M. Dolores Ivorra[1]
Miguel Valiente[1, 3]
Sonia Martínez[1]
Yolanda Madrero[1]
M. Antonia Noguera[1]
Bruce K. Cassels[2]
Eduardo M. Sobarzo[2]
Pilar D'Ocon[1]

8-NH_2-Boldine, an Antagonist of α_{1A} and α_{1B} Adrenoceptors without Affinity for the α_{1D} Subtype: Structural Requirements for Aporphines at α_1-Adrenoceptor Subtypes

Abstract

Structure-activity analysis of 21 aporphine derivatives was performed by examining their affinities for cloned human α_{1A}, α_{1B} and α_{1D} adrenoceptors (AR) using membranes prepared from rat-1 fibroblasts stably expressing each α_1-AR subtype. All the compounds tested competed for [^{125}I]-HEAT binding with steep and monophasic curves. The most interesting compound was 8-NH_2-boldine, which retains the selective affinity for α_{1A}-AR (pKi = 6.37 ± 0.21) vs. α_{1B}-AR (pKi = 5.53 ± 0.11) exhibited by 1,2,9,10-tetraoxygenated aporphines, but shows low affinity for α_{1D}-AR (pKi < 2.5). Binding studies on native adrenoceptors present in rat cerebral cortex confirms the results obtained for human cloned α_1-AR subtypes. The compounds selective for the α_{1A} subtype discriminate two binding sites in rat cerebral cortex confirming a mixed population of α_{1A}- and α_{1B}-AR in this tissue. All compounds are more selective as inhibitors of [^3H]-prazosin binding than of [^3H]-diltiazem binding to rat cerebral cortical membranes. A close relationship was found between affinities obtained for cloned α_{1A}-AR and inhibitory potencies on noradrenaline-induced contraction or inositol phosphate accumulation in tail artery, confirming that there is a homogeneous functional population of α_{1A}-AR in this vessel. On the contrary, a poor correlation seems to exist between the affinity of 8-NH_2-boldine for cloned α_{1D}-AR and its potency as an inhibitor of noradrenaline-induced contraction or inositol phosphate accumulation in rat aorta, which confirms that a heterogeneous population of α_1-AR mediates the adrenergic response in this vessel.

Key words

Aporphines · human cloned α_1-adrenoceptor subtypes · cerebral cortex · aorta · tail artery · cyclic nucleotide phosphodiesterases

Abbreviations

NA:	noradrenaline
α_1-AR:	α_1-adrenoceptor
MDO:	methylenedioxy

Introduction

The aim of the present work was to perform a systematic study of a closely related series of compounds on the three different subtypes of α_1-adrenoceptor (α_1-AR), α_{1A}, α_{1B} and α_{1D} [1], in order to determine the optimal structural elements conferring selective interaction with each α_1-AR subtype to a common skeleton. The starting point of this work was the observation that in a homolo-

Affiliation
[1] Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
[2] Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[3] Current address: Laboratorio de Biología Molecular del Cancer. Instituto de Investigaciones Citológicas, Valencia, Spain

History
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Correspondence
M. P. D'Ocon · Departament de Farmacología · Facultat de Farmacia · Universitat de Valencia ·
Avda. V. Andrés Estellés s/n · 46100 Burjassot · Spain · Phone: +34-96-354-4828 · Fax: 34-86-354-4943 ·
E-mail: m.pilar.docon@uv.es

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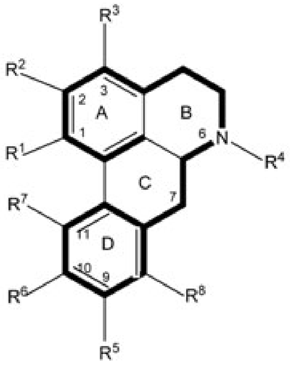
gous series of (*S*)-aporphine alkaloids, some of them (boldine and some halogenated derivatives, predicentrine and glaucine) can discriminate between α_{1A} and α_{1B} subtypes present in rat cerebral cortex [2], [3]. The fact that the introduction of a particular functional group can induce a significant increase in the selectivity for one subtype of α_1-AR suggests that these compounds might be important not only for the development of new selective agents for the α_1-AR family, but also for gaining a better understanding of the structure-activity relationships of α_1-AR subtype ligands.

We have extended our previous studies in native α_{1A} and α_{1B} rat adrenoceptors [2] to cloned human α_{1A}-, α_{1B}- and α_{1D}-AR by examining the binding behaviour of twenty-one aporphines: eleven 1,2,9,10-tetrasubstituted *(S)*-aporphines, seven 10,11-substituted *(R)*-aporphines and three *(R)*-methylenedioxyaporphines (Fig. **1**). Most compounds included in the study have been isolated from natural sources or synthesized as described later. Affinity for α_1-AR subtypes was determined by radioligand binding assays using membranes prepared from rat-1 fibroblasts stably expressing each human cloned α_1-AR subtype [4] and membranes prepared from rat cerebral cortex which contains the α_{1A} and α_{1B} subtypes [2]. We have also studied the activity of these compounds on vascular smooth muscle present in the rat tail artery and aorta where noradrenaline has been shown to predominantly mediate contraction through activation of α_{1A}-AR [5], [6] or α_{1D}-AR [6], [7], [8], [9], [10], [11], [12], [13].



No.	Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
1	(*R*)-Norapomorphine	H	H	H	H	H	OH	OH	H
2	(*R*)-Apomorphine	H	H	H	CH_3	H	OH	OH	H
4	(*R*)-*N*-*n*-Propylnorapomorphine	H	H	H	C_3H_7	H	OH	OH	H
6	(*R*)-Apocodeine	H	H	H	CH_3	H	OCH_3	OH	H
7	(*R*)-MDO-apomorphine	H	H	H	CH_3	H	$-O-CH_2-O-$		H
8	(*R*)-MDO-NPA	H	H	H	C_3H_7	H	$-O-CH_2-O-$		H
9	(*R*)-2,10,11-Trihydroxyaporphine	H	OH	H	CH_3	H	OH	OH	H
10	(*S*)-Bulbocapnine	$-O-CH_2-O-$		H	CH_3	H	OCH_3	OH	H
11	(*S*)-Glaucine	OCH_3	OCH_3	H	CH_3	OCH_3	OCH_3	H	H
12	(*S*)-3-Br-glaucine	OCH_3	OCH_3	Br	CH_3	OCH_3	OCH_3	H	H
13	(*S*)-3,8-Di-Cl-glaucine	OCH_3	OCH_3	Cl	CH_3	OCH_3	OCH_3	H	Cl
14	(*S*)-Boldine	OCH_3	OH	H	CH_3	OH	OCH_3	H	H
15	(*S*)-3-Br-boldine	OCH_3	OH	Br	CH_3	OH	OCH_3	H	H
16	(*S*)-3-Cl-boldine	OCH_3	OH	Cl	CH_3	OH	OCH_3	H	H
17	(*S*)-3-I-boldine	OCH_3	OH	I	CH_3	OH	OCH_3	H	H
19	(*S*)-3,8-Di-Br-boldine	OCH_3	OH	Br	CH_3	OH	OCH_3	H	Br
19	(*S*)-3,8-Di-Cl-boldine	OCH_3	OH	Cl	CH_3	OH	OCH_3	H	Cl
20	(*S*)-8-NH_2-boldine	OCH_3	OH	H	CH_3	OH	OCH_3	H	NH_2
21	(*S*)-8-NO-boldine	OCH_3	OH	H	CH_3	OH	OCH_3	H	NO

Fig. **1** Chemical structures of the tested compounds. The two phenylamine skeletons included in the aporphine structure and able to bind with α_1-AR are marked in bold.

Materials and Methods

Stable expression of human α_1-adrenoceptor subtypes in rat-1 fibroblasts

Transfection of each cDNA into rat-1 fibroblasts was accomplished by using the calcium phosphate precipitation method as described previously [4]. Individual colonies were isolated, subcloned and screened for high expression (1 – 2 pmol/mg of total protein). Transfected rat-1 fibroblasts stably expressing human α_{1A}, α_{1B} or α_{1D} ARs were grown and selected according to the general procedure described [4].

Radioligand binding

Human cloned α1-adrenoceptor subtypes: Membranes from Rat-1 cells expressing individual α_1-AR subtypes were prepared following the general procedure described [4]. Competition curves were determined in triplicate, with a final [^{125}I]-HEAT concentration of 100 pM as previously described [4]. Curves were fitted and pK_i calculated using non-iterative regression analysis (GraphPad, San Diego, CA).

Native adrenoceptors from rat cerebral cortex: Membranes were prepared from cerebral cortex of female Wistar rats (200 – 220 g) as reported elsewhere [2]. Competition curves were determined in triplicate, with a final [^3H]-prazosin or [^3H]-(+)-*cis*-diltiazem concentration of 0.2 nM or 3 nM, following the general procedure described previously [2], [14]. Curves were fitted and pK_i calculated using non-iterative regression analysis (GraphPad, San Diego, CA).

Functional studies in isolated organ bath

The studies were carried out according to the general procedure described [13]. Concentration-response curves of relaxation were obtained by adding cumulative concentrations of the compounds to aortic and tail artery rings in which maximal sustained contractions had been induced by NA (1 μM or 10 μM, respectively) in Ca^{2+} containing solution. Relaxations are expressed as percentages of the maximum increment in tension obtained by agonist addition. The concentration needed to produce 50% relaxation or inhibition (IC_{50}) was obtained from a non-linear regression plot (Graph Pad Software: San Diego, California, USA) and correlates well to the pA_2 values obtained by Schild analysis [15].

Accumulation of [^3H]-inositol phosphates

The determination of inositol phosphates accumulation has been described previously [13]. Aorta or tail artery rings (5 mm) were labelled with 10 μCi.ml^{-1} *myo*-[^3H]-inositol. Accumulation of [^3H]-inositol phosphates was calculated as the percentage of [^3H]-inositol-labelled lipids in each individual sample to correct for inter-experimental variations in labelling and sample sizes and was expressed as percentages of the unstimulated [^3H]-inositol phosphate accumulation (basal).

Data analysis

The results are presented as the mean ± S.E mean for *n* determinations obtained from different animals. Where ANOVA showed significant differences ($P < 0.05$), the results were further analyzed using the Student-Newman-Keuls test. Differences were considered significant when $P < 0.05$.

Drugs and solutions

IThe following drugs were used: [^3H]-prazosin (72 – 78 Ci mmol^{-1}), *myo*-[^3H]-inositol with PT6 – 271 (80 – 110 Ci mmol^{-1}) (Amersham International, Buckinghamshire, U.K.); [^3H]-HEAT (2200 Ci mmol^{-1}), [^3H]-*cis*-diltiazem (80 – 85 Ci mmol^{-1}) (DuPont-New England Nuclear, Boston; MA); acetylcholine chloride, diltiazem hydrochloride, (–)-noradrenaline bitartrate, phentolamine mesylate, prazosin (Sigma, St. Louis MO, USA). Other reagents were of analytical grade. All drugs were prepared daily in deionised water.

(R)-Apomorphine, *(S)*-apomorphine, *(R)*-norapomorphine, *(R)*-*N*-*n*-propylnorapomorphine, *(S)*-*N*-*n*-propylnorapomorphine, *(R)*-2,10,11-trihydroxyaporphine, *(R)*-apocodeine, *(R)*-MDO-*N*-*n*-propylnorapomorphine and *(S)*-bulbocapnine (compounds 1 – 6, 8 – 10) were purchased from RBI (Natick MA, U.S.A.). *(R)*-MDO-apomorphine was a gift from Prof. Abarca and Prof. Ballesteros (Dto. Química Orgánica, Univ. Valencia). *(S)*-Boldine, isolated from *Peumus boldus* (boldo) bark and crystallised in $CHCl_3$ as the 1 : 1 complex with this solvent, was methylated with diazomethane in MeOH-Et_2O to afford *(S)*-glaucine as described previously [16]. *(S)*-Boldine was halogenated with the appropriate *N*-halosuccinimide to obtain compounds 15 – 19 [17]. *(S)*-Glaucine was treated similarly to obtain compounds 12 and 13. *(S)*-8-NO-Boldine was obtained by nitrosation of *(S)*-boldine with $NaNO_2$ in AcOH, and *(S)*-8-NH_2-boldine was prepared by catalytic hydrogenation of *(S)*-8-NO-boldine [18]. Alkaloid solutions were prepared daily in deionised water with 0.1 % (w/v) ascorbic acid. In all tests carried out, the appropriate dilutions of ascorbic acid had no significant pharmacological effects.

Results and Discussion

All the compounds tested competed for [^{125}I]-heat binding in membranes from rat-1 cells stably expressing cloned α_1-AR subtypes with monophasic curves. Their pKi values are summarized in Table **1** and Fig. **2**. In the series of 10,11-dioxygenated aporphines, statistically higher affinity for α_{1D} than for α_{1B} or α_{1A} was obtained for each compound (**1 – 5**, in Fig. **2**). This selectivity depends on the presence or absence of certain hydroxy groups.

Methylation of the OH at C-10 [*(R)*-apocodeine] or introduction of a methylenedioxy group at C-10 and C-11 [*(R)*-MDO-apomorphine and *(R)*-MDO-NPA] (Fig. **1**) leads to loss of the selectivity for α_{1D}-AR subtype (Table **1**; compounds **6**, **7**, **8** in Fig. **2**). These results suggest that the interaction of the catechol group at C-10, C-11 with the binding site in the receptor is critical for imparting selectivity for the α_{1D}-AR subtype since it hinders the interaction with the other two subtypes. These results suggest that the phenylethylamine structure found in the D, C and B rings (Fig. **1**) could represent the portion of the molecule that interacts with the α_{1D} subtype.

Table **1** Affinity of the different compounds for human cloned α_1-AR subtypes as determined by competition experiments

		Human cloned α_1-AR subtypes		
No.	***Compound***	***α_{1A} (pK$_i$)***	***α_{1B} (pK$_i$)***	***α_{1D} (pK$_i$)***
1	(*R*)-Norapomorphine	4.39 ± 0.11	4.33 ± 0.04	5 28 ± 0.07
2	(*R*)-Apomorphine	4.59 ± 0.05	4.64 ± 0.08	5.63 ± 0.20
3	(*S*)-Apomorphine	5.80 ± 0.13***	5.68 ± 0.29***	6.41 ± 0.09**
4	(*R*)-*N*-*n*-Propylnorapomorphine	4.87 ± 0.06^{++}	4.87 ± 0.06^{+}	5.64 ± 0.03
5	(*S*)-*N*-*n*-Propylnorapomorphine	5.76 ± 0.05***	5.92 ± 0.16***	6.43 ± 0.06**
6	(*R*)-Apocodeine	4.70 ± 0.03	4.62 ± 0.09	4.64 ± 0.17
7	(*R*)-MDO-apomorphine	6.55 ± 0.08	5.93 ± 0.03	6.16 ± 0.19
8	(*R*)-MDO-NPA	7.04 ± 0.16	6.27 ± 0.06	6.72 ± 0.04
9	(*R*)-2,10,11-Trihydroxyaporphine	5.31 ± 0.11	5.36 ± 0.09	5.82 ± 0.17
10	(*S*)-Bulbocapnine	5.97 ± 0.05	4.99 ± 0.04	5.73 ± 0.15
11	(*S*)-Glaucine	5.79 ± 0.08	5.09 ± 0.10	4.96 ± 0.25
12	(*S*)-3-Br-glaucine	6.57 ± 0.12$^{\#\#}$	5.71 ± 0.12$^{\#\#}$	5.88 ± 0.07
13	(*S*)-3,8-Di-Cl-glaucine	6.38 ± 0.04$^{\#}$	5.23 ± 0.14	5.89 ± 0.11
14	(*S*)-Boldine	6.3± 0.17$^{\#}$	5.26 ± 0.04	4.90 ± 0.24
15	(*S*)-3-Br-boldine	7.12 ± 0.07$^{\circ\circ\circ}$	5.76 ± 0.04$^{\circ\circ}$	5.85 ± 0.16
16	(*S*)-3-Cl-boldine	7.20 ± 0.01$^{\circ\circ}$	5.82 ± 0.04°	5.58 ± 0.27
17	(*S*)-3-I-boldine	6.75 ± 0.12°	6.04 ± 0.09$^{\circ\circ\circ}$	5.70 ± 0.13
18	(*S*)-3,8-Di-Br-boldine	6.89 ± 0.08°	5.63 ± 0.08	5.12 ± 0.34
19	(*S*)-3,8-Di-Cl-boldine	6.98 ± 0.15$^{\circ\circ}$	5.78 ± 0.10$^{\circ\circ}$	6.14 ± 0.15°
20	(*S*)-8-NH_2-boldine	6.37 ± 0.21	5.53 ± 0.11	2.60 ± 0.03$^{\circ\circ\circ}$
21	(*S*)-8-NO-boldine	4.98 ± 0.05$^{\circ\circ\circ}$	3.83 ± 0.24$^{\circ\circ\circ}$	3.70 ± 0.36$^{\circ\circ}$

Data are expressed as pK$_i$ mean values of the tested agents ± S.E.M. Each experiment was performed in triplicate, with $n = 3 – 7$ individual experiments for each compound.
** $P < 0.01$, *** $P < 0.001$ vs. respective enantiomers; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. glaucine; $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ vs. *(R)*-norapomorphine; $^{\circ}$ $P < 0.05$, $^{\circ\circ}$ $P < 0.01$, $^{\circ\circ\circ}$ $P<0.001$ vs. boldine

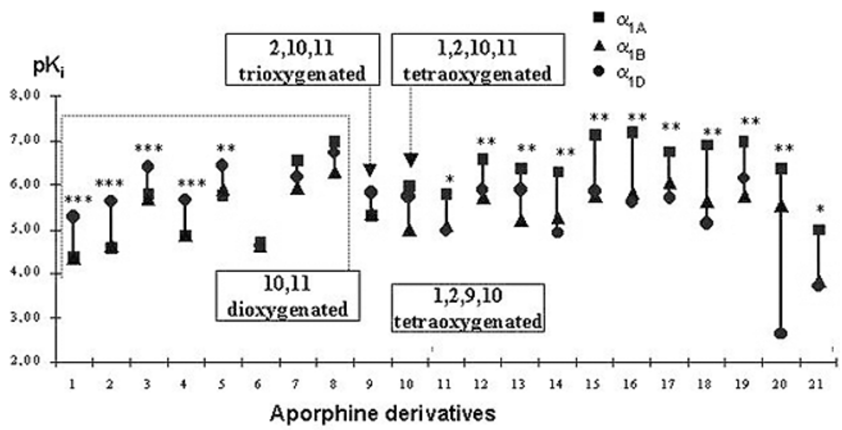


Fig. **2** Diagrammatic comparison of the affinities (pK_i values) of aporphine derivatives (**1 – 21**, see Table **1**) at the three subtypes of human cloned α_1-AR. * $P < 0.05$, **$P < 0.01$, *** $p < 0.001$ vs. other subtypes.

The presence of a hydroxy group at C-2 of the isoquinoline ring [*(R)*-2,10,11-trihydroxyaporphine] increases the affinity for α_{1A} and α_{1B}-AR subtypes. The putative interaction of this C-2 substituent with α_1-AR could involve hydrogen bond donation from the 2-hydroxy group to a relevant residue of the α_{1A}- or α_{1B}- (but not α_{1D}) AR. A similar hypothesis could be invoked to explain the role that the catechol group at C-10/C-11 plays in determining selective affinity for α_{1D}-AR. Taken together, these results suggest that a free hydroxy group located on the aromatic ring of the isoquinoline moiety of aporphines leads to a selective affinity for the α_{1A} and α_{1B} subtypes whereas a hydroxy group on the other aromatic ring affects their interaction with the α_{1D}-AR.

The absolute configuration at the chiral centre is a determining factor thereby confirming the existence of a stereoselective ligand binding pocket in the α_1-AR. *(S)*-Apomorphine showed a greater affinity for each α_1-AR subtype than its *(R)*-enantiomer. Similar results were obtained with *(R)*- and *(S)-N-n*-propilnorapomorphine (Table **1**). Interestingly, this stereoselectivity opposes the one assumed to be a general feature of the binding of apomorphine and closely related compounds to D1 and D2 dopaminergic receptors [20]. However, it is in agreement with the previously documented selectivity of *(S)*-apomorphine and *(S)-N-n*-propylnorapomorphine for α_1-AR in rat brain tissue [21]. Presumably, the absolute configuration at the chiral centre determines the orientation of the nitrogen atom lone pair or, in the protonated form, of the hydrogen atom bonded to nitrogen whereby the *(S)*-configuration provides a more suitable orientation for binding to the nitrogen binding site of the α_1-AR.

Introduction of a methyl or an *n*-propyl group at the N atom slightly increases the affinity for the three subtypes of α_1-AR in the following order: *(R)*-norapomorphine < *(R)*-apomorphine < *(R)-N-n*-propylnorapomorphine (Table **1**). It has been reported that an *N*-methyl group leads to more potent α_1-AR antagonism than either hydrogen or ethyl in the racemic nantenine series [20]. According to this, a hydrophobic chain appears to lead to better binding of aporphines to α_1-AR, possibly by stabilizing a conformation in which the nitrogen atom is in the best possible orientation for interaction with the conserved aspartate in the receptor binding site. Alternatively, it may interact with a hydrophobic pocket able to accommodate a short alkyl *N*-substituent as is believed to be the case for the D_2 dopamine receptor. In any case, these structural changes near the nitrogen atom affect all three α_1-AR subtypes in the same way, suggesting that the constitution of the receptor binding pocket complementary to this part of the aporphine molecule is similar in all three.

The series of 1,2,9,10-tetraoxygenated aporphines, glaucine, boldine and their halogenated derivatives (Table **1**, compounds **11 – 21** in Fig. **2**) corroborate the importance of the hydroxy group at C-2 for α_{1A}- and α_{1B}-AR interaction and suggest that the phenylethylamine structure included in the A and B rings (Fig. **1**) could represent the portion of the molecule that interacts with the α_{1A} and α_{1B} subtypes. The presence of this hydroxy group (**14**: boldine) vs. a methoxy group (**11**: glaucine) is associated with higher affinity for α_{1A}-AR as previous results in rat cortex have shown [2], [3]. Introduction of a halogen (Cl, Br or I) at C-3 increases the affinity of the compounds belonging to both the glaucine and boldine series for the three α_1-AR subtypes and reinforces the selectivity observed for the α_{1A}-AR with respect to both other subtypes. However, the introduction of a second halogen at C-8 has little effect, although in most cases, and at all receptor subtypes, it appears to decrease the affinity compared to the monohalo derivates while maintaining higher affinities if compared to the unhalogenated compounds.

Conversely, the affinity of *(S)*-8-NH_2-boldine for α_{1D}-AR is significantly decreased with respect to boldine while affinity for the other subtypes is not modified (Table **1**, compound **20** in Fig. **2**). These results suggest that the amino group at this position hinders binding of these molecules to the α_{1D}-AR active site. This observation has to be followed by a systematic study of the effects of introducing polar (or merely bulkier) substituents at this position. It is expected that additional data may allow an explanation of this reversed selectivity. Although the weak affinity of 8-NH_2-boldine for α_{1D}-AR is difficult to explain in terms of structure-activity relationship analysis, given the lack of a sufficient number of C-8 substituted analogues, the most interesting result of the present work is the evidence that this compound is able to discriminate between the α_{1A} and α_{1B}-AR subtypes while showing an almost complete lack of affinity for the α_{1D}-AR. At the moment, all selective α_1-AR antagonists commonly used in pharmacological studies retain appreciable affinity for each remaining subtype thereby complicating the interpretation of functional studies. A compound lacking any significant affinity for the α_{1D}-AR may be useful in determining the exact role of this subtype in a functional response as has been done using BMY 7378. It is the most com-

Table **2** Affinity of the aporphine derivatives for native α_1-AR subtypes present in membranes of rat cerebral cortex, as determined by competition experiments

No.	***Compound***	***Rat cerebral cortex*** $pK_{i\ high}$	$pK_{i\ low}$	***Hill slope***
1	(*R*)-Norapomorphine	5.32 ± 0.04		1.27 ± 0.09
2	(*R*)-Apomorphine	$5.67 \pm 0.04^{++}$		1.11 ± 0.04
3	(*S*)-Apomorphine	$6.68 \pm 0.04^{***}$		1.17 ± 0.04
4	(*R*)-*N*-*n*-Propylnorapomorphine	$5.63 \pm 0.03^{++}$		1.03 ± 0.007
5	(*S*)-*N*-*n*-Propylnorapomorphine	n. d.		
6	(*R*)-Apocodeine	5.15 ± 0.03		0.82 ± 0.06
7	(*R*)-MDO-apomorphine	6.18 ± 0.08		0.94 ± 0.04
8	(*R*)-MDO-NPA	6.33 ± 0.09		1.29 ± 0.13
9	(*R*)-2,10,11-Trihydroxyaporphine	5.85 ± 0.03		1.22 ± 0.13
10	(*S*)-Bulbocapnine	5.72 ± 0.09		0.90 ± 0.05
11	(*S*)-Glaucine$^{(1)}$	$7.12 \pm 0.10^{\circ\circ}$	$5.92 \pm 0.07^{\circ}$	0.83 ± 0.06
12	(*S*)-3-Br-glaucine	n. d.	n. d.	
13	(*S*)-3,8-DiCl-glaucine	n. d.	n. d.	
14	(*S*)-Boldine$^{(1)}$	$8.31 \pm 0.05^{\circ}$	6.50 ± 0.08	0.77 ± 0.03
15	(*S*)-3-Br-boldine$^{(1)}$	$8.93 \pm 0.09^{\circ}$	6.87 ± 0.10	0.72 ± 0.06
16	(*S*)-3-Cl-boldine$^{(1)}$	$8.65 \pm 0.16^{\circ}$	6.57 ± 0.13	0.64 ± 0.09
17	(*S*)-3-I-boldine	n. d.	n. d.	
18	(*S*)-3,8-DiBr-boldine$^{(1)}$	$8.87 \pm 0.20^{\circ}$	6.92 ± 0.12	0.78 ± 0.06
19	(*S*)-3,8-DiCl-boldine	n. d.	n. d.	
20	(*S*)-8-NH_2-boldine	$7.80 \pm 0.19^{\circ}$	6.45 ± 0.04	0.78 ± 0.03
21	(*S*)-8-NO-boldine$^{(1)}$	$6.41 \pm 0.09^{\circ\circ\circ}$	$4.93 \pm 0.07^{\circ\circ\circ}$	0.61 ± 0.01

Data are expressed as pK_i mean values of the tested agents ± S.E.M. Each experiment was performed in triplicate, with $n = 3-7$ individual experiments for each compound.
n. d. = not determined.
$^{(1)}$ Data from Madrero et al. (1996) and Martinez et al. (1999).
$^{++}$ $P < 0.01$ vs. *(R)*-norapomorphine; *** $P < 0.001$ vs. *(R)*-apomorphine; $^{\circ}$ $P < 0.05$, $^{\circ\circ}$ $P < 0.01$, $^{\circ\circ\circ}$ $P < 0.001$ vs. boldine.

monly used selective α_{1D}-AR antagonist and exhibits, in addition, partial 5-HT_{1A} serotonergic activity [22], [23].

The presence of a nitroso group at C-8 of boldine significantly decreases the affinity for all three receptor subtypes by more than one order of magnitude (Table **1**, compound **21** in Fig. **2**).

The specific binding of 0.2 nM [^3H]-prazosin to α_1-AR present in the rat cerebral cortex was completely inhibited by the alkaloids. The order of affinities obtained follows the same pattern as that obtained using human cloned α_{1A}- and α_{1B}-AR. Structural changes result in identical changes in affinity and selectivity (Table **2**).

The group of 1,2,9,10-tetraoxygenated compounds, which exhibit α_{1A}-AR selectivity in human cloned AR, give biphasic competition curves in brain membranes. The data fitted to a two site model as has been reported previously [2], [3]. The displacement affinity for the high ($pK_{i\ high}$) and low ($pK_{i\ low}$) affinity sites follows the same pattern as the one obtained with cloned α_{1A}- and α_{1B}-AR, respectively. The other aporphines tested showed monophasic displacement curves; they do not discriminate between α_{1A}- and α_{1B}-AR present in rat cerebral cortex.

In addition, these alkaloids also inhibited the binding of 3 nM [^3H]-(+)-*cis*-diltiazem to membranes from rat cerebral cortex, but with significantly ($P < 0.001$) lower affinity than that shown for [^3H]-prazosin binding (Table **3**). The presence of the hydroxy groups instead of methoxy groups at C-2 and C-9 (boldine vs. glaucine) significantly decreased affinity for the benzothiazepine binding site at the Ca^{2+} channel.

Noradrenaline, 1 μM or 10 μM, elicited maximal sustained contractile responses of rat aorta 896 ± 9.7 mg ($n = 97$) and tail artery 705.4 ± 5.6 mg ($n = 66$), respectively, in KBS at 37 °C. Addition of cumulative concentrations of alkaloids (0.001 – 100 μM) produced concentration-dependent relaxations of noradrenaline-precontracted aorta and tail artery. The pharmacological results expressed as pIC_{50} of these curves are summarized in Table **4**.

The potency of the aporphines as relaxants of noradrenaline-induced contraction in vessels follows the same structure-activity relationship pattern as found for the cloned or rat native α_1-AR subtypes.

The 1,2,9,10-tetraoxygenated aporphines with higher affinity for α_{1A}- than for α_{1B}- or α_{1D}-AR showed greater potency in tail artery than in aorta. This confirms the presence of a population of α_{1A}-AR functionally active in the tail artery as has been proposed previously [5], [6]. If the pIC_{50} obtained for each aporphine in functional studies are compared to the pK_i obtained in competition experiments on human cloned α_1-AR, a significant correlation ($P < 0.01$) between the results obtained for each compound in tail artery and cloned α_{1A}-ARs can be observed (Fig. **3**).

Table **3** Affinity (*pKi*) of the aporphine derivatives for [^3H]-(+)-*cis*-diltiazem binding sites in rat cerebral cortex membranes determined by competition experiments and inhibitory potencies (pIC_{50}) on contractions induced by 80 mM KCl in rat aorta

No.	*Compound*	*[^3H]-(+)-cis-diltiazem (pK_i)*	*KCl (pIC_{50})*
1	(*R*)-Norapomorphine	4.17 ± 0.19	4.23 ± 0.04
2	(*R*)-Apomorphine	4.36 ± 0.03	4.19 ± 0.09
3	(*S*)-Apomorphine	4.50 ± 0.10	4.37 ± 0.03
4	(*R*)-N-*n*-Propylnorapomorphine	4.49 ± 0.13	4.04 ± 0.12
5	(*S*)- N-*n*-Propylnorapomorphine	n. d.	n. d.
6	(*R*)-Apocodeine	4.59 ± 0.06	4.48 ± 0.03
7	(*R*)-MDO-apomorphine	4.50 ± 0.08	n. d.
8	(*R*)-MDO-NPA	4.60 ± 0.04	4.69 ± 0.15
9	(*R*)-2,10,11-Trihydroxyaporphine	>3	>3
10	(*S*)-Bulbocapnine	4.51 ± 0.09	4.10 ± 0.08
11	(*S*)-Glaucine	5.06 ± 0.11°	4.38 ± 0.04
12	(*S*)-3-Br-glaucine	n. d.	n. d.
13	(*S*)-3,8-DiCl-glaucine	n. d.	n. d.
14	(*S*)-Boldine	4.45 ± 0.02	4.19 ± 0.03
15	(*S*)-3-Br-boldine	>3	4.07 ± 0.11
16	(*S*)-3-Cl-boldine	>3	n. d.
17	(*S*)-3-I-boldine	n. d.	n. d.
18	(*S*)-3,8-DiBr-boldine	>3	4.05 ± 0.08
19	(*S*)-3,8-DiCl-boldine	n. d.	n. d.
20	(*S*)-8-NH_2-boldine	n. d.	>3
21	(*S*)-8-NO-boldine	>3	>3

Values are mean ± S.E.M of *n* = 4 – 10 individual experiments for each compound.
n. d. = not determined.
° P < 0.001 vs. boldine.

Table **4** Inhibitory potencies (pIC_{50}) of aporphine derivatives on contractions induced by noradrenaline (10 μM or 1 μM) in rat tail artery or aorta, respectively

No.	*Compound*	*Tail*	*Aorta*
1	(*R*)-Norapomorphine	n. d.	4.90 ± 0.05
2	(*R*)-Apomorphine	n. d.	5.09 ± 0.06
3	(*S*)-Apomorphine	n. d.	5.68 ± 0.09
4	(*R*)-*N*-*n*-Propylnorapomorphine	n. d.	5.27 ± 0.08
5	(*S*)- *N*-*n*-Propylnorapomorphine	n. d.	6.29 ± 0.10
6	(*R*)-Apocodeine	n. d.	4.68 ± 0.13
7	(*R*)-MDO-apomorphine	n. d.	n. d.
8	(*R*)-MDO-NPA	n. d.	5.78 ± 0.06
9	(*R*)-2,10,11-Trihydroxyaporphine	n. d.	5.32 ± 0.07
10	(*S*)-Bulbocapnine	5.31 ± 0.05	5.10 ± 0.07
11	(*S*)-Glaucine	6.12 ± 0.09	4.72 ± 0.08
12	(*S*)-3-Br-glaucine	6.26 ± 0.11	4.99 ± 0.14
13	(*S*)-3,8-Di-Cl-glaucine	5.80 ± 0.07	5.15 ± 0.17
14	(*S*)-Boldine	6.94 ± 0.12	4.92 ± 0.03
15	(*S*)-3-Br-boldine	7.85 ± 0.05	5.27 ± 0.06
16	(*S*)-3-Cl-boldine	7.08 ± 0.34	5.55 ± 0.10
17	(*S*)-3-I-boldine	6.77 ± 0.67	5.37 ± 0.10
19	(*S*)-3,8-Di-Br-boldine	7.53 ± 0.03	5.01 ± 0.15
19	(*S*)-3,8-Di-Cl-boldine	n. d.	n. d.
20	(*S*)-8-NH_2-boldine	6.28 ± 0.25	4.64 ± 0.32
21	(*S*)-8-NO-boldine	5.79 ± 0.09	4.44 ± 0.10

Values are mean ± S.E.M of *n* = 4 – 10 individual experiments for each compound.
n. d. = not determined.

In the aorta, a significant correlation was found regarding the α_{1D} ($P < 0.001$) and α_{1B} subtypes ($P < 0.01$) suggesting the presence of a mixed population of α_1-AR in this tissue, as has been reported previously by various authors and ourselves [6], [7], [9], [11], [13], [24].

We tested the same concentrations of compounds on the maximal contractile response induced by a depolarising solution (KCl 80 mM) in aorta. The magnitude of the contractile response obtained was 658.6 ± 23.7 mg (n = 90). No relaxant activity or a very low potency was observed in these experiments. This correlates well to the low affinity exhibited by these compounds for the [^3H]-(+)-*cis* diltiazem binding site in rat cerebral cortex (Table **3**).

The effect of some 1,2,9,10-tetraoxygenated aporphines (100 μM) on inositol phosphate accumulation induced by 10 μM noradrenaline was assayed in rat aorta and tail artery rings.

The potency of the aporphines as inhibitors of inositol phosphate accumulation in tail artery correlate with their potency as relaxants of noradrenaline-induced contraction in this tissue and with their affinity for the α_{1A}-subtype. However, in the aorta, the potency of the aporphines on inositol phosphate accumulation was higher than expected if we consider their affinity for α_{1D}-AR (Table **5**). This result corroborates the presence of a mixed population of α_1-AR functionally active in the aorta, especially if the potency of 8-NH_2-boldine in functional studies is considered.

In conclusion, the study of this series of aporphines provides information on structural requirements that determine the selec-

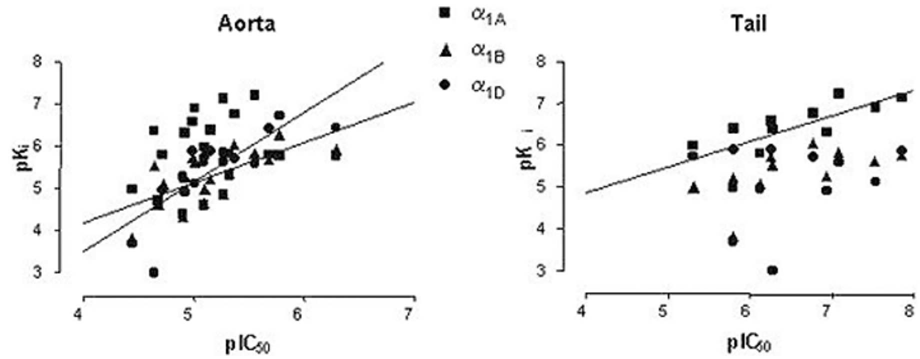


Fig. **3** Correlation plots comparing average pK_i values calculated from measurements with human cloned α_{1A}, α_{1B} or α_{1D}-AR subtypes with pIC_{50} values obtained in rat aorta or tail artery. The solid line represents the significant correlation according to the value of *P* obtained in each case: α_{1A}-AR in tail artery ($P < 0.01$), α_{1B}- and α_{1D}-AR in rat aorta ($P < 0.001$ and 0.0001, respectively).

Table **5** Functional potencies (pK_i) of compounds against noradrenaline-induced [^3H]-inositol phosphate accumulation in rat aorta and tail artery

No.	*Compound*	*Tail*	*Aorta*
11	(*S*)-Glaucine	$5.77 \pm 0.15^{*}$	5.59 ± 0.04
14	(*S*)-Boldine	$6.52 \pm 0.09^{\circ\circ}$	5.64 ± 0.09
15	(*S*)-3-Br-boldine	$6.97 \pm 0.08^{\circ\circ\circ}$	5.89 ± 0.04
19	(*S*)-3,8-DiBr-boldine	$6.75 \pm 0.12^{\circ}$	6.24 ± 0.11
20	(*S*)-8-NH_2-boldine	$6.65 \pm 0.06^{\circ\circ\circ}$	5.81 ± 0.05

Values are mean ± S.E.M. of $n = 4-6$ individual experiments for each compound.
$^{\circ}$ $P < 0.05$, $^{\circ\circ}$ $P < 0.01$, $^{\circ\circ\circ}$ $P < 0.001$ vs. aorta; * $P < 0.05$ vs. boldine.

tivity for each α_1-AR subtype. In addition, we have found that 8-NH_2-boldine is a new antagonist for α_1-AR with an interesting pharmacological profile: greater affinity for the α_{1A}- than for the α_{1B}-AR and no significant affinity for the α_{1D}-AR. Its affinity for the α_{1A}-AR is in a lower range than that of other compounds used as pharmacological references. However, it possesses a more than 100-fold lower affinity for the α_{1D}-AR. The therapeutic potential of subtype-selective compounds such as 8-NH_2-boldine should be evaluated as well.

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