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Short communication

# The effects of β-carbolines in rats trained with ibogaine as a discriminative stimulus

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**Abstract**

The structural features and hallucinogenic properties shared by ibogaine and certain β-carbolines prompted the evaluation of several representative β-carbolines in rats trained with ibogaine as a discriminative stimulus. In a previous report from our laboratory harmaline completely substituted for ibogaine (83.5%). In the present study, only 6-methoxyharmalan completely substituted (86.3%). However, partial substitution was observed with harmine, harmane, harmalol, and tetrahydro-β-carboline (THBC). Norharmane and 6,7-dimethoxy-4-ethyl–carboline-3-carboxylate (DMCM) failed to produce appreciable substitution. These results provide evidence for an ibogaine-like effect of certain β-carbolines. Whether this extends to the previously reported anti-addictive effects of ibogaine remains to be established.


*Keywords:* β-Carboline; Drug-induced stimulus control; Hallucinogen; Ibogaine

## 1. Introduction

Recent studies in both human and non-human subjects suggest a beneficial effect of the hallucinogenic alkaloid, ibogaine, in the treatment of substance abuse. In rats, ibogaine blocks self-administration of morphine (Glick et al., 1991, 1994), heroin (Dworkin et al., 1995), cocaine (Cappendijk and Dzoljic, 1993; Glick et al., 1994; Dworkin et al., 1995), and ethanol (Rezvani et al., 1995). In addition, a preliminary clinical study offers support for ibogaines anti-addictive effects in humans (Sheppard, 1994). Although several receptors including opiate, serotonergic and PCP receptors may mediate some of ibogaines effects (reviewed by Popik et al., 1995), the mechanism of action of ibogaine is not completely understood at present.

Ibogaine bears a strong structural resemblance to a group of naturally occurring hallucinogens, the Harmala alkaloids. These alkaloids are derived from several different plants including *Peganum harmala*, a plant found in Asia and the Middle East (Naranjo, 1967). Harmala alkaloids are also found in the South American vine, Banisteriopsis, from which a hallucinogenic drink called yage is prepared. Structurally, these alkaloids are β-carbolines (Fig. 1). In addition, the tremorigenic and hallucinogenic effects of harmine and harmaline are similar to those produced by ibogaine (Naranjo, 1969).

Using the technique of drug discrimination, we have conducted studies on the ibogaine-induced stimulus. In our studies, which have utilized receptor-selective agonists and antagonists as well as non-selective agents (Helsley et al., 1997, 1998a,b), only harmaline, a β-carboline hallucinogen fully substituted for ibogaine (Helsley et al., 1997). Thus, the present study represents an extension of this observation by assessing several additional β-carbolines in ibogaine-trained subjects.

## 2. Materials and methods

### 2.1. Animals

Male Fischer 344 rats were obtained from Harlan Sprague–Dawley (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

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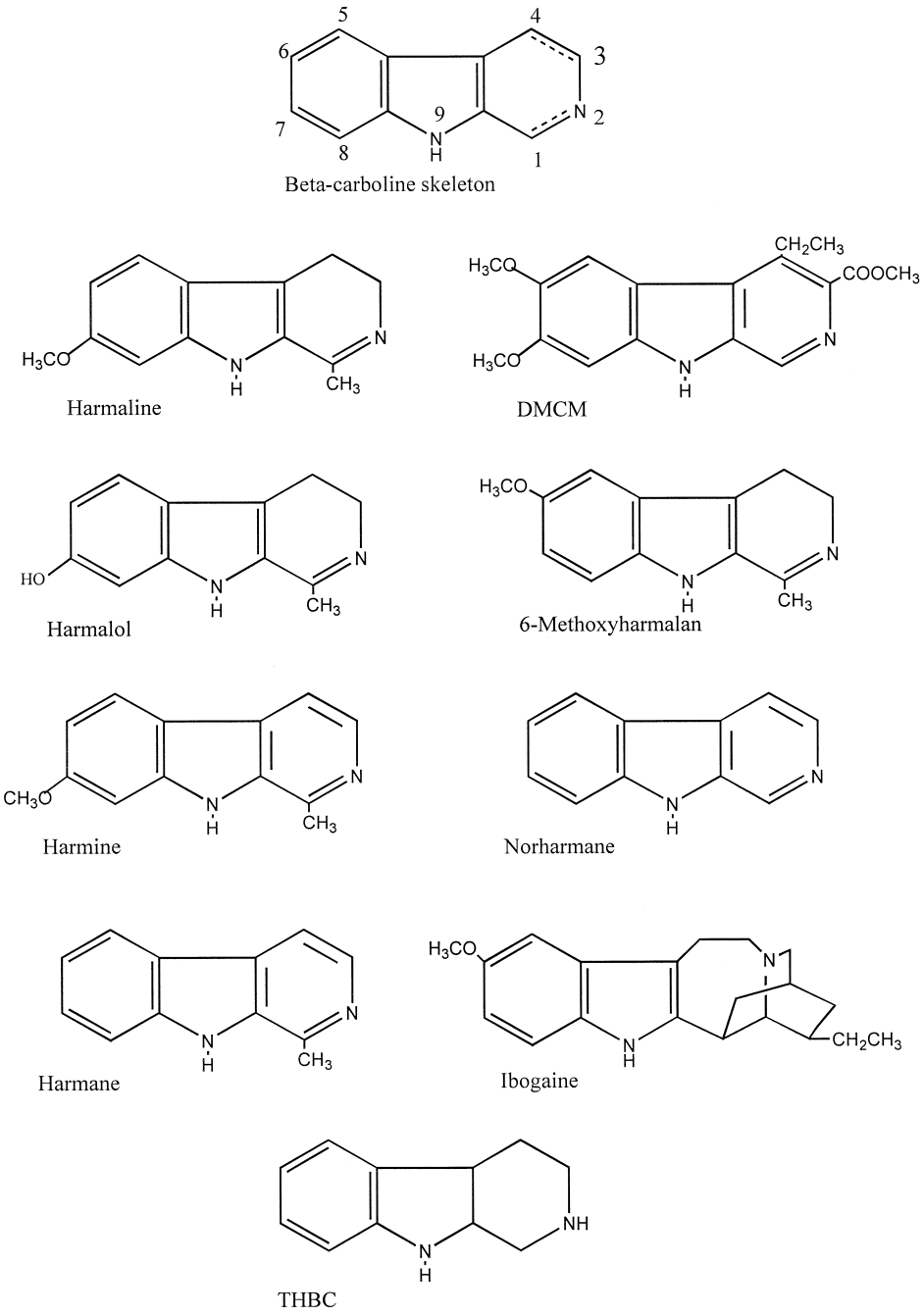


Fig. 1. Chemical structures of ibogaine and β-carbolines.

following experimental sessions. Caloric intake was controlled to yield a mean bodyweight of about 250 g.

### 2.2. 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.

### 2.3. 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 (Fiorella et al., 1995; Helsley et al., 1997). A fixed ratio 10 (FR10) schedule of reinforcement was employed. Drug-in-

duced 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. 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.

### 2.4. Test procedure

Half of the test sessions were conducted the day after vehicle training sessions with the remainder following ibogaine training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten 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 ten min test session were not considered in the calculation of percent drug-appropriate responding but were included in the calculation of rates of responding. Pretreatment time was 60 min for all agents.

### 2.5. Data analysis

The criteria for generalization and antagonism are those used in previous reports (Winter and Rabin, 1992). Complete generalization/no antagonism is said to be present when (i) a mean of 83% or more of all test responses are on the drug-appropriate lever, (ii) there is no statistically significant difference between training-drug and test-drug response distributions, and (iii) 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 $< 0.05$.

### 2.6. Drugs

Ibogaine HCl was provided by the National Institute on Drug Abuse. DMCM was purchased from Research Biochemicals (Natick, MA). Harmine HCl, harmane HCl, harmalol HCl, norharmane HCl, 6-methoxyharmalan, and THBC were purchased from Sigma (St. Louis, MO). All drugs were dissolved in deionized water with the exception of DMCM which was dissolved in water with a few drops of 8.5% lactic acid. Solutions were injected i.p. in a volume of 1.0 ml/kg bodyweight.

## 3. Results

Throughout the study, ibogaine itself produced greater than 95% ibogaine appropriate responding while less than 5% ibogaine-appropriate responding was observed following vehicle treatment (data not shown). Fig. 2 shows that of the agents tested, only 6-methoxyharmalan fully substituted (86.3%). However, partial substitution was observed with harmine (68.1%), harmane (64.7%), and harmalol (63.6%). Although the substitution elicited by THBC (31.8% at a 3.0 mg/kg dose) was statistically significant compared to vehicle, the low level of ibogaine-appropriate responding casts doubt on its biological significance. 41.3% ibogaine-appropriate responding was seen at a higher dose (10.0 mg/kg) but only five of eight subjects completed the test, thus, precluding statistical analysis. No significant substitution was elicited by DMCM (41.5%) and norharmane (41.9%).

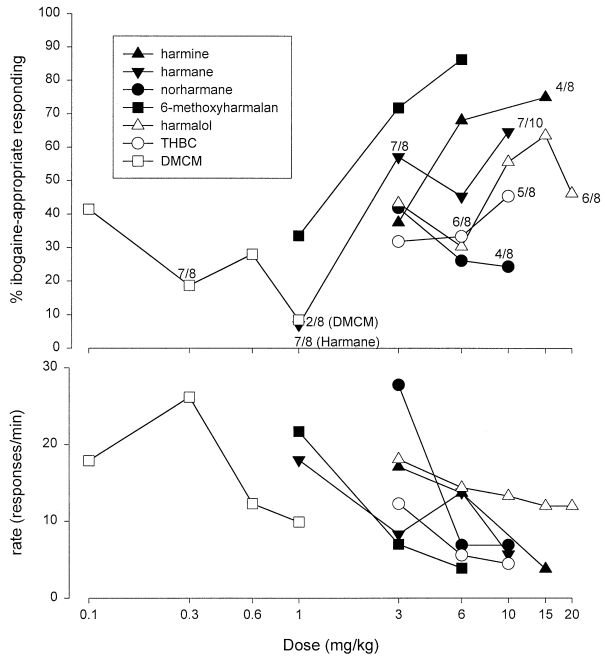


Fig. 2. Dose–response relationships for $\beta$-carbolines in rats trained with 10.0 mg/kg ibogaine as a discriminative stimulus. All agents were administered i.p. 60 min pre-session. The ratio adjacent to each of the points is the number of subjects completing the test session over the number of subjects participating in each test session. Where no ratio is shown a ratio of 8/8 is implied. Ordinate: Percent ibogaine-appropriate responding. Abscissa: Dose of test agent.

## 4. Discussion

The present study examined the ability of several representative β-carbolines to substitute for the ibogaine-induced discriminative stimulus. The results generated may provide some insight into the mechanism of action of ibogaine. Perhaps most interesting is the lack of substitution by the benzodiazepine receptor inverse agonist, DMCM (Petersen, 1983). This finding is of significance because a previous report by Trouvin et al. (1987) suggests that the iboga alkaloid, tabernanthine, which differs from ibogaine only in the position of the methoxy group (6 vs. 7), acts as a benzodiazepine inverse agonist. The lack of substitution observed in the present investigation is in agreement with the biochemical data presented by Deecher et al. (1992) in which neither ibogaine nor harmaline had an effect on GABA-stimulated chloride uptake in the mouse brain. It is noteworthy that previous reports in which DMCM was trained as a discriminative stimulus used a 15-min pretreatment time (Nielsen et al., 1985; Kirby et al., 1994). However, in the present study, DMCM did not substitute for ibogaine when given either 15 min pre-session (data not shown) or 60 min pre-session. These data suggest that ibogaine does not act as a GABA inverse agonist.

Unfortunately, with the exception of DMCM, relatively little is known regarding the mechanism(s) of action of the β-carbolines tested in the present study. However, when viewed in the context of previous studies, the present data suggest that the mechanism by which ibogaine produces its putative anti-addictive effects differs from the mechanism by which it produces its discriminative stimulus. Thus, the results of the present study may have implications regarding the putative anti-addictive effects of ibogaine. Specifically, norharmane, an agent which like ibogaine, attenuates naloxone-precipitated withdrawal from morphine (Cappendijk et al., 1994) did not substitute for ibogaine in the present study. Furthermore, Glick et al. (1994) showed that, unlike ibogaine, harmaline did not produce a sustained decrease in morphine self-administration by rats. If the self-administration paradigm used by Glick et al. (1994) is an accurate model of drug abuse in humans, then it appears that mimicry of the ibogaine discriminative stimulus is not an accurate predictor of anti-addictive activity in light of the fact that harmaline fully mimics ibogaine (Helsley et al., 1997).

Comparisons of β-carboline structures with their ability to substitute for ibogaine yield some interesting results. Only those agents with a 1-methyl group produced greater than 50% ibogaine-appropriate responding. Furthermore, only partially aromatized agents with both a 1-methyl group and a 6- or 7-methoxy group (6-methoxy harmalan, harmaline) substituted completely. Although the effects of β-carbolines in humans are not completely known, there is evidence that harmine (Pennes and Hoch, 1957), harmaline, and 6-methoxyharmalan (Naranjo, 1967) are hallucinogenic. Although all three of these produced significant substitution, no conclusions can be drawn regarding whether hallucinogenic activity in humans correlates with ibogaine-appropriate responding in the present study because the ability to produce hallucinations, or lack thereof, remains undocumented for the other agents tested. However, because other hallucinogens partially substitute for ibogaine (Helsley et al., 1998a), it is possible that the stimulus effects of ibogaine may be mediated by those same factors responsible for its hallucinogenic effects in humans. The fact that harmine produced only partial substitution taken together with the fact that this agent is known to have a short half-life in the brain (Zetler et al., 1972) raises the possibility that the pretreatment time (60 min) used in the present study was too long. However, in comparison to the 60 min pretreatment time, no differences in ibogaine-appropriate responding were observed when harmine was given 15 min pre-session (68% vs. 60%, respectively, at a dose of 6 mg/kg).

In conclusion, the present study provides evidence that Harmala alkaloids produce stimulus effects which are similar to those produced by ibogaine. Although the mechanistic components remain unknown it appears that certain structural features are required for substitution. Further studies with these agents may provide valuable insights into the neuropharmacology of hallucinogens and substance abuse.

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