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Assessment of imiloxan as a selective α_{2B}-adrenoceptor antagonist

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1 The α_2-adrenoceptor binding sites of rabbit spleen and rat kidney, labelled with [^{3}H]-rauwolscine, were characterized using a range of subtype selective ligands.

2 In rabbit spleen, the α-$_2$-adrenoceptor binding sites displayed high affinity for oxymetazoline and WB 4101 and low affinity for prazosin and chlorpromazine suggesting the presence of an α_{2A} subtype.

3 There was evidence for heterogeneity of the α_2-adrenoceptor binding sites present in rabbit spleen. The results obtained with oxymetazoline and WB 4101 indicated that at least 75% of the [^{3}H]-rauwolscine binding sites in this preparation displayed a pharmacology consistent with the presence of an α_{2A} subtype.

4 In rat kidney, the α_2-adrenoceptor binding sites displayed high affinity for prazosin and chlorpromazine and low affinity for oxymetazoline and WB 4101 suggesting the presence of an α_{2B} subtype.

5 The inclusion of guanylylimidodiphosphate (Gpp(NH)p, 0.1 mM) did not modify the pharmacology of the α_2-adrenoceptor binding sites present in the two preparations. Furthermore, when the two membrane preparations were combined, the resultant pharmacology was still consistent with the presence of two receptors that retained the characteristics of the α_{2A} and α_{2B} subtypes.

6 Imiloxan was identified as a selective α_{2B} ligand while benoxathian displayed a high degree of selectivity for the α_{2A}-adrenoceptor binding site. The selectivity of imiloxan for the α_{2B}-adrenoceptor binding site, coupled with its specificity for α_2-adrenoceptors, should make it a valuable tool in the classification of α_2-adrenoceptor subtypes.

Introduction

Direct binding studies conducted in several laboratories have now provided evidence for α_2-adrenoceptor binding site heterogeneity (Bylund, 1981; 1985; Cheung *et al.*, 1982; Summers *et al.*, 1983; Neylon & Summers, 1985). On the basis of these receptor binding studies, α_2-adrenoceptors have now been proposed to comprise α_{2A}- and α_{2B}-adrenoceptor subtypes. α_{2A}-Adrenoceptors display relatively low affinity for prazosin and high affinity for oxymetazoline while α_{2B}-receptors display the converse selectivity for these two ligands (Bylund, 1985).

While functional evidence in support of this concept has been limited (Turner *et al.*, 1984), the recent demonstration by Regan *et al.* (1988) that two α_2-adrenoceptor genes exist and that the gene products display differential sensitivity to prazosin and oxymetazoline lends considerable credence to the concept of α_2-adrenoceptor subtypes.

With the renewed interest in α_2-adrenoceptor subtypes (Bylund, 1988), the aims of the present study were two fold. Firstly, to obtain a receptor binding assay for both the α_{2A}- and α_{2B}-adrenoceptor subtypes and, secondly, to determine the α_2-adrenoceptor subtype selectivity of the α_2-adrenoceptor antagonist, imiloxan (RS 21361; Michel & Whiting, 1981). To this end we have characterized the α_2-adrenoceptors present in two tissues which appear to express homogeneous populations of the α_{2A}- and α_{2B}-adrenoceptor subtypes. The rat kidney α_2-adrenoceptor displays high affinity for prazosin relative to other α_2-adrenoceptor binding sites (Neylon & Summers, 1985) and may therefore correspond to the α_{2B}-adrenoceptor. In rabbit spleen, prazosin displays low affinity for α_2-adrenoceptor binding sites (Dickinson *et al.*, 1986) and these receptors may therefore correspond to the α_{2A}-adrenoceptor.

Methods

Membrane preparation

All membrane preparation procedures were conducted at 4°C and used ice-cold buffers. Rat kidney and rabbit spleen were obtained from Pel-Freez (AK). Tissues were homogenized for 30 s in 3 volumes (w/v) of 50 mM Tris, 5 mM EDTA homogenizing buffer (pH 7.4 at 4°C) by a Waring Blender at maximum setting. The homogenates obtained were further homogenized by a Polytron P10 tissue disrupter (setting 10; two 10 s bursts) and filtered through a double layer of cheesecloth. The homogenates were centrifuged at 500 *g* for 10 min and the supernatants reserved. The pellets were resuspended in 2 volumes of homogenizing buffer by the Polytron P10 tissue disrupter and centrifuged at 500*g* for 10 min. The supernatants from this step were combined with the original supernatants and centrifuged for 12 min at 43,500*g*. The crude membrane pellets obtained were washed by resuspending in homogenizing buffer and centrifuging at 43,500*g* for 12 min. The pellets from this step were washed twice, in a similar manner, but with ice-cold 50 mM Tris, 0.5 mM EDTA assay buffer (pH 7.4 at 4°C). The final pellets were resuspended in assay buffer (pH 7.4 at 25°C) and stored under liquid nitrogen until required.

Ligand binding assays

Membranes, [^{3}H]-rauwolscine and competing drugs were incubated in a final volume of 0.25 ml of assay buffer (pH 7.4 at 25°C) for 45 min at 25°C. In competition studies, inhibition curves were obtained by incubating a fixed concentration of radioligand (1–2 nM) and membrane (about 0.20 mg protein per tube) with 10 or 22 concentrations of the competing compound. All experiments were performed in duplicate. In saturation studies, 9 concentrations of [^{3}H]-rauwolscine ranging from 0.1 to 40 nM were used. At each concentration of radioligand, total binding was determined in triplicate and non-specific binding was determined in duplicate. In all experiments non-specific binding was defined with 10 μM phentolamine. Incubations were terminated by vacuum filtration over 0.1% PEI pretreated glass fibre filters by a Brandel cell harvester. The filters were washed for 10 s with ice-cold 0.1 M NaCl. Individual filters were placed in scintillation vials and 4 ml of Aquasol (NEN, Boston, MA) scintillation cocktail was added. The vials were shaken at room temperature for 1 h and radioactivity retained on the filters was determined by liquid scintillation spectrometry.

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Data analysis

Competition and saturation binding data were analysed as described previously by iterative curve fitting techniques (Michel & Whiting, 1988). IC_{50} values were converted to K_i values with the Cheng-Prusoff approximation (1973). In some cases, binding data from competition experiments were fitted to both one-site and two-site models. The one-site and two-site models were compared with the F value defined by the following equation:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where SS_1 is the residual sum of squares for the single site, SS_2 is the residual sum of squares for the two-site model, df_1 is the degrees of freedom for the single site model and df_2 the degrees of freedom for the two-site model (Munson & Rodbard, 1980). A two-site fit was assumed to be significantly better than a one-site fit if the calculated F value had a $P < 0.05$.

Materials

[^{3}H]-rauwolscine (specific activity 82 Ci mmol^{-1}) was obtained from New England Nuclear. Chlorpromazine, guanylylimidodiphosphate (Gpp(NH)p), noradrenaline and oxymetazoline were obtained from Sigma chemical company, as were all chemicals and reagents used. Phentolamine was obtained from Ciba-Geigy. Prazosin was obtained from Pfizer. Benoxathian and WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane) were obtained from Research Biochemical Inc. Idazoxan, imiloxan, UK 14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) and SFK 104078 (6-chloro-9-[(3-methyl-2-butenyl)oxyl-3-methyl-1H-2,3,4,5-tetrahydro-3]-benzazepine) were synthesized by Dr R. Clark, IOC, Syntex.

Results

Saturation studies

In saturation studies [^{3}H]-rauwolscine labelled homogeneous populations of binding sites in the two preparations examined. The K_D for the radioligand was marginally lower in the rabbit spleen ($K_D = 4.6 \pm 0.8$ nM; $n = 4$) than in the rat kidney ($K_D = 2.2 \pm 0.5$ nM; $n = 4$). B_{max} values were 104 ± 20 and 124 ± 9 fmol mg^{-1} protein in rat kidney and rabbit spleen, respectively. Saturation curves for the two tissues were shown in a previous study (Michel *et al.*, 1989).

Competition studies

The results of competition studies performed with a range of α-adrenoceptor antagonists are presented in Table 1. Several of the compounds examined were able to differentiate between the α_2-adrenoceptors identified in rat kidney and rabbit spleen. Of these, chlorpromazine, imiloxan (Figure 1a) and prazosin (Figure 1b) displayed greater than 10 fold higher affinity for the rat kidney than for rabbit spleen α_2- adrenoceptors ($P < 0.001$, t test), while benoxathian, UK 14304, oxymetazoline (Figure 1a) and WB 4101 (Figure 1b) displayed greater than 3 fold higher affinity for the rabbit spleen than for the rat kidney α_2-adrenoceptors ($P < 0.05$, t test).

Idazoxan and rauwolscine displayed a small (less than a 3 fold) but significant difference in affinity between rat kidney and rabbit spleen α_2-adrenoceptors ($P < 0.05$, t test), while phentolamine and SKF 104078 were not selective.

In rabbit spleen the Hill coefficients for several compounds were significantly less than unity and for both oxymetazoline and WB 4101, the data were better described by assuming the presence of two populations of binding sites (Table 2). Approximately 77% of the sites displayed high affinity for both oxymetazoline and WB 4101. The affinity of oxymetazoline for the remaining sites was similar to its affinity for rat kidney α_2-adrenoceptors. However, for WB 4101 the low affinity value in the rabbit spleen was 3.3 fold lower than the potency of this ligand in rat kidney.

Table 1 Binding parameters of adrenoceptor antagonists at the α_2-adrenoceptor of rat kidney and rabbit spleen

Ligand	*Rabbit spleen* (α_{2A}) pKi/nH	*Rat kidney* (α_{2B}) pKi/nH	*Selectivity ratio*
Benoxathian	7.65 (0.10)	6.37 (0.01)	0.05
	0.88 (0.07)	1.12 (0.05)	
Chlorpromazine	6.05 (0.02)	7.27 (0.04)	16
	1.00 (0.07)	1.05 (0.04)	
Idazoxan	7.12 (0.02)	7.29 (0.02)	1.5
	0.96 (0.11)	0.83 (0.06)	
Imiloxan	5.52 (0.03)	7.26 (0.03)	55
	0.92 (0.05)	0.97 (0.02)	
Noradrenaline	6.37 (0.10)	6.98 (0.03)	4
	0.81 (0.05)	0.78 (0.04)	
Oxymetazoline	8.11 (0.04)	6.60 (0.02)	0.03 (0.01)
	0.74 (0.04)	0.71 (0.02)	
Phentolamine	7.78 (0.06)	7.97 (0.09)	1.5
	0.92 (0.04)	1.02 (0.04)	
Prazosin	5.33 (0.08)	7.22 (0.07)	78
	0.93 (0.08)	0.96 (0.04)	
Rauwolscine	8.06 (0.10)	8.46 (0.10)	2.5
	0.92 (0.11)	0.86 (0.07)	
SKF 104078	7.00 (0.04)	6.96 (0.05)	0.91
	1.03 (0.06)	1.22 (0.03)	
UK 14304	7.46 (0.08)	6.61 (0.01)	0.14
	0.76 (0.04)	0.86 (0.07)	
WB 4101	8.17 (0.09)	7.46 (0.04)	0.19 (0.06)
	0.67 (0.04)	1.02 (0.06)	

The radioligand used was [^{3}H]-rauwolscine (1–2 nM). The values shown are the mean ($\pm$s.e.mean; $n = 4$–9) for the Hill coefficient (nH) and the negative logarithm of the K_i (pK_i). The selectivity ratio shown represents the ratio between the K_i values at the rabbit spleen and rat kidney. The selectivity ratios shown in parentheses for oxymetazoline and WB 4101 represent the ratio between the affinity of these ligands for site 1 in rabbit spleen (Table 2) and their affinity at rat kidney.

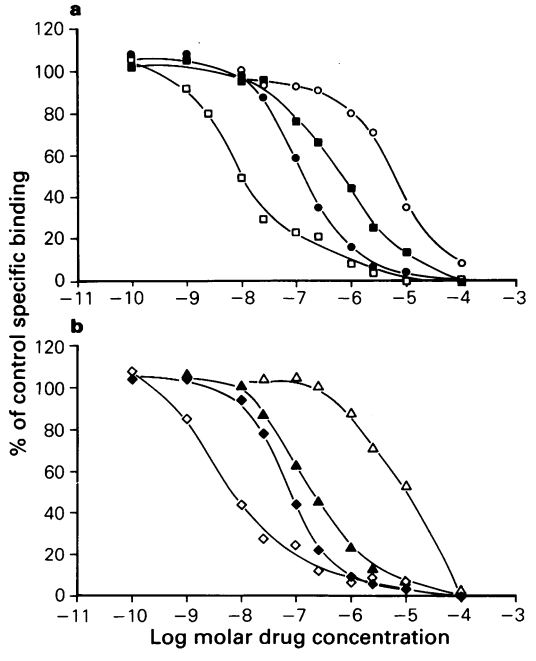

Figure 1 (a) Displacement of [^{3}H]-rauwolscine binding from rabbit spleen (open symbols) or from rat kidney (closed symbols) by either imiloxan (○, ●) or by oxymetazoline (□, ■). (b) Displacement of [^{3}H]-rauwolscine binding from rabbit spleen (open symbols) or from rat kidney (closed symbols) by either prazosin (△, ▲) or by WB 4101 (◇, ◆). In both cases membranes, [^{3}H]-rauwolscine (1–2 nM), and various concentrations of the competitors were incubated for 45 min at 25°C before bound and free ligand were separated by vacuum filtration. The data are from a single representative experiment. In rabbit spleen total binding was 1306 d.p.m. and NSB was 320 d.p.m. In rat kidney total binding was 1112 d.p.m. and NSB was 220 d.p.m.

Table 2 Two-site analysis of the interaction of WB 4101 and oxymetazoline with the α_2-adrenoceptor of rabbit spleen

	Site 1		Site 2	
Compound	pK_i	%	pK_i	%
Oxymetazoline	8.59 (0.06)	79 (5)	6.48 (0.23)	21 (5)
WB 4101	8.69 (0.08)	75 (6)	6.94 (0.17)	25 (6)

The data presented in Table 1 were analysed by assuming the presence of two populations of [^{3}H]-rauwolscine binding sites. The two-site fit was assumed to be significantly better than the single site fit if the determined F value had a $P < 0.05$ (see Methods section). The values shown represent the mean (±s.e.mean, n = 7–8) of the pK_i value or the proportion of [^{3}H]-rauwolscine binding sites displaying the indicated affinity for each ligand.

Combination studies

To determine if some factor present in, or property of the respective membrane preparations was affecting the apparent antagonist affinity estimates in the two tissues, the affinities of both prazosin and imiloxan were determined in assays where rat kidney and rabbit spleen membranes were combined in a 1:1 ratio.

With this approach, both compounds produced shallow inhibition curves that could be best analysed by assuming the existence of two populations of binding sites present in an approximately 1:1 ratio (Table 3; Figure 2). The high and low affinity estimates obtained from this analysis were similar to affinity estimates obtained at the rat kidney and rabbit spleen α_2-adrenoceptors, respectively.

Table 3 Two-site analysis of the interaction of imiloxan and prazosin with the α-adrenoceptor present in rat kidney, rabbit spleen and a 1:1 mixture of rat kidney and rabbit spleen membranes

	Rat kidney	*Rabbit spleen*	*Rat kidney plus rabbit spleen*			
			Site 1		*Site 2*	
Compound	pK_i	pK_i	pK_i	%	pK_i	%
Imiloxan	7.26 (0.03)	5.52 (0.03)	7.14 (0.18)	51 (10)	5.40 (0.13)	49 (10)
Prazosin	7.22 (0.07)	5.33 (0.08)	7.13 (0.20)	46 (3)	5.57 (0.07)	54 (3)

Values represent the mean (±s.e.mean; n = 3) pK_i value or the proportion of [^{3}H]-rauwolscine binding sites displaying the indicated affinity for each ligand. Competition curves to imiloxan and prazosin were determined in a membrane preparation that was comprised of a 1:1 mixture of the rat kidney and rabbit spleen membranes. The data were best described by a model assuming the presence of two populations of binding sites in this preparation. The pK_i values for imiloxan and prazosin in rat kidney or rabbit spleen were from Table 1.

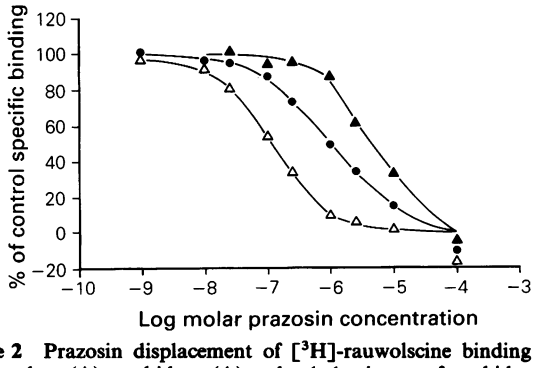

Figure 2 Prazosin displacement of [^{3}H]-rauwolscine binding from rabbit spleen (▲), rat kidney (△) and a 1:1 mixture of rat kidney and rabbit spleen membranes (●). In all cases, membranes, [^{3}H]-rauwolscine (1–2 nM), and various concentrations of the competitors were incubated for 45 min at 25°C before bound and free ligand were separated by vacuum filtration. The data are from a single representative experiment. In rabbit spleen total binding was 1426 d.p.m. and NSB was 380 d.p.m. In rat kidney total binding was 1480 d.p.m. and NSB was 420 d.p.m. In the combination study total binding was 1500 d.p.m. and NSB was 408 d.p.m.

Table 4 Effect of guanylylimidodiphosphate (Gpp(NH)p) on the interaction of ligands with the α_2-adrenoceptors of rat kidney and rabbit spleen

	Rabbit spleen (α_{2A}) pK_i		*Rat kidney* (α_{2B}) pK_i		*Selectivity ratio**	
Ligand	−	+	−	+	−	+
Noradrenaline	6.53 (0.15)	6.23 (0.14)	6.99 (0.05)	6.87 (0.05)	3.3 (1.1)	4.7 (1.1)
Oxymetazoline	8.12 (0.05)	8.14 (0.07)	6.61 (0.02)	6.52 (0.03)	0.03 (0.01)	0.03 (0.01)
WB 4101	8.25 (0.07)	8.24 (0.09)	7.48 (0.02)	7.48 (0.04)	0.17 (0.03)	0.19 (0.04)

The values represent the mean (±s.e.mean, n = 3) for the pK_i value or the selectivity ratio obtained either in the presence (+) or absence (−) of 100 μM Gpp(NH)p. In the absence of Gpp(NH)p, the Hill slope (nH) values for noradrenaline, oxymetazoline and WB 4101 were 0.80 (0.08), 0.74 (0.09) and 0.74 (0.03) respectively, in rabbit spleen, and 0.83 (0.07), 0.71 (0.04) and 1.02 (0.11) respectively in rat kidney. Addition of Gpp(NH)p did not significantly change the slope values.
* Selectivity ratio is the antilog of the difference between the pK_i value obtained in the rat kidney and the pK_i values obtained in the rabbit spleen either in the presence (+) or absence (−) of 100 μM Gpp(NH)p.

Effects of guanyl nucleotides

To examine the possible contribution of affinity states to the differences in ligand affinities seen at the α_2-adrenoceptors of the two tissues, assays in both rat kidney and rabbit spleen were conducted in the presence and absence of the non-hydrolysable GTP analogue, Gpp(NH)p (0.1 mM). As can be seen from Table 4, neither the affinity estimates nor selectivity ratios of noradrenaline, oxymetazoline or WB 4101 were affected by this assay addition.

Discussion

The major aim of the present study was to establish a receptor binding assay for the α_{2A}- and α_{2B}-adrenoceptor subtypes. To this end the rabbit spleen and rat kidney were selected, based upon data generated with prazosin in previous studies. The results obtained support the contention that these two tissues contain predominantly α_{2A}- and α_{2B}-adrenoceptors, respectively, as defined by Bylund (1988). Thus, the relatively high affinity of chlorpromazine and prazosin and the low affinity of oxymetazoline for rat kidney α_2-adrenoceptors indicated that this preparation contained mainly α_{2B}-adrenoceptors, while the converse selectivity of these compounds for rabbit spleen α_2-adrenoceptors suggested that rabbit spleen contained mainly α_{2A}-adrenoceptors.

Indeed, there was good agreement between the 78 and 16 fold selectivity of prazosin and chlorpromazine, respectively, for rat kidney α_2-adrenoceptors compared with rabbit spleen (Table 1), and the 69 and 18 fold selectivity of these compounds for the α_{2B} compared with the α_{2A}-adrenoceptor sites described by Bylund *et al.* (1988). Furthermore, oxymetazoline and WB 4101 were, respectively, 100 and 17 fold selective for rabbit spleen compared with rat kidney in the present study and have been shown to display, respectively, an 83 and 7 fold higher affinity for the α_{2A}-adrenoceptor compared with the α_{2B} sites (Bylund *et al.*, 1988).

Although these data further substantiate the classification of α_2-adrenoceptors into α_{2A}- and α_{2B}-adrenoceptor subtypes, it is possible that the differences in affinity estimates obtained in the two membrane preparations resulted from factors other than receptor heterogeneity. This, however, seemed unlikely

since in rat cortex and human platelet, Kawahara & Bylund (1985) have conducted extensive studies to rule out the role of differential metabolism of the receptors, the presence of soluble factors and membrane environment. Furthermore, in the present study when rat kidney and rabbit spleen membrane preparations were combined, biphasic inhibition curves were obtained for both imiloxan and prazosin. These curves could be resolved into both high and low affinity components and the affinities of prazosin and imiloxan for these two sites were similar to the affinity estimates at the rat kidney and rabbit spleen, respectively. This observation would suggest that differential metabolism of the ligand was not occurring.

The differences in the affinity estimates in rabbit spleen and rat kidney for agonist ligands such as oxymetazoline did not appear to be due to the presence of different proportions of high and low affinity states of the α_2-adrenoceptors, since Gpp(NH)p, a non-hydrolysable GTP analogue, did not modify either the potency or the apparent selectivity of noradrenaline, oxymetazoline or WB 4101 in the absence of Mg^{2+}. This would suggest that the ability of oxymetazoline and WB 4101 to differentiate between α_2-adrenoceptors in the two tissues was independent of affinity states.

On the basis of the present studies, then, it would appear that rabbit spleen α_2-adrenoceptors are predominantly of the α_{2A} subtype while rat kidney α_2-adrenoceptors correspond to the α_{2B} subtype. It should be noted that in rabbit spleen nH values for several compounds were low and indicated possible heterogeneity of the receptors labelled by [^{3}H]-rauwolscine. This was especially true for WB 4101 and oxymetazoline where 75–79% of the sites displayed high affinity for the competing ligand.

The major population of sites presumably represented the α_{2A}-adrenoceptor, since the selectivity of WB 4101 (17 fold) and oxymetazoline (100 fold) for these sites over the rat kidney α_{2B}-adrenoceptors was similar to the 7.5 and 83 fold selectivity of WB 4101 and oxymetazoline, respectively, for the α_{2A}- as opposed to α_{2B}-adrenoceptor (Bylund *et al.*, 1988).

The nature of the second site in rabbit spleen was uncertain. For oxymetazoline and WB 4101, the affinity for the low affinity site was possibly consistent with the presence of an α_{2B}-adrenoceptor. Although, it should be noted that for WB 4101, the low affinity K_i was 3.3 fold lower than the affinity of this compound at the α_{2B}-adrenoceptor of rat kidney. Furthermore, the α_{2B}-selective agents, chlorpromazine and prazosin, could not detect heterogeneity of binding in rabbit spleen even when more detailed 22 point competition curves were constructed (data not shown). With respect to the nature of the second site in rabbit spleen, it is relevant to note that in addition to the two α_2-adrenoceptor subtypes that have been identified to date, several other sites can be labelled by α_2-adrenoceptor ligands. Both high and low affinity binding of [^{3}H]-rauwolscine and [^{3}H]-yohimbine in rat cortex have been demonstrated by several groups (Diop *et al.*, 1983; Michel & Whiting, 1984; Boyajian & Leslie, 1987; Broadhurst *et al.*, 1988), while Bylund (1988) has proposed the existence of a third subtype of α_2-adrenoceptor, the α_{2C}-adrenoceptor. In addition, imidazolines can bind to an imidazoline binding site (Coupry *et al.*, 1987). Whether the second site in rabbit spleen corresponds to any of these other potential sites requires further study.

Several additional agents were also able to differentiate between the two receptor subtypes in rabbit spleen and rat kidney. Benoxathian (Melchiorre *et al.*, 1984), a close analogue of WB 4101, was selective for the rabbit spleen α_{2A}- adrenoceptor, while imiloxan possessed higher affinity for rat kidney α_{2B}- than for rabbit spleen α_{2A}-adrenoceptors.

The observation that imiloxan displayed a high degree of selectivity for the α_{2B}-adrenoceptor should make this compound a useful tool in the study of α_2-adrenoceptor subtypes. This is important at present since the other available subtype selective ligands such as prazosin, chlorpromazine and WB 4101 possess high affinity for other receptor systems. In contrast, imiloxan displays low affinity ($pK_i < 5$) for a wide range of receptors including dopamine, α_1-adrenoceptor, β-adrenoceptor, muscarinic and histamine receptors (A.D. Michel, unpublished observations).

In previous functional studies on the α_2-adrenoceptor in rat and rabbit vas deferens, it is interesting to note that imiloxan displayed high affinity for rat α_2-adrenoceptors but low affinity for rabbit α_2-adrenoceptors (Lattimer & Rhodes, 1985). While it is tempting to speculate that the receptors in rabbit and rat vas deferens correspond to the α_{2A}- and α_{2B}- adrenoceptors identified in the present study, it should be noted that idazoxan was marginally selective in the present study but displayed a 100 fold higher affinity for α_2-adrenoceptors of the rat vas deferens than for those present in rabbit vas deferens (Lattimer & Rhodes, 1985).

With respect to the relationship of the present binding results to functional studies it was interesting to note that SKF 104078, which, in functional studies, has been described as a selective postsynaptic α_2-adrenoceptor antagonist (Ruffolo *et al.*, 1988), was non-selective between the α_{2A}- and α_{2B}-adrenoceptors in the present study. This may indicate, as suggested by Regan *et al.* (1988), that a third subtype of α_2-adrenoceptor exists at which SKF 104078 displays low affinity and which would be the subtype functioning to mediate α_2-adrenoceptor autofeedback at sympathetic nerve terminals.

In conclusion, in the present study the α_2-adrenoceptor present in rabbit spleen has been shown to display a pharmacology consistent with the presence of an α_{2A}-adrenoceptor, while rat kidney α_2-adrenoceptors appeared to be of the α_{2B}-adrenoceptor subtype. Imiloxan displayed a 55 fold higher affinity for α_{2B}- than for α_{2A}-adrenoceptors and therefore represents a useful tool for the further characterization of α_2-adrenoceptor subtypes.

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