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Effect of Ibogaine On Serotonergic and Dopaminergic Interactions in Striatum from Mice and Rats*

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The effect of ibogaine (Endabuse®, NIH 10567) on serotonin uptake and release, and on serotonergic modulation of dopamine release, was measured in striatal tissue from rats and mice. Two hours after treatment in vivo with ibogaine (40 mg/kg i.p.), the uptake of labeled [³H]serotonin and [³H]dopamine uptake in striatal tissue was similar in the ibogaine-treated animal to that in the control. The $5HT_{1B}$ agonist CGS-12066A (10^{-5} M) had no effect on stimulation-evoked tritium release from mouse or rat striatal tissue preloaded with [³H]serotonin; however, it elevated tritium efflux from striatal tissue preloaded with [³H]dopamine. This increase was not seen in mice treated with ibogaine 2 or 18 hours previously, or in rats treated 2 hours before. Dopamine autoreceptor responses were not affected by ibogaine pretreatment in either mouse or rat striatal tissue; sulpiride increased stimulation-evoked release of tritium from tissue preloaded with [³H]dopamine. The long-lasting effect of ibogaine on serotonergic functioning, in particular, its blocking of the $5HT_{1B}$ agonist-mediated increase in dopamine efflux, may have significance in the mediation of its anti-addictive properties.

KEY WORDS: Ibogaine; dopamine; serotonin; autoreceptor; receptor-receptor-interaction.

INTRODUCTION

Ibogaine is an indole alkaloid from the Tabernanthe iboga plant, whose putative anti-addictive properties have received much interest. Ibogaine has been shown to antagonize cocaine-induced locomotor stimulation (1), preference for cocaine consumption (2), and cocaine self-administration (3). Similar antagonism of morphine-induced behavioral responses has been reported.

Ibogaine has complex effects on neurotransmitter systems, affecting noradrenergic (4), dopaminergic (1, 5, 6), cholinergic (7), and serotonergic receptors (8). It has affinity to voltage-dependent sodium channels and the kappa-opiate receptor (9). We recently found long-lasting effects of ibogaine on kappa-opiate agonist-induced decrease in stimulation-evoked release of [³H]dopamine from striatal tissue (10). In addition, $5HT_3$ modulation of dopamine release was altered by ibogaine pretreatment (10), suggesting that multiple effects of ibogaine including receptor-receptor interactions may underlie its putative anti-addictive properties.

Many of the initial studies on ibogaine were based on its similarity to serotonin. The cocaine binding site(s) in brain have been shown to be associated with both the dopamine and the serotonin reuptake carriers (11); sites that are involved in cocaine-induced locomotor activity, reward, and the reinforcing effects of cocaine. Ibogaine's effect may relate to alteration of the reuptake process, or alternatively, relate to alteration of stimulant drug-induced release of transmitter (6). The release of dopamine may be modulated at the site of releasable pools (6,12) or via interaction at other receptor sites that interact presynaptically on dopamine terminals to modulate dopamine release. Recent studies indicate that

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presynaptic serotonin sites can modulate dopamine release; for example, serotonin innervation of the anterior striatum may exert a facilitatory influence on dopamine release (13, 14).

The aim of the present study was to further characterize the site of action of ibogaine, in particular, its effect on serotonergic-dopaminergic interactions. Both rats and mice were examined, since their behavioral and biochemical responses to ibogaine have been reported to be different.

EXPERIMENTAL PROCEDURE

Animals and Treatment. Adult male C57BL/6By mice (25-30 g), and Sprague-Dawley rats (200-300 g) were used. In the experimental group, animals were treated 2 hours in advance with ibogaine HCl (40 mg/kg i.p.; Sigma Chemical Co., MO), or were given two injections of ibogaine (40 mg/kg, spaced 6 hours apart), and were killed 18 hours after the last injection. The animals were decapitated and striatal tissue was dissected and incubated for 60 min in 0.5 ml of Krebs-bicarbonate buffer (in mM: NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 2.5, glucose 11.5, Na_2EDTA 0.03, ascorbic acid 0.3) containing 1.25 μCi [^{3}H]dopamine or [^{3}H]serotonin (New England Nuclear). The reaction mixture was continuously gassed with an O_2/CO_2 mixture (95%/5%).

In Vitro Stimulation-Evoked Release. After the prelabeling with [^{3}H]dopamine or [^{3}H]serotonin, the tissue was transferred to a superfusion chamber (0.3-ml reaction chamber, Brandel Superfusion 1200, MD) and pre-perfused at a rate of 0.4 ml/min for 60 min. Effluent was discarded during this period and thereafter 4-min fractions were collected for an additional 80 min. Release was induced by electrical field stimulation (supramaximal voltage, 2-Hz frequency, 2-msec impulse, duration 1 min) applied in the 3rd (1st stimulation, S1) and 13th (2nd stimulation, S2) collection periods. Drugs were added starting with the 10th collection period and were maintained for the remainder of the perfusion. The release of tritium was expressed as fractional release, as a percentage of the radioactivity content in the tissue at the time the release was determined, and the ratio of fractional release S2 over fractional release S1 (FRS2/FRS1) was calculated. CGS-12066A, MDL-7222, and sulpiride were obtained from Research Biochemical International (Natick, MA), and ibogaine HCl was from Sigma Chemical Co. (St. Louis, MO).

In previous studies (6,12) [^{3}H]dopamine was separated from its main ^{3}H-labeled metabolites and found that enhanced tritium efflux evoked by electrical stimulation was mainly due to increase in the outflow of [^{3}H]dopamine. With [^{3}H]serotonin, radioactivity released by high-K^+ was, for the major part, due to unmetabolized [^{3}H]serotonin (15, 16). Nevertheless, for accuracy, when release is expressed as [^{3}H]dopamine or [^{3}H]serotonin, it refers to ^{3}H-labeled outflow.

RESULTS

Uptake and Release of [^{3}H]Dopamine and [^{3}H]Serotonin. [^{3}H]Dopamine and [^{3}H]serotonin uptake was similar in control and in ibogaine-treated mice and rats (data not shown). In some instances, the fractional release of transmitter was increased after ibogaine treatment (see Table I; FRS1 and FRS2); however, the data for an unknown reason were too variable to conclude whether release of transmitter was increased after ibogaine treatment.

Effect of Agonists and Antagonists on Stimulation-Evoked [^{3}H]Dopamine and [^{3}H]Serotonin Release. Table I shows the effect of a dopamine antagonist and serotonin agonist and antagonist on tritium release during electrical stimulation. The dopamine D2 antagonist sulpiride increased the evoked-release of tritium in tissue preloaded with [^{3}H]dopamine, from either rats or mice. Ibogaine (given 2 hours in advance) did not alter this D2 autoreceptor response. The $5HT_{1B}$ agonist CGS-12066A (10^{-5} M) did not affect [^{3}H]serotonin release evoked by electrical stimulation in mouse or rat striatal tissue. It increased stimulation-evoked release of [^{3}H]dopamine striatum of both mouse and rat. Pretreatment of the animals with ibogaine prevented with CGS-12066A effect in mouse and rat. This ibogaine effect persisted in mice 18 hours after ibogaine; in rats, however, the response to CGS-12066A recovered after 18 hours.

The $5HT_3$ antagonist MDL-7222 (10^{-6} M) did not affect [^{3}H]dopamine or [^{3}H]serotonin efflux in mouse or rat striatum from control or ibogaine-treated animals.

DISCUSSION

The study of neurotransmitter interactions in the modulation of transmitter release has important implications, for example, in the treatment of drug abuse. Data exist supporting either inhibitory or excitatory influences of serotonin agonists on the dopamine system. Serotonin agonists facilitate dopamine release (13, 14), and the $5HT_{1B}$ subtype may be involved (17). Release-regulating serotonin autoreceptors in rat brain belong to the $5HT_{1B}$ subtype (18, 19). Increasing 5HT tone appears to reduce the self-administration of a number of drugs of abuse. Antagonists of $5HT_3$ (20, 21) or of other 5HT subtypes (22) attenuate cocaine and amphetamine responses, suggesting that modulation (feedback control) of these systems can alter dopamine responses, or that they themselves are directly involved in the action of drugs of abuse. Studies of cocaine habituation found that cocaine binding site(s) are associated with dopamine and serotonin reuptake carriers (11). Presynaptic mechanisms (inhibition of reuptake) may mediate the discriminative stimulus, in addition to the reinforcing effects of

Table 1. Effect of Ibogaine on [^{3}H]Dopamine or [^{3}H]Serotonin Efflux During Electrically Evoked Release

	Mouse			Rat		
	FRS1	FRS2	FRS2/FRS1	FRS1	FRS2	FRS2/FRS1
[^{3}H]Dopamine						
Control	2.03 ± 0.18	2.05 ± 0.19	1.01 ± 0.10 (13)	2.80 ± 0.81	2.68 ± 0.73	1.00 ± 0.06 (6)
Ibogaine (2 h)	2.74 ± 0.31	3.24 ± 0.59	1.14 ± 0.12 (5)	1.82 ± 0.39	1.52 ± 0.23	0.91 ± 0.09 (6)
Sulpiride (10^{-5} M)	2.24 ± 0.46	6.02 ± 1.72	2.70 ± 0.14 (5)*	1.86 ± 0.29	3.53 ± 0.61	1.93 ± 0.12 (6)*
Ibogaine (2 h) + Sulpiride	1.84 ± 0.33	4.22 ± 0.65	2.40 ± 0.32 (5)*	1.84 ± 0.22	3.74 ± 0.54	2.08 ± 0.22 (6)*
CGS-12066A (10^{-5} M)	2.68 ± 0.24	3.53 ± 0.33	1.32 ± 0.06 (9)**	1.97 ± 0.27	2.68 ± 0.36	1.35 ± 0.09 (14)**
Ibogaine (2 h)+CGS-12066A	4.34 ± 0.81	4.87 ± 0.97	1.12 ± 0.09 (5)	4.07 ± 0.37	3.17 ± 0.38	0.76 ± 0.05 (11)
Ibogaine (18 h)+CGS-12066A	2.05 ± 0.38	2.39 ± 0.60	1.13 ± 0.13 (5)	1.02 ± 0.11	1.37 ± 0.29	1.33 ± 0.20 (4)**
MDL-7222 (10^{-6} M)	1.53 ± 0.20	1.79 ± 0.27	1.16 ± 0.06 (6)	1.52 ± 0.22	1.58 ± 0.36	1.04 ± 0.25 (4)
Ibogaine + MDL-7222	1.37 ± 0.28	1.32 ± 0.17	1.04 ± 0.08 (4)	3.04 ± 0.30	3.22 ± 0.30	1.11 ± 0.11 (6)
[^{3}H]Serotonin						
Control	2.68 ± 0.24	2.14 ± 0.31	0.78 ± 0.08 (6)	2.60 ± 0.46	2.03 ± 0.46	0.76 ± 0.05 (6)
Ibogaine	4.45 ± 0.46	3.93 ± 0.50	0.90 ± 0.10 (6)	2.73 ± 0.40	1.60 ± 0.34	0.58 ± 0.07 (5)
CGS-12066A (10^{-5} M)	1.54 ± 0.31	1.59 ± 0.26	1.10 ± 0.09 (10)	2.01 ± 0.20	1.36 ± 0.13	0.71 ± 0.06 (12)
Ibogaine (2 h)+CGS-12066A	2.16 ± 0.43	2.77 ± 0.69	1.25 ± 0.12 (4)	5.95 ± 0.45	3.76 ± 0.40	0.66 ± 0.10 (6)
Ibogaine (18 h)+CGS-12066A	1.53 ± 0.50	1.38 ± 0.33	1.03 ± 0.16 (5)	3.23 ± 0.31	2.16 ± 0.29	0.68 ± 0.09 (6)
MDL-7222 (10^{-6} M)	2.72 ± 0.46	2.29 ± 0.44	0.85 ± 0.08 (6)	2.51 ± 0.54	1.86 ± 0.40	0.72 ± 0.07 (5)
Ibogaine (2 h)+MDL-7222	2.63 ± 0.73	2.91 ± 0.74	1.20 ± 0.24 (6)	2.13 ± 0.58	1.34 ± 0.27	0.76 ± 0.1 (6)

Striata were incubated with [^{3}H]dopamine or [^{3}H]serotonin and electrically evoked release of tritium was measured as described in Experimental Procedure. Animals were treated either with ibogaine HCl (40 mg/kg, i.p.) and killed 2 hours later or with ibogaine (40 mg/kg, 2 times, 6 hours apart) and killed 18 hours later. The tissue was stimulated electrically during the 3rd (S1) and 13th (S2) collection period. Release was expressed as the fractional release, for example, as the percentage release of radioactivity in the tissue at the time the release was determined, and the ratio of fractional release S2 (FRS2) over fractional release S1 (FRS2/FRS1) was calculated. Values are means ± SEM; n is given in parenthesis; Student t test * $p < 0.001$; ** $p < 0.05$ versus control.

cocaine (23). Most studies have focused on the dopamine reuptake site as the site of action for cocaine. Ibogaine itself shows a weak affinity to the dopamine transporter site in striatal tissue labeled with [^{3}H]WIN 35,248 (1) or [^{3}H]GBR-12935 (24) and does not alter synaptosomal dopamine uptake (12, 24). In the present study we confirm the lack of effect of ibogaine on dopamine uptake, and the similar lack of effect on serotonin uptake into striata from ibogaine-treated animals. Our previous data indicated an effect of ibogaine on the serotonin system, with the long-term effect of reducing serotonin turnover (1) and altering responses to the $5HT_3$ agonist phenylbiguanide (10). It is not known, however, whether the altered $5HT_3$-mediated dopamine release is related to an action on serotoninergic transmission, on presynaptic modulation of dopamine release, or to changes in the releasable pool of dopamine.

Ibogaine blocks the increase in striatal dopamine induced by morphine (25, 26), but enhances the rise in extracellular dopamine induced by amphetamine (27) and cocaine (28). The prolonged effects of ibogaine that have been shown are to decrease extracellular levels of the dopamine metabolites (1, 29). Serotonin ($5HT_{1B}$) agonists can facilitate dopamine release (17), in addition to their suppression of 5HT release via autoreceptor-mediated feedback control (18). Feedback inhibition of [^{3}H]5HT release in vitro by CGS-12066A was not evident in rat or mouse striatum. This may relate to an insufficient specificity of CGS-12066A, that it is not functionally selective as an agonist for $5HT_{1B}$ receptors, or that it may act with the 5HT transporter to diminish its autoreceptor effects (30). Nevertheless, direct effects of CGS-12066A on dopamine release were evident. Ibogaine eliminated the $5HT_{1B}$ agonist-mediated increase in dopamine release, without any effect on 5HT autoreceptor function. This effect was seen in mice and rats 2 hours after ibogaine treatment; an effect persisting in mice for 18 hours after the last ibogaine treatment. In rats, CGS-12066A-mediated dopamine release was restored 18 hours later. Such differences may account for some of the variability in the observed effects of ibogaine on drug-induced behaviors between mice and rats. A difference in amphetamine-induced dopamine release and behavioral response was seen in mice and rats after

ibogaine (6, 28). Similarly, ibogaine had no effect on dopamine D2 (sulpiride) autoreceptor function. In some studies, ibogaine potentiated the motor effects induced by cocaine in rats (28) or inhibited motor activity in mice (1, 2).

The present data add additional support for the long-lasting effect of ibogaine on neurotransmitter release. The results extend beyond reduction of stimulant drug-induced transmitter release and show that the modulation of dopamine release by the kappa-opioid receptor (10) and now the $5HT_{1B}$-agonist is affected by ibogaine treatment. The reduction of the behavioral effects of stimulant drugs by ibogaine may be mediated by the blockage of the kappa-opioid and/or 5HT receptor presynaptic modulation of dopamine release. Additionally, the dopamine D2 autoreceptors themselves are not affected, further indicating an effect of ibogaine on the releasable pools of neurotransmitter subsequent to presynpatic receptor stimulation.

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