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Biochemical Pharmacology, Vol. 33, No. 9, pp. 1566–1568, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Studies of the α_2-adrenoceptor affinity and the α_2- to α_1-adrenoceptor selectivity of some substituted benzoquinolizines using receptor-binding techniques

(*Received* 10 *September* 1983; *accepted* 17 *November* 1983)

There is now considerable evidence that α-adrenoceptors exist as two pharmacologically distinct subtypes designated α_1 and α_2. Support for this classification has come from functional studies as well as from direct receptor labelling techniques [1]. Therapeutic applications of selective α_2-adrenoceptor blockers remain, as yet, a matter for speculation.

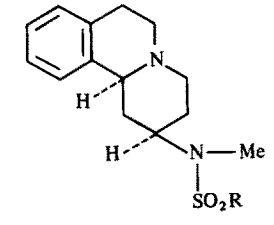
Recently, a group of substituted benzoquinolizine adrenoceptor blocking agents (Wy 25309, Wy 26392 and Wy 26703) has been identified as having greater affinity for α_2- than α_1-adrenoceptors using isolated tissue preparations [2]. The compounds have been reported to have less 5-hydroxytryptamine antagonist potency than yohimbine [3]. The purpose of the present study was to investigate further the α-adrenoceptor selectivity of these compounds using radioligand binding methods. The α_2-adrenoceptor blockers yohimbine [4] and RX 781094 [5] were included for comparison. The structures of these compounds are shown in Fig. 1.

α_1-Adrenoceptor affinity was determined from the ability of the antagonists to displace [^{3}H]prazosin, an α_1-selective ligand [6], from binding sites in rat cerebral cortex membrane fractions. [^{3}H]Rauwolscine, an α_2-selective ligand [7, 8], was similarly used to assess α_2-adrenoceptor affinity in membrane fractions of rat cerebral cortex, rat kidney cortex and of lysed human blood platelets.

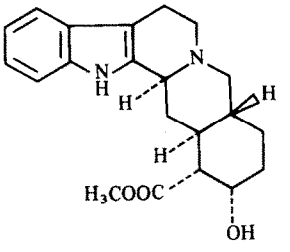
Materials and methods

Preparation of rat cerebral cortex and kidney cortex membranes. Cerebral cortex or kidney cortex tissue obtained from male Wistar rats (150–250 g) was homogenized in 20 vols. of ice-cold 5 mM Tris–HCl, 5 mM EDTA buffer with an Ultra-Turrax Homogeniser for 2 × 10 sec bursts. The suspension was then centrifuged at 27,000 *g* for 10 min at 4°. The resulting pellet was washed once more with the same buffer by resuspension, followed by centrifugation at 27,000 *g* for 10 min at 4°. One final washing was carried out by resuspending the pellet in ice-cold 'assay buffer' (50 mM Tris–HCl, 0.5 mM EDTA, 0.1% ascorbate, pH 7.5) and then centrifuging at 27,000 *g* for 10 min at 4°. The final pellet was resuspended in the appropriate volume of assay buffer (see below).

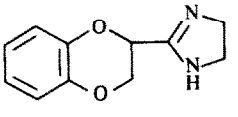
Preparation of human platelet membrane lysates. Blood was collected from human volunteers with 3% (w/v) sodium citrate as anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifuging the collected blood at 270 *g* for 15 min at 10°. PRP was then centrifuged at 27,000 *g* for 10 min at 4° and the resulting pellet resuspended in ice-cold 'lysing buffer' (5 mM Tris–HCl, 5 mM EDTA) and left for 1–2 min before being homogenized for 10 strokes with a motor-driven glass–Teflon homogeniser. The suspension was then centrifuged at 27,000 *g* for 10 min at 4°. The pellet was washed once with lysing buffer by gentle resuspension



R
Wy 25309 – CH_3
Wy 26392 – $CH_2CH_2CH_3$
Wy 26703 – $CH_2CH(CH_3)_2$



Yohimbine



RX 781094

Fig. 1. Structures of the α-adrenoceptor blockers.

and then centrifugation at 27,000 *g* for 10 min at 4°. A final washing was carried out by resuspension of the pellet in ice-cold assay buffer (50 mM Tris–HCl, 0.5 mM EDTA, 0.1% ascrobate, pH 7.5), followed by centrifugation at 27,000 *g* for 10 min at 4°. The final pellet was then resuspended in the appropriate volume of assay buffer and used in the binding assays.

Binding assays. Binding assays were performed in a final volume of 250 μl of assay buffer. A concentration range of the α-adrenoceptor blocker was incubated to equilibrium at 22° for 30 min in the presence of either 1–2 nM [^{3}H]-rauwolscine or 0.5–1 nM [^{3}H]prazosin and 300–600 μg tissue membrane fractions. Membranes were then collected by vacuum filtration on Whatman GF/B filters and rapidly washed with 3 × 5 ml ice-cold buffer. The filters were counted for radioactivity in Fisofluor 1 scintillation fluid at an efficiency of approximately 40%. Specific binding was assessed as that binding which could be displaced by 5 μM phentolamine, and represented *ca* 60–70% in brain, 70–80% in platelet and 70–80% in kidney with [^{3}H]-rauwolscine and 70–80% with [^{3}H]prazosin in brain.

Materials. [^{3}H]Rauwolscine (sp. act. 84 Ci/mmole) was obtained from New England Nuclear (Dreieich, F.R.G.); [^{3}H]prazosin (sp. act. 33 Ci/mmole) was a gift from Pfizer (Sandwich, U.K.); and yohimbine HCl was from Sigma Chemical Co., (St. Louis, MO). RX 781094 and the benzoquinolizines were synthesized by colleagues at Wyeth Laboratories.

Statistical analysis. Data were analysed by Student's *t*-tests (unpaired) to determine significance of differences between group means. Data are expressed as mean values ± S.E.M.

Results

Binding characteristics of ligands. In a series of preliminary experiments it was confirmed that both [^{3}H]prazosin and [^{3}H]rauwolscine bound specifically with high affinity and in a saturable manner to membranes from rat cerebral cortex [6, 9]. Scatchard analysis of the specific binding of [^{3}H]prazosin and [^{3}H]rauwolscine suggested that each ligand interacted with a single, though pharmacologically different, population of sites (α_1 and α_2, respectively) in this tissue. Hill plots yielded lines of slope close to unity. Analysis of [^{3}H]rauwolscine binding in rat kidney cortex and human blood platelets also suggested that specific binding is to a single population of sites which possess the characteristics of α_2-adrenoceptors in each tissue.

Effects of the α-adrenoceptor blocking agents on [^{3}H]-*prazosin and* [^{3}H]*rauwolscine binding in rat cerebral cortex.* All the antagonists had greater affinity at the α_2-([^{3}H]-rauwolscine) binding site in rat cerebral cortex than at the α_1- ([^{3}H]prazosin) site (Table 1). Wy 26392 had the highest affinity for the α_2-site and possessed an α_2:α_1 selectivity ratio (K_i [^{3}H]prazosin ÷ K_i [^{3}H]rauwolscine) of 320 in this tissue. Wy 25309 had the largest α_2:α_1 selectivity ratio (474) whereas yohimbine had the lowest affinity of this group of compounds at the α_2-site and the smallest selectivity ratio (92). With the exception of RX 781094, all compounds had Hill-plot slopes close to unity against both [^{3}H]prazosin and [^{3}H]rauwolscine in this tissue (Table 1).

The effects of α-adrenoceptor blocking agents on [^{3}H]-*rauwolscine binding with membranes from human platelet and rat kidney cortex.* Wy 26392 and yohimbine had significantly higher affinities against [^{3}H]rauwolscine using membranes from platelets than with those from rat cerebral cortex (Table 1). The difference between tissues was most marked with yohimbine, this compound having the greatest affinity of this series of compounds in the platelet preparation. All compounds produced Hill-plot slopes close to unity for displacement of [^{3}H]rauwolscine in human platelet membranes.

The K_i values for the benzoquinolizines and yohimbine against [^{3}H]rauwolscine in kidney membranes were not significantly different when values for individual compounds were compared to the values found with cerebral cortical preparations. RX 781094, however, had significantly lower affinity for this site on the rat kidney cortex (K_i = 43.4 ± 3.80 nM) than on the cerebral cortex (K_i = 9.2 ± 2.20 nM). Hill-plot slopes were close to unity for all compounds in the rat kidney cortex.

Discussion

The present studies demonstrate that the substituted benzoquinolizines Wy 25309, Wy 26392 and Wy 26703 have a much greater affinity for the [^{3}H]rauwolscine- (α_2) binding site than for the [^{3}H]prazosin- (α_1) binding site in rat cerebral cortex membranes. In this respect the benzoquinolizines resembled RX 781094 but were more selective than the α_2-adrenoceptor blocker yohimbine. These results are consistent with earlier reports [2, 5] in which these compounds were studied using isolated smooth muscle preparations.

Table 1. Affinity of substituted benzoquinolizines, yohimbine and RX 781094 at specific [^{3}H]rauwolscine binding sites in rat cerebral cortex, rat kidney cortex and human platelets and at specific [^{3}H]prazosin binding sites in rat cerebral cortex

	K_i values (nM)				
	Rat cerebral cortex			Rat kidney cortex	Human platelet
	[^{3}H]rauwolscine	[^{3}H]prazosin	α_2:α_1 Selectivity	[^{3}H]rauwolscine	[^{3}H]rauwolscine
Wy 26392	5.5 ± 0.96	1751 ± 189.5	320	6.5 ± 0.03	2.4 ± 0.26*
	(1.02)	(1.10)		(0.95)	(0.97)
Wy 25309	7.4 ± 1.62	3495 ± 265.0	474	11.31 ± 0.32	3.4 ± 0.45
	(0.98)	(0.97)		(0.95)	(0.93)
Wy 26703	9.2 ± 2.98	1951 ± 155.4	212	12.4 ± 1.17	2.2 ± 0.31
	(0.94)	(1.00)		(1.03)	(0.96)
Yohimbine	11.1 ± 1.83	1015 ± 69.5	92	11.8 ± 1.16	0.9 ± 0.07**
	(0.90)	(1.03)		(0.99)	(1.01)
RX 781094	9.2 ± 2.20	1928 ± 178.6	209	43.4 ± 3.80**	3.8 ± 0.32
	(0.79)	(1.05)		(0.89)	(0.99)

The concentration of drug which inhibited specific binding of the labelled ligand by 50% (IC_{50}) was obtained graphically and converted to K_i values using the relationship $K_i = IC_{50}/(1 + S/K_D)$, where S = the concentration of labelled ligand used and K_D = the equilibrium dissociation constant of the ligand. Values shown are the mean K_i ± S.E. of three experiments. Hill-plot slopes are shown in parentheses. Selectivity ratios (α_2:α_1) were calculated as K_i [^{3}H]prazosin ÷ K_i [^{3}H]rauwolscine. K_i values for each drug against [^{3}H]rauwolscine in rat cerebral cortex were compared with the corresponding values in other tissues using Student's unpaired *t*-test. * $P < 0.05$; ** $P < 0.01$.

In the rat cerebral cortex, the benzoquinolizines and yohimbine interacted with [^{3}H]rauwolscine and [^{3}H]prazosin yielding displacement curves consistent with simple monomolecular interactions with labelled sites. This was also the case for RX 781094 at the α_1-site but not at the α_2-site where a slope of 0.79 was obtained. Slope values of less than unity have been associated with agonist interactions at [^{3}H]yohimbine or [^{3}H]rauwolscine-labelled α_2-sites in brain and platelet [9]. This probably relates to the association of agonists with guanine-nucleotide-sensitive high and low affinity sites.

In this context it is of interest to note that agonist activity has been reported with RX 781094 in the conscious rabbit and in isolated rabbit aorta [10] though the adrenoceptors in the rabbit aorta appear to be of the α_1-type.

It has previously been observed [8] using receptor binding methods that some α-adrenoceptor blockers have significantly greater affinities for the α_2-site in human platelet membranes than in rat cerebral cortex, and this has been taken as evidence that the α_2-adrenoceptors in these two tissues are not identical. In this study both yohimbine and Wy 26392 had a greater affinity for the platelet α_2-adrenoceptor than for the α_2-adrenoceptor in rat cerebral cortex, the difference being most marked with yohimbine. Thus, in the rat cerebral cortex yohimbine had the lowest affinity of this group of antagonists at the α_2-site but in the human blood platelet preparation yohimbine had the highest affinity of the group (Table 1). This again may indicate differences between α_2-adrenoceptors in these tissues.

Of the compounds tested, only RX 781094 showed significant differences in affinity between rat cerebral cortex and rat kidney cortex. The interpretation of this observation is, however, complicated by the possible agonist activity of this compound and subsequently its heterogeneous binding at least to cerebral cortical membranes.

In interpreting apparent differences in affinity of antagonists at α_2-adrenoceptors in these tissue preparations, it must be borne in mind that the physical properties of these preparations may influence access of the ligands to the receptor. These properties may differ between the tissues studied here. Problems of this sort may in future be clarified by work on solubilized receptor preparations.

In conclusion, these receptor-binding studies support the view that this group of substituted benzoquinolizines are potent and selective displacers of the α_2-adrenoceptor ligand [^{3}H]rauwolscine in rat cerebral cortex membranes, and in this tissue have a greater α_2:α_1 adrenoceptor selectivity than yohimbine. These benzoquinolizines have a similar affinity for α_2-adrenoceptors in both rat cerebral cortex and rat kidney cortex. The observation that yohimbine had the lowest affinity of this group of compounds at the α_2-adrenoceptor of rat cerebral cortex but the greatest affinity in human platelets provides further evidence of possible tissue or species differences in the α_2-adrenoceptor subtype.

In summary, a study using receptor-binding techniques with membrane preparations from the rat cerebral cortex, rat kidney cortex and human blood platelets showed that a series of substituted benzoquinolizines had a high affinity for α_2-adrenoceptors. In the rat cerebral cortex where α_1-binding was also studied, the benzoquinolizines had a much lower affinity for this site than for the α_2-site, their α_2:α_1 selectivity being substantially greater than that of yohimbine.

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Biochemical Pharmacology, Vol. 33, No. 9, pp. 1568–1571, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Binding of benzodiazepines to blood platelets from various species

(*Received* 8 *September* 1983; *accepted* 21 *November* 1983)

Specific high affinity binding sites for benzodiazepines have been firmly established in the mammalian brain [1–4]. Comparison of receptor affinities and pharmacological effects has suggested that these receptors probably mediate the anxiolytic, muscle-relaxant and anticonvulsant properties of these compounds [2, 4–6].

Benzodiazepine binding sites have also been demonstrated in peripheral tissues such as the heart [7], kidney [8, 9], lung [9], liver [9], ileal skeletal muscle [7], peritoneal mast cells [10] and platelets [11]. The peripheral-type binding site shows a different pharmacological profile to that of the established CNS sites [8, 9, 12, 13], in that whereas the affinity of diazepam and flunitrazepam for the peripheral-type binding site is only slightly lower than that for the CNS sites clonazepam, which has a high affinity for CNS sites, has a very low affinity for peripheral sites. The benzodiazepine Ro 5-4864 has a high affinity for peripheral sites [8, 12, 13] but a very low affinity for CNS sites [1, 3, 14, 15]. Recently, specific high affinity binding sites for [^{3}H] Ro 5-4864 have also been found in the brain [16, 17] but are distinct in both regional and subcellular distribution from the established CNS sites.

Increased diazepam binding has been demonstrated to renal membranes during deoxycorticosterone/salt hyper-