 486 computer with a Med Associates interface. The intravenous self-administration system consisted of polyethylene-silicone cannulas constructed according to the design of Weeks [21], Instech harnesses and commutators, and Harvard Apparatus infusion pumps (No. 55–2222). Shaping of the bar-press response was initially accomplished by training rats to bar-press for water. Cannulas were then implanted in the external jugular vein according to procedures described by Weeks [21]. Self-administration testing began with a 16-h nocturnal session

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followed by daily 1-h sessions, 5 days (Monday–Friday) a week. A lever-press response produced a 10 μl infusion of drug solution (0.01 mg of morphine sulfate) in about 0.2 s. Since all rats generally weighed 250 ± 20 g, each response delivered approximately 0.04 mg/kg of morphine. Experiments to assess the effects of ibogaine (40 mg/kg, i.p.), alone or in combination with a κ-antagonist (norbinaltorphimine, norBNI; 10 mg/kg, s.c.) and/or a NMDA agonist (*N*-methyl-D-aspartate, NMDA; 20 mg/kg, i.p.), were begun when baseline self-administration rates stabilized (less than 10% variation from one day to the next across 5 days), usually after 2 weeks of testing. Each treatment was tested in a different group of rats; injections were made on Wednesdays. Ibogaine and NMDA were administered 15 min before a self-administration session, whereas norBNI was administered 2 h before the same test session.

The effects of the same treatments on morphine-induced locomotor stimulation were studied using the same procedures previously employed with ibogaine alone [14]. Locomotor activity was assessed using cylindrical photocell activity cages (60 cm, three crossing beams) interfaced to an IBM-compatible 486 computer. Different groups of rats were treated with different combinations of norBNI, NMDA, ibogaine or saline (norBNI administered 1 h prior to co-administration of ibogaine and NMDA or saline), and 19 h later, injected with morphine sulfate (5 mg/kg, i.p.) immediately before being placed into the activity cages. Locomotor activity was monitored for 1 h thereafter.

The microdialysis procedures used to assess the effects of the same treatments on extracellular levels of dopamine and its metabolites in the striatum have been used extensively in this laboratory, e.g., [4,5,8]. Briefly, under pentobarbital anesthesia, rats were implanted stereotaxically with guide cannulas over the striatum so that, when inserted, the tips of the dialysis probes would be located in the striatum (rostral, +0.5 mm; lateral, ±2.9 mm; ventral, −7.0 mm) [12]. The cannula was fixed firmly in the skull with dental cement.

At least 4 days after surgery, a rat was placed in a dialysis chamber, a cylindrical (30 cm diameter) Plexiglas cage providing free access to food and water. The probe (3 mm; CMA 8309563) was then lowered into the guide cannula. The dialysis probe was continuously perfused with a solution containing 146 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 1.0 mM $MgCl_2$ at a flow rate of 1 μl/min. On the next morning (15–20 h later), the dialysis experiment was carried out on a freely moving animal. NorBNI or saline was administered at time 0, and NMDA or saline, and ibogaine were co-administered 2 h later. Twenty-minute fractions were collected in vials containing 2 μl of 1.1 N perchloric acid solution (containing 5 mg/l EDTA and 5 mg/l sodium metabisulfite). Upon completion of an experiment, rats were killed and histological analysis of each brain was performed to verify the locations of the probes.

Perfusate samples were analyzed by HPLC with electrochemical detection. The HPLC consisted of a Waters pump (model 510), a WISP autosampler (model 712), a Phase

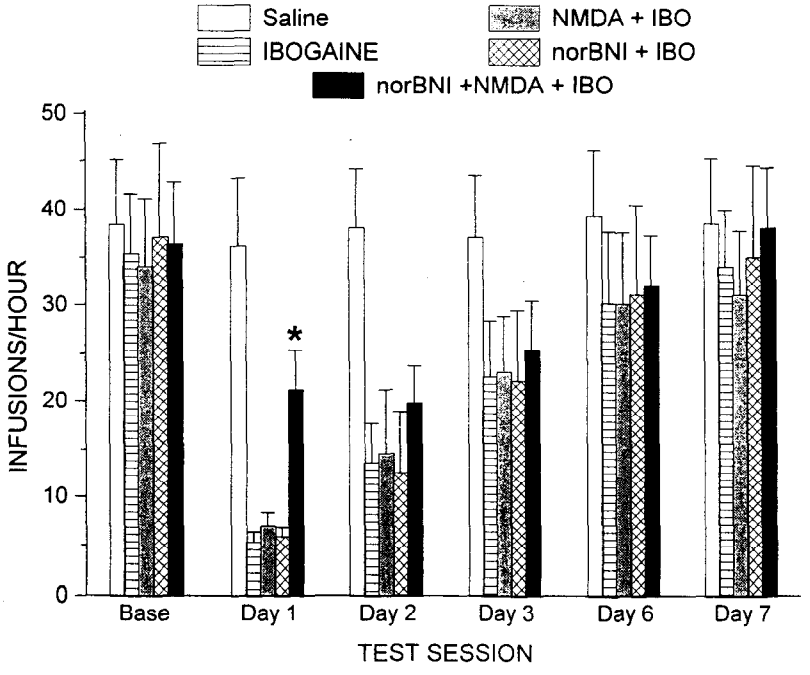

Fig. 1. Partial antagonism by a combination of NMDA and norBNI of the effects of ibogaine (IBO, 40 mg/kg, i.p.) on morphine (0.04 mg/kg/infusion) self-administration ($N = 6$ rats/group). Ibogaine (40 mg/kg, i.p.) and NMDA (20 mg/kg, i.p.) were administered 15 min before the test session on Day 1 whereas norBNI (10 mg/kg, s.c.) was administered 2 h before the same test session; the NMDA + norBNI + IBO group was significantly different from the other ibogaine groups on Day 1 (*, $P < 0.02$; see text).

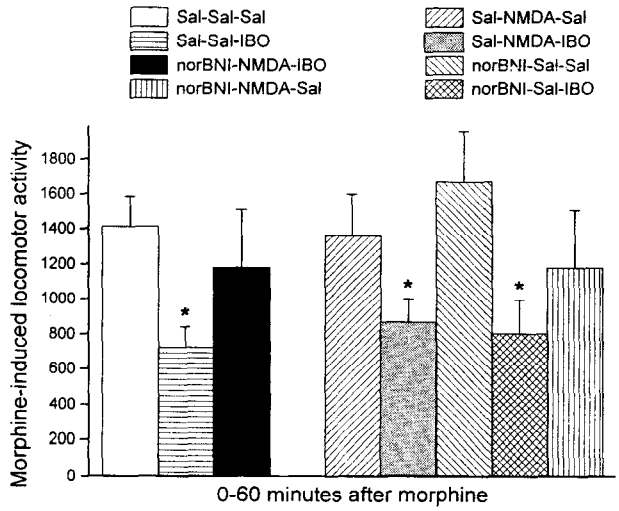

Fig. 2. Effects of norBNI (10 mg/kg, s.c.) and NMDA (20 mg/kg, i.p.) on ibogaine (40 mg/kg, i.p.) antagonism of morphine-induced (5 mg/kg, i.p.) locomotor activity ($N = 8$ rats/group). NorBNI or saline was administered 1 h before NMDA and ibogaine (or saline controls). The morphine challenge was administered 19 h after ibogaine and/or NMDA. The Sal-Sal-Ibo, the Sal-NMDA-IBO and the norBNI-Sal-IBO groups were all significantly different (*, $P < 0.05$) from the Sal-Sal-Sal group. The norBNI-NMDA-IBO group was not significantly different from the Sal-Sal-Sal group ($P > 0.05$).

Separation Spherisorb C-18 column (S3 ODS2; 10 cm × 4.6 mm) and a Waters detector (model 464). The mobile phase consisted of 6.9 g/l sodium monobasic phosphate, 450 mg/l heptane sulfonic acid, 80 mg/l disodium EDTA, and 110 ml/l methanol; the solution was adjusted with HCl to pH 3.7 and was pumped at a rate of 1.2 ml/min. Chromatograms were processed using Hewlett-Packard HPLC 2D Chem Station software.

Fig. 1 shows the morphine self-administration results. The combination of NMDA and norBNI significantly antagonized the effect of ibogaine on Day 1 (significant treatment × days interaction, ANOVA, $P < 0.01$; NMDA + norBNI + ibogaine significantly different from ibogaine, NMDA + ibogaine, and norBNI + ibogaine, $P < 0.02$, Newman–Keuls) but not thereafter. Ibogaine alone, as well as with NMDA or norBNI, significantly reduced morphine self-administration on Days 1 and 2 ($P < 0.05$–0.001) **Not shown are data from rats administered saline (in place of ibogaine) together with NMDA, norBNI or the combination: there was no effect of these treatments alone on morphine self-administration.**

Fig. 2 shows the locomotor activity results. Ibogaine inhibition of morphine-induced hyperactivity (ANOVA and LSD tests, $P < 0.05$) was partially antagonized by the combination of norBNI and NMDA. The norBNI-NMDA-IBO group did not differ (LSD test, $P > 0.05$) from the control group (Sal-Sal-Sal).

Fig. 3 shows the microdialysis results. The inhibition of dopamine release by ibogaine as well as the ibogaine-induced increases in DOPAC and HVA were antagonized by

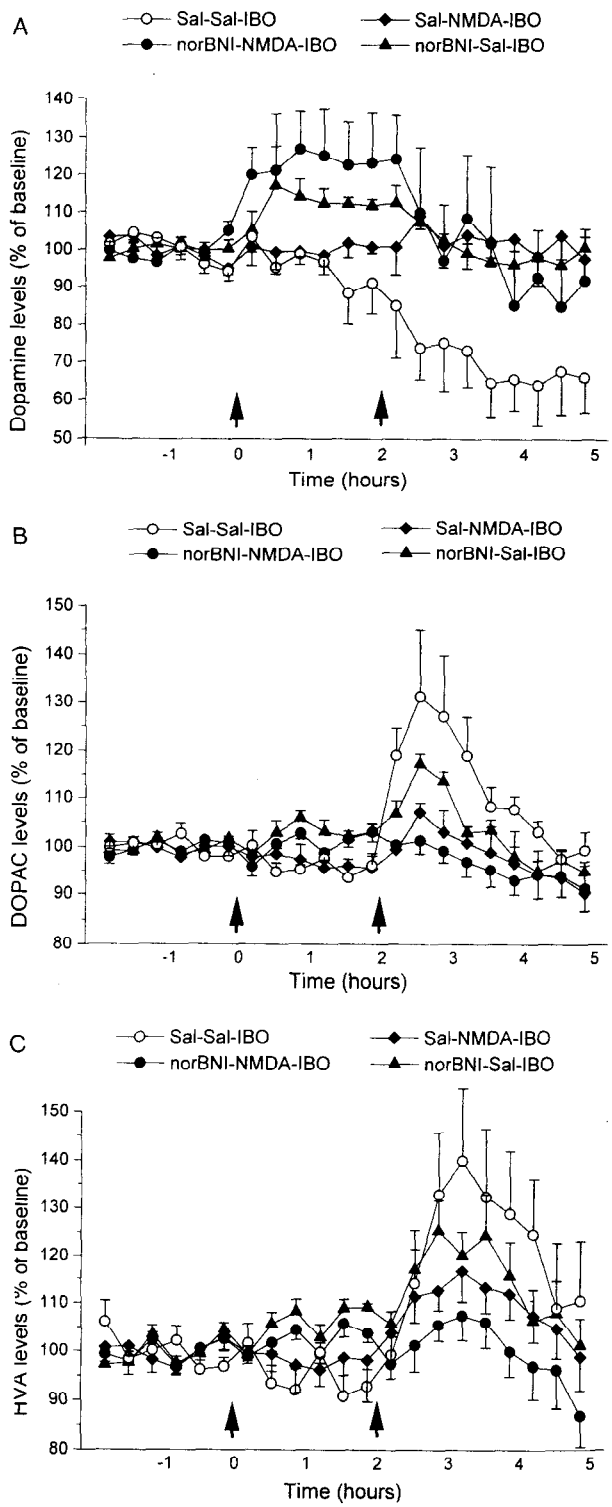

Fig. 3. Antagonism of ibogaine-induced (40 mg/kg, i.p.) changes in striatal dopamine, DOPAC and HVA by norBNI and/or NMDA. NorBNI (10 mg/kg, s.c.) or saline was administered at time 0 and NMDA (10 mg/kg, i.p.) or saline, and ibogaine (40 mg/kg, i.p.) were co-administered 2 h later ($N = 6$ for the norBNI-NMDA-IBO group and 4 for all other groups). The combination of norBNI + NMDA as well as each drug alone significantly ($P < 0.05$) attenuated the decrease in dopamine (A) and the increase in DOPAC (B); the combination of norBNI + NMDA as well as NMDA alone significantly ($P < 0.05$) attenuated the increase in HVA (C).

the combination of norBNI and NMDA as well as by each drug alone (ANOVA, $P < 0.05$). Although not significant from either drug alone, the combination of norBNI and NMDA appeared to be the most effective antagonist of ibogaine.

All of these data together indicate that both κ-opioid agonist and NMDA antagonist actions of ibogaine contribute to its putative anti-addictive effects. However, the antagonism of ibogaine by the combination of norBNI and NMDA in the behavioral studies was sometimes incomplete, suggesting that another (i.e., a third) mechanism (e.g., serotoninergic) [10] may be involved, or the dose of norBNI and/or NMDA may not have been optimal. With regard to the morphine self-administration data in particular, ibogaine and/or noribogaine or another active metabolite may have been present at a time (i.e., Day 2) when part of the combined treatment (i.e., NMDA) was no longer present.

Both κ-agonists and NMDA antagonists have been reported to decrease dopamine release in the nucleus accumbens. Because their sites of action are different their effects may be additive. κ-Agonists exert their inhibitory action in the nucleus accumbens, possibly by interacting with κ-opioid receptors located on dopaminergic terminals [19], while NMDA antagonists block the excitatory tonic control that glutamate exerts on dopamine neurons in the ventral tegmental area [20]. In addition, cortical glutamatergic neurons may be under κ-opioid inhibition [2,18], possibly increasing the efficacy of the combined actions. It appears that the combination of these actions confers ibogaine with a somewhat unique pharmacological profile. Together with data showing that ibogaine is sequestered in fat [7], the present data are consistent with the hypothesis that ibogaine's long-term effects may be mediated by slow release from fat tissue, conversion to noribogaine, and binding of both ibogaine and noribogaine to κ-opioid and NMDA receptors.

Acknowledgements

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