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Effects of some antioxidative aporphine derivatives on striatal dopaminergic transmission and on MPTP-induced striatal dopamine depletion in B6CBA mice

Felicia Loghin[a], Abdeslam Chagraoui[b], Marcelo Asencio[c], Etienne Comoy[b], Hernan Speisky[d], Bruce K. Cassels[c], Philippe Protais[b,*]

[a]*Toxicology Laboratory, Faculty of Pharmacy, University of Medicine and Pharmacy, 3400 Cluj-Napoca, Romania*
[b]*Physiology and Biochemistry Laboratories, Faculty of Medicine and Pharmacy, University of Rouen, 76183 Rouen Cedex 1, France*
[c]*Millennium Institute for Advanced Studies in Cell Biology and Biotechnology and Department of Chemistry, Faculty of Sciences, I.N.T.A., University of Chile, Santiago, Chile*
[d]*Biochemistry Unit, I.N.T.A., University of Chile, Santiago, Chile*

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Abstract

(*S*)-(+)-boldine, an aporphine alkaloid displaying antioxidative and dopaminergic properties, and six of its derivatives (glaucine, 3-bromoboldine, 3-iodoboldine, 8-aminoboldine, 8-nitrosoboldine and 2,9-*O,O′*-dipivaloylboldine) were tested for these properties in comparison with their parent compound. All the tested compounds displayed in vitro antioxidative properties equal to or slightly weaker than those of boldine, and equal to or stronger than (±)-6-hydroxy-2,5,7,8,-tetramethylchromane-2-carboxylic acid (Trolox®), a water-soluble vitamin E analogue, used as a reference compound. All the aporphine compounds tested displaced [^{3}H]SCH 23390 and [^{3}H]raclopride from their specific binding sites in rat striatum. When tested on dopamine (DA) metabolism in the striatum of B6CBA mice, all the compounds, except 8-aminoboldine, increased striatal levels of DOPAC and HVA, and the HVA/DA ratio, indicating that they cross the blood–brain barrier and that they seem to act as dopamine antagonists in vivo. B6CBA mice were sensitive to the neurotoxic action of MPTP on dopaminergic neurons as indicated by the strongly decreased striatal levels of DA, DOPAC and HVA following administration of MPTP (20 mg/kg, i.p.). Among these aporphine derivatives, only 3-bromoboldine was able to reduce the MPTP-induced decrease of striatal levels of DA and DOPAC, whereas (*R*)-apomorphine (5 mg/kg, s.c.) and acetylsalicylic acid (100 mg/kg, i.p.), used as reference compounds, were very active. These data suggest that potent in vitro antioxidative properties and the ability to cross the blood–brain barrier are not sufficient criteria to predict the inhibition of neuronal degeneration induced by MPTP.

Keywords: Aporphines; Antioxidants; Dopamine; MPTP

1. Introduction

In recent years, boldine [(*S*)-1,10-dimethoxyaporphine-2,9-diol], the diphenolic aporphine alkaloid of the Chilean boldo tree (*Peumus boldus* Mol.), has attracted attention in relation to its potent antioxidative and cytoprotective properties (Speisky et al., 1991; Speisky and Cassels, 1994; Bannach et al., 1996; Jiménez et al., 2000). Glaucine [(*S*)-1,2,9,10-tetramethoxyaporphine], the nonphenolic dimethyl ether of boldine, is almost as potent as an antioxidant in some models and, in fact, it seems likely that aporphines in general may exhibit this behaviour at low micromolar concentrations (Cassels et al., 1995). These antioxidant properties might be expected to be of interest with regard to the cellular damage caused by oxidative stress in various diseases.

Like a large number of compounds based on the aporphine skeleton, in addition to their antioxidant properties, boldine, glaucine and some halogenated boldine derivatives appear to exhibit brain antidopaminergic activities. Indeed, these boldine derivatives are able to displace [^{3}H]SCH 23390 and [^{3}H]raclopride from their specific striatal D-1 and D-2 binding sites, respectively (Asencio et al., 1999; Sobarzo-Sánchez et al., 2000). In

*Corresponding author. Tel.: +33-2-3514-8366; fax: +33-2-3566-8365.
E-mail address: philippe.protais@univ-rouen.fr (P. Protais).

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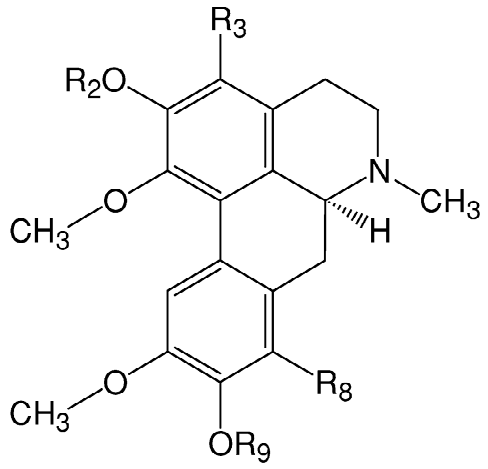

Fig. 1. General structure of the different tested compounds. For R groups, see Table 1.

addition, boldine and especially glaucine display weak in vivo antidopaminergic activities (Asencio et al., 1999). These data suggest that boldine and glaucine reach the brain, and therefore might be concentrated in brain areas containing dopaminergic terminals and receptors and could display, in these areas, their antioxidant properties. In this respect, the reference dopamine receptor agonist (*R*)-apomorphine has been recently reported as an effective aporphine derivative able to counteract the neurotoxic effect of MPTP (Grünblatt et al., 1999, 2001).

The aim of the present study was to investigate whether boldine, glaucine, halogenated (Sobarzo-Sánchez et al., 2000), nitroso- or aminoboldine derivatives (Sobarzo-Sánchez et al., 2001) or a new boldine ester prodrug (Fig. 1 and Table 1) might be effective with regard to protection against MPTP-induced striatal dopamine depletion (Burns et al., 1983; Heikkila et al., 1984; Javitch et al., 1985; Tipton and Singer, 1993) in B6CBA mice. In that model, acetylsalicylic acid and the reference dopamine agonist (*R*)-apomorphine were used as positive reference compounds (Aubin et al., 1998; Grünblatt et al., 1999, 2001; Battaglia et al., 2002). Furthermore, in preliminary phases of this work, we have compared the antioxidative properties of boldine and a number of derivatives, the potential activity of these derivatives on central dopaminergic transmission as evidence of their ability to penetrate the blood–brain barrier, a prerequisite for the prevention of oxidative stress-induced neurodegenerative diseases (Gilgun-Sherki et al., 2001), as well as the sensitivity of B6CBA mice to the neurotoxic action of MPTP.

2. Materials and methods

2.1. Chemistry

Boldine was extracted and purified from *Peumus boldus* bark, and glaucine was prepared by methylation of boldine with diazomethane (Tschesche et al., 1964). 2,9-*O*,*O*′-dipivaloylboldine was obtained by treatment of boldine with pivaloyl chloride under similar conditions to those described for the preparation of dopamine esters (Borgman et al., 1973). The halogenated boldine derivatives used in the present study were synthesized by treatment of boldine with the respective *N*-halosuccinimides (Sobarzo-Sánchez et al., 2000), 8-nitrosoboldine was obtained by nitrosation of boldine with sodium nitrite in acetic acid, and the catalytic hydrogenation of this product afforded 8-aminoboldine (Sobarzo-Sánchez et al., 2001).

2.2. Pharmacology

2.2.1. Protection of lysozyme activity

The antioxidant properties of all the boldine derivatives were assessed by the determination of the protection of lysozyme activity against degradation by free radicals generated by the thermolysis of 2,2′-azo-bis-(2-amidinopropane) (ABAP, Polysciences, Warrington, PA, USA), following the method described by Lissi et al. (1991). At each time and for each concentration of the boldine derivatives, the residual activity of lysozyme was calculated from the formula $[\Delta A_{\mathrm{lys/ABAP/antioxidant}}/\Delta A_{\mathrm{lys}} - \Delta A_{\mathrm{lys/ABAP}}] \times 100$ in which ΔA_{lys} = the change of absorbance of lysozyme alone, $\Delta A_{\mathrm{lys/ABAP}}$ = the change of absorbance of lysozyme in the presence of ABAP, and $\Delta A_{\mathrm{lys/ABAP/antioxidant}}$ = the change of absorbance of lysozyme in the presence of ABAP and an antioxidant.

2.2.2. Thiobarbituric acid reacting substances (TBARS) assay

TBARS formation was assayed according to a previously published procedure (Buege and Aust, 1978) in a liver

Table 1
Chemical substituents of the aporphine skeleton in the different compounds tested

	R_2	R_3	R_8	R_9
Boldine	H	H	H	H
Glaucine	CH_3	H	H	CH_3
3-Bromoboldine	H	Br	H	H
3-Iodoboldine	H	I	H	H
8-Aminoboldine	H	H	NH_2	H
8-Nitrosoboldine	H	H	NO	H
2,9-*O*,*O*′-Dipivaloylboldine	$COC(CH_3)_3$	H	H	$COC(CH_3)_3$

microsomal preparation from Sprague–Dawley rats (200–250 g, Instituto de Salud Pública, Santiago, Chile) undergoing lipoperoxidation by hydroxyl radicals produced by the Fe^{2+}/H_2O_2/ascorbic acid system.

2.2.3. Binding experiments

[^{3}H]SCH 23390 and [^{3}H]raclopride binding experiments were performed as previously described (Protais et al., 1992; Asencio et al., 1999; Sobarzo-Sánchez et al., 2000) on striatal membranes of male Wistar rats (200–250 g, Charles River, France).

2.2.4. Determination of tissue levels of dopamine and its metabolites

Experiments were performed on male B6CBA mice (Janvier, France) weighing 25–30 g. Striatal levels of dopamine (DA) and of its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured either 30 min after the s.c. injection of apomorphine (5 mg/kg) or of boldine derivatives (40 mg/kg) for the determination of the acute effects of the compounds, or 7 days after the i.p. administration of MPTP. For the determination of the neuroprotective effects, (*R*)-apomorphine and boldine derivatives were injected s.c. 15 min before MPTP whereas acetylsalicylic acid was injected i.p. 60 min before MPTP. In all cases, homogenates from mice striata were centrifuged (15 000× *g* for 20 min) and the supernatants were decanted. Aliquots were then analyzed directly by HPLC (Waters, Millford, MA, USA; 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) as previously described (Protais et al., 1998).

2.2.5. Solutions

Boldine and boldine derivatives were dissolved in distilled water. MPTP hydrochloride was dissolved in saline. Apomorphine hydrochloride (Sigma, L'Isle d'Abeau Chesnes, France) was dissolved in saline containing 0.1% ascorbic acid to prevent oxidation. Acetylsalicylic acid (La Cooper, Melun, France) was suspended in 1% hydroxyethylcellulose solution. All doses, expressed as the free base of the respective salts, were injected in mice in a volume of 10 ml/kg.

2.3. Statistical analysis

An analysis of variance (one-way ANOVA) followed by Bonferroni's *t*-test (INSTAT) for individual comparisons was applied to evaluate the significance of the results obtained.

3. Results

3.1. Antioxidant properties of boldine derivatives

Effective concentrations of boldine derivatives and of the water-soluble vitamin E analogue Trolox® in the lysozyme protection assay were determined in preliminary experiments with seven concentrations ranging from 1 to 100 μM. This model showed that at a concentration of 50 μM boldine was clearly much more effective than the reference antioxidant Trolox®, tested at 100 μM. In contrast, glaucine, at a concentration of 100 μM, had an efficacy roughly similar to that of Trolox®. The other boldine derivatives tested at a concentration of 50 μM were slightly less potent than boldine, but more so than Trolox® tested at 100 μM (Table 2).

In the lipid peroxidation model, glaucine showed slightly weaker activity than boldine whereas 3-bromoboldine, 3-iodoboldine and 2,9-*O*,*O*′-dipivaloylboldine were more potent than boldine, and all the aporphine derivatives were

Table 2
Effects of some boldine derivatives on the decrease of lysozyme activity induced by free radicals generated by the thermolysis of 2,2′-azo-bis-amidinopropane (ABAP)

Product	[μM]	Residual activity of lysozyme at time		TBARS assay IC_{50} (μM)
		60 min	120 min	
Boldine	50	64±5.1	47±5.3	13.3±0.5
Glaucine	100	25±5.6	15±3.4	13.5±0.8
3-Bromoboldine	50	60±4.3	56±4.9	4.0±0.3
3-Iodoboldine	50	61±4.1	40±4.0	5.6±0.3
8-Aminoboldine	50	45±4.6	16±4.9	n.t.
8-Nitrosoboldine	50	57±3.5	28±4.6	n.t.
2,9-*O*,*O*′-Dipivaloylboldine	n.t.	n.t.	4.4±1.3	
Trolox®	100	28±4.6	9±5.3	>25
Butylhydroxytoluene	100	62±5.1	38±4.6	n.t.

IC_{50} of some boldine derivatives on TBARS production in microsomal suspensions with Fe^{2+}/H_2O_2/ascorbic acid from dose–response curves with six concentrations from 1 to 50 μM.
Values are the means±S.E.M. of three to four determinations. n.t. = not tested

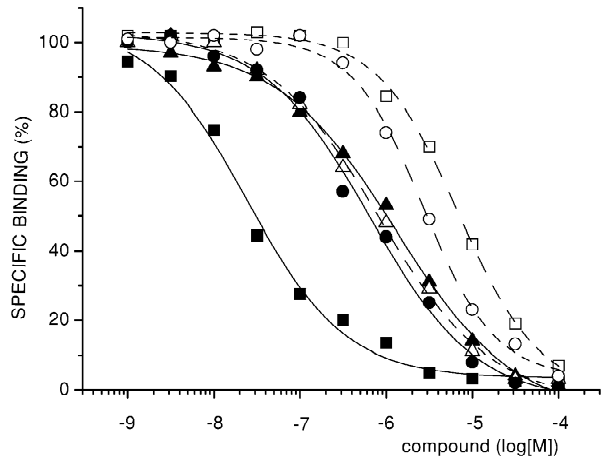

Fig. 2. Displacement curves of [^{3}H]SCH 23390 (closed symbols) or [^{3}H]raclopride (open symbols) from their specific binding sites in rat striatum by 8-aminoboldine (triangles), 8-nitrosoboldine (squares) and 2,9-*O*,*O*′-dipivaloylboldine (circles). Values are means from four to eight determinations.

more potent than Trolox® (Table 2) or α-tocopherol (results not shown).

3.2. Binding affinities at [^{3}H]SCH 23390 and [^{3}H]raclopride binding sites,

The binding affinities of boldine, glaucine and the halogenated boldine derivatives have been published previously (Asencio et al., 1999; Sobarzo-Sánchez et al., 2000). The new boldine derivatives were also able to displace [^{3}H]SCH 23390 and [^{3}H]raclopride from their respective D-1 and D-2 binding sites in rat striatum. The IC_{50} values for the displacement of [^{3}H]raclopride were in the micromolar range for all the compounds (Fig. 2 and Table 3). The rank order of potency of all the tested boldine derivatives for the displacement of [^{3}H]raclopride was boldine > 8-aminoboldine > 3-bromoboldine > 3-iodoboldine > glaucine > 2,9-*O*,*O*′-dipivaloylboldine > 8-nitrosoboldine. In contrast, strong differences were observed among the IC_{50} values for the displacement of [^{3}H]SCH 23390 with a rank order of potency of all the tested boldine derivatives 3-iodoboldine>8-nitrosoboldine>3-bromoboldine > boldine > 2,9-*O*,*O*′-dipivaloylboldine > 8-aminoboldine>glaucine.

Table 3
Comparative IC_{50} of some boldine derivatives for the displacement of [^{3}H]SCH 23390 or [^{3}H]raclopride from their specific binding sites in rat striatum

Compounds	IC_{50} (μM) on specific binding of	
	[^{3}H]SCH 23390	[^{3}H]raclopride
8-Aminoboldine	1.305±0.151	0.806±0.075
8-Nitrosoboldine	0.034±0.004	7.250±0.510
2,9-*O*,*O*′-Dipivaloylboldine	0.630±0.115	2.690±0.375

Values are means±S.E.M. from four to eight determinations.

3.3. Acute effects of boldine derivatives and apomorphine on the striatal levels of DA and its metabolites

When injected s.c. in B6CBA mice at doses of 40 mg/kg, most of the boldine derivatives tested induced significant changes in the striatal levels of DA and its metabolites 30 min later (changes were dose-dependent and less marked at lower doses, not shown; at higher doses of boldine derivatives, between 20 and 50% of the mice died in less than 30 min). Only boldine, glaucine and 3-bromoboldine decreased striatal DA levels slightly but significantly. (*R*)-Apomorphine (at a dose of 5 mg/kg) decreased both DOPAC and HVA striatal levels significantly. Boldine, glaucine and all the boldine derivatives except 8-aminoboldine enhanced striatal DOPAC and HVA levels (Fig. 3).

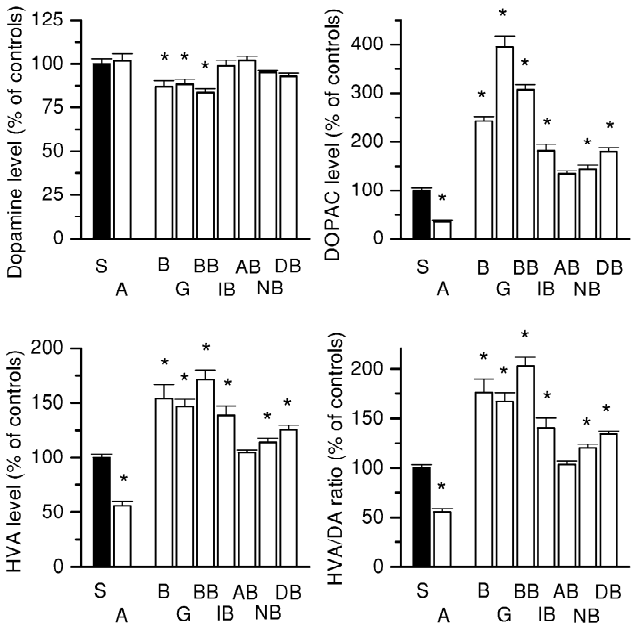

Fig. 3. Effects of aporphine derivatives on the striatal levels of dopamine (DA) and of its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in B6CBA mice. Levels of dopamine and its metabolites were measured in mice 30 min after the s.c. injection of 5 mg/kg of apomorphine (A), or 40 mg/kg of boldine (B), glaucine (G), 3-bromoboldine (BB), 3-iodoboldine (IB), 8-aminoboldine (AB), 8-nitrosoboldine (NB) or 2,9-*O*,*O*′-dipivaloylboldine (DB) and expressed as percentages of the values determined in solvent-treated mice (S). In solvent-treated mice (n=30), mean striatal levels were (nmol/mg protein) DA=1133.3±26.6, DOPAC=53.8±2.1, HVA=85.4±6.5, and the HVA/DA ratio was 0.076±0.006. Values represent the means±S.E.M. from 10 mice. * P<0.05 as compared with the respective values for animals treated with solvent.

3.4. Effects of increasing doses of MPTP, and of boldine derivatives, acetylsalicylic acid and apomorphine on MPTP-induced nigrostriatal dopaminergic toxicity in B6CBA mice

When injected in B6CBA mice 7 days before sacrifice, MPTP decreased DA, DOPAC and HVA striatal levels and increased the HVA/DA ratio. These changes were significant only for doses of 20 and 40 mg/kg MPTP (Fig. 4). However, at a dose of 40 mg/kg, between 10 and 20% of mice died before 7 days. For this reason, a 20 mg/kg dose of MPTP was used in the following experiments.

Among the various products which were tested, only 3-bromoboldine (40 mg/kg, s.c.), acetylsalicylic acid (100 mg/kg, i.p.) and (*R*)-apomorphine (5 mg/kg, s.c.) were able to protect, 7 days later, against the effects of 20 mg/kg (i.p.) MPTP administered 15 min after the products. Whereas acetylsalicylic acid was able to significantly modify both MPTP-induced changes of the striatal levels of DA, DOPAC and HVA as well as of the HVA/DA ratio, (*R*)-apomorphine significantly reduced the decrease in striatal DA, DOPAC and HVA levels but not the HVA/DA ratio, and 3-bromoboldine only reduced the DA and DOPAC levels significantly (Fig. 5). In mice receiving 4 injections of 5 mg/kg (*R*)-apomorphine (separated by a time interval of 60 min, the first injection being given 15 min before MPTP administration), the MPTP-induced decrease in striatal levels of DA and its metabolites was completely suppressed (not shown). Under similar conditions, boldine (four injections of 40 mg/kg) had no effect against MPTP-induced effects.

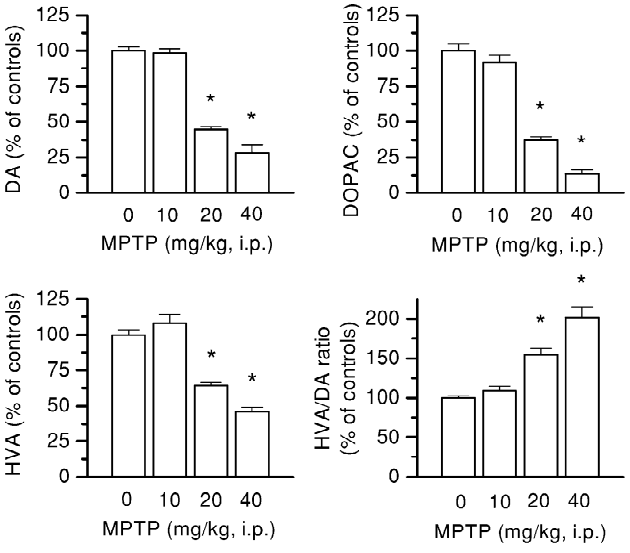

Fig. 4. Effects of increasing doses of MPTP on the striatal levels of dopamine (DA) and of its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in B6CBA mice. Mice were injected i.p. with MPTP 7 days before sacrifice. The changes in striatal levels induced by MPTP are expressed as percentages of the levels determined in mice receiving saline which were (nmol/mg protein): DA = 736.3±18.1, DOPAC = 46.4±2.2, HVA = 72.6±2.7, and the ratio HVA/DA = 0.099±0.003. Values represent the means±S.E.M. from 20 mice. * $P<0.05$ as compared with the respective values for animals treated with solvent.

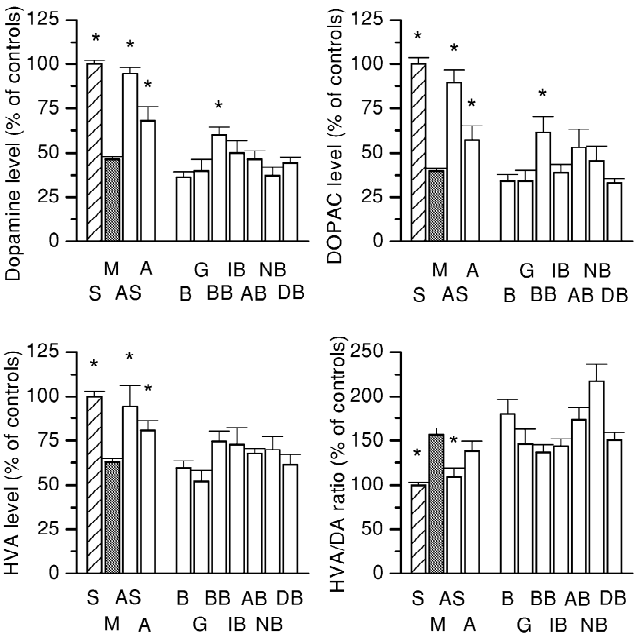

Fig. 5. Effects of aporphine derivatives and acetylsalicylic acid on MPTP-induced changes in the striatal levels of dopamine and of its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in B6CBA mice. Boldine (B), glaucine (G), 3-bromoboldine (BB), 3-iodoboldine (IB), 8-aminoboldine (AB), 8-nitrosoboldine (NB), 2,9-*O*,*O*′-dipivaloylboldine (DB) or apomorphine (A) were injected s.c. at doses of 40 mg/kg (5 mg/kg for apomorphine) 15 min before the i.p. injection of MPTP (M, 20 mg/kg). Acetylsalicylic acid (AS, 100 mg/kg) was injected i.p. 60 min before MPTP. Striatal levels of dopamine and of its metabolites were measured 7 days after the administration of MPTP and expressed as percentages of the values determined in solvent-treated mice (S). In solvent-treated mice ($n=110$), mean striatal levels were (nmol/mg protein): DA = 843.1±19.8, DOPAC = 45.2±1.6, HVA = 72.5±2.0, and the HVA/DA ratio was 0.088±0.002. Values represent the means±S.E.M. from 10 to 20 mice. * $P<0.05$ as compared with the respective values for animals treated only with MPTP.

4. Discussion

The present study confirms the antioxidant effects of boldine and glaucine previously demonstrated in other experimental models (Speisky et al., 1991; Speisky and Cassels, 1994; Cassels et al., 1995) and extends these properties to various boldine derivatives. All the phenolic substances appear to be more active than the water-soluble vitamin E analogue Trolox® (used as reference antioxidant compound) in the lysozyme protection assay and are as active as, or slightly less active than boldine, while the non-phenolic glaucine showed similar activity to that of Trolox®. In the TBARS assay, all the aporphines were

more potent than Trolox® (which did not differ significantly from its lipophilic natural analogue α-tocopherol), and the halogenated derivatives and 2,9-*O*,*O*′-dipivaloylboldine were about an order of magnitude more effective than these reference compounds. Structure–antioxidative activity relationships in benzylisoquinoline alkaloids indicate that while the potent antioxidative properties of phenolic aporphine derivatives depend upon the presence of the phenol function(s) on a biphenyl scaffold, those of non-phenolic derivatives are probably related to the abstraction of a benzylic hydrogen atom neighbouring a nitrogen lone electron pair (Cassels et al., 1995). The high antioxidative potencies of the haloboldines in the TBARS assay suggest that these relatively lipophilic phenolic compounds may be efficiently partitioned into the microsomal membranes where they exert their protective actions. The similar activity of the non-phenolic 2,9-*O*,*O*′-dipivaloylboldine might be related to an analogous concentration in the lipid phase, possibly followed by enzymatic hydrolysis to afford boldine and/or its monophenolic monopivaloyl esters in situ.

Like boldine and glaucine (Asencio et al., 1999) and haloboldines (Sobarzo-Sánchez et al., 2000), the new boldine derivatives tested (8-aminoboldine, 8-nitrosoboldine and 2,9-*O*,*O*′-dipivaloylboldine) displace [^{3}H]SCH 23390 from striatal D_1 dopamine receptors and [^{3}H]raclopride from striatal D_2 dopamine receptors with IC_{50}s in the micromolar range. When injected in mice at doses of 40 mg/kg, the various boldine derivatives appear to cross the blood–brain barrier. Indeed, as previously demonstrated for boldine and glaucine (Asencio et al., 1999), the different boldine derivatives (with the exception of 8-aminoboldine) increase the striatal release and metabolism of dopamine as indicated by the increased striatal levels of DOPAC and HVA, and HVA/DA ratio. In contrast, in accordance with many previous reports, the reference dopamine agonist (*R*)-apomorphine (5 mg/kg), which belongs to the opposite stereochemical series to (*S*)-boldine and its derivatives, decreases striatal HVA and DOPAC levels, as well as the HVA/DA ratio. These effects of apomorphine are classically attributed to the stimulation of D_2 dopamine (auto)receptors (Carlsson, 1975; Martres et al., 1977). In contrast, the increase of the striatal levels of DOPAC and HVA, and of the HVA/DA ratio is classically attributed to the activation of nigrostriatal dopaminergic neurons as a consequence of the blockade of D_2 dopamine (auto)receptors and/or the involvement of regulatory feedback loop(s) (Martres et al., 1977; Moore and Kelly, 1978; Imperato and Di Chiara, 1985; Protais et al., 1986; Zetterström et al., 1988; Santiago and Westerink, 1991). The increased dopamine metabolism observed in the present study indicates that these derivatives cross the blood–brain barrier and suggests that, like boldine and especially glaucine (Asencio et al., 1999), they seem to act as antagonists at striatal D_2 dopamine receptors. The differences in the amplitude of striatal HVA and DOPAC increases may partly reflect differences in the affinity of the various boldine derivatives for D_2 dopamine receptors and/or in their selectivity for D_1 or D_2 dopamine receptors, or again differences in their ability to cross the blood–brain barrier.

It is now well known that the administration of MPTP to mammals causes changes in the nigrostriatal dopaminergic pathway which result in a marked reduction in the levels of striatal DA and its metabolites DOPAC and HVA. MPTP causes varying degrees of dopaminergic damage in different strains of mice. For example, MPTP administration causes profound reductions in striatal levels of these compounds in C57BL mice, while similar administration produces only minimal effects in CD-1 mice (Muthane et al., 1994; Smeyne et al., 2001). In the present study, B6CBA mice were used to study the consequences of MPTP administration and the potential protection against the effects of MPTP by different compounds. As in C57BL mice, MPTP induces a marked decrease of the striatal levels of dopamine, DOPAC and HVA in B6CBA mice, associated with an increased activity of unaffected dopaminergic neurons as reflected by the increased HVA/DA ratio in MPTP-treated animals. MPTP-induced degeneration of nigrostriatal dopaminergic neurons in B6CBA mice seems to result from the same mechanism as that observed in C57BL animals, since protection is observed with acetylsalicylic acid (which blocks the inflammatory processes linked to neuronal degeneration) and with (*R*)-apomorphine as reported previously (Aubin et al., 1998; Grünblatt et al., 1999, 2001; Teismann and Ferger, 2001). Furthermore, it appears that the protection by acetylsalicylic acid as well as by (*R*)-apomorphine against MPTP-induced degeneration of nigrostriatal dopaminergic neurons is not strain-dependent.

With the exception of 3-bromoboldine, the various boldine derivatives tested by us appear to be unable to protect nigrostriatal dopaminergic neurons against the neurotoxic effects of MPTP. This might appear surprising considering the potent antioxidant properties observed with these compounds in vitro and the obvious protective effect of (*R*)-apomorphine. In fact, the main difference between (*R*)-apomorphine and the hemi-synthetic (*S*)-boldine derivatives tested here is that (*R*)-apomorphine is an agonist at dopamine receptors whereas boldine derivatives seems to act as antagonists of dopamine receptors. Since dopamine agonists decrease the activity of dopaminergic neurons and the metabolism of dopamine whereas dopamine antagonists increase dopaminergic neuronal activity and dopamine metabolism, we suggest that the increased metabolic activity elicited by boldine derivatives might counteract the neuroprotective effects associated with their antioxidative properties, whereas the decreased dopamine metabolism produced by agonists like (*R*)-apomorphine might potentiate their antioxidative properties. This hypothesis is supported by independent observations that, among dopaminergic ligands, only dopamine agonists

appear to be able to counteract MPTP neurotoxicity (Kitamura et al., 1997; Muralikrishnan and Mohanakumar, 1998; Grünblatt et al., 1999, 2001). However, recent papers indicate that apomorphine is also able to rescue nigro-striatal dopaminergic neurons and that its neuroprotective effects seem to be independent of its dopamine agonist properties since the *(S)*-enanthiomer, which is not active on DA receptors, is 10-fold more powerful than the *(R)*-enanthiomer. This protection seems to be linked to the induction of neuroprotective genes down-regulated by MPTP treatments (Grünblatt et al., 2001; Battaglia et al., 2002). It seems likely that the boldine derivatives tested by us are unable to induce these neuroprotective genes down-regulated by MPTP treatments. Finally, since toxic effects appeared at doses of boldine derivatives higher than 40 mg/kg, we cannot rule out the possibility that, at the dose of 40 mg/kg, although crossing the blood–brain barrier, boldine derivatives did not attain brain concentrations high enough for the full expression of their antioxidant properties.

In conclusion, our data suggest that potent in vitro antioxidative properties and the ability to cross the blood–brain barrier are not sufficient criteria to predict the inhibition of neuronal degeneration induced by MPTP.

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