ACCELERATED COMMUNICATION

Ibogaine: A Potent Noncompetitive Blocker of Ganglionic/Neuronal Nicotinic Receptors

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SUMMARY

Ibogaine noncompetitively blocked ($IC_{50} \sim 20$ nM) ^{22}NaCl influx through ganglionic-type nicotinic receptor channels of rat pheochromocytoma PC12 cells. The major metabolite O-desmethylibogaine was 75-fold less active, and O-t-butyl-O-desmethylibogaine was 20-fold less active. Ibogaine was relatively weak as a blocker ($IC_{50} \sim 2000$ nM) of the neuromuscular-type nicotinic receptor channels in human medulloblastoma TE671 cells. The blockade of nicotinic responses by ibogaine was only partially reversible in PC12 cells. *In vivo*, ibogaine at 10 mg/kg completely blocked epibatidine-elicited antinociception in mice, a response that is mediated by central nicotinic receptor channels. There was no significant blockade of the epibatidine response at 24 hr after the administration of 40 mg/kg ibogaine. The blockade of nicotinic channels could contribute to the antiaddictive properties of ibogaine.

During the past decade, the apparent "antiaddictive" properties of the alkaloid ibogaine toward amphetamine, cocaine, and morphine have been extensively investigated, and there are patent claims for the use of ibogaine in the treatment of alcoholism and nicotine addiction (for a review, see Ref. 1). Definition of the mechanism or mechanisms responsible for the putative antiaddictive properties of ibogaine has proved to be difficult. *In vivo*, ibogaine at pharmacologically relevant doses reaches micromolar concentrations; such concentrations affect the binding of radioligands to a variety of receptors and channels (2, 3). However, attention has been focused on three of the most potent interactions [i.e., as an apparent agonist at κ-opioid receptors (4), as a ligand at σ_2 receptors (5), and as a noncompetitive blocker of NMDA receptor channels (1, 6, 7)].

The blockade of naloxone-induced jumping in morphine-dependent mice represents one paradigm for investigation of the tolerance to and dependence on morphine; ibogaine blocks the naloxone response (8, 9). Ibogaine also blocks morphine-elicited (10) and cocaine-induced (11) hyperactivity. Currently, the most likely mechanism involved in the antiaddictive effects of ibogaine seems to be blockade of NMDA receptor channels. Thus, O-desmethylibogaine and O-t-butyl-O-desmethylibogaine have much lower affinities than ibogaine for the NMDA receptor channels, and neither of these analogs blocks naloxone-induced jumping (9). At κ-opioid receptors, O-desmethylibogaine is more potent than ibogaine (9, 12), yet it is inactive in the naloxone-jumping assay (9). O-Desmethylibogaine does have a much lower affinity for σ_2 receptors than ibogaine (13), but the O-t-butyl analog and ibogaine have similar affinities.[1] Thus, on the basis of structure-activity profiles of ibogaine analogs, a blockade of NMDA receptor channels seems most likely to be involved in the antagonism of naloxone-induced jumping in morphine-dependent mice. In addition, other noncompetitive blockers of NMDA receptor channels block naloxone-induced jumping in morphine-dependent mice (1, 9, 14). A puzzling aspect of the *in vivo* effects of ibogaine is the apparent continuance of such effects long after the alkaloid should have cleared the body; a long-lasting active metabolite has been proposed (15). O-Desmethylibogaine, a major metabolite, does exhibit some of the antiaddictive properties of ibogaine (16), but it does not reduce naxolone-induced jumping in morphine-dependent mice (9).

The possible involvement of central nicotinic pathways in the pharmacology of ibogaine has been ignored because in binding assays of agonists to central neuronal ($\alpha 4 \beta 2$) nicotinic receptors, ibogaine has low activity as an inhibitor (2, 3).

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[1] W. D. Bowen, personal communication in Layer *et al* (9)

ABBREVIATION: NMDA, *N*-methyl-D-aspartate.

However, such agonist binding assays would not have detected an activity of ibogaine as a noncompetitive blocker at nicotinic receptors. Therefore, we investigated ibogaine as a noncompetitive blocker of nicotinic receptor channels in functional assays, in both cultured cells and *in vivo*.

Materials and Methods

[³H]Nicotine (76 Ci/nmol) was obtained from New England Nuclear Research Products (Boston, MA), and ²²NaCl was from Amersham Life Science (Clearbrook, IL). (−)-Nicotine ditartrate, (−)-epibatidine L-tartrate, (−)-ibogaine, and mecamylamine were from Research Biochemicals (Natick, MA); memantine was from Merz (Frankfurt, Germany); and carbamylcholine was from Sigma Chemical (St. Louis, MO). *O*-Desmethylibogaine and *O-t*-butyl-*O*-desmethylibogaine were provided by Dr. C. M. Bertha (National Institutes of Health, Bethesda, MD). The synthesis of these ibogaine analogs has been described previously (11).

Rat pheochromocytoma PC12 cells were provided by Dr. G. Guroff (National Institutes of Health, Bethesda, MD). Human medulloblastoma TE671 cells were from the American Type Culture Collection (Rockville, MD). Cell culture and ion flux assays were conducted as described previously (17). Cells were incubated either with or without antagonists in incubation buffer for 10 min before replacement with influx buffer containing 0.7 μCi of ²²NaCl and an agonist (either carbamylcholine or nicotine) either alone or with the antagonist. After 2 min, the influx buffer was removed; after washing, the incorporation of ²²NaCl was determined (see Ref. 18 and the legends to the figures for details).

Rat brains were obtained from Pelfreez Biological (Rogers, AK). Cerebral cortical membranes were prepared and [³H]nicotine binding was assayed as described previously (17).

Adult male NIH Swiss strain mice were used for assessment of hot-plate antinociceptive activity as described previously (17). (−)-Epibatidine was administered alone, concomitant with ibogaine or memantine, or 24 hr after ibogaine, and the hot-plate reaction time was determined at preset time intervals (see Ref. 17 and the legends to the figures for details).

Results

Ibogaine was a potent inhibitor (IC$_{50}$ ~ 20 nM) of carbamylcholine-induced ²²NaCl influx in PC12 cells, which contain ganglionic-type nicotinic receptors, and was 75-fold less potent in TE671 cells, which contain neuromuscular-type nicotinic receptors (Fig. 1). Blockade by ibogaine was not completely reversed in PC12 cells (Fig. 2A) (i.e., cells that were exposed to ibogaine either with or without carbamylcholine, then washed, and subsequently stimulated by the agonist carbamylcholine showed only a partial recovery of the response to carbamylcholine). In contrast, the blockade by mecamylamine was completely reversed by 30 min after washing (Fig. 2B). The irreversible action of chlorisondamine, which has been reported *in vivo* (17–20), was clearly demonstrated in PC12 cells (Fig. 2C). The copresence of an agonist during the preincubation with chlorisondamine was required for the irreversible action (see Fig. 2C, − *carb*). Copresence of an agonist was not required for ibogaine (Fig. 2A). The inhibition of nicotinic receptor-activated influx by ibogaine in PC12 cells seemed to be noncompetitive because it was not overcome by increasing concentrations of agonist (Fig. 3). The two analogs of ibogaine were much less potent as nicotinic antagonists in PC12 cells (Table 1). The classic ganglionic blockers mecamylamine and chlorisondamine had IC$_{50}$ values of 0.86 and 0.022 μM in PC12 cells, respectively
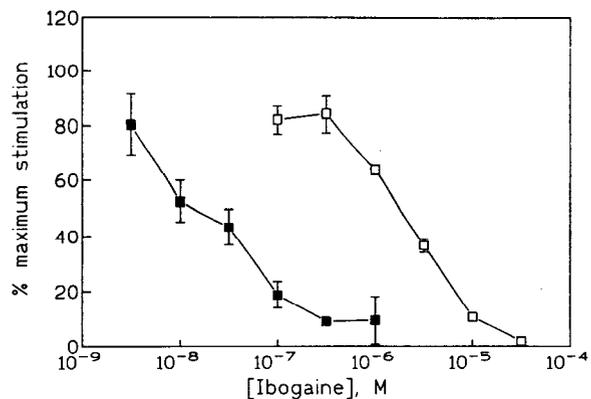

Fig. 1. Inhibition of carbamylcholine-elicited ²²NaCl influx in (■) rat pheochromocytoma PC12 cells and (□) human medulloblastoma TE671 cells in the presence of ibogaine. Assays were performed as described previously (17) with 2 mM carbamylcholine alone or with ibogaine. Each value is reported as a percentage of stimulation obtained with 2 mM carbamylcholine alone and is the mean ± standard error of three experiments.

(Table 1). Memantine, which, like ibogaine, is a noncompetitive blocker of NMDA receptor channels (14), was a relatively weak blocker at the nicotine receptor channels in PC12 cells (Table 1). A structurally similar agent, amantadine, is known to be a noncompetitive blocker of nicotinic channels (21).

Ibogaine, as expected for a noncompetitive blocker, had a relatively low affinity as an inhibitor of binding of the agonist [³H]nicotine to rat brain membranes (data not shown). The K_i value was 4000 ± 600 nM (mean ± standard error for three experiments).

In vivo, ibogaine at 10 and 40 mg/kg completely blocked the antinociceptive effect of (−)-epibatidine (Fig. 4). At 3 mg/kg, ibogaine had no significant effect. No significant blockade was manifest at 24 hr after the administration of 40 mg/kg ibogaine (Fig. 5). Memantine at 10 mg/kg had only a marginal effect on epibatidine-elicited antinociception (Fig. 6). This dose does block naloxone-elicited jumping in morphine-dependent mice (14).

Discussion

Ibogaine is under investigation as a potentially useful antiaddictive agent preclinically in animal models and with respect to molecular mechanism of action (1). Structure-activity relationships suggest that noncompetitive blockade of NMDA receptor channels may contribute substantially to some of the actions of ibogaine, such as morphine dependence (9). Indeed, the brain concentrations of ibogaine (10–20 μM) that occur after the administration of dosages purported to interfere with addiction will markedly affect the function of NMDA receptor channels (1–3). However, at such *in vivo* dosages, ibogaine will also affect κ-opioid receptors and σ_2 receptors, probably other receptors, and even the dopamine-uptake system. Thus, the *in vivo* pharmacology of ibogaine is complex, possibly involving several sites of action, and the challenge remains to determine the relative importance of each of these sites to the antiaddictive properties of ibogaine.

Another highly relevant target site for ibogaine must be considered: the potent noncompetitive blockade by ibogaine of ganglionic-type [$\alpha 3\beta 4(5)$] nicotinic receptors *in vitro* (Fig.
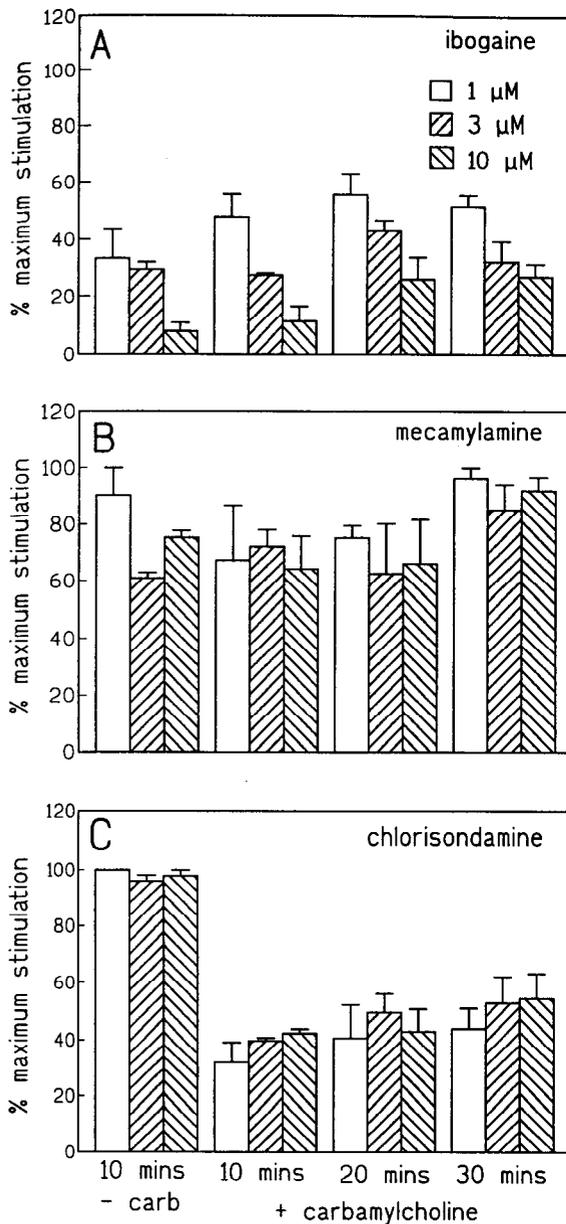
Fig. 2. Effects of (A) ibogaine, (B) mecamylamine, and (C) chlorisondamine on carbamylcholine-elicited ^{22}NaCl influx in rat pheochromocytoma PC12 cells. Preincubation with ibogaine, mecamylamine, or chlorisondamine was for 5 min in the absence (− *carb*) or presence of 1 mM carbamylcholine. Concentrations are as indicated. The cells were then washed by aspiration three times with buffer. A subsequent ^{22}NaCl influx, induced by 2 mM carbamylcholine, was assayed after a 10-, 20-, or 30-min postincubation period following the preincubation and washing. Each value is the mean ± standard error of three experiments. Values are reported as a percentage of maximum stimulation obtained with 2 mM carbamylcholine alone in both the first and second incubations.

Fig. 3. Competition curves versus nicotine-elicited ^{22}NaCl influx of ibogaine in (A) rat pheochromocytoma PC12 cells and (B) human medulloblastoma TE671 cells. Assays were performed as described previously (17) with nicotine alone or in the presence of ibogaine. Each value is reported as a percentage of maximum stimulation obtained with nicotine and is the mean ± standard error of three experiments.

TABLE 1

Inhibition of carbamylcholine-elicited influx of ^{22}NaCl in cultured cells

Assays were as described previously (17) with 2 mM carbamylcholine for 2 min alone or with an inhibitor, the latter in each case over a range of concentrations. Each value is the mean ± standard error of three experiments.

Inhibitor	IC_{50}	
	PC12 cells	TE671 cells
	µM	
Ibogaine	0.020 ± 0.007	1.6 ± 0.2
O-Desmethylibogaine	1.5 ± 0.4	13 ± 0.8
O-T-Butyl-O-desmethylibogaine	0.4 ± 0.05	1.5 ± 0.2
Chlorisondamine	0.022 ± 0.003	>100
Mecamylamine	0.86 ± 0.07	37 ± 4
Memantine	1.5 ± 0.3	3.0 ± 0.4

1) and the blockade by ibogaine (10–40 mg/kg) of a central antinociceptive nicotinic receptor-mediated response *in vivo* (Fig. 4). The antinociceptive response to (−)-epibatidine seems to involve central neuronal ($\alpha 4\beta 2$) nicotinic receptors (22), although central ganglionic-type [$\alpha 3\beta 4(5)$] nicotinic receptors may also be involved (17, 20). There is a transfected cell line with functional $\alpha 4\beta 2$ nicotinic receptors (23), but it is not yet available for functional assays of that major central
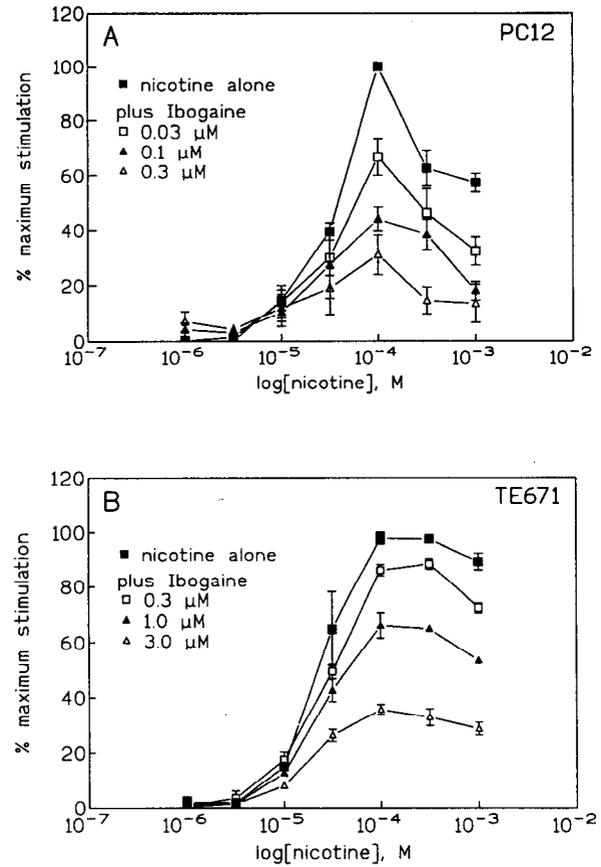
neuronal nicotinic receptor channel. The relative activities of ibogaine, O-desmethylibogaine, and O-t-butyl-O-desmethylibogaine in blocking nicotinic receptor-mediated response in PC12 cells are consonant with their effects *in vivo* on naloxone-induced jumping in morphine-dependent mice, in which the two ibogaine analogs are inactive (9). O-Desmethylibogaine and O-t-butyl-O-desmethyl-ibogaine were 75- and 20-fold less potent, respectively, than ibogaine in blocking nicotinic receptor-mediated responses in PC12 cells (Table 1). Sup-
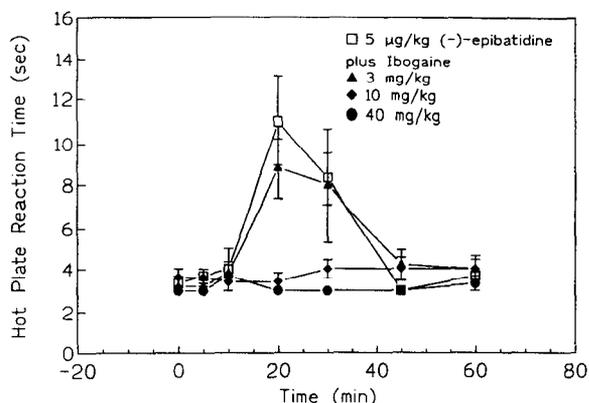
Fig. 4. Effect of various concentrations of ibogaine on (−)-epibatidine-induced antinociception. (−)-Epibatidine at 5 μg/kg was administered intraperitoneally 5 min after ibogaine (3, 10, and 40 mg/kg intraperitoneal). The hot-plate antinociceptive assay was as described previously (17). Each value is mean ± standard error for three to five animals.
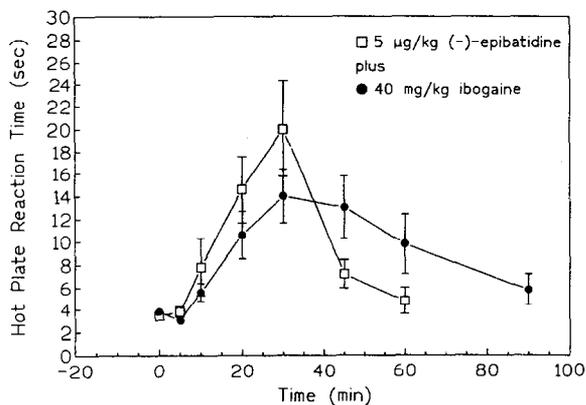

Fig. 5. Long-term effect of a single dose of ibogaine on (−)-epibatidine-induced antinociception. (−)-Epibatidine (5 μg/kg) was administered intraperitoneally 24 hr after ibogaine (40 mg/kg intraperitoneal). Each value is mean ± standard error for four or five animals.
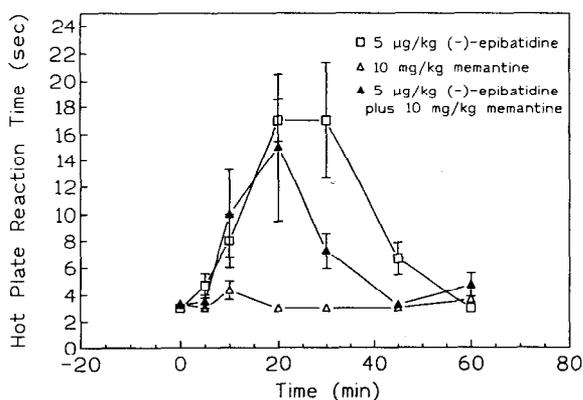

Fig. 6. Effect of memantine on (−)-epibatidine-induced antinociception. (−)-Epibatidine at 5 μg/kg was administered intraperitoneally 5 min after memantine (10 mg/kg intraperitoneal). Each value is mean ± standard error for three or four animals.

plies of the two ibogaine analogs were insufficient for testing *in vivo* versus epibatidine-elicited antinociception.

The blockade of nicotinic receptor-mediated responses by ibogaine seems to be only partially reversible in PC12 cells (Fig. 2A). Thus, the long-term effects of ibogaine on nicotinic function might be relevant to the apparent long-lasting an-

tiaddictive effects of ibogaine. However, at 24 hr after the administration of 40 mg/kg ibogaine, there was no significant effect on epibatidine-elicited antinociception (Fig. 5).

The current initial results suggest that noncompetitive blockade of central nicotinic receptors may be relevant, perhaps in consort with noncompetitive blockade of NMDA receptor channels, to the antiaddictive effects of ibogaine. It should be noted that memantine, another noncompetitive blocker of NMDA receptor channels, does block naloxone-induced jumping in morphine-dependent mice (14). Memantine is 75-fold less potent than ibogaine in block of the nicotine receptor-mediated response in PC12 cells (Table 1). *In vivo*, memantine does not significantly block (−)-epibatidine-elicited antinociception, although there is a tendency for a slight reduction in the antinociceptive response at later time points (Fig. 6). Thus, blockade of NMDA receptor channels, at least with this dose of memantine, is sufficient to antagonize naloxone-induced jumping (14) without a significant blockade of central nicotinic receptor channels (Fig. 6). In addition, blockade of NMDA receptors seems to have no major role in the complete blockade by ibogaine of epibatidine-elicited antinociception because memantine has only marginal effects on the epibatidine response.

An *in vivo* attenuation of nicotine-elicited release of dopamine in the nucleus accumbens of rats by ibogaine was reported (24) while the current investigation was under way. The nicotine response is apparently mediated by α4β2 receptors (25). It seems possible that the antiaddictive effects of ibogaine toward such diverse drugs as morphine, cocaine, and nicotine might involve, in all cases, blockade of the input to dopaminergic neurons, thereby reducing extracellular levels of dopamine and blunting activation of reward pathways. Ibogaine does antagonize σ-, NMDA-, and nicotine-induced release of dopamine (25, 26). In a recent abstract, ibogaine was reported to enhance the release of intracellular calcium and to decrease voltage-dependent influx of calcium, apparently through interaction with σ_2 receptors (27). Ibogaine has been reported to be an agonist at κ-opioid receptors (4), and activation of such receptors is inhibitory to dopamine release (28; see, however, Ref. 29). Thus, NMDA, nicotinic, κ-opioid, and σ_2 receptors may all play a role in the antiaddictive pharmacology of ibogaine, perhaps by attenuating excitatory input and release of dopamine from dopaminergic neurons.

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