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Ibogaine and noribogaine potentiate the inhibition of adenylyl cyclase activity by opioid and 5-HT receptors

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Abstract

The effects of the putative anti-addictive compound ibogaine and its principal metabolite, noribogaine, on adenylyl cyclase activity were determined in various areas of the rat brain. Neither compound altered either basal or forskolin-stimulated adenylyl cyclase activities in the frontal cortex, midbrain or striatum. However, in all three brain areas the addition of ibogaine and noribogaine significantly enhanced inhibition of adenylyl cyclase activity by a maximally effective concentration of morphine. Similarly, both compounds also potentiated the inhibition of hippocampal adenylyl cyclase activity by a maximally effective concentration of 5-hydroxytryptamine (5-HT). Although ibogaine appears to be more potent than noribogaine in augmenting opioid- and 5-HT-mediated inhibition of adenylyl cyclase activity, both compounds appear to be of comparable efficacy. Neither compound, however, modified the inhibitory action of the muscarinic acetylcholine agonist, carbachol, on adenylyl cyclase activity. The present data indicate that ibogaine and noribogaine cause a selective increase in receptor-mediated inhibition of adenylyl cyclase activity. This potentiation may be involved in the pharmacological actions of these compounds.

Keywords: Ibogaine; Noribogaine; Adenylyl cyclase; 5-HT receptors; Opioid receptors; Muscarinic acetylcholine receptors

1. Introduction

Ibogaine, an indole alkaloid found in the West African shrub *Tabernanthe iboga*, has been suggested to be efficacious in treating alcohol, opiate, stimulant (amphetamine and cocaine) and nicotine addiction (see Popik et al., 1995). In a small uncontrolled study involving individuals addicted to opiates, a single dose of ibogaine reportedly resulted in cessation of opiate use in several patients for at least 14 weeks as well as reducing alcohol and tobacco consumption (Sheppard, 1994). In animals ibogaine has been shown to decrease self-administration of morphine (Glick et al., 1991, 1994), cocaine (Cappendijk and Dzolijic, 1993; Glick et al., 1994; Sershen et al., 1994) and alcohol (Rezvani et al., 1995). In some rats the reduction in morphine intake persisted for several days or weeks (Glick et al., 1991). Ibogaine also has been shown to attenuate the naloxone-precipitated withdrawal syndrome in morphine-dependent mice (Cappendijk et al., 1994) as well as to antagonize morphine-induced locomotor activity for at least one week (Maisonneuve et al., 1992).

The mechanisms by which ibogaine produces its pharmacological actions are unclear. Because ibogaine exerts effects after the concentration of the parent compound has fallen to insignificant levels, a possible role for one or more active metabolites has been suggested (Mash et al., 1995a,b; Pearl et al., 1995). Based on radioligand binding data, putative actions of ibogaine and its primary metabolite noribogaine (12-hydroxyibogamine) at opioid, σ, 5-HT, NMDA, and acetylcholine receptors have been suggested (Deecher et al., 1992; Popik et al., 1994; Pearl et al., 1995; Mash et al., 1995b; Sweetnam et al., 1995; Bowen et al., 1995; Mach et al., 1995). Ibogaine also has been shown to bind to monoamine transporters (Mash et al., 1995a; Sweetnam et al., 1995), and in vivo alterations in extracellular 5-hydroxytryptamine (5-HT) and dopamine levels have been reported after administration of ibogaine and noribogaine (Maisonneuve et al., 1992; Mash et al., 1995a).

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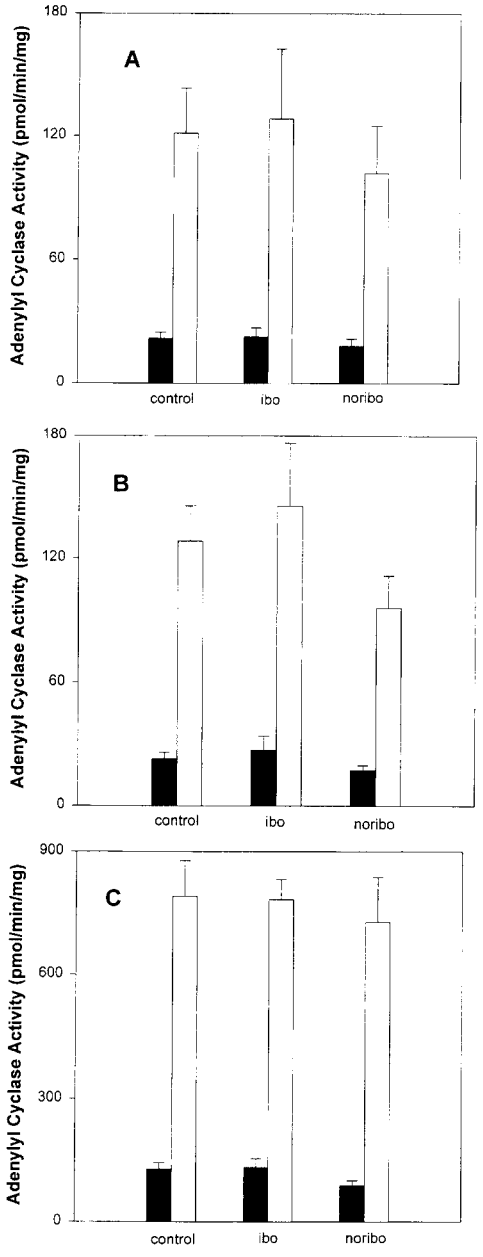

Fig. 1. Effects of ibogaine and noribogaine on basal and forskolin-stimulated adenylyl cyclase activity in the frontal cortex (A), midbrain (B) and striatum (C). Basal (solid bars) and forskolin-stimulated (10 μM; open bars) adenylyl cyclase activities were determined in the absence (control) and presence of 333 μM ibogaine (ibo) or 333 μM noribogaine (noribo). Data are plotted as mean ± S.E.M. from 4–8 animals.

Because several of the above neurotransmitters exert their cellular actions through a modulation of adenylyl cyclase activity, the present study was undertaken to investigate the effects of ibogaine and noribogaine on this enzyme. Our results show that while neither ibogaine nor noribogaine alone altered adenylyl cyclase activity, both compounds potentiated the inhibition of enzyme activity by opioid and serotonin receptors, but not muscarinic acetylcholine receptors.

2. Materials and methods

Male F-344 rats were killed by decapitation, and the brains were dissected into frontal cortex, midbrain, hippocampus and striatum (Glowinski and Iversen, 1966).

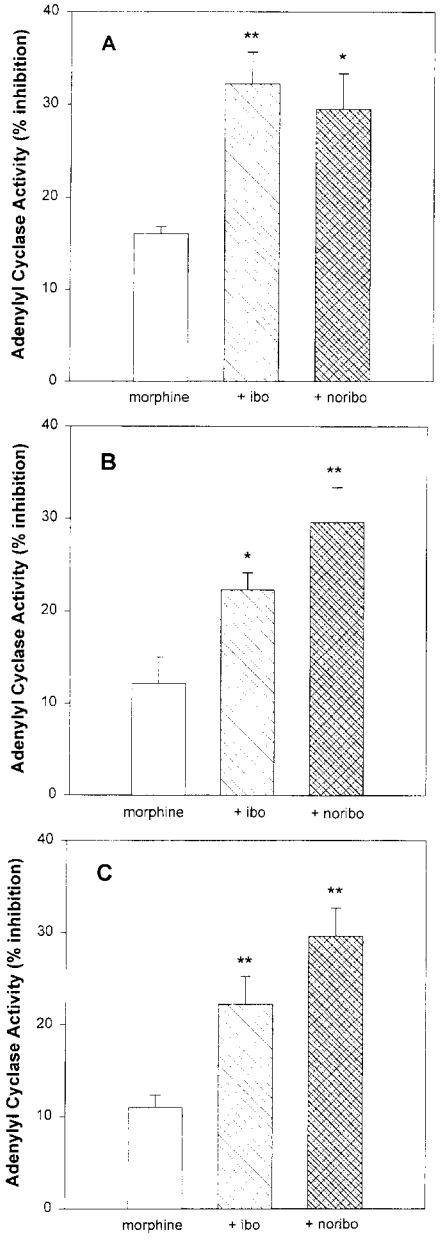

Fig. 2. Ibogaine and noribogaine potentiate the inhibition of adenylyl cyclase activity by morphine in the frontal cortex (A), midbrain (B) and striatum (C). The ability of 33 μM morphine sulfate to inhibit forskolin-stimulated (10 μM) adenylyl cyclase activity was determined in the absence (open bars) and presence of 333 μM ibogaine (+ibo; striped bars) or 333 μM noribogaine (+noribo; cross-hatched bars). Data are expressed as the percent decrease in forskolin-stimulated enzyme activity, and are plotted as mean ± S.E.M. from 4–6 animals. Forskolin-stimulated enzyme activity in the frontal cortex, midbrain and striatum were 121 ± 22.0, 128 ± 17.3 and 791 ± 87.1 pmol/min per mg, respectively. * $P < 0.05$, ** $P < 0.01$ compared to morphine alone.

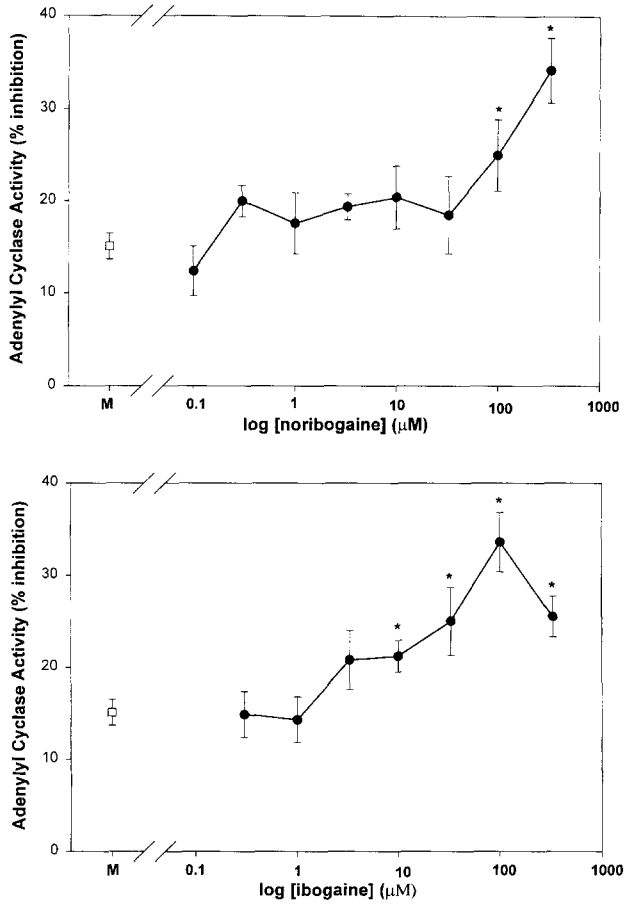

Fig. 3. Concentration–response relationships for ibogaine and noribogaine: Potentiation of morphine-induced enzyme inhibition. The ability of various concentrations of ibogaine (bottom panel) and noribogaine (top panel) to enhance the inhibition of forskolin-stimulated (10 μM) adenylyl cyclase activity by 33 μM morphine sulfate was determined in frontal cortex. Data are expressed as the percent decrease in forskolin-stimulated enzyme activity (154 ± 16.5 pmol/min per mg), and are plotted as the mean ± S.E.M. from 4–5 animals. Addition of morphine (M) alone is indicated by the open box (□). * $P < 0.05$ compared to morphine alone.

Tissues were homogenized (Dounce tissue grinder) in 2 mM Tris–HCl (pH 7.4) containing 2 mM EGTA, 300 mM sucrose and 2 mM dithiothreitol, and the homogenates were centrifuged at 4°C for 15 min at 40000 × *g*. The resulting pellets were resuspended in the above Tris buffer and again centrifuged at 40000 × *g* for 15 min. The final pellet was resuspended in the same Tris buffer (20 mg w.w./ml) and used immediately for the adenylyl cyclase assay.

Adenylyl cyclase activity was determined at 30°C in a final volume of 200 μl consisting of 50 mM Tris–HCl (pH 7.4), 5 mM cyclic AMP, 2 mM $MgCl_2$, 1 mM isomethylbutylxanthine, 100 mM NaCl, 50 μM GTP, 10 mM creatine phosphate, 0.1 mg/ml creatine kinase, 0.1 mM ATP (approximately 1 μCi α-[^{32}P]ATP and appropriate drugs. Assays were terminated after 10 min by the addition of 100 μl of 50 mM Tris–HCl (pH 7.4) containing 5 mM ATP and 10% sodium dodecyl sulfate. After a 10 min incubation in boiling water, 20000 cpm of [^{3}H]cyclic AMP were added to each tube to monitor recovery, and the [^{32}P]cyclic AMP was isolated by successive Dowex and alumina column chromatography as previously described (Rabin and Molinoff, 1983). Protein content was determined by the method of Lowry et al. (1951).

2.1. Materials

Animals were purchased from Harlan Sprague–Dawley (Indianapolis, IN). α-[^{32}P]ATP and [^{3}H]cyclic AMP were purchased from DuPont/New England Nuclear (Boston, MA, USA). Ibogaine and noribogaine were generously provided by NIDA. All other reagents were obtained from commercial suppliers.

3. Results

Basal adenylyl cyclase activity in frontal cortex, striatum and midbrain were not altered by the addition of either ibogaine or its metabolite noribogaine (Fig. 1). The possibility that these agents might inhibit adenylyl cyclase activity also was investigated; to facilitate detection of an inhibition of enzyme activity, assays were carried out in the presence of forskolin. Neither ibogaine nor noribogaine altered forskolin-stimulated enzyme activity in the different brain areas (Fig. 1). Because ibogaine has been reported to bind to opioid receptors (Deecher et al., 1992; Sweetnam et al., 1995; Pearl et al., 1995) and to antago-

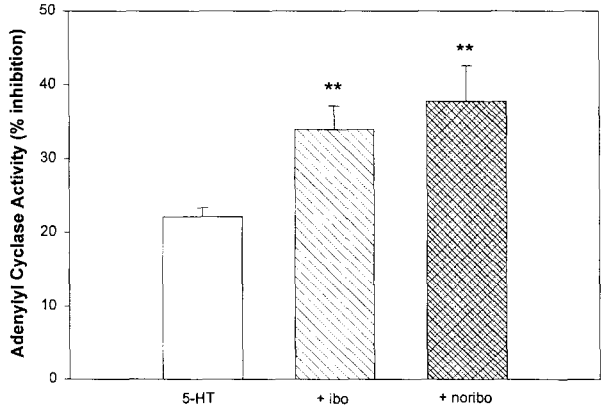

Fig. 4. Inhibition of hippocampal adenylyl cyclase activity by serotonin is enhanced by ibogaine and noribogaine. Inhibition of forskolin-stimulated (10 μM) adenylyl cyclase activity by 33 μM 5-hydroxytryptamine was determined in the absence (open bar) and presence of 333 μM ibogaine (+ibo; striped bars) and 333 μM noribogaine (+noribo; cross-hatched bars). Data are expressed as the percent decrease in forskolin-stimulated enzyme activity (136 ± 6.4 pmol/min per mg), and are plotted as mean ± S.E.M. for 5–7 animals. ** $P < 0.01$ compared to serotonin alone.

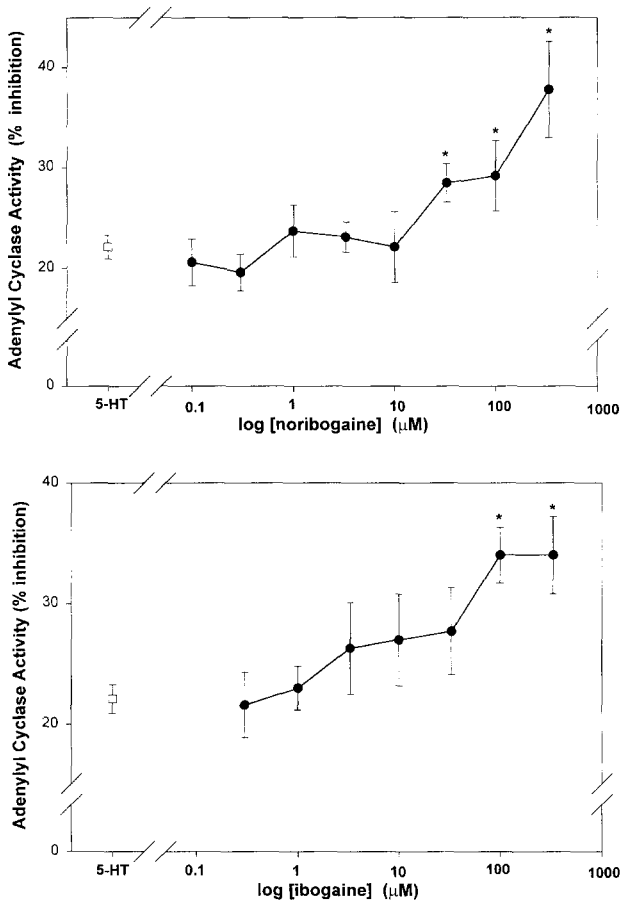

Fig. 5. Concentration–response relationships for ibogaine and noribogaine: Potentiation of 5-HT-induced enzyme inhibition. The ability of various concentrations of ibogaine (bottom panel) and noribogaine (top panel) to enhance the inhibition of forskolin-stimulated (10 μM) adenylyl cyclase activity by 33 μM 5-HT was determined in the hippocampus. Data are expressed as the percent decrease in forskolin-stimulated enzyme activity (119±8.8 pmol/min per mg), and are plotted as the mean±S.E.M. The addition of 5-HT alone is indicated by the open box (□). * $P < 0.05$ compared to 5-HT alone.

nize morphine-induced locomotor activity (Maisonneuve et al., 1992), experiments were carried out to determine whether this compound could be an opioid antagonist. In the frontal cortex, striatum and midbrain the inhibitory action of morphine on forskolin-stimulated adenylyl cyclase activity was significantly potentiated by both ibogaine and noribogaine (Fig. 2). The concentration of morphine used for these studies, 33 μM, was shown in preliminary studies to be a maximally effective concentration (data not shown), yet in all three brain areas inclusion of ibogaine or noribogaine approximately doubled the inhibitory effect of morphine on forskolin-stimulated adenylyl cyclase activity. The potentiation by ibogaine and noribogaine was concentration dependent, although ibogaine appeared to be more potent with an EC_{50} of approximately 6 μM (Fig. 3).

The ability of ibogaine and noribogaine to alter adenylyl cyclase activity was not limited to potentiating the inhibitory actions of morphine. In the hippocampus ibogaine and noribogaine also significantly enhanced the inhibition of forskolin-stimulated adenylyl cyclase activity by a maximally effective concentration of 5-HT (Fig. 4). This enhancement of 5-HT-mediated inhibition of enzyme activity by ibogaine and noribogaine was concentration dependent, but ibogaine appeared to be more potent displaying an EC_{50} of approximately 7 μM (Fig. 5).

The increase in receptor-mediated inhibition of adenylyl cyclase activity by ibogaine and noribogaine, however, does not appear to be a general phenomena. Although the muscarinic acetylcholine agonist, carbachol, inhibited forskolin-stimulated adenylyl cyclase activity in the frontal cortex, this inhibitory effect was not altered by the inclusion of ibogaine ($P = 0.52$) or noribogaine ($P = 0.17$) (Fig. 6). In these same tissue preparations, an enhanced inhibition of enzyme activity by morphine was observed in the presence of ibogaine and noribogaine (Fig. 6).

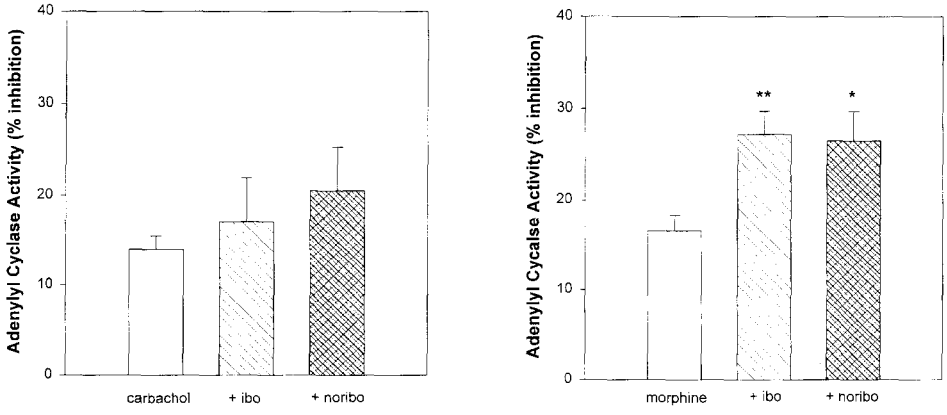

Fig. 6. Effects of ibogaine and noribogaine on muscarinic acetylcholine receptor-mediated (left) and opioid receptor-mediated (right) inhibition of adenylyl cyclase activity in frontal cortex. Inhibition of forskolin-stimulated (10 μM) adenylyl cyclase activity by 33 μM carbachol or 33 μM morphine sulfate was determined in the absence (open bars) and presence of 333 μM ibogaine (+ibo; striped bars) and 333 μM noribogaine (+noribo; cross-hatched bars). Data are expressed as the percent decrease in forskolin-stimulated enzyme activity (165 ± 10.5 pmol/min per mg), and are plotted as the mean ± S.E.M. from 8–11 animals. * $P < 0.05$, ** $P < 0.01$ compared to morphine alone.

4. Discussion

In the present study ibogaine and its principal metabolite, noribogaine, were shown to regulate adenylyl cyclase activity. However, this effect does not appear to fit the classical model by which ligands regulate adenylyl cyclase activity. Thus, neither ibogaine nor noribogaine when added alone altered adenylyl cyclase activity, but both compounds significantly increased receptor-mediated inhibition of enzyme activity. This potentiation was observed when adenylyl cyclase activity was inhibited by morphine and 5-HT, but not when enzyme activity was inhibited by carbachol. In addition, the enhanced inhibitory effect of morphine was found in various brain areas. Thus, it would appear that the potentiation of receptor-mediated inhibition of adenylyl cyclase activity by ibogaine and noribogaine is not a generalized phenomena, but rather displays a receptor specificity.

Gallagher et al. (1995) reported brain levels of ibogaine were approximately 13 μM 1 h after an i.p. injection of 40 mg/kg, a behaviorally effective dose of ibogaine. Similarly, in vitro concentration–response relationships for ibogaine indicate that the EC_{50} for enhancing receptor-mediated inhibition of adenylyl cyclase activity was less than 10 μM. Thus, it would appear that this action of ibogaine on adenylyl cyclase activity occurs over a behaviorally relevant concentration range. Ibogaine has been reported to exert long-lasting effects (Glick et al., 1991; Maisonneuve et al., 1992) that cannot be accounted for by a long biological half-life (Gallagher et al., 1995). The present data suggest that the prolonged actions of ibogaine are not due to the compound being active at low concentrations, but are consistent with noribogaine, the principal metabolite of ibogaine, being biological active. It remains to be determined whether the biological half-life of noribogaine is sufficient to account for the prolonged behavioral effects observed after administration of ibogaine.

The mechanism by which ibogaine and noribogaine elicited a concentration-dependent increase in receptor-mediated inhibition of adenylyl cyclase activity is unclear. Because maximally effective concentrations of morphine and 5-HT were used, this potentiation is not due to ibogaine and noribogaine activating additional opioid and 5-HT receptors. However, the possibility that ibogaine and noribogaine unmask previously unavailable receptors can not be excluded. Because these compounds alone did not alter adenylyl cyclase activity, the enhanced inhibition of enzyme activity cannot be attributed to activation of another receptor that is negatively coupled to adenylyl cyclase. Also, the assay conditions precluded a stimulation of phosphodiesterase activity as being responsible for the enhanced inhibitory effects on cyclic AMP production.

The pharmacological actions of ibogaine have been suggested to involve binding to the μ and κ opioid receptor subtypes (Deecher et al., 1992; Cappendijk et al., 1994; Sweetnam et al., 1995; Pearl et al., 1995; Codd, 1995). The present data, however, are not consistent with either ibogaine or noribogaine, directly binding to the ligand recognition site of the opioid receptor. Opioid receptors are negatively coupled to adenylyl cyclase (Childers, 1991), yet ibogaine and noribogaine by themselves neither inhibited enzyme activity nor antagonized morphine-induced inhibition of adenylyl cyclase activity. Thus, these compounds do not appear to be opioid receptor agonists or antagonists. The present data indicate that the reported antagonism of morphine-induced locomotor activity by ibogaine (Maisonneuve et al., 1992) is not due to blockade of the opioid receptor, but rather appears to represent functional antagonism. Also, the observed attenuation in the withdrawal syndrome in morphine-dependent mice by ibogaine (Cappendijk et al., 1994) cannot be attributed to ibogaine substituting as an agonist at the opioid receptor, but ibogaine might be expected to ameliorate the withdrawal symptoms by potentiating the effects of any residual morphine. The decreased alcohol drinking in rats (Rezvani et al., 1995) and in humans (Sheppard, 1994) after ibogaine administration also may involve an interaction with opioid receptor systems. Increasing opioid levels in the brain either by administering morphine and enkephalin or by blocking the degradation of endogenous opioids decreases alcohol consumption (George et al., 1991; Volpicelli et al., 1991; Froehlich and Li, 1993). It is possible that ibogaine may be reducing alcohol intake by potentiating the actions of the endogenous opioids.

A behavioral role for the increase in receptor-mediated inhibition of adenylyl cyclase activity in the presence ibogaine is speculative at present. Nevertheless, Schneider and McArthur (1956) found that although ibogaine did not produce analgesia, it did potentiate the analgesic effects of morphine. These in vivo results precisely mirror the in vitro effects of ibogaine on adenylyl cyclase activity. As a reduction in cyclic AMP content may be important for the antinociceptive action of μ and δ agonists (Wang et al., 1993), the observed enhanced analgesia may be due to ibogaine potentiating morphine-induced inhibition of adenylyl cyclase activity. It is interesting to note that the reduction in morphine self-administration by 18-methoxycoronaridine, a synthetic *iboga* alkaloid congener, recently was suggested to be due to an enhancement of morphine's action (Glick et al., 1996).

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