ELSEVIER

PII S0024-3205(96)00703-5

PHARMACOLOGY LETTERS
Accelerated Communication

THE EFFECTS OF NORIBOGAINE AND HARMALINE IN RATS TRAINED WITH IBOGAINE AS A DISCRIMINATIVE STIMULUS

Scott Helsley[*] , Richard A. Rabin, and J.C. Winter

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

Abstract. In the present investigation, Fischer-344 rats were trained to discriminate 10.0 mg/kg of ibogaine from water using a pretreatment time of 60 minutes. Analysis of dose response data generated an ED_{50} of 4.6 mg/kg. The time course of the ibogaine (10.0 mg/kg) cue was also determined. The stimulus reached a maximum level of 94% ibogaine-appropriate responding at the 60-min pretreatment time. This was followed by a time-dependent decrease in ibogaine-appropriate responding. At a pretreatment time of 8 hrs only 6.4% drug-appropriate responding was observed. In substitution experiments, intermediate generalization was observed with a metabolite of ibogaine, 12-hydroxyibogamine [noribogaine] (71.6%) whereas complete generalization was seen with harmaline (83.5%).

Key Words: drug-induced stimulus control, hallucinogen, ibogaine. 12-hydroxyibogamine, harmaline, time course

Introduction

Recent studies in both human and non-human subjects suggest a beneficial effect of ibogaine, in the treatment of substance abuse. In rats, ibogaine blocks self-administration of morphine (1,2), heroin (3), cocaine (2,3,4), and ethanol (5). Although clinical data in support of ibogaine's anti-addictive effects are limited (6), patents have been issued for its use in the treatment of opiate (7), cocaine (8), amphetamine (8), ethanol (9), and nicotine abuse (10). However, the mechanism by which ibogaine might produce both its antiaddictive and hallucinogenic effects remains unknown.

One of the most fascinating properties of ibogaine is its long duration of action. Indeed the psychotomimetic effects of this agent have been reported to last as long as 38 hrs (6). In addition, a single oral treatment in some individuals has been shown to diminish opiate craving for extensive periods of time (6). Because plasma levels of ibogaine drop rapidly after injection, a possible explanation for the long duration of action is that a long-acting metabolite mediates ibogaine's effects (11,12). Such a metabolite has been discovered; 12-hydroxyibogamine has been observed to persist at an appreciable level in human plasma longer than the parent compound (11). For this reason 12-hydroxyibogamine is included in the present study.

Drug-induced stimulus control has been effectively used to investigate the interoceptive states created by a variety of psychoactive drugs in animal subjects (13,14). Schechter and Gordon (15) demonstrated that ibogaine could serve as a discriminative stimulus. In the present study, we

[*] Corresponding author: S. Helsley at address above; 716 829 3063; FAX 716 829 2801.

evaluated the ability of 12-hydroxyibogamine and harmaline to substitute for the ibogaine-trained stimulus. In addition we examined the time course for the ibogaine stimulus.

Methods

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 bodyweight of about 250 grams.

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

24 subjects were trained to discriminate ibogaine (10.0 mg/kg, 60 minute pretreatment time, intraperitoneal injection) from water in a fashion similar to that used previously in our laboratory with LSD (16). The training dose for ibogaine was chosen because this dose has been previously trained in rats (15). 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 (10.0 mg/kg 60 min. pretreatment time) produced 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.

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 minute test session were not considered in the calculation of percent drug-appropriate responding. Pretreatment times were 60 minutes for ibogaine, 12-hydroxyibogamine, and harmaline except where noted during the time course study.

Complete generalization of a training drug to a test drug 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 saline-control response distributions (17). An intermediate degree of generalization is here 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 saline-control response distributions, an absence of generalization is assumed. Comparisons of data are by means of individual applications of Wilcoxon's signed

the ibogaine-trained

anapolis, IN). They
to water in the home
controlled to yield a

used in larger light-
d a house light and
`one wall. Centered
sed milk diluted 2:1

: pretreatment time,
sly in our laboratory
has been previously
:s employed. Drug-
ve sessions, 83% or
propriate lever.

:ions. The ibogaine
)ropriate responding.
ice per week in each
:orrect responding in

s with the remainder
e reinforced and the
The distribution of
onses emitted on the
ling the total number
) responses on either
vithin the constraints
ent drug-appropriate
roxyibogamine, and

when [i] a mean of
re is no statistically
is, and [iii] there is a
se distributions (17).
vhen mean response
: from distributions
wing a test-drug are
ice of generalization
f Wilcoxon's signed

ranks test. However, for the time course study at the 15 min pre-treatment time, ibogaine and 12-hydroxyibogamine were compared using the Mann-Whitney rank sum test because the same subjects were not used to generate both data points. 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.

Ibogaine HCl and 12-hydroxyibogamine HCl were provided by the National Institute on Drug Abuse. Harmaline HCl was purchased from Sigma Chemical Company (St. Louis, MO). All drugs (with the exception of the 30 mg/kg dose of 12-hydroxyibogamine) were dissolved in water and solutions were injected i.p. in a volume of 1.0 ml/kg bodyweight. 30 mg/kg of 12-hydroxyibogamine was injected in a volume of 2.0 ml/kg because of solubility problems.
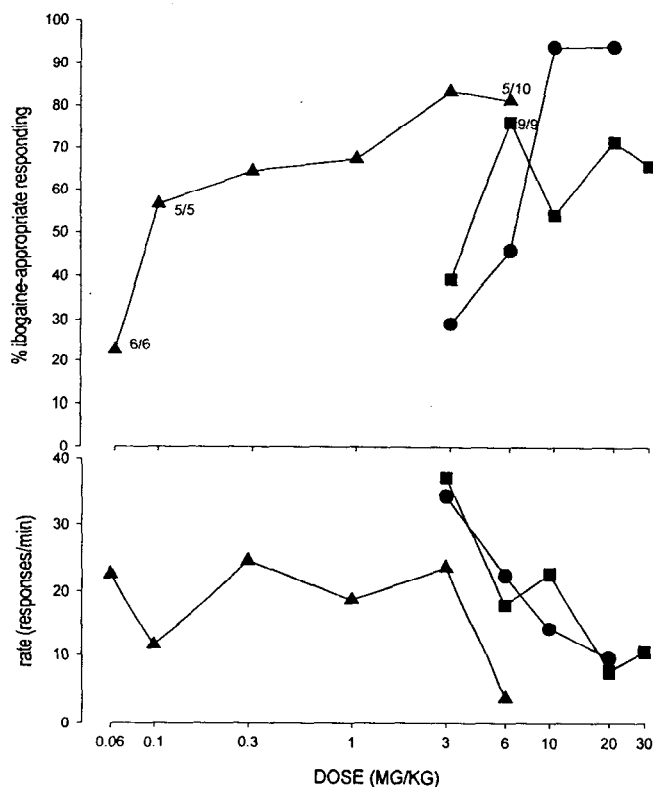
Results

Fig.1

Dose-response effects ibogaine (●), 12-hydroxyibogamine (■), and harmaline (▲) in rats trained with 10.0 mg/kg ibogaine as a discriminative stimulus. All drugs were administered i.p. 60 min before testing. Each point represents one determination in each of 10 subjects unless otherwise noted by the number of subjects completing the test over the number of subjects tested. Ordinate: Percent ibogaine-appropriate responding. Abscissa: Dose of test agent. Lower panel; Ordinate: Responses per min. Abscissa: dose of test agent.

Figure 1 shows the dose response curve for ibogaine as well as the dose dependent substitution produced by 12-hydroxyibogamine and harmaline. The ED_{50} for ibogaine was 4.6 mg/kg. 12-hydroxyibogamine produced a high level of substitution for ibogaine (71.6%) but this must be termed intermediate as it did not fulfill our criteria for full substitution. On the contrary harmaline fully substituted for ibogaine (83.5%).

Figure 2 shows the time course for the ibogaine-trained stimulus. Maximal substitution was observed at a pretreatment time of 60 min (94%). Following this, a time-dependent decrease in ibogaine-appropriate responding was observed. At a pretreatment time of 8 hours 93.6% of responding was on the vehicle-appropriate lever.
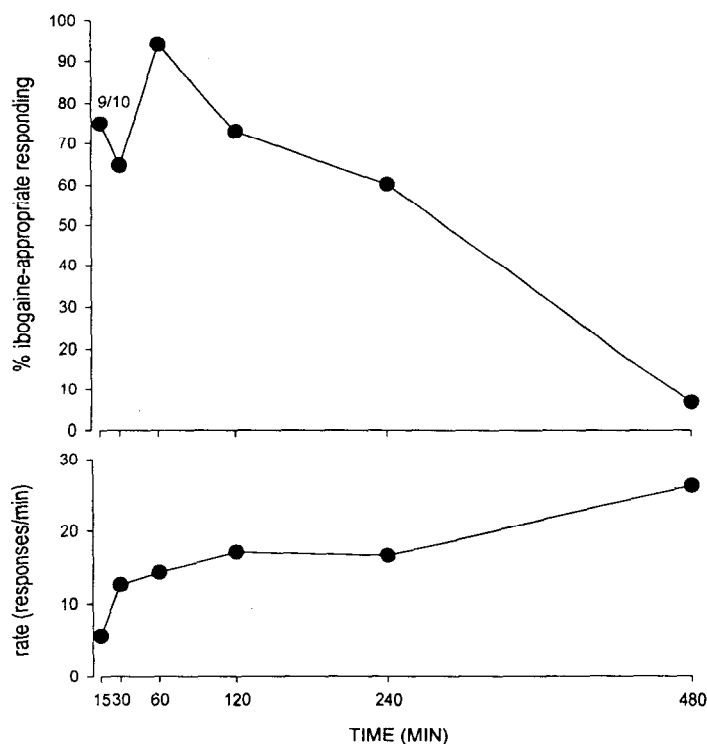

Fig.2

Time-course of ibogaine (●) in rats trained with 10.0 mg/kg ibogaine as a discriminative stimulus. Ibogaine was administered i.p. at a dose of 10.0 mg/kg. Each point represents one determination in each of 10 subjects unless otherwise noted by the number of subjects completing the test over the number of subjects tested. Ordinate: Percent ibogaine-appropriate responding. Abscissa: Ibogaine pretreatment time. Lower panel; Ordinate: Responses per minute. Abscissa: ibogaine pretreatment time.

Discussion

Although ibogaine has been known to Western medicine for more than 100 years, it is only recently that this substance has received very much attention. Sparked by reports of its anti-addictive properties, ibogaine has been the subject of more than 50 manuscripts over the past decade. Despite this, relatively little is known about how ibogaine produces its behavioral effects.

The present st
ibogaine could
a major metabo
trained stimulu
substances diff

Because we tr
this period of
12-hydroxyibo
ibogaine. Whe
a slightly low
74.9%; p=0.54
metabolite are
ibogaine diff
hydroxyibogan
shared features
relatedness (F
morphine and
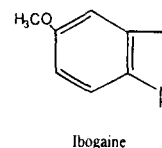
Ibogaine

On the other h
from differenc
affinity than i
serotonin trans
well as the MK

A second stru
Because this a
surprising that
mediating the
mechanisms o
interactions wi

In conclusion
effects are botl
identical to th
studies with ib
involved in the

:pendent substitution
was 4.6 mg/kg. 12-
,%) but this must be
ie contrary harmaline

mal substitution was
lependent decrease in
of 8 hours 93.6% of

The present study confirms the findings of Schechter and Gordon (15) who demonstrated that ibogaine could function as a discriminative stimulus. The discovery that 12-hydroxyibogamine is a major metabolite of ibogaine (11) raises the question as to whether it might mediate the ibogaine-trained stimulus. The present data (Fig 1) argue against that hypothesis suggesting that these substances differ somewhat in their stimulus effects.

Because we trained ibogaine at a pretreatment time of 60 min we considered the possibility that this period of time was necessary for the production of 12-hydroxyibogamine and thus a dose of 12-hydroxyibogamine given at an earlier pretreatment time would substitute completely for ibogaine. When given at a 15 min pretreatment time, 10.0 mg/kg 12-hydroxyibogamine produced a slightly lower level of substitution for ibogaine than the parent compound itself (62.5% vs. 74.9%; p=0.54). Thus our results suggest that although the stimulus effects of ibogaine and its metabolite are similar when given at a 15 min pretreatment time the overall stimulus effects of ibogaine differ somewhat from those of 12-hydroxyibogamine. Nonetheless, 12-hydroxyibogamine may contribute to the overall stimulus effects of the parent compound. The shared features of the stimulus effects produced by these agents are likely due to their structural relatedness (Fig 3). Indeed like ibogaine, 12-hydroxyibogamine has been shown to reduce morphine and cocaine self-administration (18).
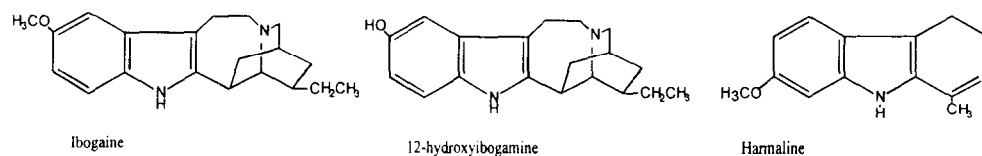

Fig.3
Structures of ibogaine, 12-hydroxyibogamine, and harmaline.

On the other hand the observed differences between ibogaine and 12-hydroxyibogamine may arise from differences in receptor binding profiles. For example 12-hydroxyibogamine possesses higher affinity than ibogaine for several receptor sites including opiate receptors (19,20,21) and the serotonin transporter (11,21). Conversely ibogaine possesses higher affinity for σ receptors (22) as well as the MK-801 binding site on the NMDA receptor complex (20,21,23).

480

A second structurally related compound, harmaline (Fig 3), was also tested in the present study. Because this agent resembles ibogaine in both hallucinogenic properties and in structure, it is not surprising that harmaline fully substitutes for ibogaine. Unfortunately, the receptors involved in mediating the harmaline cue are unknown at present and thus can shed no light on the possible mechanisms of action of ibogaine. However, for both drugs, evidence exists supporting interactions with serotonergic components of the central nervous system (11,21,24,25,26).

ibogaine as a
of 10.0 mg/kg.
iless otherwise
oer of subjects
·issa: Ibogaine
te. Abscissa:

In conclusion it appears that ibogaine serves as a reliable discriminative cue and its stimulus effects are both dose- and time-dependent. Furthermore, the ibogaine-cue seems similar but not identical to that of 12-hydroxyibogamine whereas harmaline fully mimics ibogaine. Further studies with ibogaine-trained subjects are currently underway to determine the receptor interactions involved in the ibogaine cue.

100 years, it is only
by reports of its anti-
uscripts over the past
its behavioral effects.

References

1. S.D. GLICK, K. ROSSMAN, S. STEINDORF, I.M. MAISONNEUVE, and J.N. CARLSON, Eur. J. Pharmacol. **195** 341-345 (1991).
2. S.D. GLICK, M.E. KUEHNE, J. RAUCCI, T.E. WILSON, D. LARSON, R.W. KELLER Jr., and J.N. CARLSON, Brain Res. **657** 14-22 (1994).
3. S.I. DWORKIN, S. GLEESON, D. MELONI, T.R. KOVES, and T.J. MARTIN, Psychopharmacology **117** 257-261 (1995).
4. S.L.T CAPPENDIJK and M.R. DZOLJIC, Eur. J. Pharmacol. **241** 261-265 (1993).
5. A.H. REZVANI, D.H. OVERSTREET, and Y. LEE, Pharmacol. Biochem. Behav. **52** 615-620 (1995).
6. S.G. SHEPPARD, J. Subs. Abuse Treatment **11** 379-385 (1994).
7. H. LOTSOF, U.S. Patent 4,499,096; Rapid method for interrupting the narcotic addiction syndrome. (1985).
8. H. LOTSOF, U.S. Patent 4,587,243; Rapid method for interrupting the cocaine and amphetamine abuse syndrome. (1986).
9. H. LOTSOF, U.S. Patent 4,857,523; Rapid method for interrupting the alcohol dependency syndrome. (1989).
10. H. LOTSOF, U.S. Patent 5,026,697; Rapid method for interrupting or attenuating the nicotine/tobacco dependency syndrome. (1991).
11. D.C. MASH, J.K. STALEY, M.H. BAUMANN, R.B. ROTHMAN, and W.L. HEARN, Life Sciences **57** PL 45-50 (1995).
12. L.B. HOUGH, S.M. PEARL, and S.D. GLICK, Life Sci. **58** PL 119-122 (1996).
13. F.C. COLPAERT, Methods of Assessing the Reinforcing Properties of Abused Drugs, M. A. Bozarth (Ed), 341-372, Springer, Berlin, (1990).
14. J.C. WINTER, NIDA Research Monograph Series **146** 157-182 (1994).
15. M.D. SCHECHTER and T.L. GORDON, Eur. J. Pharmacol. **249** 79-84 (1993).
16. D. FIORELLA, R.A. RABIN and J.C. WINTER, Psychopharmacology **119** 222-230 (1995).
17. J.C. WINTER and R.A. RABIN, J. Pharmacol. Exp. Ther. **263** 682-689 (1992).
18. S.D. GLICK, S.M. PEARL, J. CAI, and I.M. MAISONNEUVE, Brain Res. **713** 294-297 (1996).
19. S.M. PEARL, K. HERRICK-DAVIS, M. TEITLER, and S.D. GLICK, Brain Res. **675** 342-344 (1995).
20. R.T. LAYER, P. SKOLNICK, C.M. BERTHA, U.K. BANDARAGE, M.E. KUEHNE, and P. POPIK, Eur. J. Pharmacol. 309 159-165 (1996).
21. J.K. STALEY, Q. OUYANG, J. PABLO, W.L. HEARN, D.D. FLYNN, R.B. ROTHMAN, K.C. RICE, and D.C. MASH, Psychopharmacology **127** 10-18 (1996).
22. W.D. BOWEN, B.J. VILNER, W. WILLIAMS, C.M. BERTHA, M.E. KUEHNE, and A.E. JACOBSON, Eur. J. Pharmacol. **279** R1-R3 (1995).
23. D.C. MASH, J.K. STALEY, J.P. PABLO, A.M. HOLOHEAN, J.C. HACKMAN, and R.A. DAVIDOFF, Neuroscience Lett. **192** 53-56 (1995).

24. E.B. NIE,
    Sci. **31** 2⁴
25. P.A. PAI
26. R.A. GLI
    Pharmacc

24. E.B. NIELSEN, F.J. WHITE, A.M. HOLOHEAN, P.M. CALLAHAN, and J.B. APPEL, Life Sci. **31** 2433-2439 (1982).
25. P.A. PALUMBO and J.C. WINTER, Pharmacol. Biochem. Behav. **43** 1221-1226 (1992).
26. R.A. GLENNON, R. YOUNG, J.M. JACYNO, M. SLUSHER, and J.A. ROSECRANS, Eur. J. Pharmacol. **86** 453-459 (1983).

85 [JCW, RAR], by
ng-Plough Research
with the "Guide for
Resources, National

ıd J.N. CARLSON,

R.W. KELLER Jr.,

RTIN,

5 (1993).
ı. Behav. **52** 615-620

rcotic addiction

caine and

:ohol dependency

:nuating the

V.L. HEARN, Life

1996).
bused Drugs, M. A.

993).
19 222-230 (1995).
1992).
:s. **713** 294-297

:ain Res. **675** 342-344

E. KUEHNE, and P.

ζ.B. ROTHMAN,

JEHNE, and A.E.

'KMAN, and R.A.