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The Effects of Sigma, PCP, and Opiate Receptor Ligands in Rats Trained With Ibogaine as a Discriminative Stimulus

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HELSLEY, S., R. FILIPINK, W. D. BOWEN, R. A. RABIN AND J. C. WINTER. *Interactions of sigma, PCP, and opiate ligands with the ibogaine-induced discriminative stimulus.* PHARMACOL BIOCHEM BEHAV **59**(2) 495–503, 1998.– Although the mechanism of action of ibogaine, a hallucinogen that may be useful in the treatment of addiction, remains unknown, receptor binding studies suggest that ibogaine produces its effects via interactions with multiple receptor types. In addition to serotonergic receptors, which have been studied previously with respect to ibogaine, likely candidates include opiate, sigma (σ), and phencyclidine (PCP) binding sites. In an attempt to determine which of these receptor interactions are involved in the in vivo effects of ibogaine, ligands for σ, PCP, and opiate receptors were assessed for their ability to substitute for or to antagonize the ibogaine-induced discriminative stimulus (10 mg/kg IP, 60 min presession) in Fischer-344 rats. Intermediate levels of generalization were observed with the subtype nonselective σ ligands 3-(3-hydroxyphenyl)-N-(1-propyl)-piperidine [(+)-3-PPP] (69.0%) and 1,3-di(2-tolyl)guanidine (DTG) (73.5%) but not with the σ_1-selective agents (+)-N-allylnormetazocine [(+)-SKF 10,047] and (+)-pentazocine. These findings, along with observations that ibogaine has appreciable affinity for σ_2 receptors, suggest that these receptors may be involved in the ibogaine discriminative stimulus. With regard to opiate receptors, neither morphine, the prototypic mu agonist, nor kappa selective agonists (bremazocine,and U-50488) substituted for ibogaine. However, intermediate levels of generalization were observed with the mixed action opiates (−)-SKF 10,047 (78.9%), (±)-pentazocine (73.9%), nalorphine (70.4%), and diprenorphine (75.0%) indicating a potential role for opiate receptors in the ibogaine stimulus. Partial substitution was also observed with naltrexone (55.6%) but not with naloxone or the selective kappa antagonist nor-binaltorphimine (nor-BNI). These agents were largely ineffective as antagonists of the ibogaine cue, although naloxone produced a moderate but statistically significant antagonism (69.8%). In addition, naloxone produced complete antagonism of the ibogaine-appropriate responding elicited by both (−)-SKF 10,047 (19.7%) and nalorphine (25.8%), whereas the ibogaine-appropriate responding produced by diprenorphine was only partially antagonized (44.4%). The latter observations taken together with the finding that both nalorphine (>100 μM) and diprenorphine (30 μM) have extremely low affinity for σ_2 receptors, suggest that the ibogaine-appropriate responding produced by these agents is not mediated by σ_2 receptors. These findings imply that opiate effects may be involved in the ibogaine stimulus. In contrast to σ_2 and opiate receptors, ibogaine's reported interactions with NMDA receptors do not appear to be involved in its discriminative stimulus, as neither PCP nor MK-801 produced a significant level of ibogaine-appropriate responding. Thus, the present study offers evidence that unlike NMDA receptors, both σ_2 and opiate receptors may be involved in the ibogaine discriminative stimulus. © 1998 Elsevier Science Inc.

Ibogaine Drug discrimination Radioligand binding Sigma receptors NMDA antagonists Opiate receptors

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RECENT studies demonstrating the antiaddictive effects of ibogaine have stimulated renewed interest in an agent previously regarded as little more than a hallucinogenic curiosity (34). Indeed, the past decade has been quite fruitful, yielding over 50 reports concerning ibogaine. Despite these efforts, definitive conclusions regarding ibogaine's mechanism of action remain elusive.

Of the binding sites so far examined, ibogaine displays relatively high affinity for σ_2 receptors; reported K_i values range from 90–201 nM (4,24). Furthermore, ibogaine is proposed to be a σ_2 agonist (3). Because the affinity of ibogaine is significantly higher at σ_2 sites than those reported for other receptors, a special role for σ_2 binding in the effects of ibogaine is indicated. Of particular relevance for antiaddictive properties is the suggestion that certain σ ligands may be efficacious in the treatment of drug abuse because of their demonstrated ability to block the behavioral effects of cocaine and amphetamine in animal subjects (53). A recent study also suggests that ibogaine binds to ganglionic-type nicotinic receptor channels with nanomolar affinity (1).

In addition to σ_2 receptors, it appears that ibogaine binds with low micromolar affinity (<10 μM) to mu (6) and kappa (8,30,38,52) opiate receptors, 5-HT_{2A} and 5-HT_3 serotonergic receptors (52), M_1, M_2, and M_3 muscarinic receptors (52), σ_1 receptors (4,24), and alpha$_1$ adrenergic receptors (52). Similar affinities were observed for ibogaine at the MK-801 binding site on the NMDA channel (5,21,27,33, 49,52). These findings suggest that ibogaine's effects may be the result of low-affinity interactions with multiple receptors. This hypothesis seems especially attractive in light of the fact that ibogaine reaches high concentrations (i.e., >10 μM) in the CNS following systemic administration (20, 49,62).

Although ibogaine is purported to be broadly antiaddictive, the majority of investigations have been concerned with its anti-opiate effects. Thus, reports of ibogaine's ability to reduce opiate self-administration in laboratory animals (12,13) as well as clinical observations of its efficacy in treating opiate addiction (46) led us to examine opiate and related ligands. Another potential explanation for the reported antiaddictive effects of ibogaine involves the MK-801/PCP binding site on the NMDA receptor. Previous reports provide evidence that ibogaine interacts with this site and that this interaction may be involved in the antiaddictive properties of ibogaine (21,35).

In the present investigation, ibogaine-induced stimulus control was the dependent variable used to explore the behavioral relevance of ibogaine's multiple receptor interactions. This technique has been effectively used over the past 3 decades to investigate the interoceptive states created by a variety of psychoactive drugs in animal subjects (7,58,59). The first report of ibogaine-induced stimulus control was by Schechter and Gordon (40), who emphasized possible serotonergic interactions. In a subsequent investigation in our laboratory we found 5-HT_{2A} receptors to be involved in the ibogaine-trained stimulus as evidenced by intermediate generalization of ibogaine to 5-HT_{2A} receptor ligands. However, this interaction does not appear to be the sole component of the ibogaine discriminative cue as 5-HT_{2A} antagonists failed to block the effects of ibogaine while the ibogaine-appropriate responding produced by 5-HT_{2A} receptor agonists was completely antagonized (19). The present study examines the role played by opiate, σ, and PCP sites in ibogaine-induced stimulus control.

METHOD

Behavioral Experiments

Subjects. Male Fischer 344 rats were obtained from Harlan–Sprague–Dawley Inc. (Indianapolis, IN). They were housed in pairs under a natural light–dark cycle and allowed free access to water in the home cage. Subjects were fed following experimental sessions. Caloric intake was controlled to yield a mean weight of about 250 g.

Apparatus. Six small-animal test chambers (Coulbourn Instruments Model E10-10) housed in larger light-proof, sound-insulated boxes were used for all experiments. Each box has a house light and exhaust fan. The chamber contains two levers mounted on opposite ends of one wall. Centered between the levers is a dipper that delivers 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Ibogaine-induced stimulus control. Twenty-four subjects were trained to discriminate ibogaine (10.0 mg/kg, 60-min pretreatment time, intraperitoneal injection) from its vehicle (water) as previously described (9,18). A fixed-ratio 10 (FR10) schedule of reinforcement was employed. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever.

Ibogaine-induced stimulus control was established after 40–70 training sessions. The ibogaine training dose produced approximately 94% drug-appropriate responding. After stimulus control was established with ibogaine, tests were conducted once per week in each animal so long as performance did not fall below the criterion level of 83% correct responding in any one of the previous three training sessions.

Test procedure. Tests of generalization or antagonism were conducted in such a fashion that approximately half of the test sessions fell on days following vehicle training sessions and the remainder occurred the day after ibogaine training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on one of the levers (e.g., if eight responses were completed on one lever the session would end following the 10th response on the other lever). The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted prior to lever selection, that is, prior to the emission of 10 responses on either lever, by the elapsed time. The data for subjects failing to emit 10 responses within the constraints of the 10-min test session were not considered in the calculation of percent drug-appropriate responding but were included in the calculation of response rates.

Pretreatment times for the following agents were determined from preliminary studies in our laboratory and reports in the literature. Ibogaine or water was administered 60 min prior to each training session. The SKF isomers as well as PCP and MK-801 were given at a pretreatment time of 15 min, whereas DTG, pentazocine, nalorphine, diprenorphine, (+)-3-PPP, morphine, naloxone, BMY 14802, U-50488, and bremazocine were given 30 min presession. Naltrexone and rimcazole were given 60 min presession; in antagonism studies, the injection of these agents was followed immediately by injection of ibogaine into the peritoneal cavity on the opposite side. Nor-BNI was given 90 min presession. All solutions were injected IP in a volume of 1.0 ml/kg with the exception of morphine, nalorphine, diprenorphine, nor-BNI, and naloxonem which were injected subcutaneously.

Biochemical Studies

σ_2 binding assays. Male Fischer-344 rats weighing about 150 g were sacrificed by decapitation and their brains were removed. Tissues rostral to the midbrain as well those caudal to the cerebellum and the cerebellum itself were discarded leaving the midbrain and hindbrain regions for use in binding studies. These regions contain the highest densities of σ_2 sites in the rat brain (22).

Receptor binding assays were carried out using methods similar to those of Bowen and colleagues (16,17) with slight modifications. In brief, tissues were homogenized (Dounce tissue grinder) at a volume of 10 ml/g of tissue in ice-cold 50 mM Tris-HCl (pH = 7.4) containing 0.32 M sucrose. The resulting suspension was centrifuged at 1000 × *g* for 10 min at 4°C to remove larger pieces of tissue and the pellet was discarded. Supernatants were then centrifuged at 31,000 × *g* for 15 min at 4°C, and the resulting pellets were resuspended (3 ml/g tissue) in 10 mM Tris-HCl (pH = 7.4). Following a 30-min incubation at room temperature, the suspensions were again centrifuged at 31,000 × *g* for 15 min at 4°C. The final pellets were resuspended (1.53 ml/g tissue) in ice-cold 50 mM Tris-HCl (pH = 8.0) and were stored at −70°C until use. Competition assays were carried out for 60 min at 25°C in a final volume of 0.5 ml (50 mM Tris) containing various concentrations of the competing ligand (0–100 μM), 3.0 nM [^{3}H]DTG, and 1 μM dextrallorphan to mask σ_1 receptors. Incubations were terminated by vacuum filtration using a Brandel cell harvester. Filters were presoaked for 2 h in 0.1% PEI immediately prior to use and the filters were washed three times with ice-cold 10 mM Tris-HCl (pH = 7.4). Filters were incubated overnight with 3.0 ml of Ecoscint scintillation cocktail (National Diagnostics), and the amount of bound radioactivity was determined by liquid scintillation photometry (Beckman LS 6800 Series). Specific binding was defined as the difference in the amount of radioactivity bound in the presence and absence of 50 μM haloperidol. The data were analyzed by nonlinear regression using the program EBDA/ligand (Elsevier BIOSOFT). The method of Lowry et al. (23) was used to measure protein content.

Behavioral Data Analysis

The criteria for generalization and antagonism were as follows (60). Complete generalization/no antagonism is said to be present when (a) a mean of 83% or more of all test responses are on the drug-appropriate lever, (b) there is no statistically significant difference between training-drug and test-drug response distributions, and (3) there is a statistically significant difference between test-drug and vehicle-control response distributions. An intermediate degree of generalization/antagonism is defined as being present when mean response distributions following a test drug show a statistically significant difference from distributions following both training conditions. Finally, when response distributions following a test drug are not significantly different from vehicle-control response distributions, no generalization/full antagonism is assumed. Comparisons of data are by means of individual applications of Wilcoxon's signed ranks test. Thus, data obtained with a given drug at a given dose are compared with the immediately preceding training sessions for vehicle and training drug, respectively. Differences are considered to be significant if they would be expected to arise by random sampling alone with a probability <0.05.

Drugs

Ibogaine HCl; PCP HCl, nalorphine HCl; diprenorphine HCl; (+)-pentazocine succinate; nor-BNI dihydrochloride; and the SKF 10,047 isomers were provided by the National Institute on Drug Abuse (Rockville, MD). The following compounds were purchased from Research Biochemicals International, Natick, MA: DTG, morphine sulfate, bremazocine HCl, naloxone HCl, naltrexone HCl, MK-801 HCl, (±)-*trans*-U-50488 methanesulfonate, and (+)-3-PPP HCl. The following compounds were generously provided by the indicated organizations: (±)-pentazocine (Sterling-Winthrop Research Institute, Rennselaer, NY); rimcazole HCl (Burroughs Wellcome Co., Research Triangle Park, NC); BMY 14802 (Bristol Myers Co., Wallingford, CT). Dextrallorphan HBr was generously provided by Dr. Kenner Rice. All agents were dissolved in deionized water with the exception of DTG and racemic pentazocine, which were dissolved in water with a few drops of 8.5% lactic acid.

RESULTS

Behavioral Studies

Intermediate levels of generalization were observed with the subtype nonselective σ ligands DTG (73.5%) and (+)-3-PPP (69.0%). However, no substitution was observed with the σ_1 selective ligand (+)-pentazocine (Fig. 1). The rate-sup-

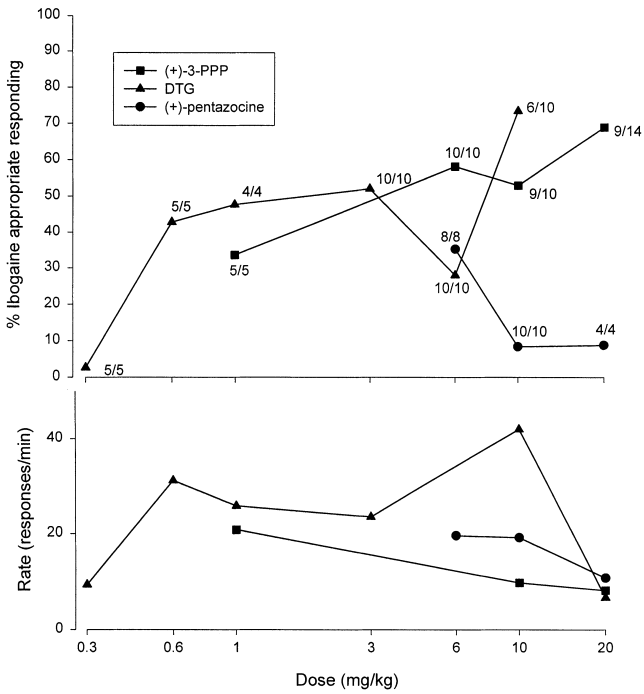

FIG. 1. The dose–response relationships for σ ligands in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60 min presession) from water. All agents were administered IP, 30 min presession. The ratio adjacent to each of the points represents the number of subjects completing the test session over the number of subjects participating in each test session. Ordinate: upper panel: mean percentage of responses on the ibogaine-appropriate lever. Lower panel: response rate expressed as responses per minute. Abscissa: dose of test agent (mg/kg).

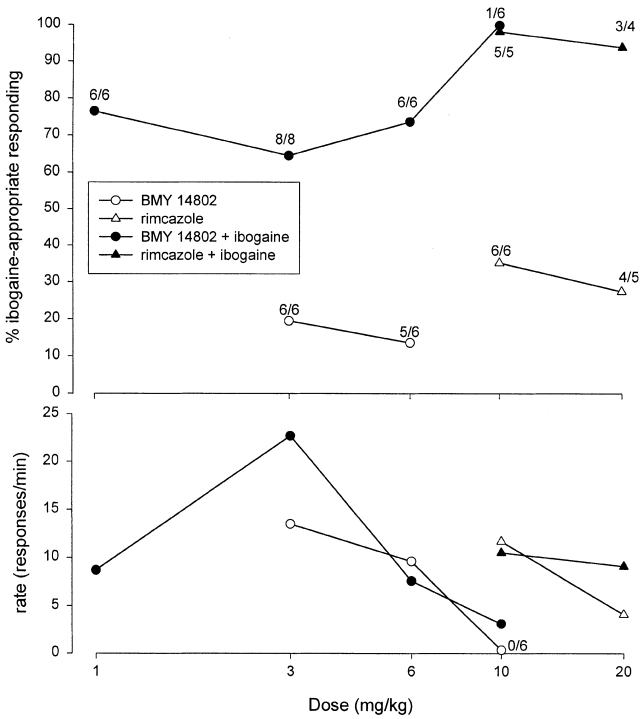

FIG. 2. The dose–response relationships for the putative σ antagonists rimcazole (IP, 60-min presession) and BMY 14802 (IP, 30-min presession) alone and in the presence of ibogaine (10 mg/kg, IP, 60-min presession). Other details are as described for Fig. 1.

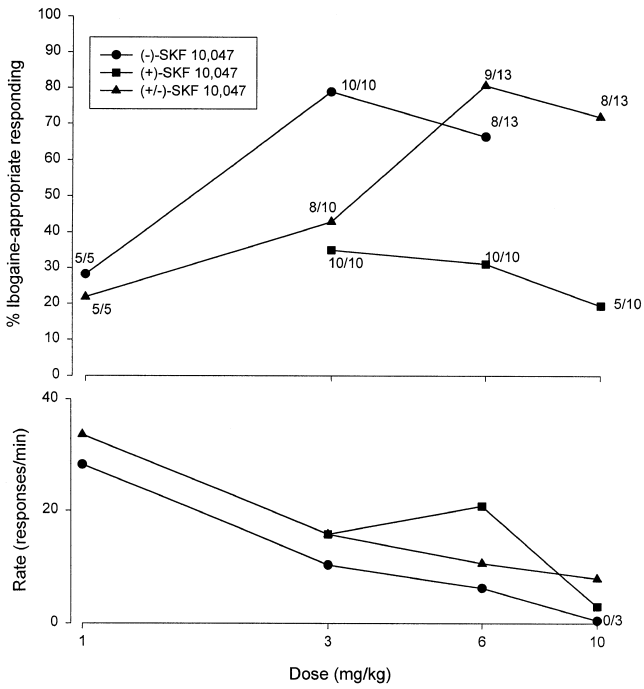

FIG. 3. The dose–response relationships for the SKF 10,047 isomers in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60-min presession) from water. All agents were administered IP, 15-min presession. All other details are the same as those for Fig. 1.

pressing effects of (+)-3-PPP and DTG precluded the testing of higher doses. Although these results provide evidence that suggests that sigma receptors (especially σ_2) are involved in the discriminative effects of ibogaine, the ibogaine cue was not antagonized by the putative sigma antagonists BMY 14802 and rimcazole (Fig. 2). To further characterize the ibogaine stimulus, the SKF 10,047 isomers were tested in our ibogaine-trained rats. (+)-SKF 10,047, a σ_1 ligand with PCP-like discriminative properties in the rat (45,47–49,51) failed to substitute for ibogaine (35%), whereas intermediate generalization was observed with both the (−) enantiomer (78.9%) as well as (±)-SKF 10,047 (80.7%) (Fig. 3). The ability of the noncompetitive NMDA antagonists, MK-801 and PCP, to substitute for ibogaine was also assessed. Although the substitution produced by both agents appears different from that produced by water, this was not a statistically significant difference. Thus, neither PCP (43.8%) nor MK-801 (49.1%) substituted for ibogaine (Fig. 4).

The mu agonist morphine and the kappa agonists bremazocine and U-50488, were also examined in tests of generalization. None of these agents produced significant substitution. Morphine produced a maximum of 41.3% ibogaine-appropriate responding, bremazocine produced 41.0% ibogaine-appropriate responding, and U-50488 produced 12.8% ibogaine appropriate responding (Fig 5). Tests of antagonism were carried out with opiate antagonists. Neither nor-BNI nor naltrexone significantly antagonized the ibogaine-induced stimulus, although naloxone (69.8%) produced partial antagonism of ibogaine and complete antagonism of the ibogaine-appropriate responding produced by (−)-SKF 10,047 (19.7%) (Table 1).

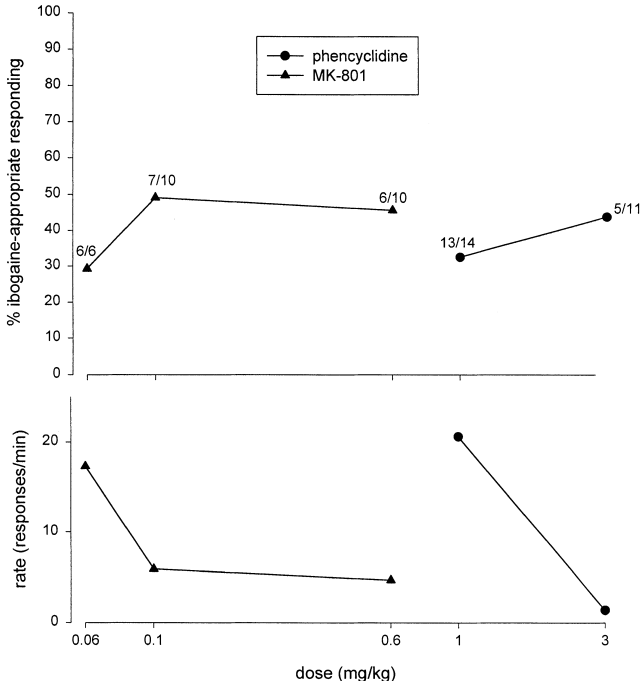

FIG. 4. The dose–response relationships for the PCP and MK-801 in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60-min presession) from water. All agents were administered IP, 15-min presession. All other details are the same as those for Fig. 1.

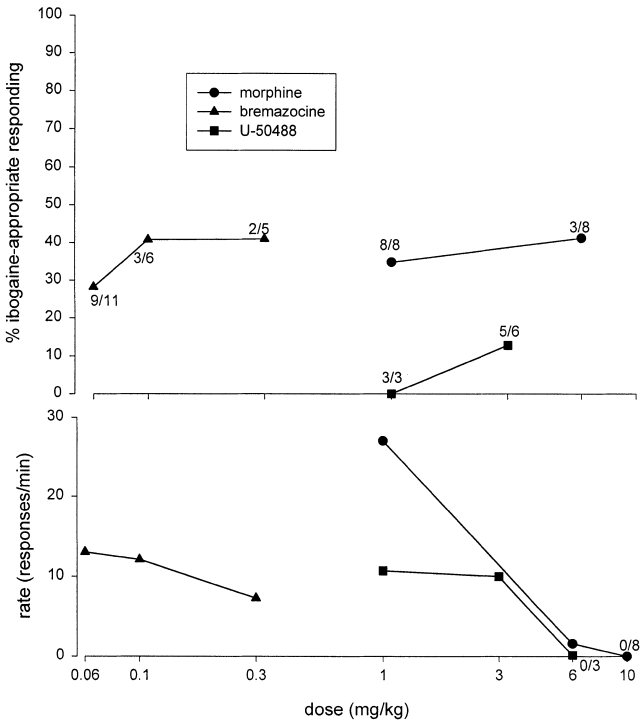

FIG. 5. The dose–response relationships for the opiate agonists morphine, bremazocine, and U-50488 in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60-min presession) from water. Morphine (30-min presession) was administered subcutaneously 15**<Q3>**, whereas U-50488 (also 30-min presession) and bremazocine (15-min presession) were administered via IP injection. All other details are the same as those for Fig. 1.

When tested alone naltrexone produced intermediate substitution (55.6%), but no substitution was produced by naloxone (37.2%) (Fig. 6) or nor-BNI (data not shown). Intermediate generalization was observed with the mixed activity opiates (±)-pentazocine (73.9%), diprenorphine (75.0%), and nalorphine (70.4%) (Fig. 7). Interestingly, the dose–response curves for diprenorphine and nalorphine are in an inverted U-shape, suggesting that higher doses are less ibogaine-like than lower doses. The ibogaine-appropriate responding produced by nalorphine was completely antagonized by naloxone (25.8%); however, this antagonism was seen at a dose more than 10-fold greater than that required to antagonize the ibogaine-appropriate responding elicited by (−)-SKF 10,047. In contrast, the ibogaine-appropriate responding produced by diprenorphine was only partially blocked by naloxone (44.4%) (Table 1).

Receptor Binding Studies

In saturation-equilibrium studies of [^{3}H]DTG binding, the data were best described by a high-affinity site characterized by a K_d of 26.9 ± 2.8 nM and a B_{max} equal to 588.0 ± 32.1 fM/mg. This K_d value for [^{3}H]DTG in rat midbrain/hindbrain is consistent with values observed in guinea pig brain (57), various cell lines (16,55), and rat liver (17). The σ_2 affinities of agents used in competition studies are shown in Table 2.

DISCUSSION

The results of the present study suggest a possible role for σ_2 as well as opiate receptors in the stimulus effects of ibogaine. The observation that the nonselective sigma ligands (+)-3-PPP and DTG (36) produced intermediate generalization in our ibogaine-trained subjects while no generalization was seen with the σ_1 ligands (+)-SKF 10,047 and (+)-penta-

TABLE 1

EFFECTS OF VARIOUS OPIATE ANTAGONISTS IN SUBJECTS TRAINED TO DISCRIMINATE 10 mg/kg IBOGAINE FROM WATER.

Drug Treatment	% Ibogaine-Appropriate Responses	Rate (Responses/min)	*n*/*N*
Ibogaine (10 mg/kg)	94.9	20.5	10/10
Ibogaine (10 mg/kg) + naloxone (10 mg/kg)	69.8	10.9	10/12
Ibogaine (10 mg/kg) + naltrexone (3.0 mg/kg)	78.4	12.6	6/7
Ibogaine (10 mg/kg) + nor-BNI (10 mg/kg)	97.8	23.4	4/4
(−)-SKF 10,047 (3.0 mg/kg) + naloxone (0.6 mg/kg)	19.7	10.1	9/10
Nalorphine (6.0 mg/kg) + naloxone (10 mg/kg)	25.8	10.4	6/6
Diprenorphine (10 mg/kg) + naloxone (10 mg/kg)	44.4	17.5	8/9

The number of animals responding (*n*) out of the number of animals tested (*N*) is expressed as the ratio *n*/*N*. Treatment sessions were compared to immediately preceding ibogaine training sessions using Wilcoxon's signed ranks test for those treatments in which six or more subjects were tested. No significant differences ($p < 0.05$) were observed compared to the ibogaine treatment condition.

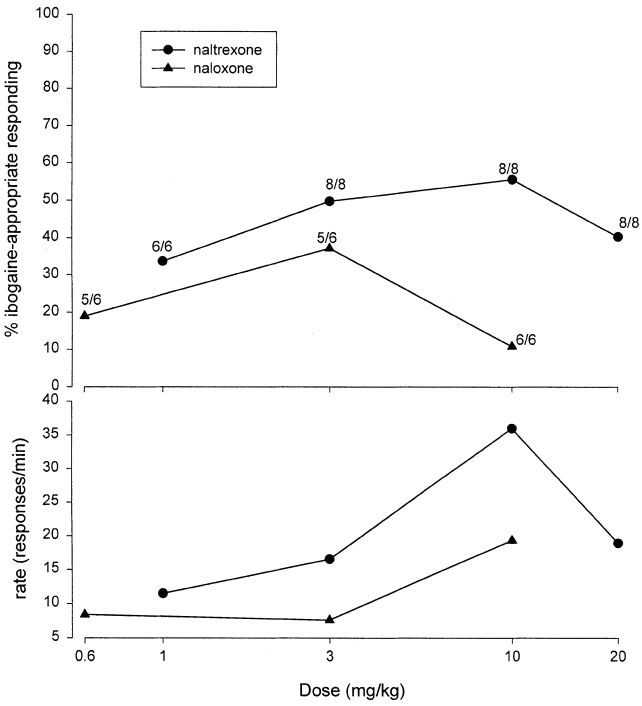

FIG. 6. The dose–response relationships for the opiate antagonists naloxone and naltrexone in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60-min presession) from water. Naltrexone (60-min presession) was administered IP, whereas naloxone was administered subcutaneously 30-min presession. All other details are the same as those for Fig. 1.

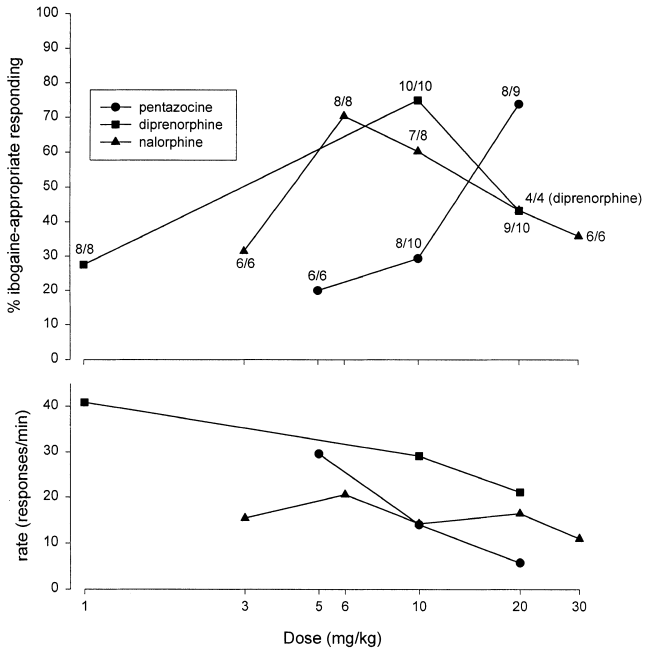

FIG. 7. The dose–response relationships for the mixed-action opiates (±)-pentazocine, nalorphine, and diprenorphine in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60-min presession) from water. Nalorphine and diprenorphine were administered via subcutaneously injection while ((±-pentazocine was given IP All agents were given 30-min presession. All other details are the same as those for Fig. 1.

zocine (36) suggests that ibogaine is σ_2 selective. Further support for the σ_2 selectivity of ibogaine comes from the fact that ibogaine has high affinity for σ_2 receptors in rat liver and guinea pig brain but not for σ_2 receptors (4,24). The present study extends these findings by demonstrating that ibogaine binds with high affinity to σ_2 receptors in the rat brain.

Whether σ receptors can account for the reported hallucinogenic and antiaddictive effects of ibogaine in man (46) remains to be determined. However, a potential role for the σ receptor in the treatment of addiction has been proposed (53). In addition, it is suggested that certain σ ligands are capable of producing psychotomimetic effects in humans, although more recently these effects have been attributed to opiate receptors (29).

A major problem in the study of σ receptors is the lack of a widely accepted functional assay for sigma activity. As a result relatively little is known regarding which σ ligands are agonists or antagonists (53). Furthermore the existence of relatively few σ_2-selective ligands further complicates the picture. The putative σ antagonists rimcazole and BMY 14802 (2) were ineffective both in tests of generalization and in tests of antagonism in our ibogaine-trained rats. Because both DTG and (+)-3-PPP were so rate suppressing, we were unable to determine whether putative σ antagonists would block the ibogaine-appropriate responding produced by these agents.

In the present study, no correlation was observed between σ_2 affinity and the ability to substitute for the ibogaine discriminative stimulus. Indeed, harmaline, the only agent tested to date in our ibogaine-trained rats that fully substitutes (18), possesses low affinity for σ_2 receptors as determined by both the present study and by previous studies (4). Furthermore, 12-hydroxyibogamine (noribogaine), a primary metabolite of ibogaine, which may mediate its antiaddictive effects (26), also has relatively low affinity for these receptors (4). Although these findings may appear to argue against a role for the σ_2 receptor in the stimulus effects of ibogaine, previous studies in our laboratory suggest that what would be considered very low in vitro binding affinity in most circumstances may be of biological significance when it comes to ibogaine and harmaline. For example, ibogaine and harmaline bind to 5-HT_{2A} receptors with low affinity in vitro (i.e., >40 μM) but both of these agents occupied 5-HT_{2A} receptors in vivo following systemic administration (19). This phenomenon is perhaps due to the fact that both ibogaine and harmaline reach high concentrations (>10 μM) in the rat brain following systemic administration. Thus, the in vitro affinities for ibogaine (194 nM) and harmaline (27.3 μM) reported in the present studies are certainly compatible with in vivo occupation of σ_2 receptors. Despite the fact that both agents possess remarkable bioavailability, the large difference in σ_2 affinity between ibogaine and harmaline suggests that σ_2 receptors may play a more prominent role in the effects of ibogaine as opposed to harmaline. Interestingly, unlike ibogaine, harmaline does not attenuate morphine or cocaine self-administration in rats (13). On the other hand, 12-hydroxyibogamine does attenuate self-administration of these drugs (15). In the present study 12-hydroxyibogamine bound to rat brain σ_2 receptors with a K_i value of 3.1 μM; this represents nearly 10-fold higher affinity

TABLE 2
AFFINITY VALUES (K_i) FOR σ_2 RECEPTORS.

Drug	K_i (SEM) in μM
Diprenorphine	30.3 (0.1)
DTG	0.031 (0.007)
Harmaline	27.3 (2.3)
12-Hydroxyibogamine	3.1 (0.05)
Ibogaine	0.194 (0.069)
Nalorphine	>100
Naltrexone	>100
(+)-Pentazocine	2.3 (0.6)
(±)-Pentazocine	0.087 (0.016)
(+)-PPP	0.118 (0.027)
(+)-SKF 10,047	14.6 (4.1)
(−)-SKF 10,047	2.5 (0.3)

K_i values were determined from in vitro competition experiments with [^{3}H]DTG in the presence of dextrallorphan (1 μM). Data are expressed as the mean of two to six separate experiments.

than that observed with harmaline. Thus, these observations suggest that σ_2 receptors may be involved in the effects of ibogaine.

Because NMDA receptor antagonists have been observed to interfere with the physical dependence and tolerance produced by a variety of addictive substances, noncompetitive NMDA receptor antagonists such as MK-801 may be clinically useful in the treatment of substance abuse disorders (54). In support of the suggestion that the PCP/MK-801 site on the NMDA receptor might mediate the effects of ibogaine, previous studies have demonstrated that ibogaine produces functional antagonism of the NMDA ionophore both in vitro (5,27,35) and in vivo (5,35). In addition, in a drug discrimination study, MK-801 partially generalized to ibogaine (35). It is noteworthy that in the present study both PCP and MK-801 produced nearly 50% ibogaine-appropriate responding. Although our criteria for intermediate generalization were not met, these values are markedly different from those observed during vehicle sessions. It should be noted that methodological differences, including animal species and operant tasks, exist between the present study and that of Popik et al. (35). Nonetheless, both studies suggest similarities between PCP-site ligands and ibogaine. However, it appears that at the present training dose these interactions play no more than a minor role in mediating the stimulus effects of ibogaine in the rat.

Reports of ibogaine's effectiveness in treating opiate addiction (46) along with reports of its ability to block opiate self-administration in animals (12,13) support a possible interaction of ibogaine with opiate receptors. Several binding studies offer evidence that ibogaine (8,30,38) and 12-hydroxyibogamine (21,30,49) display appreciable in vitro affinity (<10 μM) for kappa opiate receptors. In addition, high affinity for ibogaine at mu opiate receptors has been documented (6). In spite of these findings, no clear consensus has been reached regarding the role of opiate receptors in the effects of ibogaine. There is evidence that ibogaine may act either as an agonist or an antagonist at kappa receptors. The hypothesis that ibogaine acts an agonist at kappa receptors is supported by the observation that the kappa selective agonists U50,488 and spiradoline, effectively reduce self-administration of morphine and cocaine in rats (14). Furthermore it has been shown that ibogaine (31), like the aforementioned kappa agonists (32), antagonizes morphine-induced locomotor stimulation; in both cases this antagonism is enhanced by prior morphine exposure. Conversely, other studies support an antagonist role for ibogaine at kappa receptors (42–44), while still others present evidence suggesting that ibogaine is devoid of opiate activity (28,61).

The failure of both selective kappa and mu agonists to substitute for ibogaine, taken together with the weak antagonism by naloxone and the lack of antagonism by nor-BNI, suggest that opiate agonist effects are not a major component of the ibogaine discriminative stimulus. On the other hand, although no generalization was observed with naloxone and nor-BNI, partial generalization was observed with naltrexone. Interestingly, partial generalization was also observed with the mixed-action opiates (±)-pentazocine, nalorphine, and diprenorphine, as well as the putative kappa agonist (−)-SKF 10,047 (48). Because the present results are less than conclusive with respect to opiate receptors, we assessed whether these agents produced their ibogaine-like stimulus effects through interactions with σ_2 receptors. Although (±)-pentazocine and (−)-SKF 10,047 demonstrated appreciable σ_2 affinity, diprenorphine, nalorphine, and naltrexone did not. In addition the substitution produced by (−)-SKF 10,047 and nalorphine appear not to be σ_2-mediated as the ibogaine-like effects of these agents were completely antagonized by naloxone, which has no affinity for σ receptors (36). Thus, whatever the role played by σ_2 receptors in the ibogaine cue, it appears that σ_2 receptors do not mediate the ibogaine-like effects produced by the mixed-action opiates and (−)-SKF 10,047. The implications of these results are far from clear. Perhaps most pertinent to the present study is the observation that these agents produce psychotomimetic effects in humans that are similar to those produced by ibogaine (25,29). We are aware of no reports of hallucinations following naltrexone treatment, but naltrexone is used in the therapy of both opiate and ethanol addiction (56). Because ibogaine may also be effective in the treatment of both alcohol (39) and opiate abuse (12,13,46), it may have mechanistic features in common with naltrexone. The fact that the dose at which naltrexone produced significant ibogaine-appropriate responding was much higher than doses commonly used to produce opiate antagonism suggests that a nonopiate action of naltrexone may mediate the modest ibogaine-like effects seen in the present study. A possible explanation for this centers on the fact that opiate antagonists, at high doses have been shown to interact with GABAergic systems (11). This may also explain why naloxone partially antagonized the ibogaine cue and completely antagonized the ibogaine-appropriate responding produced by nalorphine only when given at high doses (10 mg/kg). However, a previous report by Deecher et al. (8) suggests that ibogaine does not influence GABAergic function.

The nature of ibogaine's interactions with opiate receptors is not readily explained by the present study. However, it does appear that opiate receptors may be involved in the ibogaine discriminative stimulus. The fact that ibogaine does not appear to be either an agonist or an antagonist at opiate receptors has been reported previously, both by our group and by others. For example, ibogaine is not analgesic by itself, nor does it antagonize opiate-induced analgesia; instead, it appears to potentiate opiate analgesia (10,41) . Correspondingly, in vitro studies show that unlike morphine, neither ibogaine nor 12-hydroxyibogamine inhibit adenylyl cyclase in

the rat brain, but both agents potentiate the effects of morphine on cyclase inhibition (37). These observations, taken together with those of the present study, suggest that ibogaine's interactions with opiate systems are complex in nature and may involve interactions with a novel receptor or possibly an interaction with GABAergic function. In addition, ibogaine may produce its effects via interactions with multiple receptors, as suggested by Sweetnam and colleagues (52) and, thus, the effects of ibogaine may be understood only in terms of its collective receptor interactions as opposed to its effects on any individual receptor subtype.

In summary, the present study offers evidence that functional interactions with σ_2 as well as opiate receptors may be involved in the discriminative effects of ibogaine, while the NMDA antagonist activities of ibogaine do not appear to play a major role. These observations, taken together with our previous report (19), suggest that ibogaine may mediate its discriminative effects through interactions with a variety of receptors. This may explain why receptor selective antagonists do not block the ibogaine-appropriate responding produced by ibogaine while they antagonize the ibogaine-appropriate responding produced by other compounds that are presumed to be relatively selective for the receptor in question. These findings suggest, therefore, that the unique effects of ibogaine are likely mediated by several receptors. The extent to which these receptors are involved in the putative antiaddictive effects of ibogaine awaits further study.

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