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Differential effects of ibogaine pretreatment on brain levels of morphine and (+)-amphetamine

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Previous studies in rats have shown that ibogaine inhibits neurochemical and behavioral effects of morphine yet potentiates similar effects of (+)-amphetamine. To assess whether these different functional interactions have a metabolic basis, brain levels of morphine and (+)-amphetamine were measured by gas chromatography-mass spectrometry after ibogaine pretreatment (19 h before injection of morphine or (+)-amphetamine). Ibogaine pretreatment had no effect on brain morphine levels, either at 30 min or 2 h after morphine injection; however, ibogaine significantly increased brain amphetamine levels at 30 min and, to a greater extent, at 2 h after (+)-amphetamine injection. These and other data suggest that ibogaine irreversibly inhibits an amphetamine-metabolizing enzyme. The functional interactions between ibogaine and (+)-amphetamine, but not those between ibogaine and morphine, may result from a hepatic drug–drug interaction.

It has been claimed, in two United States patents (H. Lotsof, 1985, No. 4,499,096; H. Lotsof, 1986, No. 4,587,243), that the *iboga* alkaloid ibogaine has efficacy in treating both opioid and stimulant addiction, and recent studies in animals have provided some evidence that is consistent with these claims. It has been reported that, in rats, ibogaine decreases intravenous morphine self-administration[5], reduces morphine-induced increases in motor activity[12], and blocks morphine-induced dopamine release in limbic and striatal brain regions[10]. In mice, ibogaine has been found to antagonize cocaine-induced locomotor stimulation[13]. However, data seemingly inconsistent with an anti-addictive property of ibogaine have also been reported: in rats, ibogaine has been found to enhance (+)-amphetamine-induced dopamine release in brain as well as to potentiate (+)-amphetamine-induced motor activity[11]. Although neural mechanisms that might explain these different ibogaine-drug interactions have been proposed and studied[4,10,11], metabolic or pharmacokinetic mechanisms have not been investigated. Using treatment parameters (drug doses, interval between treatments etc.) similar to those used in previous studies[10,11], we investigated whether ibogaine would alter brain levels of morphine and amphetamine.

To measure morphine brain levels, rats (female Sprague–Dawley, 250–300 g) were decapitated and their brains homogenized in 4.0 ml of ice-cold 0.05 M Tris buffer (pH 8.6) containing 600 ng of [*N*-methyl-(C^2H_3)]morphine (kindly provided by the National Institute on Drug Abuse) as the internal standard. The homogenate was then extracted, back extracted, derivatized with trifluoroacetic anhydride and analyzed by automated gas chromatography-mass spectrometry as described by Hipps et al.[7]. The system consists of a Hewlett-Packard model 5890 gas chromatograph and a Hewlett-Packard 5970 mass selective detector. Gas chromatography was performed with a 25 m cross-linked methyl silicone capillary column (0.33 μm film thickness, 0.2 mm i.d., Hewlet-Packard, Kennett Square, PA) in the splitless mode (head pressure = 16 psi, inlet = 225°, transfer line = 295°, oven pro-

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grammed from 60–250° at 70°/min) with helium as carrier. The derivatives of morphine (m/e 364, 477) and deuterated morphine (m/e 367, 480) were detected with retention times of 9.2 min. Samples containing brain homogenates from untreated animals were prepared and made to contain morphine standards (60–960 ng), as well as blanks. Each sample was injected immediately after resuspension, as the derivatives are unstable for long periods of time. Solvent injections were made between samples to ensure that no morphine derivatives were retained at the inlet or on the column. Results are expressed as ng morphine base/g brain tissue.

Brain amphetamine levels were measured by a gas chromatography-mass spectrometric method adapted from those described by Anggard et al.[1] and Jain et al.[8]. Brains were homogenized in 4 vols. of 0.2 N NH_4OH. Aliquots (3 ml) received internal standard (500 ng (+)-[phenyl-$^2H_5$]-amphetamine), a saturating amount of solid NaCl, and were extracted (15 min) with 5 ml of a mixture of chloroform-isopropyl alcohol (4 : 1). The organic phases were back extracted (20 min) with 1 ml 0.11 N HCl, and the aqueous phases evaporated to dryness under vacuum. Residues were derivatized with anhydrous ethyl acetate (50 μl) and pentafluoropropionic anhydride (50 μl, Pierce Chemical Co., Rockford, IL) with mixing at 80° for 20 min. The mixture was evaporated under N_2 at room temperature, resuspended in ethyl acetate (20 μl) and analyzed by gas chromatography–mass spectrometry. Gas chromatography was performed on a 30 m 100% methyl silicone capillary column (0.1 μm thickness, 0.25 mm i.d., DB-1, J.&W. Scientific, Folsom, CA) in splitless mode (head pressure = 8 psi, inlet = 250°, transfer line = 295°, oven programmed from 50 to 200° at 20°/min). The amphetamine derivative (m/e 91, 118) and its internal standard (m/e 96, 123) were detected with retention times of 6.7 min. Amphetamine levels, calculated from standard curves prepared in homogenates of untreated animals, are expressed as μg base/g tissue weight.

When given 19 hr earlier, ibogaine (ibogaine hydrochloride, 40 mg/kg i.p.) had no significant effect on whole brain morphine levels, assessed either 30 min or 2 h after morphine treatment (morphine sulfate, 10 mg/kg i.p.; Fig. 1). At the earlier time point there was a tendency (not statistically significant) toward higher morphine levels in the presence of ibogaine.

In contrast to its apparent lack of effect on morphine disposition, the same 19 h ibogaine pretreatment induced a pronounced enhancement of brain amphetamine levels (Fig. 2). Although this effect was small when assessed 30 min after (+)-amphetamine

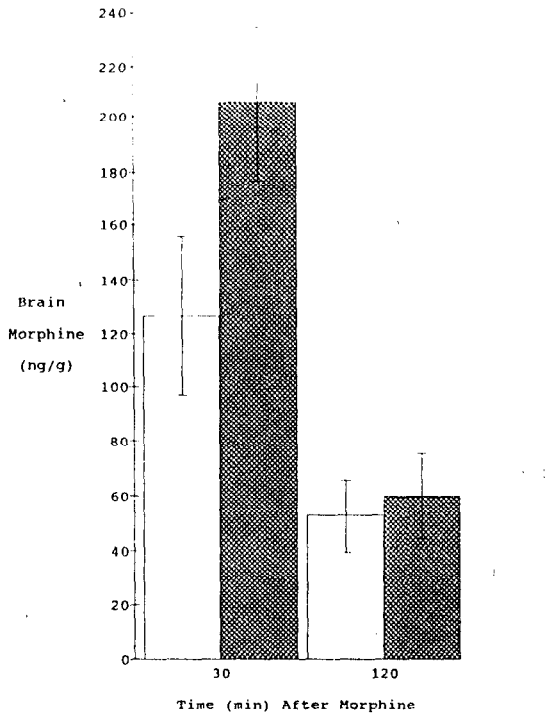

Fig. 1. Effect of ibogaine on brain morphine levels. Animals received either ibogaine hydrochloride (40 mg/kg, i.p., hatched bars) or saline (2 ml/kg, open bars), along with morphine sulfate (10 mg/kg, i.p.) 19 h later. At the intervals shown following morphine administration (abscissa), animals were decapitated, and whole brains assayed for morphine as described. The ordinate shows brain morphine levels (ng free base/g, mean ± S.E.M., $n = 6$–12). Morphine was not detected in animals receiving either saline or ibogaine alone (not shown).

administration ((+)-amphetamine sulfate, 1.25 mg/kg i.p.), ibogaine induced a fourfold increase in amphetamine levels when measured 2 h after (+)-amphetamine administration. A 30 min ibogaine pretreatment (40 mg/kg i.p.) also induced nearly a fourfold increase in brain amphetamine levels 2 h after (+)-amphetamine administration.

Brain morphine levels varied considerably after i.p. injection (Fig. 1), but the values are in agreement with previous results[6,7]. Although there may have been a slight enhancement of brain morphine levels by ibogaine 30 min after morphine, clearly such an effect was not found 2 h after morphine (Fig. 1), indicating that previous findings of ibogaine's modulation of morphine's actions observed at this time are not complicated by ibogaine-morphine drug interactions. In previous studies ibogaine pretreatment blocked morphine-induced dopamine release in three brain regions (nucleus accumbens, striatum and medial prefrontal cortex) and decreased morphine-induced locomotor activity[10,12], both kinds of effects lasting for at least 3 h after morphine administration. The present data indicate that these functional results cannot be attributed

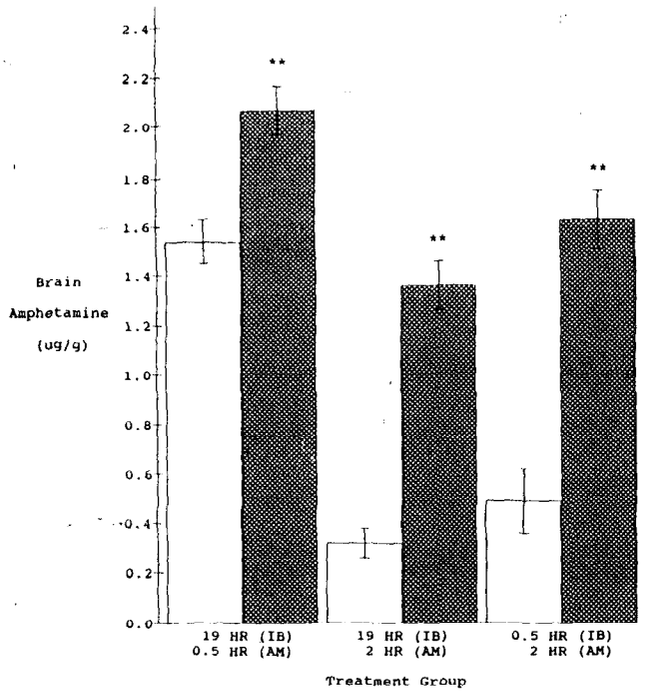

Fig. 2. Effect of ibogaine on brain amphetamine levels. Animals received either ibogaine hydrochloride (40 mg/kg, i.p., hatched bars) or saline (2 ml/kg, open bars). After the intervals shown (abscissa, labeled IB), (+)-amphetamine sulfate (1.25 mg/kg, i.p.) was administered, and the animals were decapitated either 0.5 or 2 h later, as labeled (abscissa, AM). Whole brains were assayed for amphetamine as described. The ordinate shows brain amphetamine levels (μg free base/g, mean $\pm$ S.E.M., $n = 5$–10). Amphetamine was not detected in animals receiving either saline or ibogaine alone (not shown). * $P < 0.01$ by t-test compared to respective saline control.

to a metabolic or pharmacokinetic interaction between ibogaine and morphine.

The pattern of ibogaine-induced enhancement of amphetamine levels strongly suggests that ibogaine is a potent inhibitor of amphetamine metabolism (Fig. 2). In vitro studies are required to document this. Amphetamine levels found presently are also in agreement with previous reports[9].

The present data suggest that the previously reported potentiation of (+)-amphetamine's effects (i.e., enhanced release of dopamine in nucleus accumbens and striatum, increased locomotor stimulation[11]) may be mostly or entirely due to an ibogaine-induced increase in brain amphetamine levels, probably as a result of decreased hepatic metabolism of (+)-amphetamine. Indeed, at 2 h after (+)-amphetamine injection, ibogaine pretreatment (19 h) increased brain amphetamine levels approximately fourfold (Fig. 2) and functionally, 2-3 h after (+)-amphetamine administration, sensitivity to both the neurochemical and behavioral effects of (+)-amphetamine was also increased approximately fourfold (see Figs. 1, 2 and 4 in ref. 11).

In view of ibogaine's reportedly[3] short half-life (1 h), it was previously suggested[4,10] that long-lasting effects of ibogaine might be due to an active metabolite. In the present study, we reasoned that if an active metabolite of ibogaine were responsible for the effect of the 19 h ibogaine pretreatment on brain amphetamine levels, then administering ibogaine only 30 min before (+)-amphetamine should have little or no effect on brain amphetamine levels; on the other hand, if the effect of the 19 h pretreatment was due to persistent low levels of ibogaine, then administering ibogaine only 30 min before (+)-amphetamine should have a much greater effect on brain amphetamine levels. Neither of the latter two possible results occurred; rather, the effects of the 19 h and 30 min ibogaine pretreatments were nearly identical (Fig. 2), suggesting that ibogaine might irreversibly inhibit an amphetamine-metabolizing enzyme.

The relevance of these ibogaine-amphetamine interactions in the rat to the anti-addictive claim regarding stimulant abuse in humans is unclear. There are substantial differences in amphetamine metabolism among species, particularly between rats and humans[2]. It is quite possible that ibogaine's functional interactions with (+)-amphetamine may be quite different in the absence of a hepatic drug-drug interaction; further studies will attempt to evaluate this possibility.

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