Pergamon

Life Sciences, Vol. 57, No. 3, pp. PL 45-50, 1995

0024-3205/95 $9.50 + .00

0024-3205(95)00273-1

PHARMACOLOGY LETTERS
Accelerated Communication

IDENTIFICATION OF A PRIMARY METABOLITE OF IBOGAINE THAT TARGETS SEROTONIN TRANSPORTERS AND ELEVATES SEROTONIN

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(Submitted March 17, 1995; accepted April 7, 1995;
received in final form April 19, 1995)

Abstract. Ibogaine is a hallucinogenic indole with putative efficacy for the treatment of cocaine, stimulant and opiate abuse. The purported efficacy of ibogaine following single dose administrations has led to the suggestion that a long-acting metabolite of ibogaine may explain in part how the drug reduces craving for psychostimulants and opiates. We report here that 12-hydroxyibogamine, a primary metabolite of ibogaine, displays high affinity for the 5-HT transporter and elevates extracellular 5-HT. In radioligand binding assays, 12-hydroxyibogamine was 50-fold more potent at displacing radioligand binding at the 5-HT transporter than at the DA transporter. Ibogaine and 12-hydroxyibogamine were equipotent at the dopamine transporter. *In vivo* microdialysis was used to evaluate the acute actions of ibogaine and 12-hydroxyibogamine on the levels of DA and 5-HT. Administration of 12-hydroxyibogamine produced a marked dose-related elevation of extracellular 5-HT. Ibogaine and 12-hydroxyibogamine failed to elevate DA levels in the nucleus accumbens over the dose range tested. The elevation in synaptic levels of 5-HT by 12-hydroxyibogamine may heighten mood and attenuate drug craving. The effects of the active metabolite on 5-HT transmission may account in part for the potential of ibogaine to interrupt drug-seeking behavior in humans.

Key Words: 12-hydroxyibogamine, serotonin transporters, microdialysis, desmethyl ibogaine

Introduction

Ibogaine is a psychoactive indole alkaloid derived from the rain forest shrub *Tabernanthe iboga*. The use of ibogaine for the treatment of drug dependence has been based on anecdotal reports from addict self help groups that it may decrease the signs of opiate withdrawal and reduce drug craving for cocaine and heroin (1,2). Preclinical studies have shown that ibogaine reduces morphine self-administration, ameliorates signs of opiate withdrawal, and decreases cocaine preference (3,4). Ibogaine and other indole alkaloids are centrally acting drugs that at high doses produce tremorogenic and hallucinogenic effects. While ibogaine has diverse CNS effects, the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood.

Ibogaine is not a substitute for narcotics or stimulants, it is not addicting and it reportedly promotes long-term drug abstinence after a single dose administration. These anecdotal reports of

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ibogaine treatments in humans together with the demonstrated aftereffects of ibogaine on drug self administration in rats has led to the suggestion that iboga alkaloids may persist in the body or that there may be one or more active metabolites formed (3, 4). While ibogaine is known to interact with kappa opioid, and muscarinic receptors (5) and NMDA receptor coupled cation channels (6) with potencies in the low micromolar range, none of these neuroreceptor interactions explain the putative anti-addictive aftereffects of ibogaine. We report here the identification of 12-hydroxyibogamine, a primary metabolite of ibogaine which displays high affinity for the 5-HT transporter and elevates extracellular 5-HT. We suggest that the putative anti-addictive aftereffects of ibogaine may be due to a potent action of the metabolite on 5-HT systems which modulate mood, motivation and behavioral control. A persistent targeted action of 12-hydroxyibogamine on 5-HT systems may explain in part how ibogaine treatments promote rapid behavioral changes that moderate drug abuse and dependence.

Methods

Quantitative gas chromatography/mass spectrometry: The procedure for quantifying ibogaine and a single principal metabolite was developed for assays of blood and urine samples (7). Ibogaine and its principal metabolite were identified by subjecting extracts of urine samples to full scan electron impact GC/MS on Finnigan 4521 (quadrupole) and Finnigan ITS-40 (ion trap) mass spectrometers. Compound identification was based upon comparison of retention times and fragmentation patterns obtained from authentic standards (s.a. Omnichem, Belgium). Pharmacokinetic data consisted of whole blood concentrations of parent and metabolite at time points ranging form 1 to 24 h relative to the study dose. The quantitative gas chromatography/mass spectrometry (GC/MS) analysis employs a solvent extraction under basic conditions with D_3-ibogaine as an internal standard and derivatization of the metabolite to a propyl ether. The GC/MS was operated in the full scan electron ionization mode scanning from M/Z 45 to 450 at 1 sec/scan. Ion ratios for the molecular ion of ibogaine (m/z - 310), and 12-hydroxyibogamine propyl ether (m/z - 338) to that of the internal standard O-(D_3-methyl)-ibogaine (m/z - 313) were subjected to least squares linear regression versus concentration. Standard curves were linear ($r^2 = 0.999$) and reproducible. Intraassay coefficients of variation (C.V.) were 6.4 % for ibogaine and 10.5 % for 12-hydroxyibogamine. Limits of detection were 5 ng/ml for both ibogaine and derivatized 12-hydroxyibogamine, while limits of quantitation were 5 ng/mL and 10 ng/ml, respectively.

Ligand binding assays: Ligand binding to DA and 5-HT transporters was assayed in rat striatal membranes (male Sprague-Dawley) and in human neuropathological tissue specimens obtained at autopsy (males; age range, 20-46 yrs; autolysis times, 13-21 h). Ibogaine and 12-hydroxyibogamine binding to the DA transporter was assessed using [^{125}I]RTI-121 (50 pM, S.A. = 2200 Ci/mmol) in human striatal membranes (1 mg/ml) as described by Boja et al. (8). The potency of ibogaine and 12-hydroxyibogamine for binding to the 5-HT transporter was determined using [^{125}I]RTI-55 (10 pM; S.A. = 2200 Ci/mmol) in the presence of 1 μM benztropine to occlude the DA transporter in human occipital cortex (1 mg/ml) as described by Staley et al.(9). K_i and nH values were determined from analysis of the competition curves using EBDA (DRUG)/LIGAND, Biosoft.

In vivo microdialysis: Male Sprague-Dawley rats (N=12) weighing 300-350 g were anesthetized with Equithesin (3 ml/kg). Chronic jugular catheters and intracerebral guide cannulae aimed at the nucleus accumbens (+1.6 mm anteroposterior, -1.5 mm mediolateral to bregma, -6.0 mm relative to dura) were surgically implanted. After 7 to 10 days, probes (CMA/12, 2.0 mM length, Bioanalytical Systems Inc.) were inserted into the guide cannulae, and polyethylene extensions were attached to the jugular catheters. Each rat was connected to a tethering system that allowed unrestricted movement. Ringer's solution (150 mM NaCl, 2.8 mM KCl, 2.0 mM $CaCl_2$) was perfused through the probe at 1.0 ul/min. Dialysate samples were collected at 20 min intervals beginning 3 h after probe insertion. Once 3 stable baseline samples were obtained, ibogaine or 12-hydroxyibogamine was administered via jugular catheters at a dose of 1 mg/kg followed 60 min later by a dose of 10 mg/kg. Values for DA and 5-HT were expressed as a % baseline determined from the means of 3 pre-dose samples. Aliquots (5 μl) of sample were injected onto an HPLC column (Sepstik 3 μm C18, Bioanalytical Systems Inc.) that was coupled to an electrochemical detector equipped with a glassy carbon electrode set at +650 mV relative to Ag/AgCl reference. Mobile phase containing 14.2 g monochloroacetic acid, 5.8 g NaOH, 80 mg disodium EDTA, 350 mg sodium octylsulfate, 1 ml triethylamine, 50 ml methanol and 50 ml acetonitrile (pH = 5.8) per liter H_2O was

pumped at 60 µl/min. Data were acquired on-line for peak identification, integration and analysis (MAXMA 820, Waters, Milford, MA). Unknown peak heights were compared to peak heights of known DA and 5-HT standards. The lower limit of detection (S/N=3) was routinely 0.2 pg/5 µl. Data were evaluated using ANOVA followed by Duncans' Multiple range test for post hoc comparisons.

Results

Urine and blood samples from dosed human patients were extracted under strongly basic conditions (pH > 10) with ethyl acetate. Figure 1 shows the molecular ion peaks of ibogaine (1A), O-(D3-methyl)-ibogaine (1B) and 12-hydroxyibogamine propyl-ether (1C) and respective electron impact mass spectra (1D, 1F, 1G). Underivatized extracts analyzed by GC/MS in full scan electron impact ionization mode gave total-ion chromatograms that revealed a peak identified as parent drug ibogaine by comparison with an authentic standard. All samples were found to contain a second major component eluting after ibogaine (Fig. 1E). Similar spectral characteristics of this peak to ibogaine's spectrum (ie. presence of the characteristic pattern of *M/Z* 122, 135, 136 and 149 fragments shown in Fig. 1D) define it as an ibogaine metabolite. The apparent molecular ion at *M/Z* 296 suggests that it is formed by a loss of a methyl group. The appearance of a fragment of mass 211 in place of the *M/Z* 225 fragment of the ibogaine spectrum indicates that the demethylation occurs on the indole end of the molecule (10). The probable site for metabolic demethylation of ibogaine is the methoxy group, resulting in the compound 12-hydroxyibogamine. Analysis of an authentic standard of 12-hydroxyibogamine (s.a. Omnichem) gave a single peak at the same retention time and with the same electron impact fragmentation pattern as the endogenous compound isolated from blood and urine.

At 4 h after oral administration of 20 mg kg^{-1} in male subjects, the concentrations of ibogaine measured in blood ranged from 818 to 1128 ng/ml (N = 3). The time required to eliminate the majority of absorbed ibogaine (> 90%) was 24 h postdose (Fig. 2). Preliminary studies of the pharmacokinetic profiles for ibogaine demonstrate that the amount of 12-hydroxyibogamine measured in blood at 24 h was still quite appreciable. The concentration of 12-hydroxyibogamine measured at 24 h post dose ranged from 508 to 706 ng/ml (N = 3).

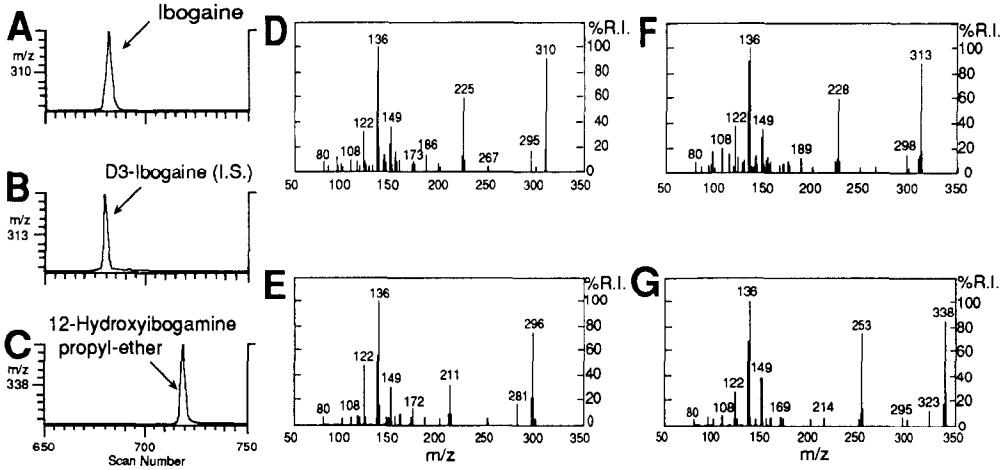

Fig. 1

Electron impact mass spectra for ibogaine and 12-hydroxyibogamine. Ion profiles for (A) ibogaine (m/z - 310), (B) O-(D3-methyl)-ibogaine (internal standard) (m/z - 313) and (C) 12-hydroxyibogamine propyl ether derivative (m/z - 338) from a quantitative analysis of blood. Full scan electron impact mass spectra of (D) ibogaine, (E) 12-hydroxyibogamine, (F) O-(D3-methyl)-ibogaine (internal standard) and (G) 12-hydroxyibogamine propyl ether derivative.

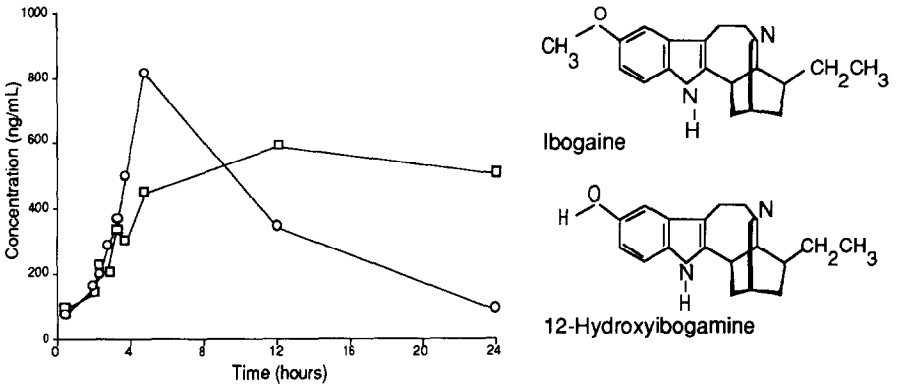

Fig. 2

Pharmacokinetics of ibogaine (○) and 12-hydroxyibogamine (□) over the first 24 hr after oral administration.

Data shown are from a representative male subject (20 mg kg^{-1}) and were measured in whole blood samples (ng per ml).

The potencies of ibogaine and 12-hydroxyibogamine in displacing ligand binding to human DA and 5-HT transporters were determined *in vitro*. Ibogaine and 12-hydroxyibogamine were equipotent with Ki values = 1.98 ± 0.46 μM (nH = 1.05 ± 0.06) and 2.05 ± 0.32 μM (nH = 1.00 ± 0.04), respectively in displacing [^{125}I]RTI-121 binding to the DA transporter (Fig. 3A). In contrast, 12- hydroxyibogamine inhibited binding of [^{125}I]RTI-55 to the 5-HT transporter (Ki = 40.7 ± 11.6 nM, nH = 0.78 ± 0.06) in human occipital cortex with at least 10-fold higher potency as compared to ibogaine (548.7 ± 29.7 nM, nH = 0.80 ± 0.07) (Fig. 3B). These results demonstrate that 12-hydroxyibogamine has high affinity for the 5-HT transporter. Similar potencies were determined for ibogaine and 12-hydroxyibogamine at DA and 5-HT transporters assayed in rat striatal membranes (data not shown).

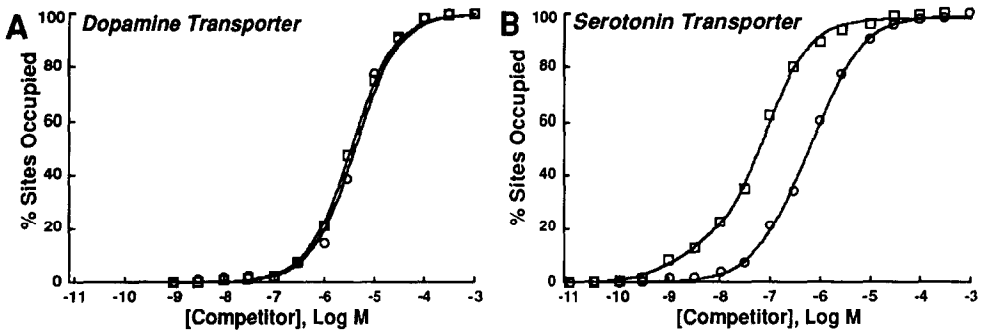

Fig. 3.

Potency of ibogaine and 12-hydroxyibogamine for binding to the DA and 5-HT transporters.

(A) Inhibition by ibogaine (○) and 12-hydroxyibogamine (□) for binding of [^{125}I]RTI-121 (50 pM) to the DA transporter in human caudate membranes. (B) Inhibition of [^{125}I]RTI-55 (10 pM) to 5-HT transporter in human occipital cortex. Data are the mean of 3 independent experiments.

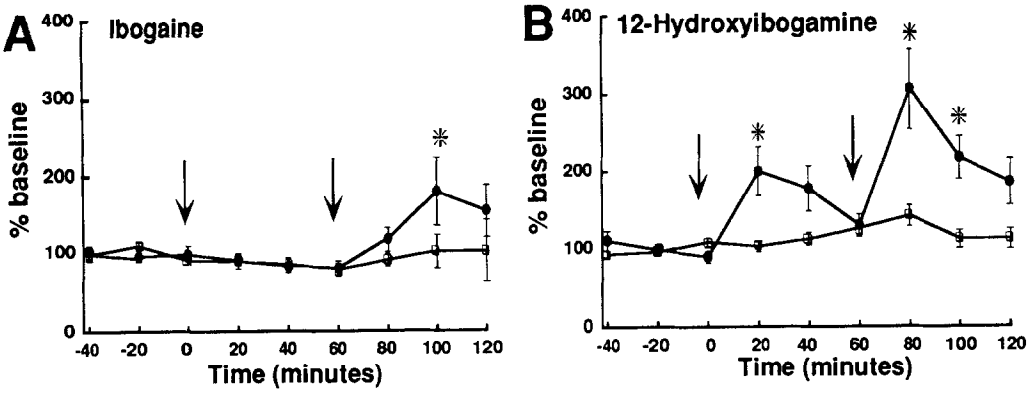

Fig. 4.

Effects of ibogaine and 12-hydroxyibogamine on extracellular DA (❑) and 5-HT (●) in the rat nucleus accumbens.

Data are expressed as the percentage of mean baseline levels from all rats (N = 12) before treatment. Each point represents the mean ± SEM of 6 independent determinations. Doses of 1 mg/kg and 10 mg/kg (i.v.) of ibogaine or 12-hydroxyibogamine were injected at time zero and 60 min, respectively.

In vivo microdialysis was used to evaluate the acute action of ibogaine and its primary metabolite on extracellular levels of DA and 5-HT. Mean baseline levels of DA and 5-HT in samples from rat nucleus accumbens (N = 12) were 1.52 ± 0.40 nM and 0.52 ± 0.08 nM, respectively. As shown in Fig 4A, ibogaine did not have a marked effect on DA at either dose tested. However, a modest and transient elevation in extracellular 5-HT ($F_{8,45} = 2.63$, $p > 0.05$) that was significant 40 min after the 10 mg/kg dose of the drug was observed consistent with its potency at the 5-HT transporter. The administration of 12-hydroxyibogamine produced a marked dose-related elevation of extracellular 5-HT ($F_{8,45} = 6.44$, $p < 0.001$). Post hoc comparisons revealed that the metabolite significantly increased 5-HT levels above baseline at 20 min after 1 mg/kg, and at 20 and 40 min after 10 mg/kg (Fig. 4B).

Discussion

The principal metabolite of ibogaine has been indentified in urine and blood specimens as 12-hydroxyibogamine by GC/MS. These studies suggest that ibogaine is primarily metabolized by O-demethylation to 12-hydroxyibogamine (7). Because the appearance of 12-hydroxyibogamine occurs soon after oral ibogaine is given, it is likely that first-pass metabolism is occurring. Preliminary pharmacokinetic analysis of the clearance rates for parent drug and its primary metabolite suggests that 12-hydroxyibogamine has a relatively long half-life in blood in drug dependent patients. While additional studies are needed, the long-term pharmacodynamic effects of ibogaine may be due in part to the active metabolite, since blood levels of ibogaine disappeared much earlier than 12-hydroxyibogamine after single oral doses.

The concentration range determined for ibogaine and 12-hydroxyibogamine in blood appears to be associated with significant pharmacological activity that may explain in part the spectrum of acute CNS effects. A comparison of 12-hydroxyibogamine to all potential targets for ibogaine determined in previous radioligand binding studies demonstrates that it has a pharmacological profile that is similar but not identical to the parent drug (data not shown). Ibogaine has demonstrated potencies in the low micromolar range for kappa opioid and muscarinic receptors, DA transporters (5) and NMDA receptor coupled cation channels (6). We have shown that 12-hydroxyibogamine binds to the 5-HT transporter in the mid-nanomolar range with 10-fold higher potency than ibogaine. 12-Hydroxyibogamine was 50-fold more potent in displacing radioligand at the 5-HT transporter than at the striatal DA transporter. Ibogaine and its metabolite were equipotent at displacing high affinity [^{125}I]RTI-121 binding to the human dopamine transporter.

Ibogaine and 12-hydroxyibogamine elevated extraneuronal 5-HT in a dose-dependent

manner. Drugs that interact with transporters act as uptake inhibitors or releasers to increase synaptic levels of neurotransmitters. Ibogaine and 12-hydroxyibogamine failed to significantly elevate DA levels, although a trend toward increased synaptic concentrations was apparent at the higher dose. In agreement with the results of radioligand binding assays, 12-hydroxyibogamine elevated 5-HT with 10-fold higher potency as compared to ibogaine. In microdialysis studies, reuptake blockers usually show a 3-5 fold elevation in neurotransmitter, whereas neurotransmitter releasers are usually distinguished by a 12 to 20-fold elevation in monoamines (11). At the highest dose tested (10 mg/kg), 12-hydroxyibogamine caused a 3-fold elevation in 5-HT. These results suggest that 12-hydroxyibogamine may act as a potent 5-HT reuptake blocker. In drug discrimination screens to test generalization of ibogaine to DA and 5-HT drugs, ibogaine was most like the potent 5-HT releaser D-fenfluramine (12). Ibogaine has been shown by *in vivo* microvoltametry to elevate extracellular 5-HT in the nucleus accumbens (13). Future studies are needed to define the complex natures of ibogaine's interaction with the 5-HT transporter and whether or not the elevation in 5-HT is due to blockade of 5-HT reuptake and/or increased 5-HT release.

Drugs that enhance 5-HT neurotransmission have been used with some success in the treatment of heroin and cocaine dependence (for review, 14). Chronic use of cocaine may lead to a "serotonin-deficit" form of 5-HT dysregulation (15). Preclinical studies demonstrate that 5-HT plays an important role in the self-administration of psychostimulants (16). The elevation of synaptic levels of 5-HT by 12-hydroxyibogamine may, by analogy with the potent 5-HT reuptake blocker fluoxetine, promote generalized anti-craving effects along with improvements in mood and cognition. The psychopharmacological targets related to 5-HT systems may be one of the mechanisms by which ibogaine and its long-acting metabolite reduce drug and alcohol abuse.

Acknowledgements

We acknowledge the excellent assistance of Q. Ouyang, M. Ayestas and J. Pablo, M.S. This work was supported by the Addiction Research Fund and grant DA09202. We thank Howard Lotsof and Dr. Edgardo Della Sera for providing residual blood samples from patient volunteers undergoing ibogaine treatments. This work is dedicated in memory to Nicola Kribus and Nancy Happel.

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