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HALOGENATED DERIVATIVES OF BOLDINE WITH HIGH SELECTIVITY FOR α_{1A}-ADRENOCEPTORS IN RAT CEREBRAL CORTEX

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Summary

The selectivity of 3-nitrosoboldine and different halogenated derivatives of boldine (3-bromoboldine, 3,8-dibromoboldine and 3-chloroboldine) for α_1 -adrenoceptor subtypes was studied by examining [^{3}H]-prazosin competition binding in rat cerebral cortex. In the competition experiments [^{3}H]-prazosin binding was inhibited completely by all the compounds tested. The inhibition curves displayed shallow slopes which could be subdivided into high and low affinity components. The relative order of affinity and selectivity for α_{1A} -adrenoceptors was 3-bromoboldine = 3,8-dibromoboldine = 3-chloroboldine > boldine > 3-nitrosoboldine. The competition curves for 3-bromoboldine remained shallow and biphasic following chloroethylclonidine treatment. Whereas the relative contribution of the high affinity sites increased, the 3-bromoboldine affinities at its high and low affinity sites remained similar to those obtained in untreated membranes. 3-Bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine did not significantly displace [^{3}H]-(+)-*cis*-diltiazem binding to rat cerebral cortex membranes. This activity was lower than that shown by boldine. Compared to boldine, halogen (bromine or chlorine) substitution at position 3 increases the α_{1A}-adrenoceptor subtype selectivity and decreases the affinity for the benzothiazepine binding site at the calcium channel. Further halogen substitution at position 8 did not significantly improve this activity with respect to 3-bromoboldine. In contrast, the NO substitution at position 3 of boldine (3-nitrosoboldine) gives a loss of affinity and selectivity for α_1-adrenoceptor subtypes.

Key Words: α_1-adrenoceptor subtypes, [^{3}H]-prazosin binding, rat cerebral cortex, (S)-aporphine alkaloids, 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine, 3-nitrosoboldine

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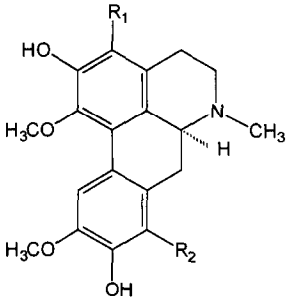
α_1-Adrenoceptors constitute a heterogenous family of receptors, and molecular biological, radioligand binding and functional studies have described several subtypes. Although differences in protocol and technique have resulted in several classification schemes, the existence of at least three subtypes with high affinity for prazosin — α_{1A}, α_{1B} and α_{1D}-adrenoceptors — is now recognized (*for review see* 1,2,3). The presence and different distribution of these subtypes of the α_1-adrenoceptors in vascular and other smooth muscles offer the opportunity for selective drug action. Therefore, a continued search for subtype-selective α_1-adrenoceptor antagonist and agonist leads is a highly attractive endeavour. Moreover, selective compounds are perhaps the most useful tools for receptor characterization and classification.

In a previous paper we have reported the selective affinity of a new class of structural lead compounds for α_{1A}-adrenoceptors in rat cerebral cortex. (S)-aporphine alkaloids (boldine and predicentrine) show high selectivity for α_{1A} with respect to the α_{1B}-adrenoceptor subtype in rat cerebral cortex (4). Our results suggest that the 2-hydroxy function could be a critical factor in the ability of these compounds to discriminate between α_1-adrenoceptor subtypes. In order to further explore the structural requirements that determine the selectivity shown by these aporphine structures, we tested the influence that the introduction of a halogen (bromine or chlorine) atom or a nitroso group ortho to the 2-hydroxy or the 2- and 9-hydroxy groups of boldine has on α_1 -subtype selectivity. The affinities of 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine (Figure 1) for the α_{1A}- and α_{1B}-adrenoceptor subtypes were evaluated by studying their ability to inhibit specific [^{3}H]-prazosin binding from rat cerebral cortex membranes.

Our previous studies also showed that these aporphine structures (boldine and glaucine) act not only at α_1-adrenoceptors but also possess Ca^{2+} entry blocking properties, and therefore inhibit [^{3}H]-(+)-*cis*-diltiazem binding in rat cerebral cortex without affecting [^{3}H]-nitrendipine interaction (5,6). However, these compounds inhibited [^{3}H]-(+)-*cis* diltiazem binding at benzothiazepine sites in the Ca^{2+} channel with lower potency than [^{3}H]-prazosin binding at α_1-adrenoceptors. In order to further examine the selectivity of action of boldine derivatives for α_1-adrenoceptors, the present work also assessed the effects of these alkaloids on [^{3}H]-(+)-*cis* diltiazem binding to rat cerebral cortical membranes.

Some of these data have been presented previously in abstract form (7)



R_1	R_2	
H	H	S(+)-BOLDINE
Br	H	S(+)-3-BROMOBOLDINE
Cl	H	S(+)-3-CHLOROBOLDINE
NO	H	S(+)-3-NITROSOBOLDINE
Br	Br	S(+)-3,8-DIBROMOBOLDINE

Fig. 1.

Chemical structures of S(+)-boldine, S(+)-3-bromoboldine, S(+)-3,8-dibromoboldine, S(+)-3-chloroboldine and S(+)-3-nitrosoboldine.

Materials & Methods

Binding study

Preparation of membranes.
Female Wistar rats (180-200 g) were decapitated and the brain was rapidly removed. The cerebral cortex was homogenized in 10 vol.(w/v) of ice-cold buffer (Tris HCl 5 mM, sucrose 250 mM and EDTA 1mM, pH 7.5 at 25°C) using an Ultra-Turrax (two, 15 s). The homogenate was centrifuged for 10 min at 1000 *g*. The pellet was discarded and the supernatant was centrifuged at 50,000 *g* for 15 min at 4°C. The pellet was resuspended in the same volume of assay buffer (Tris HCl 50 mM, pH 7.5) and centrifuged at 50,000 *g* for 15 min at 4°C. The final pellet was resuspended in assay buffer and stored at -70°C for later use. All membrane-preparation procedures were carried out at 4°C.

For pretreatment with chloroethylclonidine (CEC), aliquots of membranes were incubated for 30 min at 37°C with 10 μM CEC in assay buffer (Tris HCl 50 mM, pH 7.5). The reaction was stopped by dilution with ice-cold buffer, followed by three successive 15 min centrifugations at 50,000 *g* to extensively wash the membranes and completely remove any remaining unbound drug.

Proteins were assayed according to the method of Bradford with globulin as standard (8).

[^{3}H]-prazosin binding studies.
Binding of [^{3}H]-prazosin was measured in aliquots of diluted membranes incubated in 50 mM Tris buffer (pH 7.5) with [^{3}H]-prazosin (0.2 nM) and in the absence or presence of 17-20 concentrations of the indicated agents. The incubation volume was 1 ml (approximately 250 μg protein/tube), but displacement experiments with CEC-treated membranes were carried out in a final volume of 2 ml (approximately 500 μg protein/tube). The assay tubes were incubated for 45 min at 25°C, and then binding reactions were terminated by rapid vacuum filtration using a Brandel cell harvester (M24R) with fiberglass filters (Schleicher and Schuell, No. 30) presoaked in 0.3 % polyethyleneimine for 5 min. The filters were then washed four times with 4 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.5), and the filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as binding in the presence of 10 μ M phentolamine. Assays were carried out in duplicate.

[^{3}H]-(+)-cis-diltiazem binding studies.
Binding of [^{3}H]-(+)-*cis*-diltiazem was measured in aliquots of diluted membranes incubated in 50 mM Tris buffer containing bovine serum albumin (BSA) 1 mg ml^{-1} (pH 7.5) with [^{3}H]-(+)-*cis*-diltiazem (3-4 nM) and in the absence or presence of 5-7 concentrations of the indicated agents. The incubation volume was 1 ml (approximately 250 μg protein/tube). The assay tubes were incubated for 120 min at 25°C, and then binding reactions were terminated by rapid vacuum filtration using a Brandel cell harvester (M24R) with glass fiber filters (Schleicher and Schuell, No. 30) presoaked in 0.3 % polyethylenimine for 5 min. The filters were then washed four times with 4 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.5), and the filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as binding in the presence of 10 μM diltiazem. Assays were carried out in duplicate.

Data analysis.

Competition binding experiments were analyzed by the weighted least-squares iterative curve fitting programme, LIGAND (9). The data were first fitted to a one- and then a two-site model, and if the residual sums of squares were statistically less for a two-site fit of data than for a one-site fit, as determined by an F-test comparison, then the two-site model was accepted. The experimental results were expressed as means ± s.e.mean for n determinations obtained from different animals. When ANOVA showed significant differences ($p < 0.05$) the results were further analyzed using the Student-Newman Keuls test. p values of less than 0.05 were considered significant.

Drugs.

S(+)-3-bromoboldine, S(+)-3,8-dibromoboldine and S(+)-3-chloroboldine (Figure 1) were prepared from S(+)-boldine by treatment with the appropiate N-halosuccinimide in trifluoroacetic acid (10). In order to obtain S(+)-3-Nitrosoboldine, S(+)-boldine was selectively nitrosated at the C-3 position by adding solid sodium nitrite to a solution of the alkaloid in acetic acid (11). The identity and purity of the derivatives were confirmed by NMR and mass spectrometry and by TLC.

The following drugs (sources in parentheses) were used: [^{3}H]-prazosin (specific activity 72-78 Ci mmol^{-1}, Amersham, U.K.); [^{3}H]-(+)-*cis*-diltiazem (specific activity 60-87 Ci mmol^{-1}, New England Nuclear), phentolamine mesylate, diltiazem hydrochloride, chloroethylclonidine dihydrochloride (Research Biochemicals Inc, Natick, MA, U.S.A.).

Results

The specific binding of [^{3}H]-prazosin at a concentration of 0.2 nM in the presence of 10 μM of phentolamine represented 90% of the total binding. The specific binding of 0.2 nM [^{3}H]-prazosin to the α_1-adrenoceptors was completely inhibited by 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine (Figure 2). Displacement curves for these alkaloids were biphasic, and the LIGAND analysis fitted the data to a two site model. From the two site fits we calculated that approximately 40% of the sites had high affinity for these compounds (Table 1). This proportion of high affinity sites detected with aporphine alkaloids is similar to that obtained for α_{1A}- selective compounds like WB 4101 and benoxathian (4), and thus corresponds to the pharmacologically defined α_{1A}-adrenoceptor.

Displacement affinities (pKi) for the high and low affinity sites for the different compounds as well as the affinity ratio between subtypes ($r_{Kl/Kh}$) are shown in Table 1. 3-bromoboldine, 3,8-dibromoboldine and 3-chloroboldine showed similar effects on [^{3}H]-prazosin binding: all of them were selective for the α_{1A}- as opposed to the α_{1B}-subtype and, in addition, the mean pKi values for all three alkaloids at the same α_1-adrenoceptor subtype were equivalent. However, the three halogenated derivatives of boldine exhibited a slightly but significantly higher affinity (2-4-fold) and selectivity for the α_{1A}-adrenoceptor subtype than the parent compound boldine. They were found to be 120- , 90- and 122-fold more potent, respectively, at the α_{1A}- than at the α_{1B}-subtype, whereas boldine was 70-fold more potent. The introduction of a nitroso group at C-3 (3-nitrosoboldine) decreases the affinity of boldine for both receptor subtypes by more than an order

of magnitude. Moreover, there was also a significant loss of selectivity compared to the parent compound boldine (32-fold- *vs*.70-fold).

TABLE. I

Inhibition of [^{3}H]-prazosin binding to α_1-adrenoceptors of rat cerebral cortex membranes by the different agents.

Drug	*n*	*pKi high*	*pKi low*	*% high*	*Hill slope*	$R_{KI/Kh}$
CEC-untreated membranes						
Boldine[a]	6	8.31±0.05	6.50±0.08	33.2±1.6	0.77±0.03	70
3-bromoboldine	4	8.93±0.09*	6.87±0.10	37.8±5.6	0.72±0.06	120
3,8-dibromoboldine	3	8.87±0.20*	6.92±0.12	33.6±1.5	0.78±0.06	90
3-chloroboldine	5	8.65±0.16*	6.57±0.13	44.1±4.9	0.64±0.09	122
3-nitrosoboldine	3	6.41±0.09**	4.93±0.07**	50.7±2.8	0.61±0.01	32
CEC-pretreated membranes						
3-bromoboldine	3	9.01±0.03*	6.71±0.21	78.1±3.1°°	0.42±0.07	240

Displacement experiments were done with 0.2nM [^{3}H] prazosin and 17-20 concentrations of competing drug. Data shown are mean ± s.e.mean. *n* = number of experiments. pK_i *high* or pK_i *low*, negative log of the equilibrium dissociation constant (-log M) at high and low affinity sites for drug tested. *% high*, population binding at the high affinity site compared to the total specific binding sites. $R_{KI/Kh}$, ratio between Ki *low* and Ki *high*. *$p < 0.05$ **$p < 0.001$ *vs* boldine. °°$p < 0.001$ *vs* CEC-untreated-membranes. [a]Data from Madrero *et al.*, 1996.

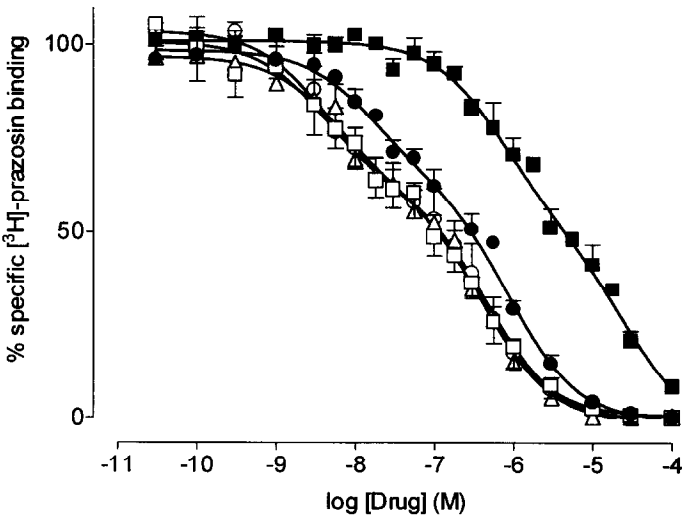


Fig. 2.

Displacement of specific [^{3}H]-prazosin (0.2 nM) binding from rat cerebral cortex membranes by boldine (●), 3-bromoboldine (○), 3,8-dibromoboldine (△), 3-chloroboldine (□) and 3-nitrosoboldine (■). Values represent the means ± s.e.mean from 3-4 individual experiments performed in duplicate.

We also tested the effect of CEC pretreatment on 3-bromoboldine inhibition curves of [^{3}H]-prazosin binding (Figure 3). CEC was used because of its alkylating effects, which are preferentially exerted towards α_{1B}- and α_{1D}-adrenoceptors relative to α_{1A}-adrenoceptors (12, 13, 14, 15, 16). As in other studies, pretreatment of rat cortical membranes for 30 min at 37°C with 10 µM CEC produced a significant reduction in the [^{3}H]-prazosin specific binding (the proportion of the remaining sites was 22.7±0.1%, n=3).

3-bromoboldine competed for [^{3}H]-prazosin binding to CEC-pretreated membranes with shallow competition curves which could be resolved into two components (Figure 3, Table 1). The affinity constants of 3-bromoboldine at its high and low affinity sites were similar to those obtained in CEC-untreated membranes, although the relative contribution of high affinity sites increased to 78% (Table 1).

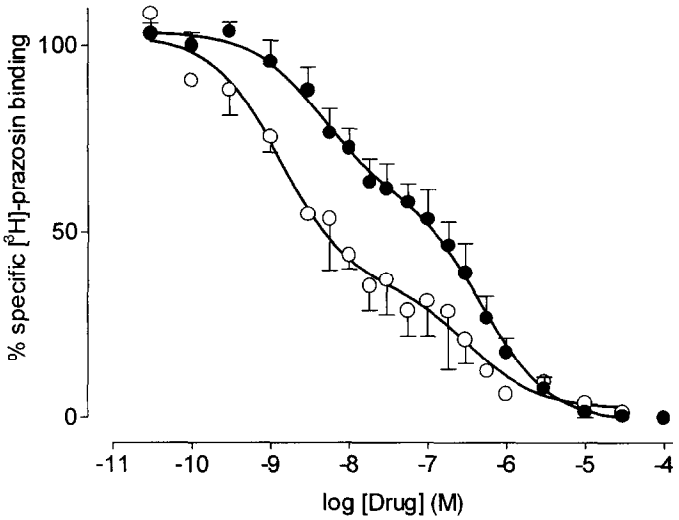


Fig. 3.

Effect of *in vitro* pretreatment of rat cerebral cortex membranes with chloroethylclonidine (CEC, 10 µM) on the inhibition of specific [^{3}H]-prazosin (0.2 nM) binding by 3-bromoboldine. Displacement experiments were performed in control membranes (●) or in CEC-pretreated membranes (○). Values represent the means ± s.e.mean from 3-4 individual experiments performed in duplicate.

The effects of 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine on [^{3}H]-(+)-*cis*-diltiazem binding were also studied. The specific binding of [^{3}H]-(+)-*cis*-diltiazem at a concentration of 3 nM in the presence of 10 µM of diltiazem represented 70% of the total binding. 3-Bromoboldine, 3,8-dibromoboldine and 3-chloroboldine did not fully displace the binding of [^{3}H]-(+)-*cis*-diltiazem to rat cerebral cortical membranes, and the highest concentration tested (100 µM) only induced 51.2 ± 3.8 % , 51.2 ± 6.4 % and 53.1 ± 2.5 (n=3) inhibition, respectively, of the binding of the radioligand. We therefore cannot calculate the IC_{50} value. [^{3}H]-(+)-*cis*-diltiazem binding was not affected by 3-nitrosoboldine in concentrations up to 100 µM. Boldine inhibited [^{3}H]-(+)-*cis*-diltiazem binding with an IC_{50} value of 33.28 ± 2.69 µM,

n=3 and the highest concentration tested (100 μM) induced 84.9 ± 2.6 % n=3, inhibition of the binding of radioligand (6).

Discussion

We have recently shown (4) that a homologous series of 1, 2, 9, 10-tetraoxygenated (S)-aporphine alkaloids can discriminate between α_{1A}- and α_{1B}-adrenoceptor recognition sites that have been labelled with [^{3}H]-prazosin in rat cerebral cortex. All the compounds tested showed a selective action for α_{1A}-adrenoceptors and the results obtained suggested that a free hydroxyl group at C-2 is associated with increased α_{1A} *vs.* α_{1B}-adrenoceptor selectivity and affinity. This hypothesis arose from the results of competition binding studies in rat cerebral cortex where [^{3}H]-prazosin was displaced biphasically by all the compounds tested but with different affinities and selectivities. The relative order of selectivity for α_{1A}-adrenoceptors was boldine = predicentrine (both 2-hydroxylated) > glaucine (2-methoxylated)

In the present study and as an additional test of the effect of the molecular environment of the aporphine C-2 oxygen substituent upon receptor affinity, we have examined the binding behaviour of several halogenated derivatives of boldine, with a chlorine or bromine atom at C-3, next to the C-2 oxygen function, and in some cases with an additional halogen atom at C-8, next to C-9, which also bears an oxygen substituent. We have also analyzed the influence of introducing another kind of group (nitroso) in the vecinity of the 2 hydroxy function.

Our present results show that 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine bound to [^{3}H]-prazosin high affinity sites in rat cerebral cortex with two different affinities, which indicates the presence of at least two α_1-adrenoceptor subtypes. Data from our laboratory have established previously (4) that in the rat cerebral cortical membranes the prazosin-high affinity sites are known to be composed of α_{1A} and α_{1B} subtypes at a ratio of approximately 37:63 as previously reported in the literature (17, 18, 19, 20, 21). On the basis of pharmacological criteria, binding sites for [^{3}H]-prazosin with high affinity for WB-4101 and phentolamine (19), as well as oxymetazoline (22), benoxathian (23), 5-methylurapidil (24), and S(+)-niguldipine (25) were designated α_{1A}-adrenoceptors, whereas binding sites with low affinity for these ligands were designated α_{1B}-adrenoceptors. The fact that the affinity constants of the different selective antagonists tested in rat cerebral cortex (4) at their high (α_{1A}) and low affinity sites (α_{1B}) are consistent with those obtained in previous studies (16, 17, 18) as well as with their affinities at cloned α_{1A} or α_{1B}-adrenoceptors (*for review see* 3, 26) respectively, confirms the existence of an α_{1A}/ α_{1B} mixed population of adrenoceptors in rat cerebral cortex. All boldine derivatives recognized a similar proportion of high affinity sites, that is, about 40%. Since this proportion is similar to the percentage of high affinity sites for selective α_{1A}-adrenoceptor agents (4, 17, 18, 19), the sites with high affinity for boldine derivatives probably belong to the α_{1A}-adrenoceptor subtype. This implies that 3-bromoboldine, 3,8-dibromoboldine, 3-chloroboldine and 3-nitrosoboldine are selective for α_{1A}-adrenoceptors relative to α_{1B}-adrenoceptors, which confirms our early impression that the 1, 2, 9, 10-tetraoxygenated (S)-aporphine skeleton leads to some α_{1A} selectivity (4).

The results obtained also showed that all three halogenated derivatives of boldine were able to act at α_1-adrenoceptor subtypes with similar degrees of affinity and selectivity but exhibited a slightly but significantly greater affinity and selectivity for α_{1A}-adrenoceptors than did boldine.

Boldine is approximately 70 fold α_{1A}-selective, whereas the selectivities of 3-bromoboldine, 3,8-dibromoboldine and 3-chloroboldine are 120-fold, 90-fold, and 122-fold, respectively. The fact that the halogenated compounds have a similar profile on α_1-adrenoceptor subtypes suggests that the introduction of a bromine or chlorine atom at position 3 of the (S)-aporphine nucleus increases the selectivity of the compound formed (3-bromoboldine or 3-chloroboldine), whereas the introduction of a second bromine atom at position 8 (3,8-dibromoboldine) did not significantly modify this activity respect to 3-bromoboldine. These results indicate that the presence of a single halogen atom ortho to the free hydroxyl group at position 2 increases the selectivity for the α_{1A}-adrenoceptor subtype, whereas the presence of a second bromine atom ortho to the free hydroxyl group at position 9 did not improve this selectivity. This in turn suggests a specific interaction of the free hydroxyl group at position 2 but not that at position 9, with the α_{1A}-adrenoceptor subtype. The present results corroborate and extend the hypothesis postulated in our previous study (4) that in this (S)-aporphine series, the 2-hydroxy function seems to be a critical factor in the interaction of these compounds with the α_{1A}-adrenoceptors while the 9-hydroxyl group does not. In fact, the 9-methoxylated predicentrine retains practically all the α_{1A}-selectivity of boldine (4).

We have also tested the influence of introducing a different group (nitroso) at C3. The resulting compound, 3-nitrosoboldine, showed a loss of more than an order of magnitude in the affinity for both α_1-adrenoceptors subtypes (α_{1A}- and α_{1B}) present in rat cerebral cortex, as compared to boldine. The introduction of a nitroso group at C-3 also leads to a great loss of selectivity for α_{1A}-adrenoceptors since 3-nitrosoboldine was only 35-fold α_{1A}-selective.

The putative interaction of the C-2 substituent with α_1-adrenoceptors could most reasonably involve hydrogen bonding. The introduction of a halogen group in the vecinity of the C-2 hydroxy function improves the interaction between this function and α_{1A}-adrenoceptor for slight but significant increases in both affinity and selectivity for α_{1A}-subtype were obtained. A possible explanation is that the C-3 halogen atom is making one of the lone electron pairs on the C-2 oxygen atom more accesible for hydrogen bonding. The reduced affinity of 3-nitrosoboldine for α_{1A}-adrenoceptors is consistent with this interpretation. In this compound, the C-2 hydroxyl group should be chelated with the C-3 nitroso oxygen atom, effectively anchoring its electron lone pairs in an orientation which would presumably be unfavourable for the suggested hydrogen bonding. This hypothesis should be explored both experimentally and with quantum-chemical and molecular modelling studies.

In order to corroborate the selective action of 3-bromoboldine on the α_{1A}-adrenoceptor subtype, we tested its effect on [^{3}H]-prazosin binding to rat cerebral cortical membranes pretreated with CEC, which has been reported to preferentially inactivate the α_{1B}- and α_{1D}-subtype relative to α_{1A}-subtype (12, 13, 14, 15, 16). In rat cortical membranes, the pharmacological profile of CEC-insensitive sites appeared to be indicative of α_{1A}-adrenoceptors, as selective antagonists like WB 4101, benoxathian, phentolamine, (+)niguldipine, or 5-methylurapidil, displayed potencies comparable to those obtained with the high affinity sites in competitive studies in normal membranes (17, 18, 27, 28) and with cloned α_{1A}-adrenoceptors (*for review see* 3, 26). In our experimental conditions it seems that CEC pretreatment is unable to completely eliminate low affinity sites for 3-bromoboldine. The competition curves for 3-bromoboldine remained shallow and biphasic following CEC treatment but the relative contribution of high affinity sites increased to 78%. Moreover, the 3-bromoboldine affinities at its high and low affinity sites remained

similar to those obtained in non-treated membranes. As far as the results with CEC are concerned, this compound is known to be an irreversible inhibitor of α_{1B}- or α_{1D}-adrenoceptors but has no effect on α_{1A}-adrenoceptors (12, 13, 14). Since it is known that the extent of CEC alkylation depends on several factors, such as temperature, concentration and time of incubation (3), we think the most likely explanation for the data obtained is that in our experimental conditions CEC pretreatment does not completely inactivate the α_{1B}-subtype. It is also possible that in the in rat cerebral cortex there is an additional CEC-insensitive subtype with an affinity for 3-bromoboldine similar to that obtained for the α_{1B} subtype. Nevertheless, the present results demonstrate that we were able to enrich the preparation in α_{1A}-adrenoceptors with CEC-pretreatment and have corroborated the greater affinity of 3-bromoboldine for this population.

In the present study we have also analyzed the effect of boldine derivatives on Ca^{2+} channels. We used [^{3}H]-(+)-*cis*-diltiazem binding to label the Ca^{2+} channel because in previous papers we had demonstrated that neither boldine (6) nor glaucine (5) or other structurally related compounds (29) interact with [^{3}H]-nitrendipine binding sites. The results obtained show that, as compared to boldine, the activity of the halogenated derivatives and that of 3-nitrosoboldine on [^{3}H]-(+)-*cis*-diltiazem binding were lower. As a result of the differences in the influence of halogenation on α_1-adrenoceptor and calcium channel, the selectivity of these (S)-aporphine derivatives with respect to α_1 -adrenoceptors was improved.

In summary the present study shows that, as compared to the parent compound boldine, bromine or chlorine substitution at position 3 increases the selectivity for the α_{1A} with respect to the α_{1B}-adrenoceptor subtype and decreases the effect on the benzothiazepine binding site of voltage-gated calcium channels, thereby improving the selectivity of the compound. However, further halogen substitution at position 8 of the (S)-aporphine nucleus (3,8-dibromoboldine) did not improve this activity with respect to 3-bromoboldine. In contrast, nitroso substitution at C-3 (3-nitrosoboldine) decreased both the affinity and selectivity for α_{1A}-adrenoceptors. On the basis of these results and our earlier ones, therefore, one can assume that the free hydroxyl group at position 2 plays an important role in determining the selectivity of boldine and its derivatives for the α_{1A}-adrenoceptor. Moreover, 3-bromoboldine or 3-chloroboldine exhibits one of the highest α_{1A}-adrenoceptor *versus* α_{1B}-adrenoceptor selectivities recorded when compared with previously described α_{1A}-selective compounds.

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