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Short communication

Functional characterisation of the pharmacological profile of the putative α_{1B}-adrenoceptor antagonist, (+)-cyclazosin

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Abstract

We studied the functional pharmacological profile of (+)-cyclazosin, which has been characterised as a selective, high-affinity ($pK_i = 9.68$) α_{1B}-adrenoceptor ligand in binding experiments with rat liver membranes. The pK_B/pA_2 values for antagonism of contractions mediated via $\alpha_{1A/L}$-adrenoceptors of rat small mesenteric artery, α_{1D}-adrenoceptors of rat aorta and α_{1B}-adrenoceptors of rat spleen were 7.78 ± 0.04, 6.86 ± 0.07 and 7.96 ± 0.08, respectively. Furthermore, in mouse spleen, which is also regarded as an α_{1B}-adrenoceptor preparation, (+)-cyclazosin displayed low potency and did not act as a competitive antagonist. Thus, in contrast with results obtained in radioligand binding experiments, (+)-cyclazosin does not behave as a selective α_{1B}-adrenoceptor antagonist in functional tissues. Whether this discrepancy has consequences for the classification of α_1-adrenoceptors requires further investigation.

Keywords: α_1-Adrenoceptor; Aorta, rat; (+)-Cyclazosin; Small mesenteric artery, rat; Spleen, mouse; Spleen, rat; Tamsulosin

1. Introduction

Radioligand binding studies and molecular biology experiments have demonstrated the existence of at least three α_1-adrenoceptor subtypes, now referred to as α_{1A}, α_{1B} and α_{1D} (Hieble et al., 1995). Functional studies suggest the existence of an additional α_{1L}-adrenoceptor subtype displaying low affinity for prazosin (Hieble et al., 1995). Recently, it was postulated that the α_{1L}-adrenoceptor might represent a low affinity state of the α_{1A}-adrenoceptor (Ford et al., 1997). Selective competitive antagonists for α_{1A}- and α_{1D}-adrenoceptors, have been described in detail (see Hieble et al., 1995; Stam et al., 1996; Ford et al., 1997). Although the preferential susceptibility to irreversible inactivation by chloroethylclonidine has been used to subclassify α_{1B}-adrenoceptors (Hieble et al., 1995), the lack of a selective competitive antagonist has impeded a precise quantitative characterisation of α_{1B}-adrenoceptors. Initially, some data obtained in radioligand binding experiments suggested that spiperone and risperidone were competitive, selective α_{1B}-adrenoceptor antagonists, but functional studies were not able to confirm this (Burt et al., 1995; Eltze, 1996b). Recently, however, Giardina et al. (1996) have described a potent competitive α_{1B}-adrenoceptor antagonist, (+)-cyclazosin, which displays a 90- to 130-fold selectivity for binding to rat α_{1B}-adrenoceptors compared to α_{1A} and α_{1D} subtypes ($pK_i = 9.68$, 7.73 and 7.57 for rat liver α_{1B}, hippocampus α_{1A} and cloned α_{1d}-adrenoceptors, respectively). The selectivity of (+)-cyclazosin on functional responses mediated by the α_1-adrenoceptor subtypes has however not yet been studied. Therefore, in the present study we examined the effect of (+)-cyclazosin on the contractile responses to noradrenaline and phenylephrine in rat small mesenteric artery, rat aorta and rat and mouse spleen, responses which are believed to be mediated mainly by $\alpha_{1A/L}$- (Stam et al., 1996), α_{1D}- (Hieble et al., 1995) and α_{1B}-adrenoceptors (Burt et al., 1995; Hieble et al., 1995; Eltze, 1996a), respectively.

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2. Materials and methods

2.1. Tissue preparation

The mesentery, aorta and spleen were isolated from male Wistar rats (250–350 g) and spleen from white mice (25–30 g) which had been killed by cervical dislocation. Rats received prior anaesthesia (sodium pentobarbitone, 60 mg kg^{-1}, i.p.). Tissues were placed in ice-cold modified Krebs–Henseleit solution (KHS) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5.5, $CaCl_2$ 2.5 and ethylenediaminetetraacetic acid 0.026. The Ca^{2+} concentration ($CaCl_2$ = 0.25 mM) used for rat aorta was one tenth of that of standard KHS (see Van der Graaf et al., 1996a). Tissues were mounted in thermostatically controlled (37°C) organ baths to measure isometric contractions. The bath medium was continuously gassed with 95% O_2 and 5% CO_2.

Rat small mesenteric arteries were isolated from the arterial tree and mounted as ring segments (~2 mm in length) in a myograph (J.P. Trading, Aarhus, Denmark), as described by us previously (Van der Graaf et al., 1996b). After a 30 min stabilization period, the preparations were challenged five times with noradrenaline (10 μM) with washouts after each challenge. The endothelium was left intact, since its removal turned out to be technically difficult and was found to be associated with a substantial decrease in the functional reactivity (unpublished observation). The integrity of the endothelium was confirmed after the first challenge with noradrenaline by using acetylcholine (10 μM), which produced at least 60% relaxation in all tissues.

After removal of the endothelium by gentle rubbing with a polyethylene tube, rat aortic ring segments (3 mm) were mounted in 15-ml organ baths and equilibrated at 20 mN for 90 min. Subsequently, a calibration contraction was obtained to 30 μM 5-hydroxytryptamine (5-HT) and the absence of the endothelium was then confirmed by the lack of relaxation in response to acetylcholine (10 μM).

Rat and mouse splenic strips, obtained after longitudinal bisection, were mounted in 15 ml organ baths and equilibrated at a tension of 15 mN for 90 min and at 8 mN for 60 min, respectively.

2.2. Experimental protocol

Tissues were incubated with desipramine (10 μM), timolol (6 μM) and corticosterone (10 μM) to block neuronal uptake, β-adrenoceptors and non-neuronal uptake, respectively. Sixty minutes later and in the presence of these substances, agonist (noradrenaline in rat small mesenteric artery and mouse spleen; phenylephrine in rat aorta and spleen) concentration-effect ($E/$[A]) curves were recorded. In the ase of rat small mesenteric artery, cocaine (30 μM) replaced desipramine, corticosterone was omitted and SCH-23390 (10 nM) was added to block dopamine D_1 receptors (Van der Graaf et al., 1996b).

A multiple-curve design was used in experiments with rat small mesenteric artery and rat and mouse spleen. After the first (rat small mesenteric artery and spleen) or third (mouse spleen) agonist $E/$[A] curve was recorded, each tissue segment was washed (rat small mesenteric artery: 30 min, rat spleen: 120 min, mouse spleen: 60 min) and equilibrated (60 min) with vehicle or antagonist at different concentrations. Subsequently, another agonist $E/$[A] curve was obtained and the responses were expressed as a percentage of those of the preceding agonist curve.

A single curve design was used for rat aorta. Thus, after the calibration contraction in response to 5-HT, separate segments from each vessel were incubated with either vehicle or different antagonist concentrations. Subsequently, a single $E/$[A] curve was obtained for phenylephrine. Data are expressed as percentages of the calibration contraction.

2.3. Analysis

Individual agonist curve data were fitted to the Hill equation by using an iterative, least-squares method to calculate the midpoint location (pEC_{50}), Hill slope (n_H) and upper asymptote (α). The effect of drug treatment on these parameters was assessed by one-way analysis of variance (ANOVA) or Student's *t*-test, as appropriate. Values of $P < 0.05$ were considered to be significant.

When minimum criteria for competitive antagonism were satisfied, that is the antagonist produced a parallel rightward shift of the agonist $E/$[A] curve with no change in the upper asymptote, antagonist affinity was estimated by fitting the individual pEC_{50} values obtained in the absence and presence of antagonist to the Schild equation as described previously (Van der Graaf et al., 1996a). When the Schild plot slope parameter (b) was not significantly different from unity, the data were re-fitted with b constrained to unity so that the antagonist dissociation equilibrium constant, K_B, could be estimated (Jenkinson et al., 1995). When the criteria of competitive antagonism were not completely satisfied, an empirical pA_2 value was estimated by using the Schild equation, with b constrained to unity. All data are presented as means ± S.E.M.

2.4. Compounds

Compounds were obtained from the following sources: cocaine hydrochloride, 5-hydroxytryptamine creatine sulphate (5-HT), (−)-noradrenaline hydrochloride, acetylcholine chloride, (−)-phenylephrine hydrochloride, desipramine, corticosterone were from Sigma, The Netherlands; SCH-23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) was from Research Biochemicals, USA; timolol maleate was from ICN Biomedicals, The Netherlands; tamsulosin hydrochloride was a gift from Yamanouchi

Pharmaceutical, Japan; (+)-cyclazosin (+[4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]-furan-2-ylmethanone) was a gift from Dr. A. Leonardi, Recordati, Italy. (+)-Cyclazosin was dissolved in dimethylsulfoxide to give a 0.1 M stock solution and further diluted in distilled water. Corticosterone was dissolved in ethanol to give a stock solution of 30 mM. All other drugs were dissolved in distilled water.

3. Results

3.1. Effect of (+)-cyclazosin on noradrenaline-induced contraction of rat small mesenteric artery

Noradrenaline produced concentration-dependent contractions of rat small mesenteric artery (Fig. 1A). Hill parameters of the control noradrenaline $E/[A]$ curves ($n = 7$) were: midpoint location (pEC_{50}) = 6.40 ± 0.17, Hill slope (n_H) = 3.34 ± 0.45 and upper asymptote (α) = 95 ± 2% of that of the first noradrenaline $E/[A]$ curve (20.6 ± 2.1 mN). (+)-Cyclazosin (0.1–1 μM) produced a parallel, rightward shift of the noradrenaline $E/[A]$ curve. Schild analysis (Fig. 1B) yielded a slope parameter not different from unity (1.15 ± 0.11, $df = 18$) and a pK_B of 7.78 ± 0.04 was estimated.

3.2. Effect of (+)-cyclazosin on phenylephrine-induced contraction of rat aorta

Phenylephrine produced concentration-dependent contractions of rat aortic rings: $pEC_{50} = 6.76 \pm 0.05$, $n_H = 0.62 \pm 0.04$ and $\alpha = 108 \pm 7\%$ of that of the 5-HT calibration contraction (9.3 ± 0.1 mN, $n = 5$). (+)-Cyclazosin (0.1–3 μM) concentration dependently shifted the phenylephrine $E/[A]$ curve to the right (Fig. 1C). However, the criteria for competitive antagonism were not completely satisfied, since (+)-cyclazosin produced a concentration-dependent steepening of the $E/[A]$ curves ($n_H = 0.75 \pm 0.02$, 0.82 ± 0.06, 0.89 ± 0.02 and 0.96 ± 0.06 for 0.1, 0.3, 1 and 3 μM (+)-cyclazosin, respectively ($P < 0.001$). Notwithstanding this complexity, Schild analysis was per-

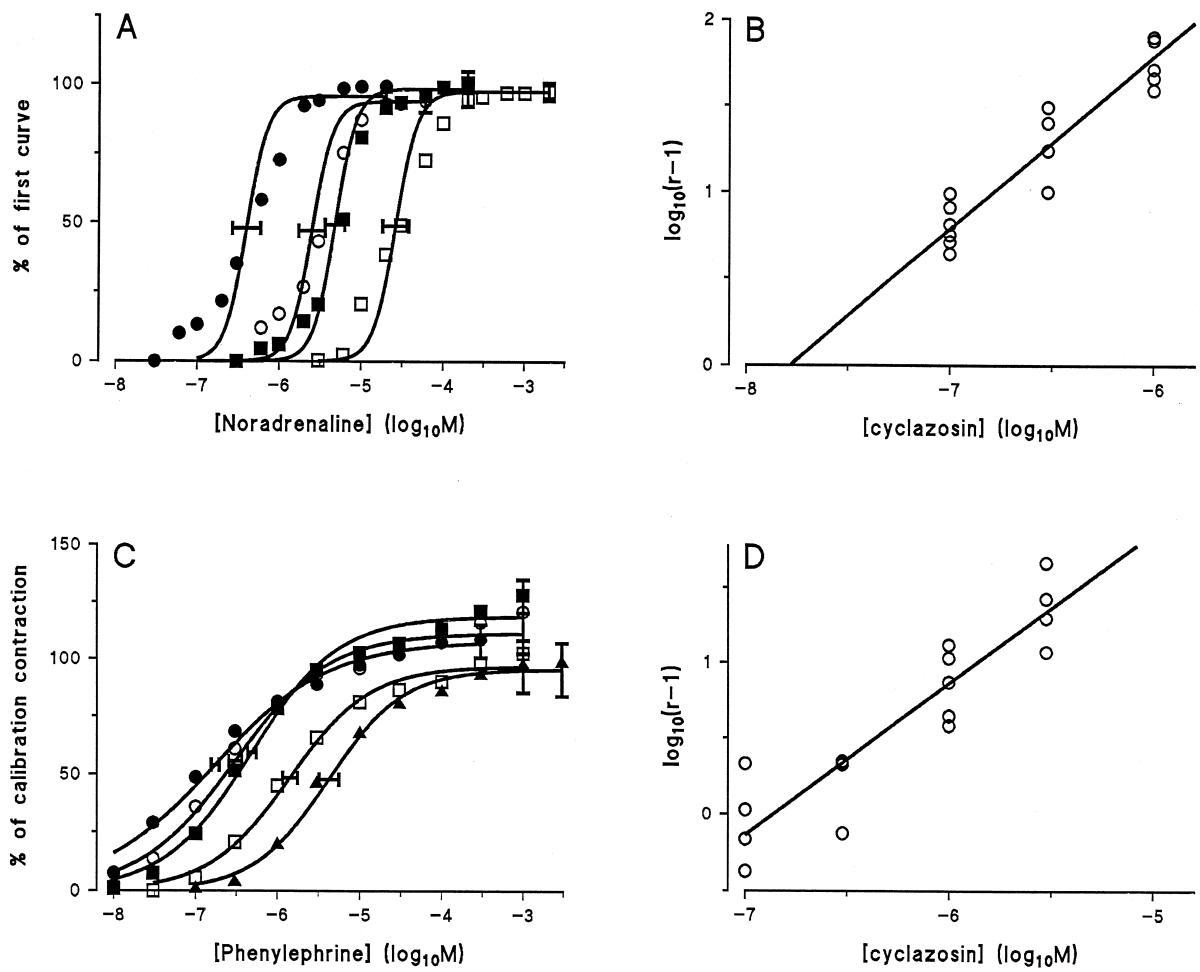

Fig. 1. $E/[A]$ curves of noradrenaline in rat small mesenteric artery (panel A) and phenylephrine in rat aorta (panel C) in the absence (●) or presence of 0.1 (○), 0.3 (■), 1 (□) and 3 (▲) μM (+)-cyclazosin. The corresponding Schild plots are shown in panels B and D. The lines superimposed on the data points were determined by using parameters obtained from the constrained model fit.

formed (Fig. 1D). The Schild slope parameter was not different from unity (1.07 ± 0.09, $df = 21$) and a pA_2 value of 6.86 ± 0.07 was estimated.

3.3. Effect of (+)-cyclazosin on phenylephrine-induced contraction of rat spleen

Hill parameters for the phenylephrine-induced contraction of vehicle-treated rat spleen ($n = 6$) were $pEC_{50} = 5.22 \pm 0.04$, $n_H = 0.75 \pm 0.05$ and $\alpha = 127 \pm 9\%$ of that of the first phenylephrine $E/[A]$ curve (3.5 ± 0.1 mN). (+)-Cyclazosin at concentrations of 0.03–0.3 μM produced a parallel, rightward displacement of the phenylephrine $E/[A]$ curve (Fig. 2A). In the presence of a higher concentration of (+)-cyclazosin (1 μM), the maximum of the agonist $E/[A]$ curve could not be attained with the highest concentration of phenylephrine (10 mM), and the phenylephrine $E/[A]$ curve flattened ($n_H = 0.45 \pm 0.01$, $P < 0.05$). Schild analysis was performed only for the concentrations of (+)-cyclazosin (0.03–0.3 μM) that met the criteria of competitive antagonism: $b = 1.02 \pm 0.20$ ($df = 11$) and $pK_B = 7.96 \pm 0.08$ (Fig. 2B).

For comparison, the affinity of the reference α_1-adrenoceptor antagonist, tamsulosin, was estimated. Tamsulosin (10 nM) produced a parallel rightward displacement of the phenylephrine $E/[A]$ curve and yielded a pA_2 of 9.16 ± 0.14 ($n = 3$), similar to that reported by Noble et al. ($pA_2 = 8.9$; Noble et al., 1997).

3.4. Effect of (+)-cyclazosin on noradrenaline-induced contraction of mouse spleen

As shown in Fig. 2C, noradrenaline produced concentration-dependent contractions of mouse spleen and the Hill parameters in the control ($n = 8$) tissue were $pEC_{50} = 6.44 \pm 0.07$, $n_H = 0.74 \pm 0.04$, $\alpha = 107.3 \pm 0.4\%$ of that of the third $E/[A]$ curve (2.7 ± 0.02 mN). (+)-Cyclazosin (0.1 μM) produced a parallel, rightward shift of the noradrenaline $E/[A]$ curve, with an associated pA_2 value of 7.38 ± 0.08. Higher concentrations of (+)-cyclazosin (0.3 and 1 μM), however, did not produce any further shift (Fig. 2C). A slight decrease in the maximal response ($\alpha = 89.4 \pm 0.6\%$, $P < 0.05$) was produced by (+)-cyclazosin (1 μM). The affinity estimate determined from

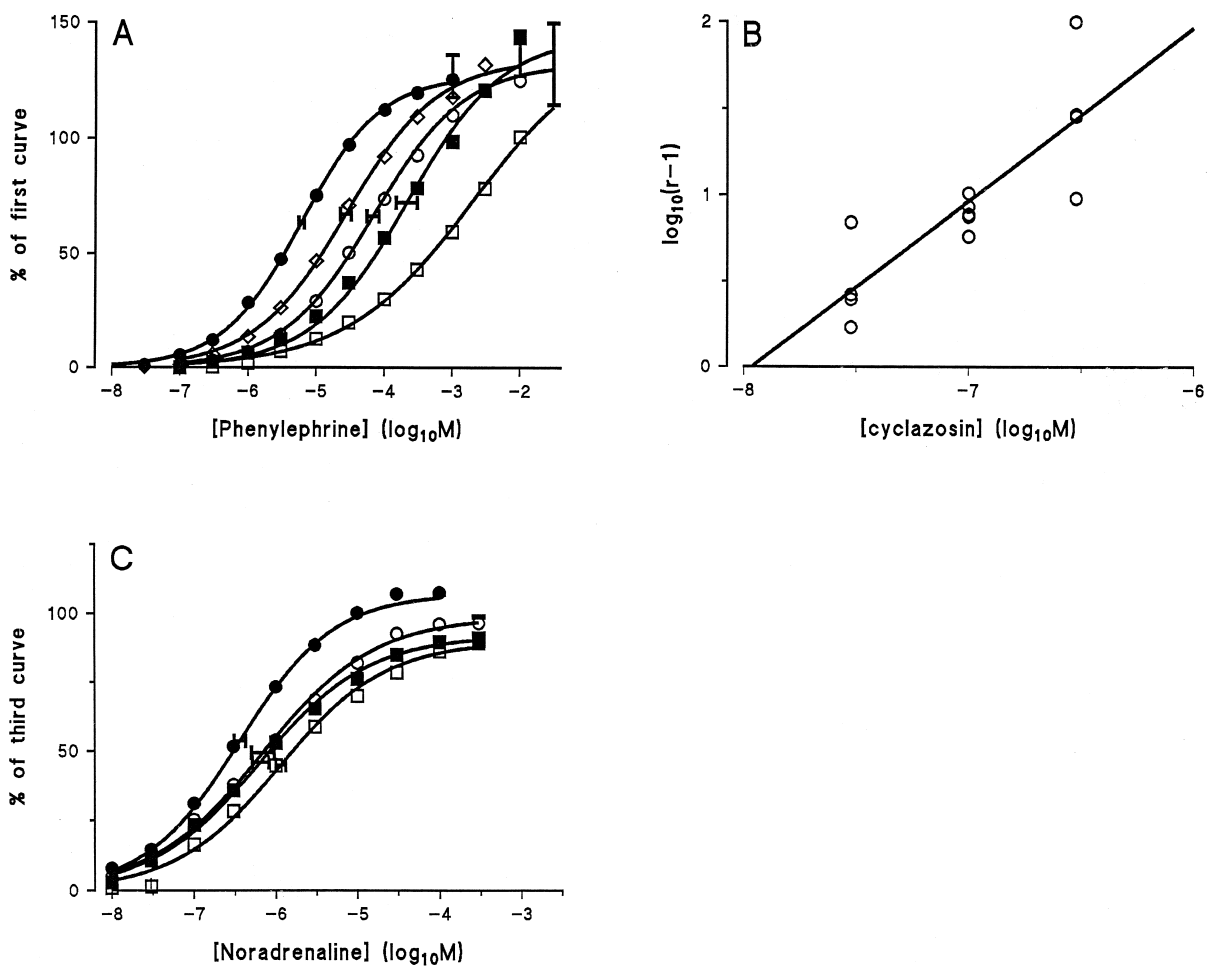

Fig. 2. $E/[A]$ curves of phenylephrine in rat spleen (panel A) and noradrenaline in mouse spleen (panel C) in the absence (●) or presence of 0.03 (◇), 0.1 (○), 0.3 (■) and 1 (□) μM (+)-cyclazosin. The corresponding Schild plot in the rat spleen is shown in panel B. The line superimposed on the data points was determined by using parameters obtained from the constrained model fit.

the rightward shift produced by 10 nM tamsulosin ($pA_2 = 8.44 \pm 0.14$, $n = 3$) was in good agreement with that of a previous report ($pK_B = 8.62$; Eltze, 1996a).

4. Discussion

In this study, we characterised the potency of the putative α_{1B}-selective antagonist (+)-cyclazosin in tissues expressing different subtypes of functional α_1-adrenoceptors. The affinity estimate of (+)-cyclazosin in rat small mesenteric artery ($pK_B = 7.78 \pm 0.04$) was in agreement with the reported binding affinity for α_{1A}-adrenoceptors in rat hippocampus, human cloned α_{1a}-adrenoceptors and α_{1L}-adrenoceptors ($pK_i/pK_B = 7.1–7.7$; Giardina et al., 1996; Kava et al., 1998).

In rat aorta, which is considered to be a functional α_{1D}-adrenoceptor correlate (Hieble et al., 1995), the rightward displacement of the phenylephrine $E/[A]$ curves by (+)-cyclazosin was accompanied by a concentration-dependent steepening of the phenylephrine $E/[A]$ curve. This phenomenon has also been reported for other antagonists and is suggested to be due to the expression of two closely related forms of the α_{1D}-adrenoceptor in rat aorta (Van der Graaf et al., 1996a). The functional potency in rat aorta of (+)-cyclazosin ($pA_2 = 6.86$), however, was within the range of its affinity for rat cloned α_{1d}-adrenoceptors ($pK_i = 7.57$; Giardina et al., 1996).

On the basis of the high sensitivity to inactivation by chloroethylclonidine, the receptors mediating contraction of rat spleen in response to phenylephrine have been classified as α_{1B}-adrenoceptors (Han et al., 1987; Burt et al., 1995). However, the pA_2 value of 7.96 estimated from the competitive antagonism displayed by (+)-cyclazosin (0.03–0.3 μM) is incompatible with its affinity for rat liver α_{1B}-adrenoceptors ($pK_i = 9.68$; Giardina et al., 1996). This discrepancy with radioligand binding data led us to study the antagonism of (+)-cyclazosin in mouse spleen, a tissue where an even better correlation of antagonist affinities with cloned α_{1b}-adrenoceptors has been observed (Burt et al., 1995; Eltze, 1996a). Surprisingly, the rightward displacement of the noradrenaline $E/[A]$ curve by (+)-cyclazosin was only small and was not concentration dependent (Fig. 2C). It should be noted that in our hands tamsulosin, the reference α_1-adrenoceptor antagonist, yielded affinity estimates in rat and mouse spleen that were in accordance with those of previous studies (Eltze, 1996a; Noble et al., 1997). Thus, in contrast with the results of radioligand binding experiments (Giardina et al., 1996), (+)-cyclazosin appears not to behave as a selective α_{1B}-adrenoceptor antagonist in functional studies. Whether this discrepancy has consequences for the classification of α_1-adrenoceptors requires further investigation.

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