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Effect of Ibogaine on Cocaine-Induced Efflux of [^{3}H]Dopamine and [^{3}H]Serotonin From Mouse Striatum

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SERSHEN, H., A. HASHIM AND A. LAJTHA. *Effect of ibogaine on cocaine-induced efflux of [^{3}H]dopamine and [^{3}H]serotonin from mouse striatum.* PHARMACOL BIOCHEM BEHAV 53(4) 863-869, 1996. – Ibogaine, an indole alkaloid with proposed antiaddictive properties, has structural similarity to serotonin and has been shown to have affinity to the κ-opioid binding site. In addition to the dopamine system, the serotonin system is a major target for cocaine action and the opioid system can affect the serotonin system. Therefore, the present study examined the effect of ibogaine on cocaine-induced, electrically evoked efflux of [^{3}H]dopamine and [^{3}H]serotonin from striatal tissue incubated in vitro, and their modulation by the κ-opioid system. Cocaine (10^{-6} M) added in vitro increased the fractional efflux of both [^{3}H]dopamine (FRS_2/FRS_1 = 2.42 ± 0.36) and [^{3}H]serotonin (FRS_2/FRS_1 = 1.31 ± 0.06). Mice treated in vivo with ibogaine (40 mg/kg or 2 times 40 mg/kg, IP) and killed 2 or 18 h later still showed the cocaine-induced increase in [^{3}H]dopamine, but [^{3}H]serotonin efflux was not increased. The 5-HT_{1B} agonist CGS-12066A (10^{-6} M, added in vitro) increased [^{3}H]dopamine release, but did not alter cocaine-induced efflux of [^{3}H]dopamine. CGS-12066A did not affect [^{3}H]serotonin release, but the cocaine-induced increase in [^{3}H]serotonin was inhibited. CGS-12066A (1 mg/kg, SC) potentiated cocaine (25 mg/kg, SC)-induced locomotor activity. Ibogaine pretreatment reduced both the cocaine and the CGS-12066A cocaine-induced increase in locomotor activity. The κ-opioid agonist U-62066 (10^{-6} M, added in vitro) reduced both [^{3}H]dopamine and [^{3}H]serotonin release. This inhibitory effect was blocked by in vivo administration of ibogaine. U-62066 did not alter cocaine-induced [^{3}H]dopamine efflux, but reduced cocaine-induced [^{3}H]serotonin efflux. In striatal tissue from ibogaine-pretreated mice, U-62066 restored the cocaine-induced increase in [^{3}H]serotonin release. U-62066 (1 mg/kg, SC) potentiated cocaine-induced behavior and maintained an increased locomotor activity after ibogaine treatment. The results suggest that ibogaine may block the cocaine-mediated effects on serotonergic transmission, that subsequently modulate dopamine release. The κ-opioid modulation of serotonergic transmission is also involved.

Ibogaine Cocaine Serotonin Dopamine κ-Opioid

IBOGAINE (Endabuse; NIH 15067), an indole alkaloid, has been proposed to have antiaddictive properties. It has been previously shown that ibogaine can antagonize several cocaine-induced behavioral responses in C57BL/6By mice, blocking cocaine-induced locomotor stimulation (30,31) preference for cocaine consumption (32), and self-administration (6). Blockade of morphine-induced behavioral responses (21,22) and self-administration (12) have also been reported.

Although the dopamine system is the primary substrate underlying the action of cocaine, multiple receptor interactions are involved in the mediation of cocaine-induced behaviors: for example, dopamine-serotonin (4,5), dopamine-glutamate (19), or dopamine-κ-opioid receptor interactions (14,15) mediating stereotypy, sensitization, and locomotor activities. Ibogaine also has apparently complex effects on neurotransmitter systems, affecting noradrenergic (10), dopaminergic (13,26,31), cholinergic (27), and serotonergic sites (33, 35). Recently, ibogaine has been shown to have a weaker affinity to the dopamine transporter (30) than dopamine itself or cocaine, and to inhibit the binding of [^{3}H]U-69593 (κ-

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agonist) to κ-opioid receptors, [^{3}H]batrachotoxin A 20-α-benzoate binding to the voltage-dependent sodium channels (9), and [^{3}H]MK-801 (NMDA antagonist) binding to NMDA receptors (25). Long-term effects of ibogaine do not appear to act at the dopamine transporter, because labeled dopamine uptake or binding to the dopamine transporter ([^{3}H]WIN 35,248 or [^{3}H]GBR-12935) were not altered (4,30). Therefore, we examined whether effects of ibogaine at other sites may alter cocaine-mediated response. Because ibogaine was shown to have affinity to the κ-opioid site (9), and κ-agonists decrease dopamine release (15) and can attenuate behavioral sensitization produced by cocaine, we further investigated ibogaine's effect on κ-agonist-induced dopamine release. In mice, the antinociceptive effects of κ-opioid agonists are due, in part, to the activation of serotonin receptor (20). Its effect on serotonin release was also examined.

We recently reported long-lasting blockade of ibogaine on κ-opioid agonist-induced inhibition and 5-HT$_3$ agonist-induced increase of dopamine release from striatal tissue (34). Whether interaction of ibogaine at such sites is involved in altering cocaine-induced responses was further investigated in the present study. Although much emphasis has been placed on the effects of cocaine on the dopaminergic system, there is evidence that a serotonin pathway is also involved in cocaine's action (38). Because cocaine binding sites in brain have been associated with both dopamine and serotonin uptake carriers (28), and ibogaine is structurally related to serotonin, the effect of ibogaine on cocaine-induced changes in these neurotransmitters was investigated.

Therefore, the present study is an attempt to further characterize the effect of ibogaine on serotonergic-κ-opioid-dopaminergic interactions that mediate cocaine-induced behaviors.

METHODS

Animals

Adult male C57BL/6By mice (25–30 g) were used. The animals were bred in our animal facility and housed under conditions of controlled temperature (21 ± 0.4°C) and a 12 L : 12 D cycle.

Locomotor Activity and Drug Administration

We measured locomotor activity using a Columbus Instruments Auto-Track System (Columbus, OH). The system consists of a host controller, an interface box, and 15 × 15 infrared beam-based activity monitors (Opto-Varimex units). Animals were housed within individual transparent cages (27 × 17 × 12 cm), through which infrared beams passed in a horizontal plane, for at least 24 h before the drug treatment and activity measurements. Total ambulatory counts (TAC) were calculated in 10-min segments after drug administration. TACs represent the total number, on both the X and Y axes, of beams interrupted by the animals in their ambulatory activity. All mice were initially given an injection of saline (0.1 ml, SC) followed 30 min later by injection of the 5-HT$_{1B}$ agonist CGS-12066A, the 5-HT$_{1A}$/5-HT$_{1B}$ antagonist (±)proprandol, κ-agonist U-62066, or saline. Thirty minutes later, cocaine (10 or 25 mg/kg, SC) was administered and locomotor activity was monitored for an additional 2 h. Two injections of ibogaine: HCl (40 mg/kg, SC) were given 6 h apart, and cocaine-induced behavior was monitored the next day (18 h after the first ibogaine injection). Two injections of ibogaine were given, because this produced the maximal inhibition of cocaine-induced motor stimulation (30,31).

[^{3}H]Dopamine and [^{3}H]Serotonin Release In Vitro

The mice were decapitated and the striatal tissue was dissected out 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_2 EDTA 0.03, ascorbic acid 0.3) containing 1.25 μCi [^{3}H]dopamine [dihydroxyphenylethylamine 3,4(7-[^{3}H]), 20.3 Ci/mmol] or [^{3}H]serotonin [hydroxytryptamine 5-(1,2-[^{3}H]), 25.5 Ci/mmol] (New England Nuclear, Boston, MA) (final concentration about 0.1 μM). The reaction mixture was continuously gassed with an O_2/CO_2 mixture (95% : 5%). After the prelabeling, the tissue (approximately 8–10 mg wet wt.; amount accumulated was approximately 7 and 2 pmol per total tissue wet wt. for [^{3}H]dopamine and [^{3}H]serotonin, respectively) was transferred to a superfusion chamber (0.3-ml chamber; Brandel Superfusion 1200, MD) and preperfused 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 ms impulse, duration 1 min) applied in the third (first stimulation, S_1) and 13th (second stimulation, S_2) collection periods. Drugs were added starting with the 10th collection period and were maintained for the rest of the perfusion. The release was expressed as fractional release, as a percentage of the amount of radioactivity in the tissue at the time the release was determined. The ratio of fractional release S_2 over fractional release S_1 (FRS_2/FRS_1) was calculated. Previous studies, in which [^{3}H]dopamine or [^{3}H]serotonin was separated from its main [^{3}H]-labeled metabolites in the superfusate, found that enhanced tritium efflux evoked by electrical stimulation was mainly due to an increase in the outflow of [^{3}H]dopamine (13,31) or [^{3}H]serotonin (26,29). In these experiments, when release is expressed as [^{3}H]dopamine or [^{3}H]serotonin, it refers to [^{3}H]-labeled outflow.

For the slice-release experiments, mice were treated, either 2 or 18 h before decapitation, with a single or double injection of ibogaine (40 mg/kg, SC).

Drugs

Cocaine HCl and ibogaine HCl were obtained from Sigma Chemical (St. Louis, MO), and CGS-12066A, (±)-propranolol, and U-62066 were from Research Biochemicals (Natick, MA). Ibogaine HCl was dissolved in warm distilled water. CGS-12066A and (±)proprandol were dissolved in saline and U-62066 was first dissolved in ethanol.

Statistical Evaluations

Separate groups of animals were used for each dose of cocaine or treatment protocol. Comparisons of behavioral data between groups were done by the paired Student's *t*-test. Multiple group comparisons for the release data were done by one-way analysis of variance followed by paired Student's *t*-test; results are expressed as mean ± SEM.

RESULTS

Effect of CGS-12066A and (±)Propranolol on Cocaine-Induced Locomotor Activity (Fig. 1)

An acute cocaine injection (10 or 25 mg/kg, SC) produced an increase in locomotor activity for approximately 2 h. Pretreatment with the 5-HT$_{1B}$ agonist CGS-12066A (1.0 mg/kg, SC) potentiated the cocaine-induced locomotor response.

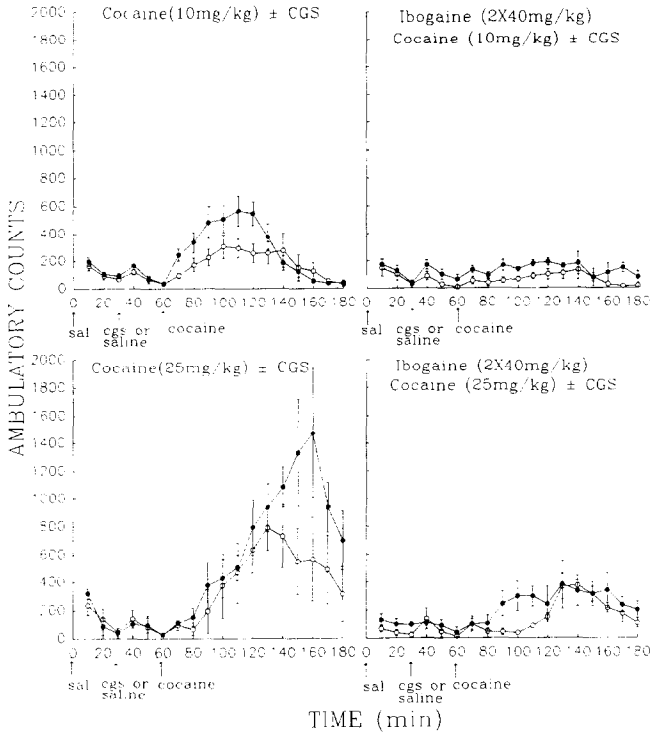

FIG. 1. Mice were first given an injection of saline, followed 30 min later by an injection of CGS-12066A (1.0 mg/kg, SC) (● – ●) or saline (○ – ○) and then cocaine (upper panels, 10 mg/kg; lower panels, 25 mg/kg). (Right) Mice were treated with ibogaine (2 × 40 mg and tested 18 h later). (Left) CGS-12066A-treated (● – ●) mice were significantly different from cocaine control (○ – ○), $p < 0.05$. Ibogaine-treated mice (right panels) were significantly different from nontreated ones (left panel), $p < 0.05$. Values are means ± SEM ($n = 8$) of ambulatory counts measured in 10-min segments after drug administration (arrows).

CGS-12066A alone did not increase locomotor activity (not shown).

Ibogaine pretreatment (2 × 40 mg/kg, IP, 18 h ahead) antagonized the cocaine-induced locomotor stimulation. The response to CGS-12066A was also eliminated.

The nonselective 5-HT_{1A}/5-HT_{1B} receptor antagonist (±)propranolol HCl (3.0 mg/kg, SC) did not affect cocaine-induced locomotor activity, and did not alter the effect of ibogaine (not shown).

Effect of U-62066 on Cocaine-Induced Locomotor Activity (Fig. 2)

The κ-agonist U-62066 (1 mg/kg), given 30 min before cocaine (25 mg/kg), potentiated the locomotor response. U-62066 itself had no effect on locomotor response. In ibogaine-pretreated mice (2 × 40 mg/kg) tested 18 h after the last injection, cocaine-induced locomotor activity was reduced. U-62066 given before the cocaine partially reversed the ibogaine-induced inhibition, and locomotor activity was similar to that with cocaine alone; no further potentiation of locomotor activity was seen.

Effect of Ibogaine on Agonist- and Cocaine-Induced Efflux of [^{3}H]Dopamine and [^{3}H]Serotonin (Table 1)

Efflux of dopamine and serotonin was similar in mice pretreated either 2 or 18 h ahead with ibogaine (1 or 2 × 40 mg/kg, SC). Cocaine (10^{-6} M) added in vitro to the perfusion fluid increased the efflux of [^{3}H]dopamine and [^{3}H]serotonin. Ibogaine pretreatment prevented the cocaine-induced increase in [^{3}H]serotonin release. Similarly, in mice treated with cocaine in vivo (2 × 25 mg/kg per day for 4 days) followed by ibogaine (2 × 40 mg/kg), the increased release of [^{3}H]serotonin induced by cocaine added in vitro was eliminated; cocaine-induced increase in efflux of [^{3}H]dopamine was not affected.

The 5-HT_{1B} agonist (CGS-12066A; 10^{-5} M) increased the release of [^{3}H]dopamine but not [^{3}H]serotonin. When CGS-12066A was added in vitro before the in vitro cocaine, the cocaine-induced release of serotonin was prevented.

The κ-agonist (U-62066; 10^{-6} M) reduced the electrically evoked release of both [^{3}H]dopamine and [^{3}H]serotonin. Ibogaine pretreatment reduced the inhibitory effect of U-62066 on both [^{3}H]dopamine and [^{3}H]serotonin release. U-62066 itself prevented the cocaine-induced release of serotonin. In ibogaine-pretreated mice, the inhibitory effect of U-62066 on cocaine-induced serotonin release was eliminated.

DISCUSSION

Several recent behavioral and biochemical studies support the proposed antiaddictive properties of ibogaine: in particular, its effect on cocaine-mediated behaviors. The mechanisms involved in these putative properties are currently under investigation. Research into the mechanisms by which cocaine reinforces self-administration and craving behaviors has focused predominantly on the dopaminergic system. However, it is increasingly clear that dopamine release is under the regulatory control of multiple excitatory and inhibitory neurotransmitters affecting presynaptic receptors for excitatory amino acids – acetylcholine, dopamine, dynorphins, GABA, and serotonin – on dopaminergic cells. As such, selective agonists and antagonists at each of these sites can be expected to alter cocaine-induced behaviors. These functional interactions add

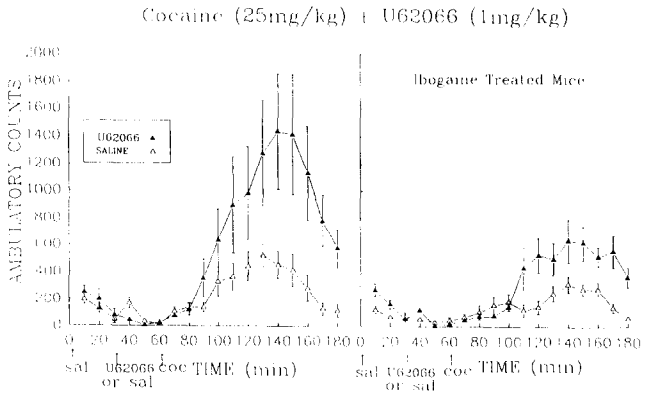

FIG. 2. Mice were first given an injection of saline, followed 30 min later by an injection of U-62066 (▲ – ▲) (1 mg/kg, SC) or saline (△ – △) and then cocaine (25 mg/kg, SC) 30 min later (left panel). Ambulatory counts were measured throughout in 10-min segments and measured for a period of 2 h after cocaine. Ibogaine-treated mice (2 × 40 mg/kg; 18 h prior) were injected similarly (right panel). U-62066 (▲ – ▲)-treated mice were significantly different from cocaine controls (△ – △), $p < 0.05$. U-62066 alone had no effect on ambulatory counts (data not shown). Values are the means ± SEM ($n = 8$) of ambulatory counts measured in 10-min segments after drug administration (arrows).

TABLE 1

EFFECT OF IBOGAINE ON COCAINE- AND AGONIST-INDUCED RELEASE OF [^{3}H]DOPAMINE AND [^{3}H]SEROTONIN

	Fractional Release (FRS_2/FRS_1)	
	[^{3}H]Dopamine	[^{3}H]Serotonin
Control	1.13 ± 0.08	0.97 ± 0.09
Ibogaine (2 h)	1.07 ± 0.09	0.71 ± 0.05
Ibogaine (18 h)	1.21 ± 0.13	0.85 ± 0.14
Cocaine (10^{-6} M)	2.42 ± 0.36*	1.31 ± 0.06*
Ibogaine (2 h) + cocaine	2.02 ± 0.27*	0.91 ± 0.03†
Ibogaine (18 h) + cocaine	2.38 ± 0.18*	1.15 ± 0.16†
Cocaine (in vivo) + cocaine	2.04 ± 0.30*	1.55 ± 0.19*
Cocaine (in vivo) + ibogaine (18h) + cocaine	1.86 ± 0.23	0.97 ± 0.14†
CGS-12066A (10^{-5} M)	1.32 ± 0.06*	1.10 ± 0.09
CGS-12066A + cocaine	2.11 ± 0.17‡	0.88 ± 0.05‡
U-62066 (10^{-6} M)	0.57 ± 0.09*	0.54 ± 0.04*
Ibogaine (2 h) + U-62066	0.90 ± 0.13†	0.72 ± 0.10†
Ibogaine (18 h) + U-62066	1.10 ± 0.11†	0.89 ± 0.10†
U-62066 + cocaine	1.93 ± 0.21‡	0.72 ± 0.06†
Ibogaine (18 h) + U-62066 + cocaine	1.76 ± 0.15‡	1.29 ± 0.07‡

Striata were incubated with [^{3}H]dopamine or [^{3}H]serotonin and electrically evoked release of tritium was measured as described in METHODS. Animals were treated with ibogaine (40 mg/kg, IP) and killed 2 h later or with (40 mg/kg, twice, 6 h apart) and killed 18 h later. The tissue was stimulated electrically during the third (S_1) and 13th (S_2) collection period. Cocaine (10^{-6} M) was added starting at fraction 10, and agonists (CGS-12066A (10^{-5} M) or U-62066 (10^{-6} M) during fraction 7. Cocaine (in vivo) was given twice a day (25 mg/kg, SC) for 4 days; on day 7 ibogaine (2 × 40 mg/kg) was given and animals killed 18 h later. The ratio of fractional release S_2 (FRS_2) over fractional release S_1 (FRS_1) (FRS_2/FRS_1) was calculated. Values are means ± SEM; n = 5–12.

Student's t-test *$p < 0.05$ vs. control; †vs. group control; ‡vs. cocaine (10^{-6} M).

to the complexity of understanding the mechanism of behavioral responses to abused drugs. In addition, the apparently complex pharmacology of ibogaine makes it more difficult to determine its site(s) of action. Therefore, the present study attempted to further localize the site(s) of action of ibogaine by studying its effect on cocaine-induced transmitter release in the presence of κ-opioid and 5-HT agonists.

Although ibogaine was shown to inhibit [^{3}H]WIN 35,248 (29) binding to the dopamine transporter (IC_{50} = 1.5 μM), this affinity is a 10th of that of dopamine or cocaine (120 nM). In vivo ibogaine treatment did not affect the binding of [^{3}H]WIN 35,248 in striatal tissue (29). Ibogaine did not significantly inhibit [^{3}H]GBR-12935 binding and did not affect striatal dopamine uptake (4). At the nerve terminal level, ibogaine added in vitro released dopamine from the cytoplasmic pool, but this release was not subject to presynaptic autoreceptor regulation (9). Similarly, release of dopamine from striatal tissue of animals treated in vivo with ibogaine did not show altered autoreceptor responses (33). Therefore, it is unlikely that ibogaine has a direct effect on the dopamine transporter, because release in the absence of agonists was not affected (Table 1). Other than the acute effect of ibogaine to release cytoplasmic dopamine, it does not appear that its long-term effects are related to an action on the dopamine terminal, for example, altering affinity to the uptake carrier or dopamine autoreceptor.

We further investigated the effect of ibogaine on cocaine-induced changes in the "release-uptake blockade" of [^{3}H]dopamine and [^{3}H]serotonin. Because it is difficult to differentiate uptake inhibition from release in vitro, the phrase "increase efflux" is used to represent the increase in extracellular dopamine or 5-HT during the second stimulation. This may reflect an actual increase in release of transmitter or, in the case of cocaine, may represent predominantly an increase in extracellular dopamine resulting from a block in reuptake, although as a caution, the ability of cocaine to influence release should not be excluded (38). In the present study, the cocaine-induced increase in efflux of dopamine was not affected by prior in vivo treatment with ibogaine; however, cocaine-induced serotonin efflux was inhibited (Table 1). Because cocaine is a known uptake blocker of dopamine and serotonin, it is not surprising that no effect by ibogaine was noted with cocaine-induced enhanced efflux of dopamine (Table 1). Both autoregulation and reuptake of released amines should be considered when interpreting the release data (8). It is unlikely that autoregulation was affected, as sulpride was still able to increase the release of dopamine in striatum from ibogaine-treated mice (33).

Cocaine is known to have affinity to the 5-HT transporter (28) and 5-HT can modulate dopamine release (1,2,24). Ibogaine is structurally related to serotonin, and striatal serotonin turnover was reduced after ibogaine treatment (30). The effects of the $5\text{-}HT_3$-agonist on [^{3}H]dopamine release were altered by ibogaine (34) and the $5\text{-}HT_{1B}$-agonist (CGS-12066A)-mediated facilitation of dopamine release is attenuated by ibogaine (33). In the present study, CGS-12066A did not alter the cocaine-induced dopamine release, but it did prevent the cocaine-induced increase in 5-HT release (Table 1). CGS-12066A also potentiated the cocaine-induced increase in locomotor activity; this effect was eliminated by ibogaine adminis-

tration (Fig. 1). It has been suggested that the hyperlocomotor effects of cocaine may also involve stimulation of 5-HT receptors (10). Behavioral effects of cocaine may be mediated either by the enhancement of 5-HT neurotransmission or by interactions between the serotonergic and dopaminergic systems. The potentiation by CGS-12066A of cocaine-induced locomotor activity could be due to enhanced 5-HT activity and/or the serotonergic potentiation of dopaminergic activity. Serotonin has also been shown to have a modulatory role in cocaine-induced behaviors (37). The results suggest that ibogaine action on serotonergic transmission results in altered modulation of dopamine release in response to cocaine, or possibly in altered effects of cocaine on serotonergic transmission directly. Broderick et al. (5) reported different effects of cocaine on serotonin release: decreasing or increasing serotonin release after IP or SC administration of cocaine, respectively. Ibogaine administration potentiated both effects of cocaine on serotonin release, further suggesting that ibogaine acts on serotonin mechanisms.

We have shown that ibogaine is able to release dopamine in vivo (30) or in vitro (13). The present study does not indicate a change in the FRS_2/FRS_1 from ibogaine-treated controls. This is not unexpected, as ibogaine was administered in vivo 2 or 18 h previously. Second, the ratio of FRS_2/FRS_1 would not show a change, because ibogaine was administered in vivo before the two electrical stimulations. Nevertheless, the fractional release measured—for example, the FRS_1 (data not shown)—was similar between control and ibogaine-treated tissue, further indicating no long-lasting effect of ibogaine directly on dopamine release.

Since Deecher et al. (9) showed that ibogaine has affinity to the κ-opioid receptor (K_i = 2 μM) and that the κ-agonist-mediated inhibition of dopamine release was prevented by ibogaine treatment (32), the action of a κ-agonist on cocaine-induced transmitter responses was studied further. κ-Agonists can modulate dopamine release (18); a tonically active κ-opioidergic system functions presynaptically to reduce dopamine release (23). Ibogaine eliminated the κ-agonist-mediated inhibition of [^{3}H]dopamine and [^{3}H]serotonin release (Table 1). In the presence of U-62066, cocaine increased the release of dopamine, but serotonin release was still reduced. After ibogaine treatment in the presence of U-62066, cocaine increased the efflux and release of both dopamine and serotonin. This would suggest that the modulation of serotonin release by the κ-opioid system is also affected by ibogaine treatment.

The reported attenuation of some of the behavioral effects of cocaine by κ-agonists is possibly related to the inhibition of dopamine release (14,15). In our study, U-62066 (1 mg/kg) potentiated the effect of cocaine and locomotor activity was not completely inhibited by ibogaine (Fig. 2), which suggests that the action of the κ-agonist to increase locomotor activity may not be solely on the dopamine terminal. There have been only a few studies of the effect of κ-agonists on 5-HT release. The dynorphins can also act as feedback inhibitors of excitatory amino acid release (7). It has been reported that κ-agonists enhance serotonergic activity (17,36). An unexpected finding was that the κ-agonist also inhibited 5-HT release (Table 1). It is not known whether there are species and/or strain differences, or whether the differences are due to the different agonists used. Opposite locomotor responses to amphetamine were observed in ibogaine-treated mice and rats (31). Possibly the high dose of U-62066 used in vivo stimulated dopamine release. Although dynorphins are predominantly inhibitory on NMDA receptors, excitatory action may also be mediated via the NMDA receptor complex, depending on the dose or the action on nonopioid excitatory sites (7). Popik et al. (25) showed that ibogaine is a competitive inhibitor at the NMDA receptor ([^{3}H]MK-801 binding site), further indicating the possible multiple sites of action of ibogaine.

Multiple receptor interactions are involved in the mediation of cocaine-induced behaviors: for example, dopamine–serotonin (4), dopamine–glutamate (19), or dopamine–κ-opioid receptor interactions (14,15) mediating stereotypy, sensitization, and locomotor activities. In addition to the complexity of these presynaptic transmitter interactions, there is the possibility of multiple sites of action for ibogaine, as represented in Fig. 3. At present, ibogaine has been shown to affect the dopamine, serotonin, κ-opioid, and NMDA receptor sites. Finally, κ-opioids can inhibit calcium currents and calcium action potentials, and this mechanism may mediate κ-opioid in-

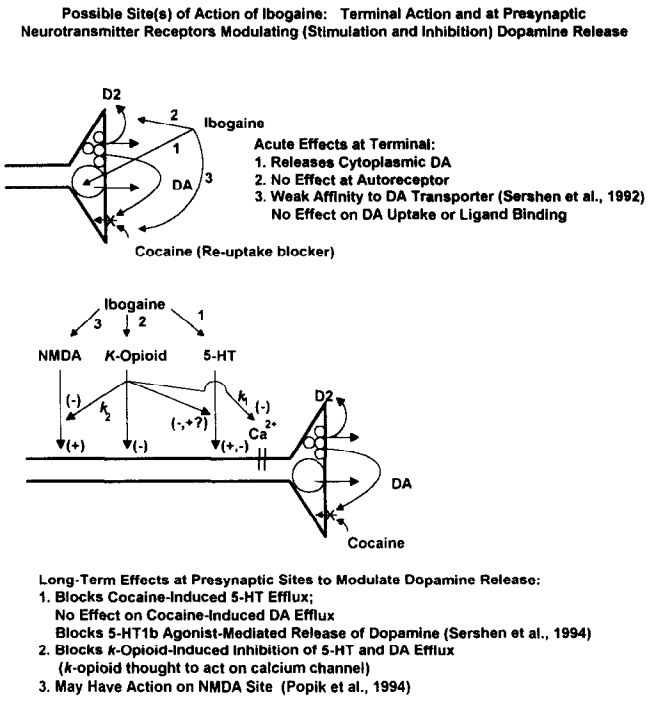

FIG. 3. Diagrammatic representation of possible sites of action of ibogaine and the regulatory control of dopamine release by multiple excitatory (+) and inhibitory (−) neurotransmitters, by activation of colocalized presynaptic receptors for dopamine excitatory amino acids (NMDA), dynorphins (κ-opioid), and serotonin. Acutely, ibogaine releases dopamine from the cytoplasmic pool (13) (upper panel). The long-term effect of ibogaine is not related to its action at the dopamine terminal; dopamine uptake and reuptake and D_2 autoreceptor function are not altered. There are indications that the serotonergic, κ-opioid, and NMDA receptors may be directly or indirectly involved in the action of ibogaine, altering cocaine-induced dopamine release, or more likely, altering the serotonergic modulation of dopamine release via inhibiting cocaine's effects on the serotonergic system. The κ-opioid receptor can also modulate the release of excitatory amino acids (NMDA) (7), serotonin (35), and dopamine (18), which can subsequently modulate cocaine effects. The present study indicates that ibogaine may act directly at the serotonin receptor and/or the κ-opioid receptor to change dopaminergic function in response to stimulant drugs. It has also been reported that ibogaine may interact at the NMDA site (25); however, its action on NMDA-mediated dopamine release was not examined in the present report.

hibition of transmitter release (37). Whether ibogaine acts at the calcium channel to modulate dopamine release is unknown, but it has been shown to have affinity to the voltage-dependent Na^+ channel (9). Binding studies may relate only to the acute effects of ibogaine, and may not give an indication of the mechanism of long-term action of ibogaine; for example, an ibogaine metabolite with a long half-life that inhibits receptor binding could participate in the long-term effects of ibogaine.

Clearly, the complexity of dopamine release modulation and the multiplicity of sites of action of ibogaine are all related to the neurochemical action of this putative antiaddiction drug.

REFERENCES

1. Benloucif, S.; Galloway, M. P. Facilitation of dopamine release in vivo by serotonin agonists: Studies with microdialysis. Eur. J. Pharmacol. 200:1-8; 1991.
2. Benloucif, S.; Keegan, M. J.; Galloway, M. P. Serotonin-facilitated dopamine release in vivo: Pharmacological characterization. J. Pharmacol. Exp. Ther. 265:373-377; 1993.
3. Broderick, P. A. In vivo electrochemical studies of gradient effects of (SC) cocaine on dopamine and serotonin release in dorsal striatum of conscious rats. Pharmacol. Biochem. Behav. 46:973-984; 1993.
4. Broderick, P. A.; Phelan, F. T.; Berger, S. P. Ibogaine alters cocaine-induced biogenic amine and psychostimulant dysfunction but not [^{3}H]GBR-12935 binding to the dopamine transporter protein. NIDA Research Monograph Series 119:285; 1992.
5. Broderick, P. A.; Phelan, F. T.; Eng, F.; Wechsler, R. T. Ibogaine modulates cocaine responses which are altered due to environmental habituation: In vivo microvoltammetric and behavioral studies. Pharmacol. Biochem. Behav. 49:711-728; 1994.
6. Cappendijk, S. I. T.; Dzolic, E. D. Effects of ibogaine on cocaine self-administration in rats. Eur. J. Pharmacol. 241:261-265; 1992.
7. Caudle, R. M.; Chavkin, C.; Dubner, R. κ_2 Opioid receptors inhibit NMDA receptor-mediated synaptic currents in guinea pig CA3 pyramidal cells. J. Neurosci. 14:5580-5589; 1994.
8. Chen, N.-H.; Reith, M. E. A. [^{3}H]Dopamine and [^{3}H]serotonin release in vitro induced by electrical stimulation in A_9 and A_{10} dopamine regions of rat brain: Characterization and responsiveness to cocaine. J. Pharmacol. Exp. Ther. 267:379-389; 1993.
9. Deecher, D. C.; Teitler, M.; Soderland, D. M.; Bornmann, W. G.; Kuehne, M. E.; Glick, S. D. Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies. Brain Res. 571:242-247; 1992.
10. Essman, W. D.; Singh, A.; Lucki, I. Serotonergic properties of cocaine: Effects on a 5-HT_2 receptor-mediated behavior and on extracellular concentrations of serotonin and dopamine. Pharmacol. Biochem. Behav. 49:107-113; 1994.
11. Gershon, S.; Lang, W. J. A psych-pharmacological study of some indoles alkaloids. Arch. Int. Pharmacodyn. 135:31-56; 1962.
12. Glick, S. D.; Rossman, K.; Steindorf, S.; Maisonneuve, I. M.; Carlson, J. N. Effects and after effects of ibogaine on morphine self-administration in rats. Eur. J. Pharmacol. 195:341-345; 1991.
13. Harsing, L. G., Jr.; Sershen, H.; Lajtha, A. Evidence that ibogaine releases dopamine from the cytoplasmic pool in isolated mouse striatum. J. Neural Transm. (in press).
14. Heidbreder, C. A.; Goldberg, S. R.; Shippenberg, T. S. The κ-opioid receptor agonist U-69593 attenuates cocaine-induced behavioral sensitization in the rat. Brain Res. 616:335-338; 1993.
15. Heidbreder, C. A.; Shippenberg, T. S. U-69593 prevents cocaine sensitization by normalizing basal accumbens dopamine. Neuroreport 5:1797-1800; 1994.
16. Heijna, M. H.; Hogenboom, F.; Mulder, A. H.; Schoffelmeer, A. N. M. Opioid receptor-mediated inhibition of [^{3}H]-dopamine and [^{14}C]-acetylcholine release from rat nucleus accumbens slices. Naunyn-Schmied. Arch. Pharmacol. 345:627-632; 1992.
17. Ho, B. T.; Takemori, A. E. Release by U-50,488H of [^{3}H]serotonin from brain slices and spinal cord synaptosomes of U-50,488H-tolerant and nontolerant mice. J. Pharmacol. Exp. Ther. 254:8-12; 1990.
18. Jackisch, R.; Hotz, H.; Hertting, G. No evidence for presynaptic opioid receptors on cholinergic, but presence of κ-receptors on dopaminergic neurons in the rabbit caudate nucleus: Involvement of endogenous opioids. Naunyn-Schmied. Arch. Pharamcol. 348: 234-241; 1993.
19. Karler, R.; Calder, L. D.; Lam, H. T.; Bedingfield, J. B. A dopaminergic-glutamatergic basis for the action of amphetamine and cocaine. Brain Res. 658:8-14; 1994.
20. Kunihara, M.; Ohyama, M.; Nakano, M. Central monoaminergic mechanisms in mice and analgesic activity of spiradoline mesylate, a selective κ-opioid receptor agonist. Eur. J. Pharmacol. 214: 111-118; 1992.
21. Maisonneuve, I. M.; Keller, R. W., Jr.; Glick, S. D. Blockade of morphine induced stimulation of mesolimbic and striatal dopamine release by ibogaine. Soc. Neurosci. Abstr. 382:15; 1990.
22. Maisonneuve, I. M.; Rossman, K. L.; Keller, R. W., Jr.; Glick, S. D. Acute and prolonged effects of ibogaine on brain dopamine metabolism and morphine-induced locomotor activity in rats. Brain Res. 575:69-73; 1992.
23. Mudler, A. H.; Wardeh, G.; Hogenboom, F.; Frankhuyzen, A. L. κ- and δ-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. Nature 308:278-280; 1984.
24. Palfreyman, M. G.; Schmidt, C. J.; Sorensen, S. M.; Dudley, M. W.; Kehne, J. H.; Moser, P.; Gittos, M. W.; Carr, A. A. Electrophysiological, biochemical and behavioral evidence for 5-HT_2 and 5-HT_3 mediated control of dopaminergic function. Psychopharmacology 112:S60-S67; 1993.
25. Popik, P.; Layer, R. T.; Skolnick, P. The putative anti-addictive drug ibogaine is a competitive inhibitor of [^{3}H]MK-801 binding to the NMDA receptor complex. Psychopharmacology 114:672-674; 1994.
26. Raiteri, M.; Maura, G.; Folghera, S.; Cavazzani, P.; Andrioli, G. C.; Schlicker, E.; Schalnus, R.; Gothert, M. Modulation of 5-hydroxytryptamine release by presynaptic inhibitory α-adrenoceptors in the human cerebral cortex. Naunyn-Schmied. Arch. Pharmacol. 342:508-512; 1990.
27. Raymond-Hamet Pharmacodynamics. Difference between the pharmacological action of ibogaine and that of cocaine. C. R. Acad. Sci. (Paris) 211:285-288; 1940.
28. Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem. Pharmacol. 35:1123-1129; 1986.
29. Schlicker, E.; Brandt, F.; Classen, K.; Gothert, M. Serotonin release in human cerebral cortex and its modulation via serotonin receptors. Brain Res. 331:337-341; 1985.
30. Sershen, H.; Hashim, A.; Harsing, L. G., Jr.; Lajtha, A. Ibogaine antagonizes cocaine-induced locomotor stimulation in mice. Life Sci. 50:1079-1086; 1992.
31. Sershen, H.; Harsing, L. G., Jr.; Hashim, A.; Lajtha, A. Ibogaine reduces amphetamine-induced locomotor stimulation in C57BL/6By mice, but stimulates locomotor activity in rats. Life Sci. 51:1003-1011; 1992.
32. Sershen, H.; Hashim, A.; Lajtha, A. Ibogaine reduces preference for cocaine consumption in C57BL/6By mice. Pharmacol. Biochem. Behav. 47:13-19; 1993.
33. Sershen, H.; Hashim, A.; Lajtha, A. Effect of ibogaine on serotonergic and dopaminergic interactions in striatum from mice and rats. Neurochem. Res. 19:1463-1466; 1994.

34. Sershen, H.; Hashim, A.; Lajtha, A. The effect of ibogaine on κ-opioid- and 5-HT_3-induced changes in stimulation-evoked dopamine release in vitro from striatum of C57BL/6By mice. Brain Res. Bull. 36:587–591; 1995.
35. Sloviter, R. S.; Drust, E. G.; Damino, B. P.; Connor, J. D. A common mechanism for lysergic acid, indolealkylamine and phenethylamine hallucinogens: Serotonergic mediation of behavioral effects in rats. J. Pharmacol. Exp. Ther. 214:231–238; 1980.
36. Von Voigtlander, P. F.; Lewis, R. A.; Neff, G. L. Kappa opioid analgesia is dependent on serotonergic mechanisms. J. Pharmacol. Exp. Ther. 231:270–274; 1984.
37. Werz, M. A.; MacDonald, R. L. Dynorphin and neoendorphin peptides decrease dorsal root ganglion neuron calcium-dependent action potential duration. J. Pharmacol. Exp. Ther. 234:49–56; 1985.
38. Wolf, W. A.; Kuhn, D. M. Cocaine and serotonin neurochemistry. Neurochem. Int. 18:33–38; 1991.