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Behavioral and Biochemical Evidence for a Nonessential 5-HT_{2A} Component of the Ibogaine-Induced Discriminative Stimulus

SCOTT HELSLEY, DAVID FIORELLA, R. A. RABIN AND J. C. WINTER

Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, 102 Farber Hall, Buffalo, NY 14214-3000

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HELSLEY, S., D. FIORELLA, R. A. RABIN AND J. C. WINTER. *Behavioral and biochemical evidence for a nonessential 5-HT_{2A} component of the ibogaine-induced discriminative stimulus.* PHARMACOL BIOCHEM BEHAV **59**(2) 419–425, 1998.—In the present investigation, the ability of two known hallucinogens, lysergic acid dimethylamide (LSD) and (−)-2,5-dimethoxy-4-methyl-amphetamine (DOM), to substitute for the ibogaine-induced discriminative stimulus (10 mg/kg IP, 60 min presession) was assessed in Fischer-344 rats. In these subjects, intermediate levels of generalization were observed to both agents (LSD, 63%; DOM, 66.4%). This intermediate generalization was completely blocked by pretreatment with the 5-HT_{2A} antagonist pirenpirone, suggesting that the ibogaine-like effects of these agents are mediated by the 5-HT_{2A} receptor. However, pirenpirone did not antagonize ibogaine itself, nor did it antagonize the ibogaine-like effects of harmaline and 12-hydroxyibogamine (noribogaine). To further evaluate the serotonergic properties of ibogaine, in vivo protection assays and in vitro binding assays were employed. Micromolar 5-HT_{2A} affinity was observed with ibogaine (92.5 μM), 12-hydroxyibogamine (34.5 μM), and harmaline (42.5 μM). Despite the apparently low affinity of these agents, both ibogaine and harmaline, but not 12-hydroxyibogamine, produced significant protection from receptor alkylation by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) when given 60 min prior to this alkylating agent. The results of these studies suggest that although ibogaine may produce some of its effects via interactions with 5-HT_{2A} receptors, these do not appear to be essential to the ibogaine-induced discriminative stimulus. © 1998 Elsevier Science Inc.

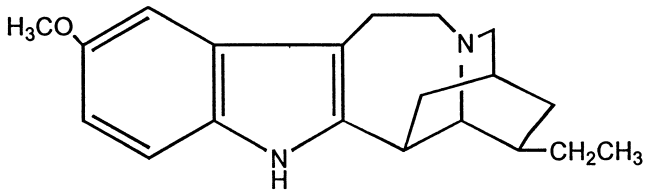
Ibogaine Drug discrimination Serotonin Radioligand binding Hallucinogens

THE stimulant and hallucinogenic properties of ibogaine (12-methoxyibogamine) have been known to Western medicine for over 100 years, but relatively little is known regarding its mechanism of action (24). Recent findings that suggest a role for ibogaine in the treatment of substance abuse have stimulated renewed interest in this agent (28). Because of its hallucinogenic properties and its structural similarity to serotonin (5-hydroxytryptamine, 5-HT; Fig. 1), previous studies have attempted to determine whether interactions with serotonergic receptors are involved in the effects of ibogaine.

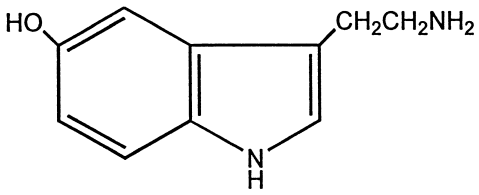
Although ibogaine has poor affinity for 5-HT receptors in vitro (3,25,31,33), some studies suggest that ibogaine acts as a 5-HT receptor agonist (21,30). In addition, recent findings indicate that ibogaine, along with its metabolite 12-hydroxyibogamine, may increase synaptic 5-HT via interactions with the 5-HT transporter (16,31). Thus, it appears that ibogaine may act at serotonergic receptors either directly or indirectly via a metabolite or through 5-HT release.

Drug-induced stimulus control has been used to investigate the interoceptive states created by a variety of psychoactive drugs in animal subjects (2,32,35,37). Recent studies in our laboratory provide evidence that the 5-HT_{2A} receptor is the primary mediator of DOM- and LSD-induced stimulus control, while the 5-HT_{2C} receptor plays at most a modulatory role (4–6). Consequently, ibogaine's structural similarity to serotonin taken together with its hallucinogenic properties suggests that the psychotropic effects of this agent may be mediated via serotonergic receptors of the 5-HT_{2A} subtype. Thus, in the present study, the classical hallucinogens, LSD and DOM, were evaluated for their ability to substitute for

Requests for reprints should be addressed to S. Helsley, SUNY–Buffalo, School of Med. and Biomedical Sciences, Dept. of Pharmacology and Toxicology, 102 Farber Hall, Buffalo, NY 14214-3000.



Ibogaine



Serotonin (5-HT)

FIG. 1. Structural similarity between ibogaine and 5-HT.

the ibogaine-trained discriminative stimulus. Furthermore 5-HT_{2A} antagonists were tested for their ability to block the ibogaine-appropriate responding elicited by ibogaine itself as well as that produced by LSD and DOM.

Biochemical studies were included to complement our behavioral experiments. Specifically, the affinities of ibogaine and related agents for 5-HT_{2A} receptors were assessed using in vitro binding studies. Furthermore, protection assays using the irreversible receptor alkylating agent EEDQ were employed to determine whether ibogaine and certain related compounds (harmaline, 12-hydroxyibogamine) bind to 5-HT_{2A} receptors in vivo. Thus, the present study represents our attempts to determine the involvement of the 5-HT_{2A} receptor in the ibogaine-trained discriminative stimulus using behavioral (drug discrimination) and biochemical techniques (in vivo protection assays and in vitro binding).

METHOD

Behavioral Experiments

Subjects. Male Fischer 344 rats were obtained from Harlan–Sprague–Dawley Inc. (Indianapolis, IN). They were housed in pairs in clear plastic cages (46.6 × 25.6 × 20.2 cm) with wood shavings for bedding under a 12 L:12 D cycle and allowed free access to water in the home cage. Subjects were fed following experimental sessions (Lab Diet rat chow, St. Louis, MO). 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 (which is activated at the beginning of each session) and an 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. Sessions are initiated manually immediately after placement of the subject into the chamber. Solid-state programming and electromechanical counters are used to control and record the sessions.

Ibogaine-induced stimulus control. Twenty-four subjects were trained to discriminate ibogaine (10.0 mg/kg, 60-min pretreatment time, intraperitoneal injection) from water as previously described (11). A fixed ratio 10 (FR10) schedule of reinforcement was employed. Levers were not reset after incorrect responses. Training sessions were 10 min in duration, after which responses on either lever were without consequence. 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 94% drug-appropriate responding. Training sessions were conducted M-F, vehicle, and drug sessions alternated on an every other day basis (i.e., ibogaine-vehicle-ibogaine-vehicle-ibogaine). After stimulus control was established with ibogaine, tests were conducted once per week (usually on Thursday) 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. Thus, a typical week's schedule might be as follows ibogaine-vehicle-ibogaine-test-vehicle. Behavioral sessions were usually conducted between 0800–1200 h. A previous report from our laboratory includes both the time course and the dose–response curves for ibogaine (11).

Test procedure. Tests of generalization were conducted in such a fashion that approximately half of the test sessions fell on days following saline 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 10 responses on either 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 minute test session were not considered in the calculation of percent drug-appropriate responding but were included in the calculation of response rates.

Ibogaine, harmaline, and 12-hydroxyibogamine and water were injected 60 min prior to testing (11). Pretreatment times for LSD and DOM were 15 min (4) and 75 min (7), respectively. Pirenpirone, pizotylene, and mesulergine, either alone or in combination with other drugs were given at a pretreatment time of 75 min (4). Drugs were tested in the following order: LSD, DOM, ibogaine/pirenpirone, ibogaine/mesulergine, ibogaine/pizotylene, LSD/pirenpirone, DOM/pirenpirone, harmaline/pirenpirone, 12-hydroxyibogamine/pirenpirone. In cases where more than one dose was used, lower doses were tested first.

Biochemical Studies

Binding assays. Receptor binding assays were carried out as previously described by Fiorella et al. (8). Frontal cortex from Fischer 344 rats was removed and homogenized in 40 ml 50 mM Tris-HCl (pH = 7.4). The homogenate was then centrifuged at 40,000 × *g* for 15 min at 4°C. The resulting pellets were resuspended in the TRIS buffer and suspensions were incubated for 10 min in a 37°C water bath to remove endoge-

nous 5-HT. The suspensions were once again centrifuged at 40,000 × *g* for 15 min and the resulting pellets were washed an additional time in ice-cold 50 mM TRIS-HCl. The final pellets were resuspended at a concentration of 6.7 mg tissue per ml in 50 mM Tris-HCl containing 4.0 mM $MgCl_2$, 10 μM pargyline, and 0.1% ascorbate. Binding assays were carried out for 30 min at 30°C in a final volume of 0.3 ml. For saturation equilibrium experiments [^{3}H]ketanserin was used at a concentration range of 0.1–5.0 nM. For competition assays [^{3}H]ketanserin was used at a concentration of 3.0 nM and the concentration for competing ligands ranged from 0–100 μM. Reactions were terminated with a Brandel cell harvester using Whatman GF/B filters that were presoaked with 0.1% polyethyleneimine. Immediately following filtration, filters were washed twice with 3 ml of ice cold Tris-HCl (pH .4). Filters were incubated overnight in Liquiscint scintillation cocktail (National Diagnostics) and the amount of bound radioactivity was determined by liquid scintillation spectrophotometry. Specific binding was defined as the difference between the amount of radioactivity bound in the presence and absence of 100 μM cinanserin. The data were analyzed by nonlinear regression using the EBDA/ligand program (Elsevier BIOSOFT). The method of Lowry et al. (15) was used to measure protein content.

In vivo protection assays. Because EEDQ has been shown to produce an irreversible inactivation of radioligand binding at several receptors including 5-HT_{2A} (1,10,17,18,22), this agent was employed as an irreversible receptor alkylating agent. It has been shown that this 5-HT_{2A} inactivation can be prevented by pretreatment with ligands specific for this receptor (10,17). It is believed that the occupation of the receptor in question (5-HT_{2A}) prevents binding and subsequent alkylation by EEDQ. Thus, in the present study, the density of 5-HT_{2A} receptors (B_{max}) in frontal cortex from Male Fischer 344 rats was determined following in vivo administration of either EEDQ alone or a potential protecting agent followed by EEDQ. EEDQ was administered IP at a dose of 8 mg/kg, and rats were sacrificed either 1 h or 24 h later (17). Experimental compounds were administered 60 min prior to EEDQ. This treatment time was chosen partly because Matsubara et al. (17) administered most of their test compounds 60 min prior to EEDQ. In addition, this pretreatment time was used for ibogaine and related compounds (harmaline and 12-hydroxyibogamine) in drug discrimination studies (11). B_{max} values were determined using saturation equilibrium binding assays described above.

Data Analysis

Behavioral experiments. The criteria for generalization and antagonism were as follows (38). 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 (c) there is a statistically significant difference between test-drug and saline-control response distributions. An intermediate degree of generalization/antagonism is defined as being present when mean response distributions following a test

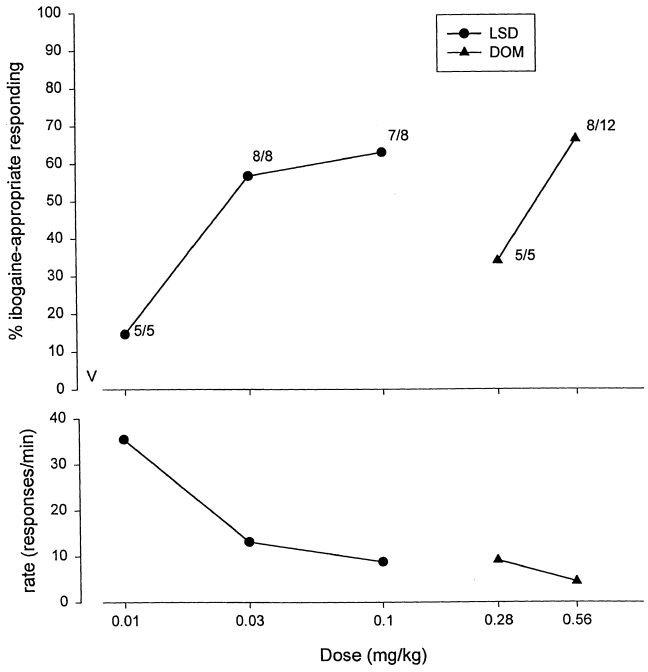


FIG. 2. The dose–response relationships for LSD (15-min presession) (●) and DOM (75-min presession) (▲) in rats trained to discriminate ibogaine (10.0 mg/kg, IP, 60 min presession) from saline. In addition, the lack of substitution by the vehicle is shown (V). The ratio adjacent to each of the points 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).

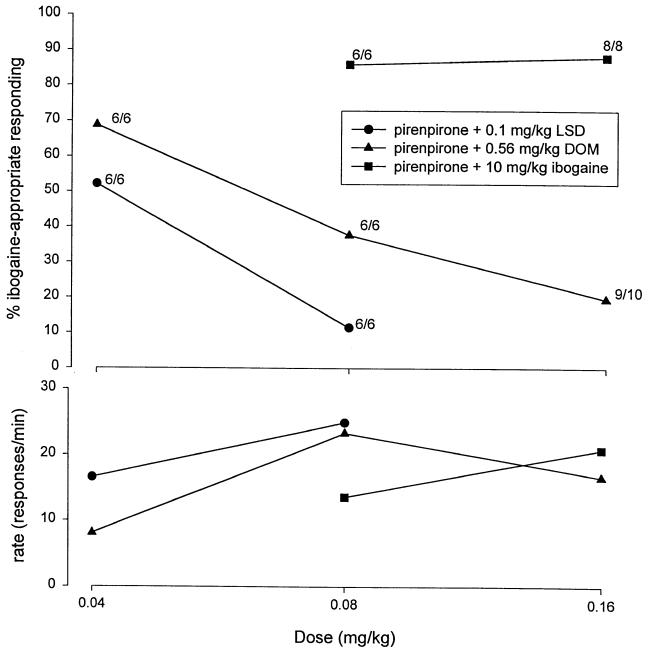


FIG. 3. The dose–response relationships for pirenpirone (75 min presession) in the presence of LSD (●), DOM (▲), and ibogaine (■). The ratio adjacent to each of the points represents the number of subjects completing the test session over the number of subjects participating in each test session. Ordinate: 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).

TABLE 1

TESTS OF GENERALIZATION AND ANTAGONISM IN IBOGAINE-TRAINED RATS

Drug Treatment	% Ibogaine-Appropriate Responses	Rate (Responses/min)	*n/N*
Ibogaine* (10 mg/kg)	94.0	14.3	10/10
Ibogaine (10 mg/kg) + pizotylene (10 mg/kg)	94.3	15.2	3/3
Ibogaine (10 mg/kg) + mesulergine (20 mg/kg)	98.5	9.7	6/6
12-Hydroxyibogamine* (20 mg/kg)	71.6	7.9	10/10
Noribogaine (20 mg/kg) + pirenpirone (0.16 mg/kg)	65.5	9.6	8/8
Harmaline* (3.0 mg/kg)	83.5	23.6	10/10
Harmaline (3.0 mg/kg) + pirenpirone (0.16 mg/kg)	85.5	9.0	6/8

The ratio *n/N* represents the number of animals responding (*n*) out of the number of animals tested (*N*). Treatment sessions were compared to immediately preceding ibogaine training sessions using Wilcoxon's signed ranks test. No significant differences ($p < 0.05$) were observed, with the exception of noribogaine, which was significantly different from both the ibogaine and the water condition. This intermediate generalization was not antagonized by pirenpirone.

*Refers to previously reported data (11).

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 saline-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 saline 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.

Biochemical experiments. B_{max} and K_D values were compared between test (protecting agent + EEDQ) and control groups (EEDQ) using a one-way ANOVA followed by planned comparisons using the Bonferroni *t*-procedure. Significance is indicated by *p*-values less than 0.05.

Drugs

(−)-DOM, (+)-LSD-(+)-tartrate, ibogaine HCl, and 12-hydroxyibogamine HCl were provided by the National Institute on Drug Abuse (Rockville, MD). The following compounds were purchased from commercial sources: EEDQ (Aldrich Chemical Co., Milwaukee, WI); mesulergine (Research Biochemicals International, Natick, MA); harmaline (Sigma, St. Louis, MO). The following compounds were generously provided by the indicated organizations: racemic fenfluramine (Robins Research Laboratories, Richmond, VA); pizotylene (Sandoz Pharmaceuticals, E. Hanover, NJ); pirenpirone (Janssen Pharmaceuticals, Belgium); cinanserin (Squibb, New Brunswick, NJ). All agents were dissolved in sterile deionized water with the exception of pizotylene, pirenpirone, and EEDQ. Both pizotylene and pirenpirone were dissolved in water with a few drops of 8.5% lactic acid. EEDQ was dissolved in a 50% ethanol–water solution. All solutions as well as water were injected IP in a volume of 1.0 ml/kg with the exception of ibogaine, which was injected in a volume of 5.0 ml/kg in the protection assays due to solubility problems.

RESULTS

Behavioral Experiments

Both LSD (63%) and DOM (66.4%) elicited intermediate levels of generalization in ibogaine-trained subjects (Fig. 2). These effects were completely antagonized by the 5-HT_{2A} antagonist pirenpirone (Fig. 3). In contrast, when pirenpirone (1.6 mg/kg) was given in combination with ibogaine, 88% ibogaine-appropriate responding was observed (Fig. 3). In addition, pirenpirone failed to block the ibogaine-appropriate responding produced by harmaline and 12-hydroxyibogamine (Table 1). Likewise, other 5-HT_{2A} antagonists (pizotylene and mesulergine) failed to antagonize the ibogaine cue (Table 1). None of the antagonists tested in the present study substituted for ibogaine when given alone (data not shown).

Biochemical Experiments

In saturation equilibrium experiments, [^{3}H]ketanserin bound to 5-HT_{2A} receptors in the frontal cortex with a K_d of 1.5(±0.2) nM and a B_{max} equal to 376.2(±19.6) fM/mg. In vitro, ibogaine, harmaline, and 12-hydroxyibogamine displayed micromolar affinity for frontal cortical 5-HT_{2A} receptors labeled with [^{3}H]ketanserin (Table 2).

In the protection assays, B_{max} values from EEDQ-treated subjects (123.9 ± 14.3 fM/mg) were significantly lower than those from controls (376.2 ± 19.6 fM/mg). Despite their relatively low affinities for 5-HT_{2A} receptors in vitro, both ibogaine and harmaline protected 5-HT_{2A} receptors from EEDQ-induced alkylation in vivo (Fig. 4). However, this effect was not seen with 12-hydroxyibogamine or fenfluramine in subjects sacrificed either 1 (Fig. 5) or 24 h (Fig. 4) after treatment with EEDQ. Thus, it appears that temporal effects are not responsible for the lack of protection seen with these agents. Furthermore, the failure of these agents to protect

TABLE 2

IN VITRO RECEPTOR AFFINITY VALUES (K_i) AT 5-HT_{2A} RECEPTORS LABELED WITH [^{3}H]KETANSERIN

Drug	K_d (SEM) in μM
Harmaline	42.5 (10.9)
Ibogaine	92.5 (8.6)
12-Hydroxyibogamine	34.5 (3.5)

Data are expressed as the mean of three separate experiments.

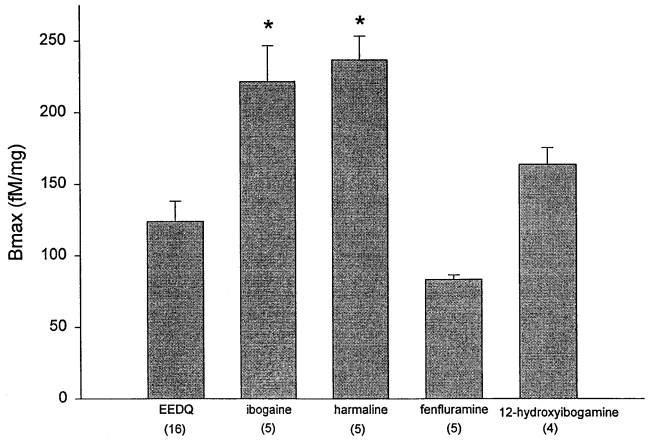


FIG. 4. The effects of ibogaine and related compounds on 5-HT_{2A} receptor inactivation by EEDQ (8 mg/kg, IP). Ibogaine (50 mg/kg), harmaline (20 mg/kg), fenfluramine (2 mg/kg), and 12-hydroxyibogamine (50 mg/kg) were administered 1 h prior to EEDQ treatment, and subjects were sacrificed 24-h following EEDQ. The number of subjects tested at each treatment condition is indicated in parentheses below the treatment. *Reflects a statistically significant difference from EEDQ ($p < 0.05$).

cannot be attributed to irreversible binding as neither agent reduced B_{max} values when given alone (Fig. 6).

K_d values for treatment groups did not differ significantly from controls with the exception that the ibogaine + EEDQ group had a significantly higher K_d than control (3.7 nM vs. 1.5 nM, control).

Ibogaine and 12-hydroxyibogamine were given at a dose of 50 mg/kg because this dose has been shown to produce high levels of ibogaine in the rat brain (31). However, the 12-hydroxyibogamine/EEDQ combination was lethal in approximately

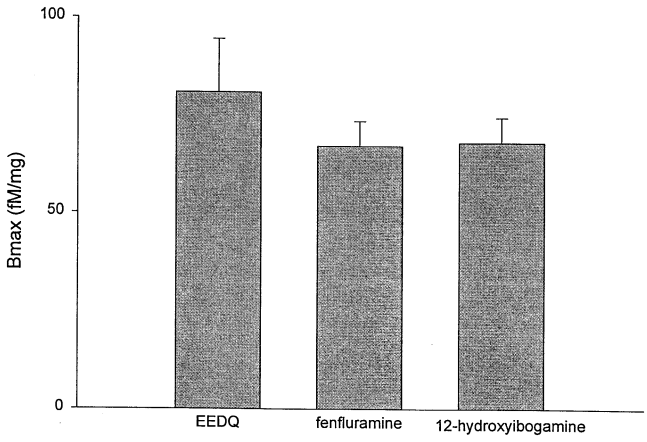


FIG. 5. The effects of fenfluramine (2 mg/kg) and 12-hydroxyibogamine (25 mg/kg) on 5-HT_{2A} receptor inactivation by EEDQ (8 mg/kg, IP). Subjects were sacrificed 1 h after treatment with EEDQ. Because higher doses of 12-hydroxyibogamine produced significant lethality (~50%) when given in combination with EEDQ, 12-hydroxyibogamine was given at a dose of 25 mg/kg. The number of subjects tested at each treatment condition is indicated in parentheses below the treatment. *Reflects a statistically significant difference from EEDQ ($p < 0.05$).

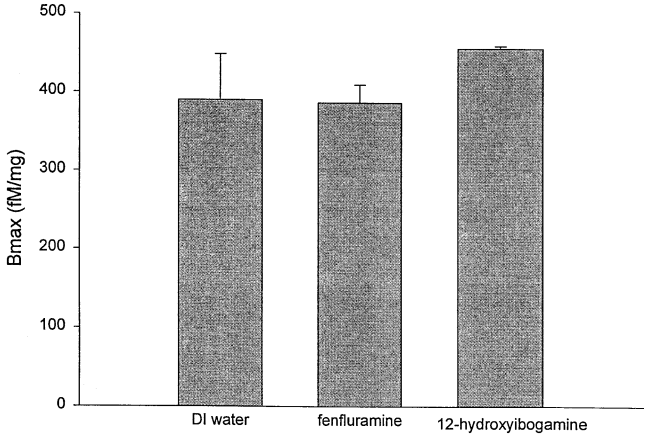


FIG. 6. The effects of fenfluramine (2 mg/kg) and 12-hydroxyibogamine (25 mg/kg) on 5-HT_{2A} receptor Bmax values. Subjects were sacrificed 24 h after drug treatment. Three subjects were used to generate each data point. The number of subjects tested at each treatment condition is indicated in parentheses below the treatment.

50% of subjects; therefore, in subsequent studies (Figs. 5 and 6) 12-hydroxyibogamine was given at a dose of 25 mg/kg. Harmaline and fenfluramine were given at 20 and 2.0 mg/kg, respectively, because higher doses were sometimes fatal when given with EEDQ.

DISCUSSION

The structural similarity between ibogaine and serotonin, taken together with the hallucinogenic effects of ibogaine, suggests that interactions with serotonergic receptors may play a role in the overall effects of this agent. The present study offers support for this hypothesis. The fact that ibogaine generalized partially to hallucinogens that produce their discriminative cues through agonist interactions with 5-HT_{2A} receptors along with the observation that ibogaine protects these receptors, in vivo, from EEDQ alkylation confirms the existence of a 5-HT_{2A}–mediated component in the ibogaine-trained stimulus. The partial generalization observed with LSD appears to conflict with the findings of Schecter and Gordon (27), who observed a maximum of 34.5% ibogaine-appropriate responding with LSD. However, the fact that these authors trained ibogaine using a pretreatment time of 30 min while the present study used 60 min could account for this apparent discrepancy. In addition, Schechter and Gordon used Sprague–Dawley rats while the present study utilized Fischer 344 rats.

The findings of the present study are in agreement with those of Palumbo and Winter (21), who demonstrated that ibogaine elicits intermediate generalization in DOM- and LSD-trained rats. In their LSD-trained subjects, this generalization was blocked by the 5-HT_{2A} antagonist pizotylene. In the present study, when the selective 5-HT_{2A} antagonist pirenpirone was given in combination with ibogaine no antagonism was observed. Likewise, other 5-HT_{2A} antagonists (pizotylene and mesulergine) failed to block the ibogaine stimulus in the present study. In addition, the ibogaine-appropriate responding produced by harmaline (full substitution) and 12-hydroxyibogamine (partial substitution) (11), was not antagonized by pirenpirone. Conversely, pirenpirone fully antagonized the ibogaine-appropriate responding elicited by both LSD and DOM. Thus, it appears that 5-HT_{2A} agonism is required for

these agents to mimic ibogaine, whereas this appears nonessential to the ibogaine-appropriate responding produced by harmaline, 12-hydroxyibogamine, and by ibogaine itself. The existence of nonessential serotonergic stimulus components has been documented for other agents. For example, in rats trained with para-methoxyamphetamine (PMA), LSD produced intermediate levels of substitution; however, the PMA cue was not blocked by pizotylene. Conversely, in LSD-trained animals the partial substitution produced by PMA is completely antagonized by pizotylene (36).

Like ibogaine, harmaline is a known hallucinogen bearing a structural resemblance to 5-HT (19). Correspondingly, harmaline produces partial substitution in both DOM- (9) and LSD-trained rats (20). These findings, taken together with our observation that ibogaine generalizes completely to harmaline (11), suggest that the interoceptive cues produced by these agents are similar to one another and may involve a serotonergic component.

Previous investigations along with the present report show that ibogaine (3,25,31,33) and harmaline (3) have low affinity for the 5-HT_{2A} receptor. In light of these findings, it is somewhat surprising that ibogaine interacts functionally with these receptors. However, our in vivo protection assays offer evidence that ibogaine and harmaline bind to 5-HT_{2A} receptors. Nonetheless, it remains that this protection was observed when ibogaine was given at a dose fivefold higher than that used in the behavioral studies. Because lower doses of ibogaine were not tested, this data alone does not prove but does support (when taken together with the behavioral results) the hypothesis that 5-HT_{2A} receptor interactions play a major role in the effects of ibogaine at the training dose used in the present study (10 mg/kg).

If functional interactions with 5-HT_{2A} receptors are indeed a component of the ibogaine discriminative stimulus as trained in the present study, two possible explanations can be envisioned to reconcile the low affinity of ibogaine and harmaline for the 5-HT_{2A} receptor with their hallucinogen-like discriminative effects and the observed 5-HT_{2A} protection. First, these agents may elevate 5-HT levels. Both harmaline (13,14,23,29,34) and ibogaine (16) have been reported to elevate 5-HT levels in vivo. This is supported by the observation that the 5-HT releasing agent, fenfluramine elicits partial generalization to the ibogaine stimulus (27). However, fenfluramine's lack of in vivo protection suggests that ibogaine and harmaline do not produce their 5-HT_{2A} receptor interactions indirectly by elevating 5-HT levels.

A second explanation is that ibogaine and harmaline reach high enough concentrations in the rat brain to overcome their apparently low affinity. Previous studies provide evidence that when given at doses similar to those used in the present study, both ibogaine (12,31,39) and harmaline (26,39) achieve high ($\geq$10) micromolar concentration in the rodent brain. This could account for the protection produced by these agents, however, it is somewhat surprising that protection was not observed with 12-hydroxyibogamine, especially because this agent had the highest affinity of the three for the 5-HT_{2A} receptor in vitro. In addition, this agent elicits intermediate levels of generalization when tested in ibogaine-trained subjects (11). Although the lack of protection with 12-hydroxyibogamine is not readily explainable by the present study, it is possible that 12-hydroxyibogamine's failure to protect is the result of lower bioavailability compared to ibogaine or harmaline following IP injection. The fact that the combination of 12-hydroxyibogamine and EEDQ was sometimes lethal to our subjects adds to our difficulty in interpreting these data.

In summary, it appears that both ibogaine and harmaline produce functional interactions with 5-HT_{2A} receptors as evidenced by the partial substitution elicited by LSD and DOM. However, this interaction does not seem to be an essential component of the discriminative stimulus produced by ibogaine because pirenpirone, while able to antagonize the ibogaine-appropriate responding produced by both LSD and DOM, was ineffective as an antagonist of the ibogaine discriminative stimulus. Furthermore, it appears that ibogaine and harmaline, following systemic injection, reach high enough levels in the rat brain to compensate for low receptor binding affinities because these agents protected 5-HT_{2A} receptors from EEDQ inactivation in vivo. The present behavioral data indicate that ibogaine's effects on 5-HT_{2A} receptors are not required for ibogaine-induced stimulus control but are revealed when cross tests are conducted with drugs such as LSD and DOM, whose stimulus effects are clearly mediated by these receptors. This taken together with the observation that ibogaine and harmaline bind to 5-HT_{2A} receptors in vivo (albeit at higher doses than those used in the drug discrimination studies) suggests that these receptors may play a role in mediating the effects of ibogaine and harmaline.

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