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Biochemical and Behavioral Effects of Boldine and Glaucine on Dopamine Systems

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ASENCIO, M., B. DELAQUERRIÈRE, B. K. CASSELS, H. SPEISKY, E. COMOY AND P. PROTAIS. *Biochemical and behavioral effects of boldine and glaucine on dopamine systems.* PHARMACOL BIOCHEM BEHAV **62**(1)7–13, 1999.—The aporphine alkaloids boldine and glaucine have been reported to show "neuroleptic-like" actions in mice, suggesting that they may act as dopamine antagonists. We have found that in vitro boldine displaces specific striatal [^{3}H]-SCH 23390 binding with IC_{50} = 0.4 μM and [^{3}H]-raclopride binding with IC_{50} = 0.5 μM, while the affinities of glaucine at the same sites are an order of magnitude lower. In vivo, however, 40 mg/kg boldine (IP) did not modify specific striatal [^{3}H]-raclopride binding and only decreased [^{3}H]-SCH 23390 binding by 25%. On the other hand, 40 mg/kg glaucine (IP) displaced both radioligands by about 50%. Behaviors (climbing, sniffing, grooming) elicited in mice by apomorphine (0.75 mg/kg SC) were not modified by boldine at doses up to 40 mg/kg (IP) but were almost completely abolished by 40 mg/kg glaucine (IP). In the apomorphine-induced (0.1 mg/kg SC) rat yawning and penile erection model, boldine and glaucine appeared to be similarly effective, inhibiting both behaviors by more than 50% at 40 mg/kg (IP). Boldine and glaucine, injected IP at doses up to 40 mg/kg, were poor modifiers of dopamine metabolism in mouse and rat striatum. These data suggest that boldine does not display effective central dopaminergic antagonist activities in vivo in spite of its good binding affinity at D_1- and D_2-like receptors, and that glaucine, although less effective in vitro, does appear to exhibit some antidopaminergic properties in vivo. © 1998 Elsevier Science Inc.

Boldine Glaucine D_1/D_2 antagonists Neuroleptic-like Affinity Climbing Sniffing Grooming Yawning Penile erection Dopamine metabolism

A large number of compounds based on the aporphine skeleton are known to exhibit dopaminergic activities. Some, like the prototypical apomorphine [(*R*)-aporphine-10,11-diol], are agonists, while others antagonize some of the actions of dopamine, with strong agonist activity apparently being associated with the (*R*) configuration at C-6a and the presence of a hydroxyl group at C-11 (9,10,21). Thus, (*R*)-apomorphine elicits stereotypical behaviors such as sniffing, licking, gnawing (8,13), and climbing (22). On the other hand, the natural 11-hydroxyaporphine (*S*)-bulbocapnine has long been known to induce catalepsy (7), and is also able to antagonize stereotypies elicited by apomorphine or (+)-amphetamine in rats (30). Bulbocapnine, corytuberine, boldine, and glaucine, all aporphine alkaloids that share the (*S*) configuration and thus have a twisted biphenyl skeleton enantiomeric with that of apomorphine and its dopamine agonist congeners (2), have been reported to show "neuroleptic-like" actions in mice upon subcutaneous injection, which would seem to suggest that they may be acting as central dopamine antagonists (35). Both bulbocapnine and corytuberine bear phenolic hydroxyl groups at C-11. As neither boldine nor glaucine carry any substituent at C-11, however, this could be interpreted as an indication that a hydrogen-bonding group at this position is not a requirement for aporphine derivatives to exhibit reasonably high affinity (although perhaps not intrinsic efficacy) for dopamine receptors.

In recent years the diphenolic boldine [(*S*)-1,10-dimethoxyaporphine-2,9-diol] has attracted attention in relation to its potent antioxidative and cytoprotective properties (3,4,28). This alkaloid is present in high concentrations in the

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bark of the Chilean boldo tree (*Peumus boldus* Mol., Monimiaceae), which makes it an interesting candidate for development as a natural drug. Although it does not carry a hydroxyl group at C-11, its diphenolic structure does incorporate the *meta*-hydroxy-phenethylamine moiety of dopamine in two different restrained conformations, at least one of which might reasonably be assumed to interact strongly with the crucial anionic center and one of the characteristic serine residues of the active site(s) of one or more subtypes of dopamine receptors (11,29). Glaucine [(*S*)-1,2,9,10-tetramethoxyaporphine], the nonphenolic dimethyl ether of boldine, is almost as potent as an antioxidant, and in fact, it seems likely that aporphines in general may exhibit this behavior at low micromolar concentrations (6). Nevertheless, its complete lack of hydroxyl groups would seem to indicate the possibility of some mode of binding, other than that postulated for agonists, to explain its hypothetical dopaminergic activity.

If antioxidative activity were present at concentrations capable of eliciting behavioral changes, aporphines might be valuable as cytoprotective CNS drugs. On the contrary, if no CNS activity were discernible at central neuroprotective concentrations, some of these substances might be interesting candidates for development as antioxidants per se. The ready availability of boldine and glaucine, their demonstrated strong antioxidative properties, as well as the published data suggestive of dopamine antagonist activity, led us to delve deeper into the interactions of these alkaloids with dopaminergic systems in order to further evaluate the potential of aporphines as drug leads.

METHOD

Animals

Experiments were performed on male Wistar rats (Charles River, France) weighing 220 ± 40 g, and on male Swiss albino mice (CD1, Charles River) weighing 28 ± 3 g. Animals were housed in groups of 4 rats or 15 mice per cage (L = 38 cm, W = 24 cm, H = 18 cm) and maintained under standard laboratory conditions (22 ± 1°C, 12 L:12 D cycle with light on at 0800 h, food and water ad lib) for at least 7 days before use. The animals were separated and placed in small individual cages without food and water 30 min before the beginning of the experiments, which were carried out between 9000 and 1900 h in a diffusely illuminated room maintained at 22 ± 1°C.

In Vitro Binding

Binding experiments were performed on rat striatal membranes as previously described (24). Each striatum was homogenized in 2 ml ice-cold Tris-HCl buffer (50 mM, pH = 7.4 at 22°C) with a polytron (4 s, maximal scale) and immediately diluted with Tris buffer. The homogenate was centrifuged either twice ([^{3}H]-SCH 23390 binding experiments) or four times ([^{3}H]-raclopride binding experiments) at 20,000 × *g* for 10 min at 4°C with resuspension in the same volume of Tris buffer between centrifugations. For [^{3}H]-SCH 23390 binding experiments, the final pellet was resuspended in Tris buffer containing 5 mM $MgSO_4$, 0.5 mM EDTA, and 0.02% ascorbic acid (Tris-Mg buffer), and the suspension was briefly sonicated and diluted to a protein concentration of 1 mg/ml. A 100μl aliquot of freshly prepared membrane suspension (100 μg of striatal protein) was incubated for 1 h at 25°C with 100 μl Tris-Mg buffer containing [^{3}H]-SCH 23390 ([^{3}H]-(*R*)(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride, 85.5 Ci/mmol, NEN, Paris, France, 0.25 nM final concentration) and 800 μl of Tris buffer containing the required drugs. Nonspecific binding was determined in the presence of 30 μM SK&F 38393 and represented around 2–3% of total binding. For [^{3}H]-raclopride binding experiments, the final pellet was resuspended in Tris buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid (Tris-ions buffer), and the suspension was treated as described above. A 200-μl aliquot of freshly prepared membrane suspension (200 μg of striatal protein) was incubated for 1 h at 25°C with 200 μl of Tris-ions buffer containing [^{3}H]-raclopride (86.5 Ci/mmol, NEN, Paris, France, 0.5 nM final concentration) and 400 μl of Tris-ions buffer containing the drug being investigated. Nonspecific binding was determined in the presence of 50 μM apomorphine and represented around 5–7% of total binding. In both cases, incubations were stopped by the addition of 3 ml of ice-cold buffer (Tris-Mg buffer or Tris-ions buffer as appropriate) followed by rapid filtration through Whatman GF/B filters. Tubes were rinsed with 3-ml ice-cold buffer, and filters were washed three times with 3-ml ice cold buffer. After the filters had been dried, radioactivity was counted in 4-ml BCS scintillation liquid (Amersham, Paris, France) at an efficiency of 45%. Filter blanks corresponded to approximately 0.5% of total binding and were not modified by drugs.

In Vivo Binding

[^{3}H]-SCH 23390 and [^{3}H]-raclopride were injected into a tail vein at a tracer dose (4 μCi diluted in saline, corresponding to dosages of 0.5 μg/kg SCH 23390 and 0.7 μg/kg raclopride devoid of effects on dopamine and its metabolite levels), 20 min after the injection of boldine or glaucine and 20 min before the animals were killed. Immediately after killing, the striatum, the olfactory bulbs and the cerebellum were dissected on ice and homogenized by sonication (TC4C sonotrode, range 1) in ice-cold saline (1 ml for striatum and olfactory bulbs, 4 ml for cerebellum). The radioactivity of a 400-μl sample of each homogenate was directly counted in a minivial containing 4 ml of BCS scintillation liquid (Amersham, Les Ulis, France). Proteins were determined on a 10-μl sample of each homogenate according to the method of Bradford (5) using bovine serum albumin (Sigma) as the standard, and the results are expressed in dpm/mg protein. Specific binding (binding to dopamine receptors) was calculated as corresponding to the radioactivity retained in striatum or olfactory bulbs (dpm/mg protein) minus the radioactivity retained in cerebellum (dpm/mg protein), a brain structure devoid of dopaminergic innervation and dopamine receptors (1,12,15).

Determination of Tissue Levels of DA, DOPAC, HVA, 5-HT, and 5-HIAA

A 500-μl sample of each striatal homogenate prepared for in vivo binding experiments was sonicated in an ice-cold solution containing trichloracetic acid 5%, ethylenediamine tetraacetic acid 0.025%, and Na metabisulfite 0.05% (final concentrations). The resulting homogenate was centrifuged (15,000 × *g* for 20 min), and the supernatant was decanted. Aliquots were then analyzed directly by HPLC (Waters, Millford, MA; Wisp sample processor, pump model 510, Maxima 820 Chromatography Workstation) with a CLINREP "catecholamines in plasma" column (Recipe, München, Germany) and electrochemical detection (Waters 460, potential = +0.70 V). The mobile phase consisted of 50 mM CH_3COONa, 20 mM citric acid, 3.75 mM octanesulfonic acid, 5.9 mM dibuty-

lamine, and 7% methanol; pH was adjusted to 3.5 with CH_3COOH.

Behavioral Testing

Mice were introduced into individual cylindrical cages (12 cm diameter, 14 cm high) with a wall of vertical metal bars (2 mm diameter, 1 cm apart) and smooth ends. After 5 min, the behavior of each animal was observed every 2 min (approximately during 4–5 s, as 24–28 mice were simultaneously tested) by a single observer. Climbing behavior was scored as reported by Marçais et al. (16); mice that had all four paws on the floor were scored 0, mice that stood upright and gripped the vertical bars with their forepaws were scored 1, and mice that gripped the vertical bars with all four paws were scored 2. Sniffing, licking (repeated protrusions of the tongue) and gnawing (repeated biting of the cage bars) were also assessed: mice were scored 0 if they did not show such behavior, 1 if they did so in a discontinuous manner or with low intensity, and 2 if they showed such behavior continuously or with high intensity. Grooming was assessed as reported by Vasse and Protais (32) by attributing, at each observation, a score of 1 to mice that displayed the behavior and a score of 0 to mice that did not. The scores assigned to each animal during the observation period (60 min) were added and the individual scores were averaged for each group.

Rats were individually introduced into square cages (L = 25 cm, W = 18 cm, H = 30 cm) with vertical walls of wire netting. Yawns (slow and sustained opening of the mouth) and penile erections (rats in an upright position presenting an emerging and engorged penis) were counted by direct observation during a 60 min period starting from the injection of apomorphine.

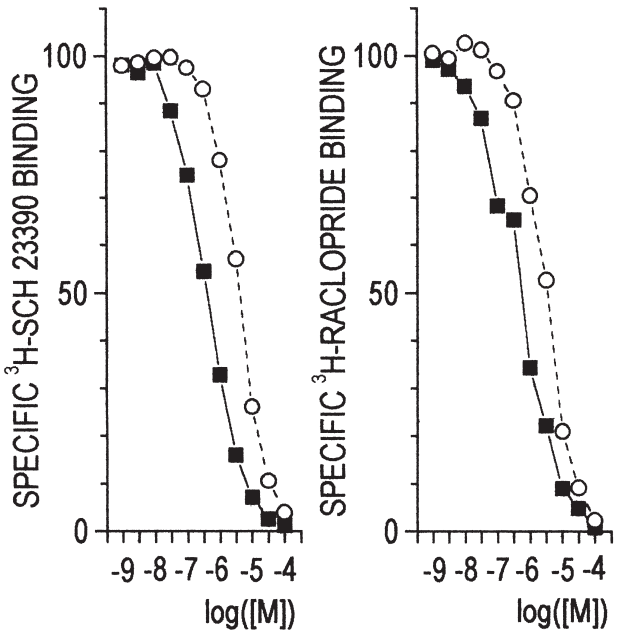

FIG. 1. Displacement curves of [^{3}H]-SCH 23390 and [^{3}H]-raclopride from rat striatal binding sites by increasing concentrations of boldine (filled squares) or glaucine (open circles). Values represent the means of eight determinations.

Drugs and Solutions

Boldine [isolated from boldo bark as previously described; (26)] HCl, and glaucine HCl (Sigma) were dissolved in saline. Apomorphine HCl (Sigma) was dissolved in saline containing 0.1% ascorbic acid to prevent autoxidation. Chlorpromazine HCl (Largactil®) was diluted in saline. All doses, expressed as the free base of respective salts, were injected in a volume of 5 ml/kg.

Statistics

An analysis of variance (ANOVA), followed by Student's *t*-test, was applied to evaluate the significance of means.

RESULTS

Binding Studies

In in vitro binding studies, increasing concentrations of boldine and glaucine were able to completely displace [^{3}H]-SCH 23390 or [^{3}H]-raclopride from their specific binding sites

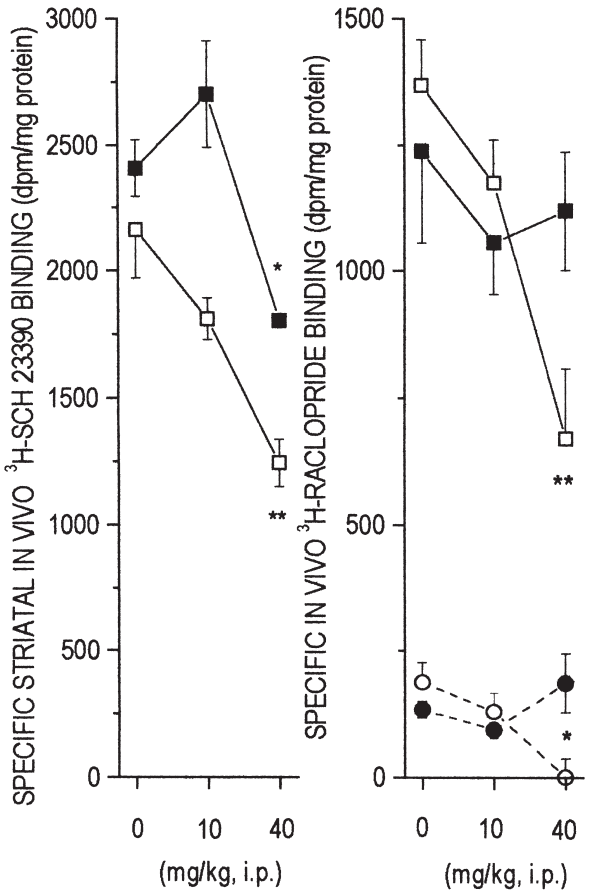

FIG. 2. Specific in vivo [^{3}H]-SCH 23390 and [^{3}H]-raclopride binding in striatum (squares) or in olfactory bulbs (circles) of mice treated with boldine (filled symbols) or glaucine (open symbols). Brain structures were dissected 40 min after IP injections of boldine or glaucine, and 20 min after SC injections of [^{3}H]-SCH 23390 or [^{3}H]-raclopride (4 μCi, 0.2 ml in saline). Values represent the means ± SEM of four mice per dose. *$p < 0.05$; **$p < 0.01$ compared to mice treated with solvent instead of boldine or glaucine.

in rat striatum. Boldine was about 10 times more potent than glaucine because the IC_{50} values (μM) were 0.40 ± 0.03 and 3.90 ± 0.23 for boldine and glaucine, respectively, at [^{3}H]-SCH 23390 binding sites, and 0.52 ± 0.11 and 3.02 ± 0.30 for boldine and glaucine, respectively, at [^{3}H]-raclopride binding sites (Fig. 1).

In in vivo binding studies boldine and glaucine were tested at 10 and 40 mg/kg (IP). The specific [^{3}H]-SCH 23390 binding in mice striatum was partially inhibited by boldine, $F(2, 9) = 10.88$, $p < 0.005$, and glaucine, $F(2, 9) = 12.50$, $p < 0.005$; at a 40 mg/kg dose (IP), the inhibition was about 25% for boldine and 50% for glaucine. The specific in vivo [^{3}H]-raclopride binding was not modified by boldine in either striatum, $F(2, 9) = 0.44$, $p > 0.05$, or olfactory bulbs, $F(2, 9) = 1.63$, $p > 0.05$, but it was inhibited by 40 mg/kg (IP) of glaucine by about 50% in striatum, $F(2, 9) = 11.33$, $p < 0.005$, and completely in olfactory bulbs, $F(2, 9) = 6.71$, $p < 0.05$ (Fig. 2).

Striatal Levels of Dopamine and Its Metabolites

In mice, at doses of 10 and 40 mg/kg (IP), 40 min after administration, boldine did not modify striatal levels of dopamine, $F(2, 21) = 0.04$, $p > 0.05$, or the HVA/DA ratio, $F(2, 21) = 3.03$, $p > 0.05$, while increasing striatal DOPAC, $F(2, 21) = 17.44$, $p < 0.001$, and HVA, $F(2, 21) = 5.37$, $p < 0.05$, levels significantly at 40 mg/kg. In contrast, under the same conditions, glaucine did not modify striatal levels of dopamine, $F(2, 21) = 2.25$, $p > 0.05$, but significantly increased striatal levels of DOPAC, $F(2, 21) = 14{,}47$, $p < 0.001$, and HVA, $F(2, 21) = 5.98$, $p < 0.01$, as well as the HVA/DA ratio, $F(2, 21) = 8.43$, $p < 0.01$, at 40 mg/kg.

Somewhat similar results were obtained in rats, 60 min after the IP administration of boldine or glaucine at doses of 2.5, 10, and 40 mg/kg (IP). Boldine did not significantly modify the striatal levels of dopamine, $F(3, 20) = 0.72$, $p > 0.05$, of DOPAC, $F(3, 20) = 2.54$, $p > 0.05$, of HVA, $F(3, 20) = 1.37$, $p > 0.05$, or the HVA/DA ratio, $F(3, 20) = 1.28$, $p > 0.05$. Glaucine did not modify the striatal levels of dopamine, $F(3, 20) = 0.14$, $p > 0.05$, but increased the striatal levels of DOPAC, $F(3, 20) = 3.91$, $p < 0.05$, of HVA, $F(3, 20) = 4.05$, $p < 0.05$, and the HVA/DA ratio, $F(3, 20) = 7.66$, $p < 0.01$, in a significant manner at 40 mg/kg. Under the same experimental conditions, doses of 0.25, 1, and 4 mg/kg (IP) chlorpromazine increased the striatal levels of dopamine metabolites, only attaining significance at 4 mg/kg (Table 1): DOPAC, $F(3, 20) = 6.01$, $p < 0.01$, HVA, $F(3, 20) = 4.67$, $p < 0.01$, and the HVA/DA ratio, $F(3, 20) = 10.76$, $p < 0.001$.

Behavioral Testing

When administered IP in mice, doses of boldine from 2.5–40 mg/kg were unable to modify significantly apomorphine-

TABLE 1

EFFECTS OF BOLDINE, GLAUCINE AND CHLORPROMAZINE ON THE STRIATAL LEVELS OF DOPAMINE AND ITS METABOLITES

Compound	Striatal levels (pmoles/mg proteins) of dopamine	DOPAC	HVA	ratio HVA/DA
Mice				
Boldine (mg/kg, IP)				
0	1190 ± 94	49.4 ± 2.9	86.0 ± 6.5	0.075 ± 0.009
10	1162 ± 62	59.3 ± 5.5	82.0 ± 7.2	0.072 ± 0.008
40	1175 ± 48	92.5 ± 7.0‡	118.9 ± 11.6*	0.103 ± 0.012
Glaucine (mg/kg, IP)				
0	1335 ± 102	62.3 ± 6.9	88.5 ± 14.3	0.064 ± 0.007
10	1240 ± 47	70.8 ± 6.6	95.7 ± 17.3	0.075 ± 0.012
40	1119 ± 55	176.7 ± 27.4‡	166.2 ± 20.5*	0.142 ± 0.021†
Rats				
Boldine (mg/kg, IP)				
0	1257 ± 103	51.7 ± 5.5	90.4 ± 17.5	0.074 ± 0.007
2.5	1191 ± 47	41.1 ± 2.8	105.6 ± 3.9	0.090 ± 0.006
10	1160 ± 88	50.9 ± 9.5	105.4 ± 23.5	0.092 ± 0.014
40	1319 ± 85	73.3 ± 12.7	138.8 ± 18.5	0.107 ± 0.017
Glaucine (mg/kg, IP)				
0	1373 ± 154	53.7 ± 7.5	94.1 ± 20.1	0.070 ± 0.009
2.5	1299 ± 202	64.5 ± 9.6	100.3 ± 16.3	0.079 ± 0.004
10	1436 ± 111	66.1 ± 11.1	126.6 ± 29.1	0.090 ± 0.013
40	1344 ± 136	108.4 ± 18.0*	194.5 ± 23.9*	0.147 ± 0.019†
Chlorpromazine (mg/kg, IP)				
0	1639 ± 219	72.7 ± 12.7	86.5 ± 19.0	0.055 ± 0.008
0.25	1894 ± 243	99.2 ± 19.6	114.0 ± 23.5	0.062 ± 0.010
1	1555 ± 242	102.9 ± 15.6	137.4± 41.1	0.090 ± 0.014
4	1627 ± 185	194.0 ± 32.8†	259.7 ± 52.3*	0.162 ± 0.023†

Levels of dopamine and its metabolites were measured in mice 40 min after the IP injection of drugs, and in rats 60 min after the IP injection of drugs. Values represent the means ± SEM from eight mice or six rats.

* = $p < 0.05$; † = $p < 0.01$; ‡ = $p < 0.001$ compared to respective animals treated with solvent.

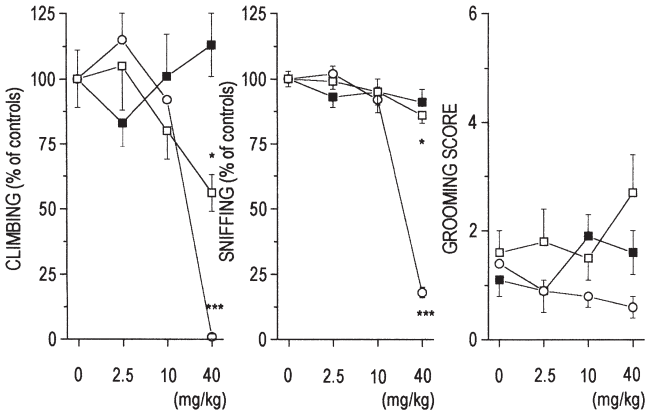

FIG. 3. Effects of increasing doses of boldine (squares) or glaucine (circles) on apomorphine-induced behaviors in mice. Apomorphine (0.75 mg/kg) was administered SC 30 min after the IP (filled squares) or s.c. (open squares) injection of boldine or the IP injection of glaucine. Values represent the means ± SEM of 14–15 mice per dose. $*p < 0.05$; $***p < 0.001$ compared to mice treated with solvent instead of boldine or glaucine.

induced climbing, $F(3, 56) = 0.94$, $p > 0.05$, sniffing, $F(3, 56) = 0.79$, $p > 0.05$, or grooming, $F(3, 56) = 1.85$, $p > 0.05$ behaviors. Administered SC at the same dosages, boldine weakly (but significantly only at 40 mg/kg) inhibited apomorphine-induced climbing, $F(3, 52) = 3.55$, $p < 0.05$, and sniffing, $F(3, 52) = 5.24$, $p < 0.01$, and did not modify grooming, $F(3, 52) = 1.02$, $p > 0.05$. In contrast, injected IP at the same doses, glaucine almost completely antagonized apomorphine-induced climbing, $F(3, 56) = 27.19$, $p < 0.0001$, and sniffing, $F(3, 56) = 137.76$, $p < 0.0001$, at 40 mg/kg, while leaving grooming unchanged, $F(3, 56) = 1.37$, $p > 0.05$ (Fig. 3).

Apomorphine-induced yawning in rats was inhibited by boldine, $F(3, 36) = 5.02$, $p < 0.01$, and glaucine, $F(3, 36) = 3.14$, $p < 0.05$, with a significant effect at 40 mg/kg (IP). The same doses of boldine and glaucine, injected IP, also inhibited apomorphine-induced penile erections in rats, reaching significance at 40 mg/kg, $F(3, 36) = 4.42$ and 7.13 for boldine and glaucine, respectively, $p < 0.01$] (Fig. 4).

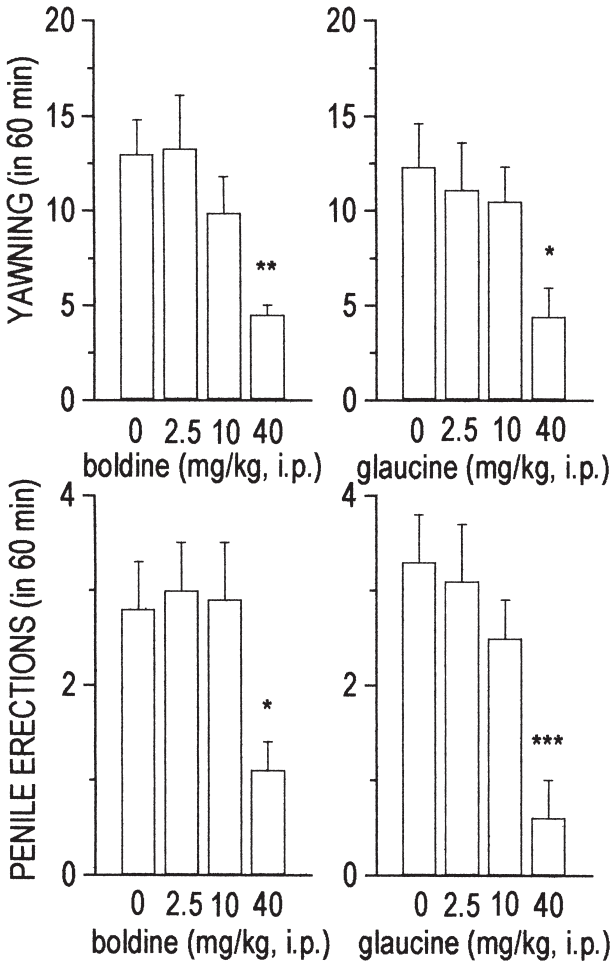

FIG. 4. Effects of increasing doses of boldine or glaucine on apomorphine-induced yawning and penile erections in Wistar rats. Apomorphine (0.1 mg/kg) was administered SC 30 min after the IP injection of boldine or glaucine. Values represent the means ± SEM of 10 rats per dose. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to rats treated with solvent instead of boldine or glaucine.

DISCUSSION

In vitro binding data indicate that boldine and glaucine (Fig. 5) are able to recognize striatal dopamine D_1 and D_2 binding sites labeled respectively with [^{3}H]-SCH 23390 and [^{3}H]-raclopride (12,15). Although boldine is about 10 times more potent than glaucine, each of the two products appears to be equally active at dopamine D_1 and D_2 binding sites. The affinities of boldine and glaucine for D_1 and D_2 dopamine receptors appear consistent with previous studies on aporphine derivatives (25), but are rather low compared to those of classical dopamine antagonists (18).

Several of our results indicate that in vivo, glaucine acts as a weak but effective dopamine antagonist at D_1 and D_2 receptors, especially at a dose of 40 mk/kg (IP): 1) it partially inhibits in mice the in vivo striatal binding of [^{3}H]-SCH 23390 and [^{3}H]-raclopride, and completely inhibits the binding of [^{3}H]-raclopride in olfactory tubercles; 2) like classical dopamine antagonists acting at dopamine D_2 receptors (17), and like chlorpromazine, it increases in mice and rats the release of dopamine in striatum, as evidenced by the increased levels of dopamine metabolites and HVA/DA ratio; 3) it antagonizes apomorphine-induced climbing and sniffing in mice, two behaviors produced by the simultaneous stimulation of dopamine D_1 and D_2 receptors (31,33); 4) it does not allow, in apomorphine-treated mice, the reappearance of grooming, a behavior induced by the isolated stimulation of dopamine D_1 receptors (20,32); and 5) in rats treated with a low dose of apomorphine, it antagonizes yawning and penile erection re-

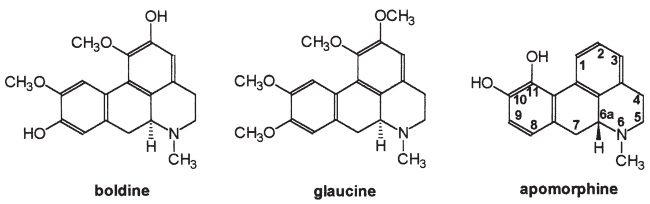

FIG. 5. Structures of aporphines used in this work.

sulting from the stimulation of dopamine D_2 receptors in the paraventricular nucleus of the hypothalamus (19,20).

Compared to glaucine, boldine administered IP is able 1) to more weakly inhibit striatal [^{3}H]-SCH 23390 binding in mice, with no effect on [^{3}H]-raclopride binding; and 2) to increase the levels of dopamine metabolites in mice less than glaucine, and to an even lesser extent in rats. Nevertheless, both boldine and glaucine are able to decrease D_2 receptor-dependent apomorphine-induced yawning and penile erections in rats. Because the in vitro binding studies indicate that boldine displays a 10-fold greater affinity for dopamine receptors than does glaucine, these data suggest that boldine 1) does not easily cross the blood–brain barrier, or 2) is metabolized and/or excreted extensively before it can reach the brain, or 3) does not act as an antagonist at some dopamine receptor subtypes. In the understanding that the changes observed in striatal dopamine metabolite levels are a consequence of postsynaptic dopamine receptor blockade, and considering that boldine is about 10 times more potent than glaucine in in vitro binding studies, it may be postulated that at similar doses more glaucine than boldine is made available to the brain. Conversely, the same potency ratio and the fact that boldine and glaucine inhibit yawning and penile erections to similar extents may be construed to indicate that boldine reaches the D_2 receptors of the paraventricular nucleus of the hypothalamus at effective concentrations about 10 times lower than those attained by glaucine in the same area.

Preliminary data from our group (27) indicate that boldine is rapidly cleared from plasma in rats. Comparison of the administration pathway-associated effects of boldine on apomorphine-induced behaviors indicates that at 40 mg/kg, boldine is completely ineffective as an inhibitor of apomorphine-induced climbing and sniffing when administered IP, but partially effective to inhibit these behaviors when administered SC. Therefore, although boldine may act as an antagonist at dopamine receptors, its poor access, at least to certain regions of the CNS, added to its very short plasma half-life, do not allow this property to be easily revealed in some in vivo experiments. As an alternative, it might be suggested that boldine could act as a partial agonist at dopamine receptors. Indeed, in the present in vitro binding studies, the displacement of [^{3}H]-raclopride is best fitted using a two-site model as described for agonists (14). Furthermore, the contention that boldine is poorly effective in inhibiting in vivo [^{3}H]-SCH 23390 and [^{3}H]-raclopride binding is consistent with this hypothesis, because dopamine agonists are known to be able to inhibit in vivo binding at dopamine receptors only at very high doses. For instance, apomorphine doses of 3 mg/kg are necessary to achieve 50% inhibition of the in vivo binding at D_1 and D_2 dopamine receptors, while doses lower than 1 mg/kg are sufficient for the induction of obvious and long-lasting behaviors (1,22,23,31,34).

Taken together, these data suggest that glaucine and especially boldine display weak in vivo antidopaminergic activities compared to classical dopamine antagonists (18,22,23,31,34). Nevertheless, insofar as the lack of potency of boldine may be due in part to its unfavorable pharmacokinetics, appropriate prodrugs might overcome this limitation. On the other hand, because in vivo results indicate that these aporphine alkaloids are able to reach the brain at concentrations approaching those at which they exhibit antioxidative activity, they might be valuable leads for the development of cytoprotective drugs for use in stroke or trauma.

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