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Receptor binding profile of cyclazosin, a new α_{1B}-adrenoceptor antagonist

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Abstract

The binding profile of cyclazosin, a new prazosin-related α_1-adrenoceptor antagonist, at α_1-, α_2-adrenoceptors, dopamine D_2 and 5-HT_{1A} receptors was compared to that of 5-methylurapidil, spiperone, risperidone and other prazosin-related ligands. In addition, cyclazosin was investigated at native and cloned α_1-adrenoceptor subtypes. Cyclazosin showed high specificity for α_1-adrenoceptors and a 10–15-fold selectivity for α_{1B} (α_{1b})-adrenoceptors with respect to the α_{1A} (α_{1a}) subtype (pK_i values of 9.23–9.57 and 8.18–8.41, respectively). However, it failed to discriminate between cloned α_{1b} and α_{1d}-adrenoceptors (pK_i values of 9.23 and 9.28, respectively).

Keywords: Cyclazosin; α_1-Adrenoceptor antagonist; Prazosin-related antagonist; α_1-Adrenoceptor subtype

1. Introduction

Two native α_1-adrenoceptor subtypes, α_{1A} and α_{1B}, have been characterized in functional and binding assays (Bylund et al., 1994). However, cloning studies have shown a greater heterogeneity and three distinct α_1-adrenoceptors have been cloned, namely the α_{1b} (Cotecchia et al., 1988), α_{1c} (Schwinn et al., 1990) and α_{1d} (Lomasney et al., 1991) subtypes. The cloned α_{1b}-adrenoceptor corresponds to the native α_{1B} type whereas the α_{1c}-adrenoceptor may correspond to the pharmacologically defined α_{1A}-adrenoceptor (Faure et al., 1994). The other cloned α_{1d}-adrenoceptor seems to represent a novel subtype. Ford et al. (1994) proposed to name the cloned α_{1c}-adrenoceptor α_{1a} owing to the observed pharmacological correspondence between native α_{1A}- and cloned α_{1c}-adrenoceptors. On this basis, α_1-adrenoceptors have been subdivided into α_{1A} (α_{1a}), α_{1B} (α_{1b}) and α_{1d} subtypes (Ford et al., 1994).

We report here on the binding profile of the competitive α_1-adrenoceptor antagonist, cyclazosin [[4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-ylmethanone hydrochloride] (Fig. 1) (Giardinà et al., 1993), a novel prazosin-related compound, at α-adrenoceptors, dopamine D_2 and 5-HT_{1A} receptors. Furthermore, we investigated the selectivity of cyclazosin at native and cloned α_1-adrenoceptor subtypes. Prazosin and related antagonists such as abanoquil, alfuzosin and terazosin, as well as the α_{1A}-selective antagonist, 5-methylurapidil (Bylund et al., 1994), and the α_{1B}-selective antagonists, spiperone (Bylund et al., 1994) and risperidone (Sleight et al., 1993), were used as standard compounds.

2. Materials and methods

Male rats (Crl: CD(SD)BR, 200–300 g, Charles River, Italy) were killed and their hippocampus, striatum, cerebral cortex and liver were dissected, frozen on dry ice and then stored at −70°C until used.

Drugs were obtained from the following sources: abanoquil (Pfizer, Sandwich, England), risperidone (Janssen, Beerse, Belgium), 5-methylurapidil and chloroethylclonidine (RBI, Natick, USA), prazosin and spiperone (Sigma Chemical), whereas alfuzosin, tera-

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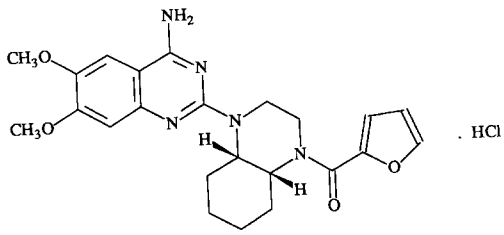

Fig. 1. Chemical structure of cyclazosin.

zosin and cyclazosin were synthesized in one of our laboratories.

The radioligands [^{3}H]prazosin (specific activity 89.2 Ci/mmol), [^{3}H]rauwolscine (specific activity 80.5 Ci/mmol), [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (specific activity 162.9 Ci/mmol) and [^{3}H]spiperone (specific activity 17.7 Ci/mmol) were obtained from Dupont NEN, Milan, Italy.

2.1. Radioligand binding assays at native receptors

Binding studies at α_1- and α_2-adrenoceptors, 5-HT$_{1A}$ and dopamine D_2 receptors were carried out in rat cerebral cortex (α_1 and α_2), hippocampus (5-HT$_{1A}$) and striatum (D_2) membranes as previously described (Foreman et al., 1994). The respective radioligands were [^{3}H]prazosin, [^{3}H]rauwolscine, [^{3}H]8-OH-DPAT and [^{3}H]spiperone. Chloroethylclonidine-pretreated rat hippocampus and liver membranes were used to determine [^{3}H]prazosin binding to native α_{1A}- and α_{1B}-adrenoceptor subtypes, respectively, as previously described (Testa et al., 1993).

2.2. Radioligand binding assays at cloned receptors

[^{3}H]Prazosin binding to cloned α_1-adrenoceptor subtypes was performed in COS-7 cells (CV-1 monkey kidney epithelial cells, SV 40) expressing transiently bovine α_{1a}- (previously named α_{1c}-), hamster α_{1b}- and rat α_{1d}-adrenoceptors. Construction and transfection of individual α_1-adrenoceptor subtypes were carried out by Dr. S. Cotecchia (Université de Lausanne, Switzerland) as previously described (Cotecchia et al., 1988; Schwinn et al., 1990; Lomasney et al., 1991). COS-7 cell membranes (35–70 μg proteins) were incubated in 50 mM Tris-HCl, pH 7.4, containing 10 μM pargyline and 0.1% ascorbic acid, with 0.3–0.6 nM [^{3}H]prazosin, in absence or presence of the inhibiting drug, in a final volume of 0.22 ml. Non-specific binding was determined in the presence of 100 μM phentolamine. The reaction mixture was incubated for 30 min at 25°C and incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through Whatman GF/B filters.

2.3. Data analysis

The inhibition of specific binding of the radioligands by the drugs tested was analyzed to estimate the IC_{50} value using a non-linear curve-fitting program, Allfit (De Lean et al., 1988). The IC_{50} value was converted to an affinity constant (K_i) with the equation of Cheng and Prusoff (1973).

3. Results

Cyclazosin inhibited [^{3}H]prazosin binding at α_1-adrenoceptors and [^{3}H]rauwolscine binding at α_2-adrenoceptors of rat cerebral cortex with pK_i values of 9.25 ± 0.09 and 6.17 ± 0.05, respectively. Its affinity for 5-HT$_{1A}$ and dopamine D_2 receptors in hippocampus and striatum rat membranes was markedly lower, with pK_i values of 5.16 ± 0.08 and < 5, respectively (Table 1).

The ability of cyclazosin to inhibit α_1-adrenoceptor subtypes was compared with that of the reference compounds by determining the inhibition of [^{3}H]-

Table 1
Affinity of cyclazosin for α_1- and α_2-adrenoceptors, 5-HT$_{1A}$ and dopamine D_2 receptors in comparison to that of reference compounds. The data represent the pK_i values and are the means ± S.E. of three to four different experiments, each performed in triplicate. The pseudo-Hill coefficients of inhibition curves, evaluated according to De Lean et al. (1978), were close and not significantly different from unity, with the exception of those for 5-methylurapidil and spiperone as well as prazosin and terazosin on α_1- and α_2-adrenoceptors, respectively. N.T. = not tested

Antagonist	Receptor			
	α_1	α_2	5-HT$_{1A}$	D_2
Cyclazosin	9.25 ± 0.09	6.17 ± 0.05	5.16 ± 0.08	< 5
Abanoquil	9.75 ± 0.04	N.T.	N.T.	5.66 ± 0.02
Alfuzosin	7.93 ± 0.01	6.44 ± 0.02	< 5	< 5
Prazosin	9.13 ± 0.02	6.83 ± 0.07	5.63 ± 0.07	< 5
Terazosin	8.47 ± 0.17	6.76 ± 0.06	< 5	< 5
5-Methylurapidil	7.55 ± 0.06	6.36 ± 0.09	8.92 ± 0.12	5.98 ± 0.05
Risperidone	8.03 ± 0.06	7.84 ± 0.02	6.43 ± 0.04	8.18 ± 0.02
Spiperone	8.16 ± 0.01	6.87 ± 0.15	7.60 ± 0.11	9.24 ± 0.03

Table 2
Affinity of cyclazosin for the native and cloned α_1-adrenoceptor subtypes, in comparison to reference compounds. The data represent the pK_i values and are the means ± S.E. of three to four different experiments, each performed in triplicate. The pseudo-Hill coefficients of inhibition curves, evaluated according to De Lean et al. (1978), were always close and not significantly different from unity

Antagonist	Receptor				
	α_{1A}	α_{1B}	α_{1a}	α_{1b}	α_{1d}
Cyclazosin	8.41 ± 0.03	9.57 ± 0.01	8.18 ± 0.14	9.23 ± 0.04	9.28 ± 0.05
Abanoquil	9.60 ± 0.16	9.85 ± 0.09	10.10 ± 0.05	9.89 ± 0.11	10.22 ± 0.18
Alfuzosin	7.70 ± 0.06	8.35 ± 0.10	7.63 ± 0.08	7.93 ± 0.16	7.81 ± 0.15
Prazosin	9.03 ± 0.09	9.44 ± 0.12	9.14 ± 0.04	9.34 ± 0.12	8.86 ± 0.05
Terazosin	8.23 ± 0.16	8.45 ± 0.06	7.58 ± 0.09	7.52 ± 0.05	7.46 ± 0.16
5-Methylurapidil	8.33 ± 0.16	6.66 ± 0.10	8.69 ± 0.12	6.10 ± 0.03	6.80 ± 0.05
Risperidone	8.33 ± 0.04	8.51 ± 0.17	8.56 ± 0.02	8.11 ± 0.15	7.60 ± 0.15
Spiperone	7.42 ± 0.07	8.81 ± 0.08	7.87 ± 0.11	8.15 ± 0.01	7.66 ± 0.13

prazosin-specific binding both in chloroethylclonidine-pretreated rat hippocampus (native α_{1A}) and liver (native α_{1B}) membranes and in COS-7 cell membranes expressing α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes. Cyclazosin showed the highest affinity at native α_{1B}-adrenoceptors with a pK_i value of 9.57 ± 0.01 which was similar to that observed at cloned α_{1b}- and α_{1d}-adrenoceptors (pK_i = 9.23 ± 0.04 and 9.28 ± 0.05, respectively), whereas its affinity was 10- to 15-fold lower at native α_{1A}- and cloned α_{1a}-adrenoceptors (Table 2).

Prazosin exhibited a similar affinity at both native, α_{1A}- and α_{1B}-, and cloned α_{1a}- and α_{1b}-adrenoceptor subtypes, and a slightly lower affinity at α_{1d}-adrenoceptors. Similarly, prazosin analogues, abanoquil, alfuzosin and terazosin, were not able to discriminate markedly between native and cloned α_1-adrenoceptor subtypes, the only exception being the 4-fold selectivity for α_{1B}-adrenoceptors, compared to the native α_{1A} subtype, displayed by alfuzosin.

The antipsychotic drugs, spiperone and risperidone, showed opposite results. Spiperone was 24-fold more potent at native α_{1B}- than at α_{1A}-adrenoceptors and approximately equiactive at cloned α_1-adrenoceptors, whereas risperidone exhibited a similar affinity both at native, α_{1A}- and α_{1B}-, and at cloned α_{1a}- and α_{1b}-adrenoceptor subtypes, and a slightly lower affinity at the α_{1d}-adrenoceptor.

Among the ligands investigated, 5-methylurapidil displayed the best affinity profile with a marked selectivity (50–500-fold) for native α_{1A}- and cloned α_{1a}-adrenoceptors compared to the other α_1-adrenoceptor subtypes (Table 2).

4. Discussion

Cyclazosin is a new prazosin-related compound which proved to be a potent and highly selective antagonist at α_1-adrenoceptors of isolated rat vas deferens (Giardinà et al., 1993). It was even more potent (pA_2 = 8.97 vs. 8.54) and selective (α_1/α_2 selectivity ratio = 7800 vs. 1200) than prazosin. The purpose of the present investigation was to evaluate the affinity profile of cyclazosin in binding assays, particularly at native and cloned α_1-adrenoceptor subtypes.

Cyclazosin, like prazosin, displayed a high specificity for α_1-adrenoceptors owing to poor affinity for the other receptor systems investigated, namely 5-HT_{1A} and dopamine D_2 receptors (Table 1). Furthermore, cyclazosin showed a markedly high affinity for α_1-adrenoceptors, as revealed by the α_1/α_2 selectivity ratio value of 1200 (Table 1), which parallels the selectivity observed in functional assays (Giardinà et al., 1993).

However, the most interesting result of the present investigation was the higher affinity displayed by cyclazosin for native α_{1B}- and cloned α_{1b}-adrenoceptors (pK_i = 9.57 ± 0.01 and 9.23 ± 0.04, respectively), similar to that observed at α_{1d}-adrenoceptors (pK_i = 9.28 ± 0.05), compared to the affinity for native α_{1A}- and cloned α_{1a}-adrenoceptors (pK_i = 8.41 ± 0.03 and 8.18 ± 0.14, respectively). This resulted in a significant selectivity (10–15-fold) for α_{1B}- (α_{1b}-) and α_{1d}-adrenoceptors in comparison with the α_{1A}(α_{1a}) subtype (Table 2). On the contrary, prazosin as well as the other related analogues, terazosin, alfuzosin and abanoquil, did not display any significant selectivity for native or cloned α_1-adrenoceptor subtypes. This is in agreement with previous results (Bylund et al., 1994; Forray et al., 1994) but in contrast to the 100-fold higher affinity for α_{1A}-adrenoceptors than for α_{1B}- in rat membranes and 70-fold higher affinity for cloned α_{1d}-adrenoceptors, in comparison with cloned hamster α_{1b}- or bovine α_{1c}-adrenoceptors, reported for abanoquil by other authors (Greengrass et al., 1991; Marshall et al., 1992).

Spiperone, which is considered a selective α_{1B}-adrenoceptor antagonist, showed an about 10-fold lower affinity than cyclazosin at α_{1B}-adrenoceptors and only a slightly higher (2-fold) α_{1B}/α_{1A} selectivity at native receptors, in agreement with previous results (Bylund et al., 1994). However, spiperone may not represent a useful tool for the characterization of α_{1B}-

adrenoceptors because of the lack of receptor specificity owing to its high affinity for dopamine D_2 ($pK_i = 9.24 \pm 0.03$) and 5-HT_{1A} receptors as well ($pK_i = 7.60 \pm 0.11$) (Table 1). In our hands, risperidone did not display at either native or cloned α_1-adrenoceptors the claimed high selectivity for the α_{1B} subtype (α_{1B}/α_{1A} selectivity ratio = 120) observed in rat hippocampus (Sleight et al., 1993).

In conclusion, we demonstrated that cyclazosin is a potent and selective α_1-adrenoceptor ligand which can distinguish the α_{1B}- and α_{1d}-adrenoceptors from the α_{1A} subtype.

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