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Acute and prolonged effects of ibogaine on brain dopamine metabolism and morphine–induced locomotor activity in rats

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Ibogaine, an indolalkylamine, proposed for use in treating opiate and stimulant addiction, has been shown to modulate the dopaminergic system acutely and one day later. In the present study we sought to systematically determine the effects of ibogaine on the levels of dopamine (DA) and the dopamine metabolites 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in tissue at several time points, between 1 h and 1 month post-injection. One hour after ibogaine-administration (40 mg/kg i.p.) a 50% decrease in DA along with a 37–100% increase in HVA were observed in all 3 brain regions studied: striatum, nucleus accumbens and prefrontal cortex. Nineteen hours after ibogaine-administration a decrease in DOPAC was seen in the nucleus accumbens and in the striatum. A week after administration of ibogaine striatal DOPAC levels were still reduced. A month after ibogaine injection there were no significant neurochemical changes in any region. We also investigated the effects of ibogaine pretreatment on morphine-induced locomotor activity, which is thought to depend on DA release. Using photocell activity cages we found that ibogaine pretreatment decreased the stimulatory motor effects induced by a wide range of morphine doses (0.5–20 mg/kg, i.p.) administered 19 h later; a similar effect was observed when morphine (5 mg/kg) was administered a week after ibogaine pretreatment. No significant changes in morphine-induced locomotion were seen a month after ibogaine pretreatment. The present findings indicate that ibogaine produces both acute and delayed effects on the tissue content of DA and its metabolites, and these changes coincide with a sustained depression of morphine-induced locomotor activity.

INTRODUCTION

Ibogaine is the main alkaloid found in the root of Tabernanthe iboga, a shrub that grows in West Central Africa. Ibogaine has been used, at low doses, as a stimulant and, at high doses, for its hallucinogenic properties. In 1985 and 1986 two US patents[10,11] described the potential efficacy of ibogaine in treating opiate and stimulant addiction. Possibly consistent with those claims, recent studies have shown that ibogaine pretreatment interferes with the neurochemical and behavioral responses of rats to morphine[6,12] and D-amphetamine[13].

Using microdialysis, it has been reported[12] that ibogaine acutely increases extracellular dopamine (DA) levels in the prefrontal cortex, decreases extracellular DA levels in the striatum and has no significant effect in the nucleus accumbens. Furthermore, 19 h after its administration, ibogaine has been shown to decrease extracellular levels of the DA metabolites 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic acid (HVA) in the prefrontal cortex, striatum and nucleus accumbens. The mechanism of action by which ibogaine modulates DA neurons is unclear. In radioligand binding assays ibogaine has been reported to interact with kappa receptors and with voltage-dependent sodium channels[3]. However, it is difficult to explain the effects of ibogaine by those 2 direct interactions alone. That is, ibogaine appears to have prolonged effects (at least 19 h) which are hard to reconcile with its short half-life (1 h in rodent[4]); the formation of one or more active metabolites, possibly with a different pharmacological profile, has been postulated. In the present study, in an effort to gain further insights into ibogaine's mechanisms of action, we examined its effects on postmortem tissue content of DA and its metabolites at several time points, between 1 h and 1 month. Since locomotor stimulatory effects of morphine are thought to be mediated via DA release[9], changes induced by ibogaine in the activity of DA pathways could presumably be translated into locomotor changes. Therefore, using photocell activity cages, we examined the effects of ibogaine pretreatment on the locomotor effects induced by morphine at different time points.

MATERIALS AND METHODS

Drugs

Ibogaine HCl was obtained from Sigma Chemical Co. and was dissolved in water at a concentration of 20 mg/ml. Morphine sul-

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fate, obtained from Mallinckrodt, was dissolved in saline. Both drugs were injected intraperitoneally (i.p.) in doses expressed as the salt.

Animal preparation and tissue dissection

Female Sprague-Dawley rats (weighing 250–275 g) were pretreated with either ibogaine HCl (40 mg/kg) or saline (2 ml/kg) 1 h, 19 h, 1 week or 1 month before being decapitated. (Ibogaine's effects were studied at 19 h and not 24 h in order to match studies already published with ibogaine[12]). After decapitation, the brains were removed and frozen by immersion in ice-cold *n*-pentane. The brains were then placed in a rat brain matrix (Zivic-Miller) and 3 slices were taken. Prefrontal cortex was dissected from the first slice (3–6 mm from the anterior pole of the brain), nucleus accumbens from the second (6–8 mm), and striatum from the third (8–11 mm). Tissue from both hemispheres was pooled for each region.

Assay of dopamine and metabolites

Tissue samples were homogenized in perchloric acid (0.1 M) containing 3,4-dihydroxybenzylamine as an internal standard. Homogenates were centrifuged for 20 min at 14,000 rpm. The supernatant was placed in filters (0.2 μm, Schleicher Sch centrifuged for 5 min at 2500 rpm. The filtered supern then analyzed by HPLC-EC. The HPLC system includ pump (PM 30A), a BAS amperometric detector (LC-4 glassy carbon electrode set at a potential of 0.77 V with a silver-silver chloride reference electrode. The analytic was a 25 cm BAS biophase ODS 5 μm column. The mo consisted of 300 ml of 0.1 M citric acid, 160 ml of 0.1 M 1 10 mg of sodium octyl sulfate and 50 ml of methanol. Ti of DA and its metabolites, DOPAC and HVA, were d by means of internal and external standards and express of wet tissue. The limit of detection of our assay was 1 DA, 18.9 pg for DOPAC and 27.3 pg for HVA.

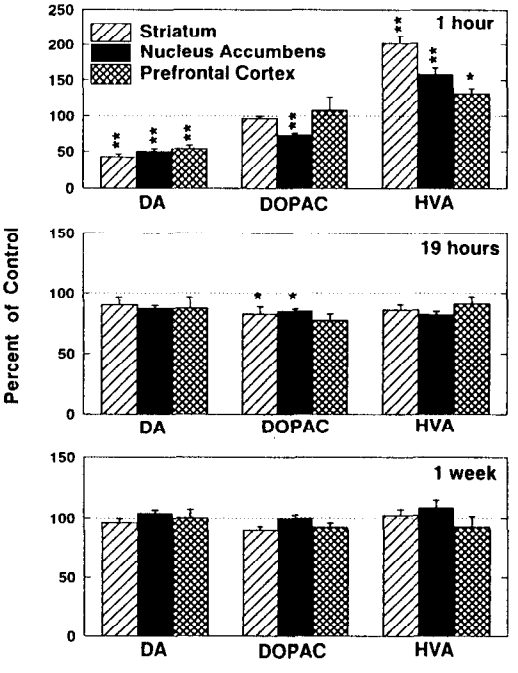

Fig. 1. Changes, expressed as percent of control ± S.E.M., in postmortem tissue levels of DA, DOPAC and HVA in the striatum, nucleus accumbens and prefrontal cortex 1 h (top panel, n = 6), 19 h (central panel, n = 6) or a week (bottom panel, n = 12) after injection of ibogaine (40 mg/kg). $^*P < 0.05$ and $^{**}P < 0.01$ as compared to saline pretreatment (Least Significant Difference test). Control values, expressed as μg/g of wet tissue (± S.E.M.), were at 1 h post-ibogaine administration in the striatum DA 10.47 ± 0.63, DOPAC 2.56 ± 0.16, HVA 0.97 ± 0.041; in the nucleus accumbens DA 8.48 ± 0.38, DOPAC 3.82 ± 0.17, HVA 0.83 ± 0.073; and in the prefrontal cortex DA 0.085 ± 0.011, DOPAC 0.039 ± 0.005, HVA 0.040 ± 0.006. At 19 h there were in the striatum DA 9.27 ± 0.70, DOPAC 2.22 ± 0.12, HVA 0.75 ± 0.034; in the nucleus accumbens DA 7.94 ± 0.39, DOPAC 3.56 ± 0.16, HVA 0.72 ± 0.038; and in the prefrontal cortex DA 0.107 ± 0.033, DOPAC 0.041 ± 0.006, HVA 0.031 ± 0.004. At 1 week there were in the striatum DA 9.75 ± 0.34, DOPAC 2.43 ± 0.069, HVA 0.85 ± 0.046; in the nucleus accumbens DA 8.20 ± 0.44, DOPAC 3.54 ± 0.18, HVA 0.74 ± 0.053; and in the prefrontal cortex DA 0.074 ± 0.005, DOPAC 0.037 ± 0.002, HVA 0.032 ± 0.003.

Motor activity monitoring

Locomotor activity was measured in photocell activi Interruptions of the infrared photocell beams were recor Apple IIe computer. Rats were pretreated with saline (2 ibogaine (40 mg/kg) 19 h before the administration of sa kg) or morphine (0.5–20 mg/kg); in addition, other rats treated with saline (2 ml/kg) or ibogaine (40 mg/kg) 1 month before the administration of saline (1 ml/kg) or tory dose (5 mg/kg) of morphine. Immediately after the injection rats were placed in activity boxes and locom measured for 3 h. Rats were maintained on a normal ligl cle (lights on/off at 7:00 a.m./7:00 p.m.) and experim conducted during the light phase in a quiet room.

Statistical analysis

For the chemical data a 3-way analysis of variance was performed to evaluate for pretreatment and time eff regions for each amine. When appropriate ANOVAs wer in order to test for pretreatment and time effects in e for each amine. Any significant interaction was subseq

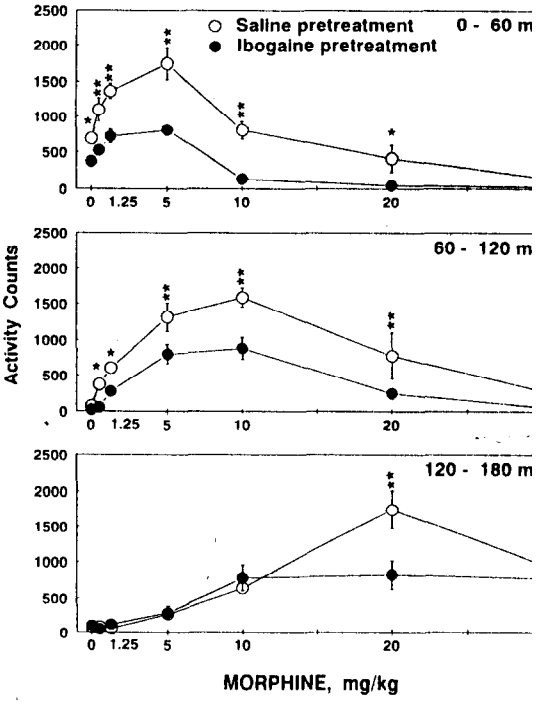

Fig. 2. Effects of a saline or ibogaine (40 mg/kg) pretr the dose-response curve of morphine. Morphine was ad at time zero and the pretreatments 19 h before. Each p sents the average activity counts (± S.E.M.) of 6 rats f of 1 h. $^*P < 0.05$ and $^{**}P < 0.001$ as compared to salir ment (Least Significant Difference test).

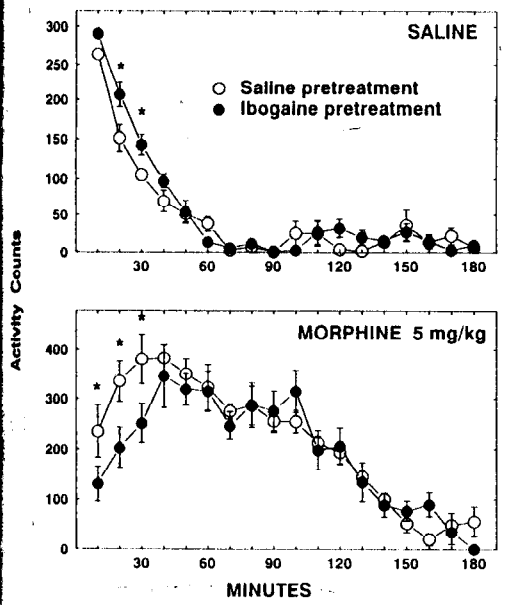

Fig. 3. Effects of saline (n = 6) or ibogaine (40 mg/kg, n = 6) on locomotor activity induced by saline or by morphine (5 mg/kg) 1 week after their administration. Each point represents the average activity counts (± S.E.M.) for a period of 10 min. *$P < 0.05$ as compared to saline pretreatment (Least Significant Difference test).

composed using the Least Significant Difference (LSD) test. For the behavioral data an ANOVA was used to test for pretreatment, dose and time effects. Each rat received 1 pretreatment and 1 dose of morphine. Post-hoc analyses were carried out using the LSD multiple comparison tests.

RESULTS

Effects of ibogaine pretreatment on DA and its metabolites

One hour after injection of 40 mg/kg of ibogaine (Fig. 1, top panel) significant effects were observed in all 3 regions studied. Ibogaine decreased DA levels (54% of control in the prefrontal cortex, $P < 0.0006$; 51% in the nucleus accumbens, $P < 0.00001$; 42% in the striatum, $P < 0.00001$) and increased HVA levels (137% of control in the prefrontal cortex, $P < 0.02$; 158% in the nucleus accumbens, $P < 0.00001$; 200% in the striatum, $P < 0.00001$). In addition, in the nucleus accumbens only, a decrease in DOPAC (73% of control, $P < 0.0002$) was noted.

Nineteen hours after administration (Fig. 1, central panel) ibogaine induced a decrease in DOPAC in the nucleus accumbens (85% of control, $P < 0.05$) and in the striatum (83%, $P < 0.05$); this effect in the prefrontal cortex was not significant.

One week after ibogaine (Fig. 1, bottom panel) no effects in the prefrontal cortex or in the nucleus accumbens were significant; however, there was a marginal decrease in striatal DOPAC (90% of control, $P < 0.06$). Because of the latter unexpected result, this experiment was repeated and a decrease in striatal DOPAC was found again ($P < 0.05$, t-test for the repeated experiment and $P < 0.025$, t-test for both experiments combined). No significant effects were detectable in any region 1 month after ibogaine injection (data not shown).

Effects of ibogaine on behavioral response to morphine

Ibogaine pretreatment 19 h prior to measuring motor responses induced by a wide range of morphine doses (Fig. 2) led to significant decreases in locomotor activation, mainly during the first 2 h (pretreatment × time, $P < 0.00001$), for all doses of morphine except 30 mg/kg. During the third hour only the activity induced by morphine 20 mg/kg was reduced ($P < 0.00001$). It should be noted that ibogaine itself had an inhibitory effect on locomotion, but only during the first h ($P < 0.015$). In contrast, 1 week after its administration, ibogaine had a stimulatory effect on locomotion (first h, $P < 0.03$), yet still inhibited morphine (5 mg/kg)-induced locomotion during the same time period ($P < 0.019$) (Fig. 3). A month after its administration, ibogaine had no effects on locomotion and did not alter the morphine induced locomotor activity (data not shown).

DISCUSSION

Using in vivo microdialysis, we had previously found that acute ibogaine treatment produced markedly different changes in extracellular DA levels depending on the region studied (see Introduction). In the present investigation, we have studied the effects of ibogaine on postmortem DA tissue levels. Although tissue levels correspond to a combination of intra- and extracellular levels, the extracellular fraction of DA is infinitesimally small and thus tissue levels are primarily a reflection of intracellular stores.

The pervasive and substantial effects of acute ibogaine administration, namely a 50% decrease in DA levels, could be explained by a large release of DA either in the cytoplasmic environment or in the extracellular fluid. The lack of increase in DOPAC levels argues against an intraneuronal DA release. The increase in HVA, but not in DOPAC, may reflect a change in the balance between intraneuronal and extraneuronal metabolism of DA and could correspond to a release of DA in the extracellular fluid coupled with a blockade of the uptake system. However, if such a large DA release occurred, an increase in extracellular DA should have been noticed immediately after ibogaine administration in our previous studies[12]. Although differences between in vivo extracellular measurements and postmortem tissue levels are not

unusual and have been noted by other authors[8], it is unfortunate that our assay did not allow us to measure 3-methoxytyramine, since an increase in this extraneuronal metabolite might have clarified this enigma. The action of ibogaine on voltage dependent sodium channels[3] could partially account for an extraneuronal DA release. Since the affinity of ibogaine for this channel is 8.1 μM[3], and the brain concentration of ibogaine is estimated to be about 10 μM 1 h after i.p. administration of 50 mg/kg ibogaine[4], it is not likely that this interaction would lead to full depolarization; however, it could increase the intracellular Na^+ concentration, reducing the inward Na^+ gradient and thus causing DA release through the transport carrier and impeding the DA uptake[14]. It is doubtful that this action alone could explain the 50% decrease in DA levels. A neurotoxic effect is unlikely, since it has been shown[15] that lesions of the nigrostriatal DA pathway (medial forebrain bundle) increase striatal DA levels for at least 20 h before producing a marked decrease 48 h later. Intracerebroventricular injections of 6-hydroxydopamine have led to the same results[2].

Depletion of intracellular DA levels has been shown to enhance tyrosine hydroxylase activity[5] which could explain why DA levels return to their basal values 19 h after an ibogaine challenge. At that time the decrease in DOPAC could result from the diversion of newly synthesized DA, from which DOPAC originates[16], into DA storage pools. A parallel decrease in metabolites has also been observed in the extracellular fluid[12]. The fact that a single injection of ibogaine can still affect, a week later, the levels of DOPAC in the striatum was surprising and we were skeptical at first. However, this finding was readily replicated and suggests that ibogaine either induces the formation of a long-active metabolite and/or induces a persistent change in neuronal activity.

Concurrent with the neurochemical changes, ibogaine also affected locomotor activity, decreasing it 19 h post-injection and increasing it a week later. The decreases in tissue DOPAC levels observed at these 2 time points cannot, alone, explain the behavioral changes, but are suggestive of a long-term ibogaine-induced alteration of DA systems which is translated into locomotor changes. A month after ibogaine administration no changes in DA metabolism and no changes in locomotion were observed. The motor changes induced by ibogaine could presumably be due to effects on DA release which would not be detected in the brain homogenates used in this study. In a previous study[12], basal DA levels, 19 h after ibogaine administration, were not altered in the nucleus accumbens, but were markedly decreased in the striatum. It is possible that ibogaine-induced changes in striatal function might alter morphine-induced rigidity (i.e. catalepsy) and thus secondarily affect locomotion, which is thought to be primarily mediated by the nucleus accumbens.

A single dose of ibogaine injected 19 h prior to morphine decreased the locomotor activity induced by a wide range of morphine doses. Morphine affects locomotion in a time- and dose-dependent manner: low doses increase activity and high doses initially inhibit and then stimulate activity[1]. Ibogaine pretreatment seems to affect mainly the hyperactive phase, although it is possible that ibogaine reduces the hyperactivity by potentiating morphine's inhibitory effects.

In conclusion, the present data indicate that ibogaine produces both acute and delayed effects on the brain content of DA and its metabolites, and these changes coincide with a sustained depression of morphine-induced activity. Further studies are needed in order to understand the underlying mechanisms of ibogaine's actions and its interactions with morphine.

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