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(+)-Cyclazosin, a selective α_{1B}-adrenoceptor antagonist: Functional evaluation in rat and rabbit tissues

Gabriella Marucci [a], Piero Angeli [a], Michela Buccioni [a], Ugo Gulini [a], Carlo Melchiorre [b], Gianni Sagratini [a], Rodolfo Testa [c], Dario Giardinà [a,*]

[a] Department of Chemical Sciences, University of Camerino, Via S.Agostino, 1, 62032 Camerino, Italy
[b] Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy
[c] Pharmaceutical R&D Division, Recordati SpA, Milano, Italy

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Abstract

To shed light on the discrepancy between reported binding and functional affinity and selectivity at $\alpha_{1b/B}$-adrenoceptors, the antagonist (+)-cyclazosin was reinvestigated in rat and rabbit tissues. It displayed a competitive antagonism at α_{1A} and α_{1D}-adrenoceptors of rat prostatic vas deferens and aorta with pA_2 values 7.75 and 7.27, respectively.

In rabbit thoracic aorta (+)-cyclazosin competitively antagonized noradrenaline-induced contractions at α_{1B}-adrenoceptors with a pA_2 value of 8.85, whereas its affinity at α_{1L}-adrenoceptors was markedly lower (pA_2 = 6.75 − 7.09).

In conclusion, these data confirmed that (+)-cyclazosin is a selective α_{1B}-adrenoceptor antagonist also in functional assays, showing 13- and 38-fold selectivity for the α_{1B}-adrenoceptor over α_{1A}- and α_{1D}-subtypes, respectively. Furthermore, (+)-cyclazosin displayed a significant selectivity for α_{1B}-adrenoceptors relative to the α_{1L}-subtype.

Keywords: α_1-Adrenoceptor subtype; α_{1B}-Adrenoceptor; Rabbit aorta; α_1-Adrenoceptor antagonist; α_{1B}-Adrenoceptor antagonist; (+)-Cyclazosin

1. Introduction

α_1-Adrenoceptors are members of the G-protein coupled superfamily of receptors, which play critical roles in the regulation of a variety of physiological processes. At present, three native subtypes, α_{1A}, α_{1B}, and α_{1D}, have been pharmacologically detected, which are recognized to correspond to the cloned α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors expressed in various cell lines (Bylund et al., 1994). In addition, also a fourth α_1-adrenoceptor, α_{1L}, displaying low affinity for prazosin (Muramatsu et al., 1995) and conformationally related to the α_{1A}-subtype (Ford et al., 1997), has been reported.

α_1-Adrenoceptors are of therapeutic interest because of their important role in the control of blood pressure and contraction and growth of smooth and cardiac muscle (Zhong and Minneman, 1999). Furthermore, current medical treatment of lower urinary tract symptoms, associated with or suggestive of benign prostatic hyperplasia, is based both on α_1-adrenoceptor non-selective antagonists (Moreland et al., 2004) and α_{1A}-adrenoceptor selective antagonists because of their uroselective character (Bock and Patane, 2000).

However, the functional role of specific α_1-adrenoceptor subtypes is not completely defined because of the lack of any very potent and subtype-selective α_1-adrenoceptor ligands. There is a particular need for α_{1B}-selective antagonists which, to date, have been discovered in very restricted numbers, in comparison to the α_{1A}- and α_{1D}-selective ligands. In fact, WB4101 (Morrow and Creese, 1986), (+)-niguldipine (Boer et al., 1989), Rec 15/2739 (Testa et al., 1997) and tamsulosin (Rabasseda and Fitzpatrick, 1996), are examples of α_{1A}-selective antagonists whereas compounds such as BMY-7378 (Goetz et al., 1995), A-119637 (Carroll et al., 2001), and (±)-domesticine (Indra et al., 2002) are selective for the α_{1D}-adrenoceptor.

* Corresponding author. Tel.: +39 0737 402265; fax: +39 0737 637345.
E-mail address: dario.giardina@unicam.it (D. Giardinà).

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Concerning the α_{1B}-adrenoceptor ligands, the alkylating agent chloroethylclonidine (CEC) was originally identified as a selective antagonist (Han et al., 1987). However, the recent finding that its receptor selectivity is localization-dependent instead of affinity-dependent (Hirasawa et al., 1997) made it the subject of controversy, and led to its falling into disfavor. Only a few reversible antagonists have been reported to be α_{1B}-selective, including spiperone (Hancock, 1996), risperidone (Sleight et al., 1993), (±)-cyclazosin (Giardina' et al., 1995), L-765,314 (Chang et al., 1998; Patane et al., 1998), and AH11110A (King et al., 1994), but their moderate selectivity, observed in radioligand binding assays, was not confirmed, with the exception of L-765,314, in functional experiments (Eltze et al., 2001).

The quinazoline chiral compound (+)-cyclazosin, (+)-([4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-(4a*R*,8a*S*)-perhydro-1-quinoxalinyl])(2-furyl) methanone) hydrochloride (Giardina' et al., 2004) (Fig. 1), was synthesized in our laboratory and characterized as an α_{1b}-selective ligand in binding assays both in animal and human cloned α_1-adrenoceptor subtypes (Giardina' et al., 1996). In contrast to this finding, a functional study, carried out on $\alpha_{1A,}$ $\alpha_{1B,}$ and α_{1D} adrenergic receptor of mesenteric artery, spleen, and aorta rat tissues, respectively, reported a low affinity for (+)-cyclazosin at all three α_1-adrenoceptors and, unexpectedly, no selectivity for the α_{1B}-subtype (Stam et al., 1998).

Given the sharp discrepancy between binding and functional affinity estimates at $\alpha_{1b/B}$-adrenoceptors (pK_i=9.16−9.87 and pK_B=7.96, respectively), we planned to reinvestigate the functional antagonism and selectivity of (+)-cyclazosin using two classical and widely utilized rat models for α_1-adrenoceptor subtypes, that is the prostatic vas deferens preparation for the α_{1A}-adrenoceptor (Eltze et al., 1991) and the aorta preparation for the α_{1D}-subtype (Ko et al., 1994).

The affinity of (+)-cyclazosin at α_{1B}-adrenoceptor was assessed on rabbit thoracic aorta preparation because in this tissue noradrenaline-induced contractions are mediated by a mixture of α_{1B} and α_{1L} adrenoceptors (Oshita et al., 1993) or by the α_{1B} subtype, as recently reported (Eltze et al., 2001).

In addition, the rabbit aorta preparation allowed us to assess also the (+)-cyclazosin affinity at α_{1L}-adrenoceptors by performing experiments with chloroethylclonidine pre-treated tissues or using methoxamine as agonist (Oshita et al., 1993).

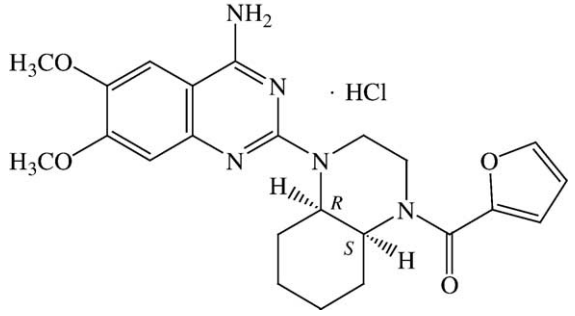

Fig. 1. Chemical structure of (+)-([4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-(4a*R*,8a*S*)-perhydro-1-quinoxalinyl]) (2-furyl) methanone) hydrochloride, (+)-cyclazosin.

2. Materials and methods

2.1. Drugs and chemicals

(+)-Cyclazosin [(+)-([4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-(4a*R*,8a*S*)-perhydro-1-quinoxalinyl])(2-furyl) methanone) hydrochloride] was synthesized in the Department of Chemical Sciences of Camerino University (Italy) as previously described (Giardina' et al., 1996) and dissolved in dimethylsulfoxide (DMSO) to prepare 0.01 M stock solution, which was then diluted with water to the concentration required for the experiments. In all experiments, the maximal concentration of DMSO in the bathing solution did not exceed 0.5% and had no effect on tissue contraction.

Chemicals, (−)-noradrenaline bitartrate, methoxamine hydrochloride, cocaine hydrochloride, normetanephrine hydrochloride, and (±)-propranolol hydrochloride were purchased from Sigma-Aldrich S.r.l. (Milano, Italy). Chloroethylclonidine dihydrochloride was obtained from RBI (Natick, MA, USA).

2.2. Functional experiments

Required tissues were taken from male Wistar rats (275–300 g; Charles River, Como, Italy) and male New Zealand white rabbits (2.7–3.2 kg; Bettinardi, Brescia, Italy). Rabbits were raised with fodder not containing particular additive for disease prevention.

All animal testing was carried out according to the European Community Council Directive of 24 November 1986 (86/609/EEC).

Rats were killed by cervical dislocation and the required organs were isolated. Vas deferens prostatic portion and aorta were freed from adhering connective tissue and set up rapidly, under a suitable tension, in 20-ml organ baths. Thoracic aortas were isolated also from rabbits, killed under pentobarbitone anaesthesia, then cleaned of adherent connective tissue. The bath medium, containing physiological salt solution (pH 7.4), was kept at 37 °C and aerated with 5% CO_2:95% O_2.

Concentration–response curves were constructed by cumulative addition of agonist. The agonist concentration in the bath was increased approximately 3-fold at each step, with each addition being made only after the response of the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer connected to the MacLab System PowerLab/800.

In all experiments a control agonist concentration–response curve (vehicle) was constructed in the presence of the maximum DMSO concentration (0.5%), which was not different from the previous one. The agonist-elicited concentration–response curves obtained in the presence of the indicated concentrations of antagonist were related to the vehicle control curve, of which the maximal response was taken as 100%.

Parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

The experimental conditions used for the pharmacological investigation at α_1-adrenoceptor subtypes are procedures taken from quoted literature.

2.3. Prostatic rat vas deferens

Affinity at α_{1A} adrenoceptors was evaluated on prostatic rat vas deferens according to Eltze et al. (1991). Prostatic portions of 2 cm length were mounted under 0.35 g tension at 37 °C in Tyrode solution of the following composition (mM): NaCl, 130; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 0.89; NaH_2PO_4, 0.42; $NaHCO_3$, 25; glucose, 5.6. To prevent the neuronal uptake of noradrenaline, cocaine hydrochloride (10 μM) was added to the Tyrode solution 20 min before the agonist cumulative concentration–response curve. Vas deferens were equilibrated for 45 min with washing every 15 min. After the equilibration period, tissues were primed twice by addition of 10 μM of the agonist noradrenaline in order to obtain a constant response. After another washing and equilibration period of 45 min, a cumulative isotonic noradrenaline concentration–response curve was constructed to determine the relationship between agonist concentrations and contractile response. When measuring the effect of the antagonist, it was allowed to equilibrate with the tissue for 30 min before constructing a new concentration–response curve to the agonist. The noradrenaline solution contained 0.05% $Na_2S_2O_5$ to prevent oxidation.

2.4. Rat and rabbit aortae

Affinity at rat aorta α_{1D} adrenoceptors was evaluated using a procedure adapted from that reported by Ko et al. (1994), whereas the affinity at rabbit aorta α_{1B} and α_{1L} adrenoceptors was assessed according to Oshita et al. (1993).

Two strips (15 mm × 3 mm) were cut helically from each rat thoracic aorta beginning from the end most proximal to the heart. The endothelium was removed by rubbing with filter paper: the absence of 100 μM acetylcholine-induced relaxation to preparations contracted with 1 μM noradrenaline was taken as an indicator that the vessel was denuded successfully. The strips were then tied with surgical thread and suspended in an organ bath containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 1.9; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7.

Cocaine hydrochloride (10 μM), normetanephrine hydrochloride (1 μM), and propranolol hydrochloride (1 μM) were added to prevent the neuronal and extraneuronal uptake of the agonist noradrenaline and to block the β-adrenoceptors, respectively. In the absence of these inhibitors the noradrenaline concentration–response curve was significantly displaced to the right (data not shown).

After an equilibration period of at least 2 h under an optimal tension of 1 g, cumulative noradrenaline concentration–response curves were recorded isometrically at 1-h intervals, the first being discarded and the second one taken as control.

After inspection of vehicle activity, the antagonist was allowed to equilibrate with the tissue for 30 min before generation of the third cumulative concentration–response curve to the agonist. Noradrenaline solutions contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

The cleaned rabbit thoracic aorta was cut helically, then the endothelial cells were removed by rubbing them with filter paper (Oshita et al., 1993). The strips were mounted in an organ bath containing a Krebs solution of the following composition (mM): NaCl, 112; KCl, 5.9; $MgCl_2$, 1.2; $CaCl_2$, 2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; glucose, 11.5. Cocaine hydrochloride (10 μM), normetanephrine hydrochloride (1 μM) and propranolol hydrochloride (3 μM) were added to prevent the neuronal and extraneuronal uptake of the agonist noradrenaline and to block the β-adrenoceptors, respectively. A resting tension of 1.5 g was applied and the responses were recorded isometrically through force-displacement transducers. Tissues were equilibrated for 90 min before the experiments were begun.

Concentration–response curves to noradrenaline (α_{1B}- and α_{1L}-mediated contractions) and to methoxamine (α_{1L}-mediated contractions) were cumulatively obtained, the first two being discarded and the third one taken as control. After inspection of vehicle activity, preparations were treated with the antagonist (+)-cyclazosin for 30 min before a new concentration–response curve of the suitable agonist was constructed.

In the experiments with chloroethylclonidine, tissues were pre-incubated for 20 min with 50 μM chloroethylclonidine, then extensively washed with the drug-free solution (40 min) before constructing the concentration–response curve to noradrenaline. The preparation was then treated, for 30 min, with various concentrations of (+)-cyclazosin before a new noradrenaline concentration–response curve was constructed. The noradrenaline solution contained 0.05% K_2EDTA in 0.9% NaCl to prevent oxidation.

2.5. Data analysis

In evaluating the antagonist activity of (+)-cyclazosin, the results were calculated as a percentage of the maximum response of the concentration–effect curve taken as control for each agonist used. Each response was plotted graphically as a mean from at least four separate experiments with vertical bars representing standard error of mean (S.E.M.). Curves were fitted to all the data by a non-linear regression using a Prism 3.0 program (GraphPad Software, San Diego, CA, USA) to calculate pEC_{50} values. In all cases, 50% of the maximum for each concentration–response curve was used to calculate the EC_{50} value. The EC_{50} value in presence and in absence of antagonist in a single tissue was used to determine the concentration ratio.

Schild plots were constructed to estimate the pA_2 values and the slope of the regression line using experimental series obtained from at least three different concentrations (Arunlakshana and Schild, 1959; Tallarida and Murray, 1987). The Schild diagrams were constructed by plotting the log (concentration ratio −1) against the log [antagonist] and deriving it from a linear regression using the Prism 3.0 program. Since the Schild plot slope was not significantly

different from unity ($P>0.05$), the regression was recalculated with a constrained slope of 1 and the result given as a pA_2 value.

Data were compared by Student's t test, a probability of less than 0.05 being assumed to denote a significant difference, and are presented as means±S.E.M. of 4 to 6 experiments.

3. Results

3.1. Effect on prostatic rat vas deferens α_{1A}-adrenoceptor

Noradrenaline induced reproducible contractions of the tissue with a pEC_{50} value of 5.89±0.01 and a maximum contraction of 1.09±0.12 g ($n=30$).

In the 0.1–1 μM concentration range, (+)-cyclazosin gave a parallel shift to the right of the agonist concentration–response curve without affecting the maximum response of the control curve (Fig. 2A). The Schild analysis gave a linear plot with a slope of 0.99±0.13 (not significantly different from 1.00, $P>0.05$), and a pA_2 value of 7.78±0.18 (7.77±0.05 from the regression line constrained to 1), indicating competitive antagonism (Fig. 2B).

3.2. Effect on rat aorta α_{1D}-adrenoceptor

Noradrenaline produced concentration-dependent contractions of the rat aorta with a pEC_{50} value of 7.85±0.01 and a maximum contraction of 0.96±0.18 g ($n=30$).

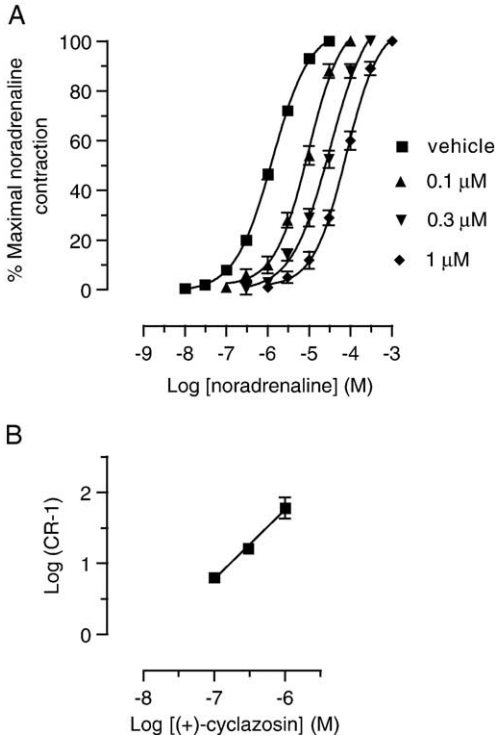

Fig. 2. (A) Concentration–response curves of noradrenaline-induced contractions in prostatic rat vas deferens in absence (vehicle) and presence of 0.1, 0.3, and 1 μM (+)-cyclazosin. (B) The Schild plot for the antagonism to noradrenaline response by (+)-cyclazosin was constructed with the mean concentration ratios (CR) of performed experiments. Data are the mean±S.E.M. of four separate experiments.

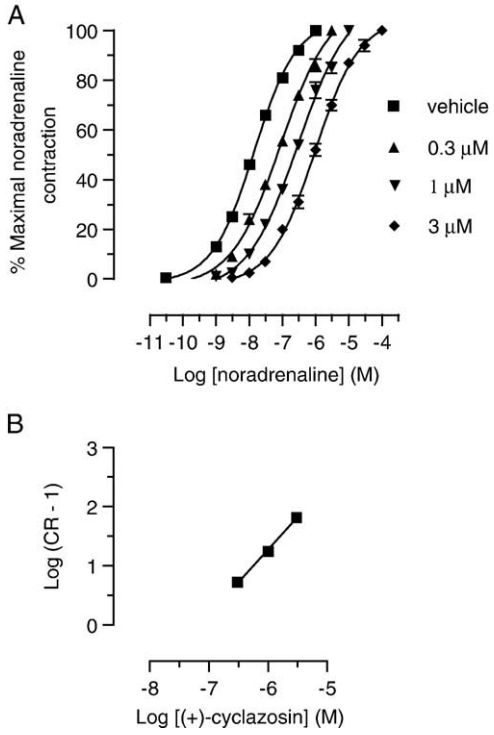

Fig. 3. (A) Concentration–response curves of noradrenaline-induced contractions in rat aorta in absence (vehicle) and presence of 0.3, 1, and 3 μM (+)-cyclazosin. (B) The Schild plot for the antagonism to noradrenaline response by (+)-cyclazosin was constructed with the mean concentration ratios (CR) of performed experiments. Data are the mean±S.E.M. of four separate experiments.

(+)-Cyclazosin (0.3-3 μM) inhibited noradrenaline-induced contractions in this tissue, producing a parallel shift of the agonist concentration–response curve without any appreciable reduction of the maximum response (Fig. 3A). The Schild plot gave a pA_2 value of 7.16±0.08 with a slope of 1.09±0.07, not significantly different from 1.00, $P>0.05$, indicating competitive antagonism (Fig. 3B). A pA_2 value of 7.27±0.03 was obtained by constraining the slope to 1. At 10 μM concentration, (+)-cyclazosin lost its competitive character as the rightward shift of the agonist concentration–response curve was greater than expected (data not shown).

3.3. Effect on rabbit aorta α_{1B}- and α_{1L}-adrenoceptors

Following the finding of Oshita et al. (1993), as confirmed by Eltze et al. (2001), we tested (+)-cyclazosin against noradrenaline-induced contraction of rabbit aorta, as a functional model for determining its α_{1B}-adrenoceptor antagonist activity. At the same time, this tissue was used to evaluate the antagonist potency of (+)-cyclazosin towards the α_{1L}-adrenoceptor by performing experiments both using noradrenaline as agonist on chloroethylclonidine pretreated tissue, and in the presence of methoxamine as agonist (Oshita et al., 1993).

Noradrenaline produced concentration-dependent contractions of the rabbit aorta with a pEC_{50} value of 7.18±0.02 and a maximum contraction of 1.48±0.11 g ($n=30$).

In the 0.003–0.1 μM concentration range, in a concentration-dependent manner and with no reduction of the agonist maximal response, (+)-cyclazosin antagonized noradrenaline-induced contractions with a parallel rightward displacement of concentration–response curves, characteristic of a competitive antagonist mechanism (Fig. 4A).

The pA_2 value calculated from a constrained Schild plot was 8.85±0.05 (pA_2=8.96±0.07, slope=0.90±0.05, not different from 1, P>0.05) (Fig. 4B). The antagonist competitive character was lost at higher concentrations as the shift of the agonist curve was not proportional to (+)-cyclazosin concentration (data not shown).

To test the affinity of (+)-cyclazosin at α_{1L}-adrenoceptors, experiments were performed by pretreating (20 min) the rabbit aorta tissue with 50 μM chloroethylclonidine, an α_{1B}-selective irreversible antagonist that was shown to mask α_{1B}-mediated noradrenaline contractions (Oshita et al., 1993).

In this situation, the noradrenaline curve showed a significant rightward displacement relative to the control (pEC_{50}=6.48±0.08 (n=6) vs. 7.18±0.02), without maximum depression, and a log (concentration ratio −1) value of 0.37±0.07.

(+)-Cyclazosin competitively blocked noradrenaline-induced contractions in chloroethylclonidine pretreated aorta, as indicated by the concentration-dependent rightward shift of the agonist curves (Fig. 5A) and the slope of the Schild plot of 0.91±0.14 (not significantly different from unity, P>0.05) (Fig. 5B), displaying a pA_2 value of 7.19±0.19 (7.09±0.05 with the slope constrained to 1).

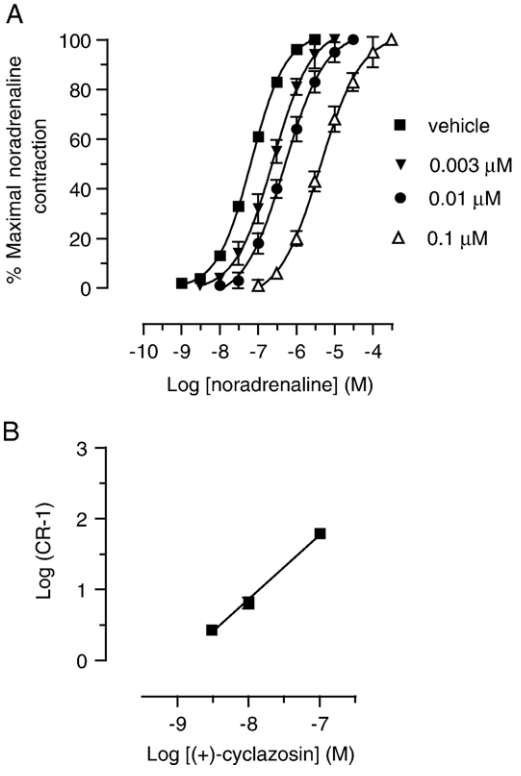

Fig. 4. (A) Concentration–response curves of noradrenaline-induced contractions in rabbit aorta in absence (vehicle) and presence of 0.003, 0.01, and 0.1 μM (+)-cyclazosin. (B) The Schild plot for the antagonism to noradrenaline response by (+)-cyclazosin was constructed with the mean concentration ratios (CR) of performed experiments. Data are the mean±S.E.M. of four to six separate experiments.

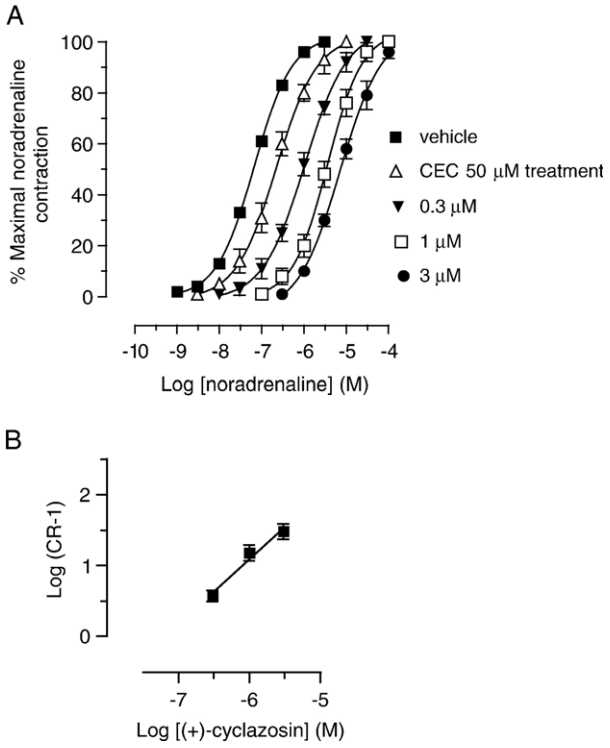

Fig. 5. (A) Concentration–response curves of noradrenaline-induced contractions in rabbit aorta in absence (vehicle), after pretreatment with 50 μM chloroethylclonidine (CEC) and in presence of 0.3, 1, and 3 μM (+)-cyclazosin. (B) The Schild plot for the antagonism to noradrenaline response by (+)-cyclazosin was constructed with the mean concentration ratios (CR) of performed experiments. Data are the mean±S.E.M. of four separate experiments.

To further confirm the antagonism of (+)-cyclazosin towards rabbit aorta α_{1L}-adrenoceptors, experiments using methoxamine as agonist were performed, as reported by Oshita et al. (1993).

Methoxamine gave a pEC_{50} value of 5.74±0.01 and a maximum contraction of 1.37±0.15 g (n=20).

In the 0.3–3 μM concentration range, (+)-cyclazosin competitively antagonized methoxamine-induced responses with a pA_2 value of 6.92±0.11 and a slope of 0.81±0.09, not significantly different from unity (P>0.05) (Fig. 6). The pA_2 value calculated from the constrained Schild plot was 6.75±0.05.

The pharmacological profile of (+)-cyclazosin is summarized in Tables 1 and 2.

4. Discussion

In a previous work (Giardina' et al., 1996), we reported the binding affinity of (+)-cyclazosin at α_1-adrenoceptor subtypes and a significant selectivity for α_{1b}-adrenoceptor. This compound, in fact, displayed 48- and 91-fold selectivity for hamster (pK_i=9.16) and human (pK_i=9.87) cloned α_{1b}-adrenoceptor relative to bovine (pK_i=7.48) and human (pK_i=7.91) α_{1a}-

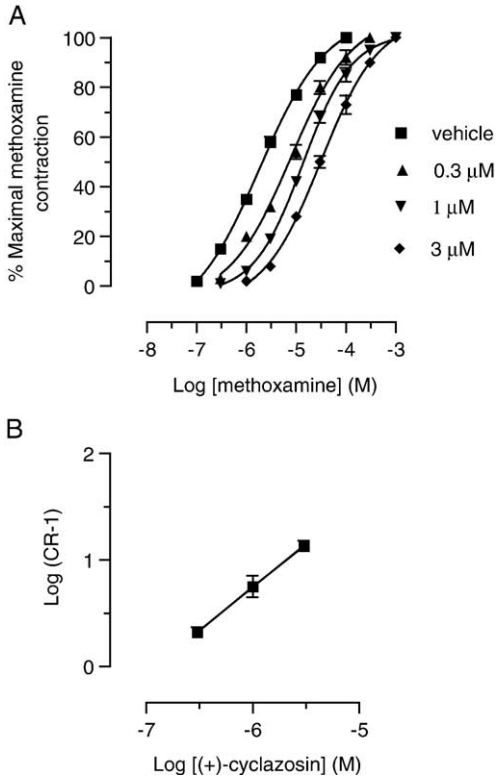

Fig. 6. (A) Concentration–response curves of methoxamine-induced contractions in rabbit aorta in absence (vehicle) and in presence of 0.3, 1, and 3 μM (+)-cyclazosin. (B) The Schild plot for the antagonism to methoxamine response by (+)-cyclazosin was constructed with the mean concentration ratios (CR) of performed experiments. Data are the mean±S.E.M of four separate experiments.

adrenoceptor subtypes. In addition, an 89-fold selectivity was also determined for native rat liver α_{1B}-adrenoceptor over the α_{1A}-subtype of the chloroethylclonidine-pretreated rat hippocampus (pK_i=9.68 vs. 7.73). Similarly, 39- and 24-fold higher affinity for hamster and human cloned α_{1b}-adrenoceptor, respectively, over the rat (pK_i=7.57) and human (pK_i=8.49) α_{1d} cloned subtypes, was found.

However, in a subsequent study (Stam et al., 1998), (+)-cyclazosin did not show any selectivity for α_1-adrenoceptor subtypes. In fact, antagonist potencies (pA_2/pK_B) of 7.78, 7.96 and 6.86 in the rat α_{1A}, α_{1B}, and α_{1D} adrenoceptors were reported. These data prompted us to reinvestigate the (+)-cyclazosin functional affinity both on rat α_{1A}- and α_{1D}-adrenoceptors, and on rabbit thoracic aorta α_{1B}-adrenoceptors.

Table 2
Cumulative selectivity ratios of (+)-cyclazosin in binding and functional experiments at α_1-adrenoceptor subtypes

Binding selectivity ratio [a]		Functional selectivity ratio [b]		
α_{1b}/α_{1a}	α_{1b}/α_{1d}	α_{1B}/α_{1A}	α_{1B}/α_{1D}	α_{1B}/α_{1L}
48	39	13	38	126

[a] Derived from data reported in Giardina' et al., 1996.
[b] An α_{1A}/α_{1L} selectivity ratio of 10 was also calculated.

(+)-Cyclazosin displayed competitive antagonism both in rat prostatic vas deferens (pA_2=7.77) and aorta (pA_2=7.27) as revealed by the slopes (0.99 and 1.09, respectively) of the Schild plot, which were not significantly different from unity. The antagonist potency towards the α_{1A}-adrenoceptor was not different from the affinity found in binding experiments (pK_i=7.48–7.91) and in the previous functional report (pK_B=7.78, Stam et al., 1998). Similarly, the potency of (+)-cyclazosin at the α_{1D}-adrenoceptor (pA_2=7.27) was very close to the rat binding affinity estimated value (pK_i=7.57) and the Stam value (pA_2=6.86), although it was 16-fold lower than that found at cloned human α_{1d} receptor (pK_i=8.49).

It was suggested that two distinct α_1-adrenoceptor subtypes (α_{1B} and α_{1L}) are involved in the noradrenaline-induced contractions of rabbit thoracic aorta, whereas only the α_{1L}-subtype predominantly mediates the methoxamine-induced effect (Oshita et al., 1993). In those experiments, the antagonist prazosin inhibited noradrenaline induced contractions by two distinct affinity constants (pK_B/pA_2=9.71 and 8.74), but by only one (pA_2=8.50) in chloroethylclonidine-pretreated experiments. On the contrary, methoxamine-induced contractions were blocked by prazosin with a potency close to the lower affinity value (pA_2=8.22).

On rabbit aorta (+)-cyclazosin behaved like prazosin, confirming that two functional α_1-adrenoceptors are activated by noradrenaline, but only one subtype by methoxamine.

When tested in a range of low concentrations (0.003–0.1 μM) against noradrenaline, (+)-cyclazosin evidenced a competitive antagonism, with a pA_2 value of 8.85, that was not observed at higher concentrations. Taking into account the

Table 1
Functional affinities (pA_2) of (+)-cyclazosin at α_1-adrenoceptor subtypes of rat and rabbit tissues in comparison with the binding affinities (pK_i) at animal cloned α_1-subtypes

pK_i [a]			pA_2 [b] (slope)			
Bovine	Rat	Hamster	Rat		Rabbit	
Brain (α_{1a})	Brain (α_{1d})	Smooth muscle (α_{1b})	Prostatic vas deferens (α_{1A})[c]	Thoracic aorta (α_{1D})[c]	Thoracic aorta (α_{1B})[c]	Thoracic aorta (α_{1L})[d]
7.48±0.05	7.57±0.00	9.16±0.02	7.75±0.05 (0.99±0.13)	7.27±0.03 (1.09±0.07)	8.85±0.05 (0.90±0.05)	6.75±0.05[e] (0.81±0.09)

All pA_2 values derive from constrained Schild plots for competitive antagonism (the slopes of regression lines are in parentheses).
[a] From Giardiná et al., 1996. [b] Data are reported as mean±S.E.M. of 4–6 experiments for each of the three tested concentrations. [c] In the presence of noradrenaline as agonist. [d] In the presence of methoxamine as agonist. [e] A pA_2 value of 7.09±0.05 was also obtained in chloroethylclonidine pretreated tissue contracted with noradrenaline.

finding by Oshita et al. (1993), this pA_2 value may be related to the blockade of α_{1B}-adrenoceptors, whereas the lack of competitive antagonism at concentrations higher than 0.1 μM may indicate the inhibition of a mixed population of α_{1B}/α_{1L}-adrenoceptors.

The decreased potency displayed by (+)-cyclazosin in chloroethylclonidine pretreated tissues (pA_2=7.09 vs. 8.85) confirms that the high affinity is related to the blockade of α_{1B}-adrenoceptors. On the contrary, the pA_2 value of 7.09 gives an estimate of (+)-cyclazosin affinity towards α_{1L}-adrenoceptors, which is coincident with the affinity (pK_B=7.10, slope=1) reported for rabbit bladder neck α_{1L}-adrenoceptors (Kava et al., 1998), and similar to the pA_2 value of 6.75 here obtained, by using methoxamine as the agonist.

A global analysis of the data obtained in the present work (Table 1) reveals that (+)-cyclazosin is a competitive antagonist at all three α_1-adrenoceptor subtypes, displaying a very high potency at α_{1B}-adrenoceptors, with a pA_2 value of 8.85, and a lower potency at α_{1A} and α_{1D} subtypes (pA_2=7.75 and 7.27, respectively). In comparison, a still lower affinity was observed at the putative α_{1L}-adrenoceptor, with pA_2 values of 6.75 and 7.09.

In conclusion, the present study has shown that (+)-cyclazosin is a potent α_{1B}-adrenoceptor antagonist. In addition, its binding selectivity for cloned α_{1b}-adrenoceptor, over the α_{1a} and α_{1d} subtypes, has been confirmed also in the functional experiments on isolated tissues, as indicated by the α_{1B}/α_{1A}- and α_{1B}/α_{1D}-selectivity ratio values of 13 and 38, respectively (Table 2). (+)-Cyclazosin was also decidedly more potent (126-fold) at the α_{1B}-adrenoceptors than at the α_{1L}-subtype. Finally, it distinguished, although to a lesser extent, α_{1A}- and α_{1L}-adrenoceptors, being 10-fold less potent at the latter receptor.

Taken together, the present results indicate that (+)-cyclazosin can be a useful tool for characterizing the α_{1B}-adrenoceptor subtype both in binding and functional studies.

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