Identification of a single α_1 -adrenoceptor corresponding to the α_{1A} -subtype in rat submaxillary gland

¹Anton D. Michel, Dana N. Loury & Roger L. Whiting

Institute of Pharmacology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA, 94303, U.S.A.

- 1 The α_1 -adrenoceptors present in membranes of rat liver, cortex and submaxillary gland were labelled with [³H]-prazosin and the affinity of 15 ligands for these receptors was determined.
- 2 In saturation studies, [³H]-prazosin bound with high affinity ($K_d = 30\text{--}39\text{ pM}$) to a single population of sites in all three preparations.
- 3 In competition studies using rat cortex, evidence for heterogeneity of the α_1 -adrenoceptor binding sites was obtained. Displacement isotherms for amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 were biphasic and were consistent with the presence of both α_{1A} - and α_{1B} -adrenoceptor subtypes as described by Morrow & Creese (1986) and Han *et al.* (1987).
- 4 The rat liver and submaxillary gland membrane preparations both possessed homogeneous populations of α_1 -adrenoceptors. However, there were pharmacological differences between the receptors in these two preparations. Rat submaxillary gland α_1 -adrenoceptors displayed high affinity for amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 and therefore appeared to represent α_{1A} -adrenoceptors. Rat liver α_1 -adrenoceptors possessed lower affinity for these ligands (6–65 fold) suggesting that these receptors were of the α_{1B} -subtype.
- 5 Spiperone exhibited 12.9 fold higher affinity for rat liver α_{1B} -adrenoceptors than for rat submaxillary gland α_{1A} -adrenoceptor and may therefore represent the first α_{1B} -adrenoceptor selective ligand.

Introduction

The concept of α_1 -adrenoceptor subtypes has arisen from both ligand binding and functional studies (See review by Minneman, 1988) although at present there is no consensus as to the number of subtypes or their definition (McGrath & Wilson, 1988).

In functional studies Rufollo *et al.* (1982), have provided evidence that there is heterogeneity of post-junctional α_1 -adrenoceptors in mammalian aortae, although in these studies the existence of post-synaptic α_2 -adrenoceptors may have complicated interpretation of the results (Minneman, 1988). McGrath (1982) initially proposed that postsynaptic α_1 -adrenoceptors were heterogeneous and that α_{1A} and α_{1B} -adrenoceptors existed. More recently, however, McGrath & Wilson (1988) have questioned the validity of subdividing α_1 -adrenoceptors. Indirect evidence for the presence of α_1 -adrenoceptor sub-

types has been suggested by the observation that there are considerable differences in the pA_2 values of antagonists for α_1 -adrenoceptors of various tissues. Thus, depending on the preparation and agonist chosen, the reported pA_2 values for a single antagonist often extend over 2 log orders (Drew, 1985). Docherty (1987) has, however, suggested an alternative explanation for variations in antagonist pA_2 values.

In contrast to the difficulty in subclassifying the α_1 -adrenoceptors identified in functional studies, in radioligand binding studies there is now relatively strong support for the concept of α_1 -adrenoceptor subtypes. In rat cerebral cortex, both phentolamine and WB 4101 have been shown to produce biphasic displacement isotherms (Morrow *et al.*, 1985; Han *et al.*, 1987). Morrow & Creese (1986) have suggested that this phenomenon is due to the presence of α_{1A} - and α_{1B} -adrenoceptor subtypes.

The recent demonstration by Han *et al.* (1987), that the α_1 -adrenoceptors of rat vas deferens, which

¹ Author for correspondence at present address: Department of Neuropharmacology, Glaxo Group Research Limited, Park Road, Ware, Hertfordshire, SG12 0DP.

mediate contractile responses through a calcium entry blocker-sensitive mechanism, display the pharmacology of the α_{1A} -adrenoceptor identified in binding studies, while the α_1 -adrenoceptors of rat spleen, which mediate contractile responses through a calcium entry blocker-insensitive mechanism, display the pharmacology of the α_{1B} -adrenoceptor, provides additional support for the existence of α_1 -adrenoceptor subtypes.

In receptor binding studies on the α_1 -adrenoceptor, while rat liver and spleen α_1 -adrenoceptors have been shown to be exclusively of the α_{1B} -adrenoceptor subtype (Han *et al.*, 1987), there have been no preparations described to date in which α_1 -adrenoceptors are exclusively of the α_{1A} -adrenoceptor subtype. In the present study we have conducted a detailed ligand binding characterization of the α_1 -adrenoceptors present in rat cortex, liver and submaxillary gland and find that the rat submaxillary gland α_1 -adrenoceptors are exclusively of the α_{1A} -adrenoceptor subtype.

Methods

Membrane preparation

All membrane preparation procedures were conducted at 4°C and used ice cold buffers. Livers, cerebral cortices and submaxillary glands from adult Sprague-Dawley rats were obtained from Pel Freeze (Arkansas, U.S.A.).

Rat cortices were homogenized in a polytron P10 tissue disrupter (setting 10; two 10 s bursts) and an EDTA-washed membrane fraction was prepared as described by Cheung *et al.* (1982). Briefly, the cortical homogenate was centrifuged at 43,000 *g* for 12 min at 4°C and the crude membrane pellet obtained was washed by resuspending in homogenizing buffer and centrifuging at 43,000 *g* for 12 min. The pellet from this step was 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 pellet was resuspended in assay buffer (pH 7.4 at 25°C) and stored under liquid nitrogen until required.

The livers and submaxillary glands were separately homogenized for 30 s in 5 volumes (w/v) of 50 mM Tris, 5 mM EDTA homogenizing buffer (pH 7.4 at 4°C) in a Waring Blender at maximum setting. The homogenates obtained were further homogenized in 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 and rehomogenized in 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 membrane fractions were prepared exactly as described above for the rat cortex.

Ligand binding assays

Membranes, [3 H]-prazosin and competing drugs were incubated in a final volume of 1 ml of assay buffer (pH 7.4 at 25°C) for 1 h at 25°C. In competition studies the final assay concentration of radioligand was 0.03–0.07 nM while in saturation studies the concentration of [3 H]-prazosin was varied between 0.005 and 2 nM. In all experiments non-specific binding was defined by use of 10 μ M phentolamine. Incubations were terminated by vacuum filtration over 0.1% PEI pretreated glass fibre filters using a Brandel cell harvester. The filters were washed for 10 s with ice cold 0.1 M NaCl and radioactivity retained on the filters was determined by liquid scintillation spectrometry.

Protein assay

Protein was measured by the dye binding method (Bio-Rad) with bovine gamma globulin used as standard.

Data analysis

Competition and saturation binding data were analysed as described previously using iterative curve fitting techniques (Michel & Whiting, 1988). IC₅₀ values were converted to K_i values by use of the Cheng-Prusoff approximation (1973).

Materials

[3 H]-prazosin (specific activity 82 Ci mmol⁻¹) was obtained from New England Nuclear. Phentolamine was obtained from Ciba-Geigy. Prazosin was obtained from Pfizer. Benoxathian, spiperone, spiroxatrine and WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminoethyl-1,4-benzodioxane) were obtained from Research Biochemical Inc. Amidephrine was synthesized by Dr R. Clark, IOC, Syntex. All other chemicals and reagents were obtained from Sigma chemical company.

Results

Kinetic studies

In rat cortex, liver and submaxillary gland, [3 H]-prazosin binding achieved equilibrium within 15–30 min at a concentration of 0.03 nM (the lowest con-

Table 1 Affinity of ligands for α_1 -adrenoceptors of rat cortex, liver and submaxillary gland

Ligand	Submaxillary gland pK _i /nH	Cortex pK _i /nH	Liver pK _i /nH	Selectivity ratio*
Amidephrine	6.25 (0.02) 0.81 (0.01)	5.20 (0.10) 0.62 (0.02)	4.74 (0.04) 0.99 (0.03)	32.4
Benoxathian	9.67 (0.04) 0.97 (0.08)	8.67 (0.04) 0.69 (0.01)	8.35 (0.06) 1.02 (0.05)	20.9
Chlorpromazine	9.03 (0.14) 1.11 (0.11)	ND	9.46 (0.11) 1.03 (0.04)	0.4
Clonidine	6.90 (0.10) 0.96 (0.06)	6.45 (0.13) 0.79 (0.08)	6.32 (0.05) 1.04 (0.07)	3.8
Corynanthine	7.47 (0.02) 0.98 (0.06)	ND	7.28 (0.02) 0.90 (0.02)	1.5
Idazoxan	6.54 (0.16) 1.01 (0.06)	6.38 (0.08) 0.85 (0.09)	6.29 (0.05) 1.00 (0.02)	1.8
Imiloxan	5.14 (0.06) 0.80 (0.02)	ND	5.17 (0.17) 0.80 (0.03)	0.9
Noradrenaline	6.59 (0.05) 0.95 (0.02)	6.09 (0.10) 0.66 (0.08)	6.53 (0.10) 0.88 (0.02)	1.1
Oxymetazoline	8.49 (0.04) 0.82 (0.03)	7.34 (0.13) 0.54 (0.02)	6.68 (0.05) 1.02 (0.04)	64.6
Phentolamine	8.85 (0.13) 0.94 (0.01)	8.33 (0.10) 0.72 (0.02)	8.07 (0.05) 1.07 (0.05)	6.0
Rauwolscine	6.33 (0.15) 1.01 (0.08)	6.29 (0.06) 0.90 (0.06)	6.63 (0.05) 0.99 (0.02)	0.5
Spiperone	8.14 (0.01) 0.98 (0.01)	ND	9.25 (0.04) 1.01 (0.02)	0.08
Spiroxastrine	7.41 (0.10) 1.06 (0.08)	ND	7.54 (0.10) 1.01 (0.08)	0.7
WB 4101	9.98 (0.08) 0.85 (0.03)	9.19 (0.17) 0.69 (0.07)	8.78 (0.05) 1.04 (0.04)	15.8
Xylometazoline	7.87 (0.08) 0.96 (0.08)	ND	6.58 (0.07) 1.08 (0.09)	19.5

Values are the mean ($\pm$ s.e.mean) of the Hill coefficient (nH) or the negative logarithm of the K_i (pK_i). * Selectivity ratio is the antilog (pK_i submaxillary gland - pK_i liver). ND = not determined.

centration used in competition studies) and was stable for up to 2 h of further incubation.

In more detailed kinetic studies carried out with rat cortical and liver membranes, the rate constants for association were 3.35×10^8 and $5.73 \times 10^8 \text{ min}^{-1} \text{ M}^{-1}$, respectively. Dissociation of the radioligand from these preparations was slow with half lives of 29 min and 38 min in rat cortex and rat liver, respectively. Both the association and dissociation data were consistent with the presence of only a single population of binding sites. The kinetically derived K_d values for rat cortex and rat liver were 0.07 and 0.03 nM, respectively.

Saturation binding studies

The binding of [³H]-prazosin in rat cortex, liver and submaxillary gland was saturable and of high affinity. Binding was consistent with the presence of a single population of sites in each of the three preparations examined. In rat cortex, liver and submax-

illary gland K_d values of $59 \pm 8.2 \text{ pM}$, $31 \pm 3.5 \text{ pM}$ and $39 \pm 2.7 \text{ pM}$, respectively, were obtained. The values in rat liver and cortex were in good agreement with the kinetically derived K_d values obtained above. In the rat cortex, liver and submaxillary gland, B_{max} values were $70 \pm 7 \text{ fmol mg}^{-1} \text{ protein}$, $192 \pm 10 \text{ fmol mg}^{-1} \text{ protein}$ and $193 \pm 9 \text{ fmol mg}^{-1} \text{ protein}$, respectively.

Competition studies

Fifteen ligands were evaluated for their affinity at α_1 -adrenoceptors present in rat cortex, liver and submaxillary gland. The data are presented in Table 1 and representative competition curves for amidephrine, oxymetazoline and WB 4101 are shown in Figure 1. In rat liver and rat submaxillary gland the nH values for all compounds were close to unity. Amidephrine, benoxathian, oxymetazoline, phentolamine and WB4101 displayed 6–65 fold higher affinity in rat submaxillary gland than in rat liver. In

Figure 1 Competition of WB 4101 (a), amidephrine (b) and oxymetazoline (c) for [^3H]-prazosin binding sites present in rat cortex ($\circ$), liver ($\bullet$) and submaxillary gland ($\blacksquare$) membranes. In rat cortex, liver and submaxillary gland, total binding was 1230, 1401 and 980 d.p.m., respectively, while non-specific binding was 54, 43 and 65 d.p.m., respectively.

contrast, spiperone displayed 12.9 fold higher affinity in rat liver than in rat submaxillary gland. In Figure 2 affinity data obtained in the rat liver and rat submaxillary gland are compared. This analysis reveals that the correlation between affinity estimates at the two α_1 -adrenoceptor binding sites was poor ($r = 0.82$).

In rat cortex the nH values for amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 were significantly less than unity and the data could be better described by assuming the presence

Figure 2 Correlation between ligand affinity estimates obtained at the α_1 -adrenoceptors of rat liver and rat submaxillary gland labelled with [^3H]-prazosin. The dashed line represents the line of identity, while the solid line represents the regression line. The data were best described by the expression $y = 0.826x + 0.868$ ($r = 0.82$).

of two populations of [^3H]-prazosin binding sites for which these compounds displayed affinities that differed 19–58 fold (Table 2). The two populations of sites were present in approximately equal proportions at the concentration of [^3H]-prazosin used in this study and consequently it was difficult to determine which of the two sites corresponded to the α_{1A} - and α_{1B} -adrenoceptors previously described. Consequently, in Table 2 the sites displaying high affinity for the compounds were termed site 1 while the remaining low affinity sites were termed site 2.

Comparing the affinity estimates of amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 at the two sites identified in rat cortex with the affinity data obtained in rat submaxillary gland and liver, it would appear that the affinity estimates of these compounds for site 1 in rat cortex were in good agreement with the affinity estimates obtained in rat submaxillary gland, while affinity estimates for site 2 in rat cortex were in good agreement with the affinity estimates obtained in rat liver.

Even though the nH values of displacement curves for noradrenaline, clonidine and idazoxan were less than unity in rat cortex, it should be noted that these data could not be consistently fitted to a two site model of binding.

Table 2 Binding parameters of ligands at the α_1 -adrenoceptor binding sites of rat cortex

Ligand	Hill model pK _i nH	2 Site model	
		Site 1 pK _i (% of sites)	Site 2 pK _i (% of sites)
Amidephrine	5.20 (0.01) 0.62 (0.02)	5.97 (0.13) 52.6 (5.20)	4.49 (0.06) 47.4 (5.20)
Clonidine	6.45 (0.13) 0.79 (0.08)		ND
Noradrenaline	6.09 (0.10) 0.66 (0.08)		ND
Oxymetazoline	7.34 (0.13) 0.54 (0.02)	8.32 (0.05) 48.0 (5.40)	6.55 (0.05) 52.0 (5.40)
Benoxathian	8.67 (0.04) 0.69 (0.01)	9.69 (0.25) 42.3 (3.30)	8.26 (0.09) 56.7 (3.30)
Idazoxan	6.38 (0.08) 0.85 (0.09)		ND
Phentolamine	8.33 (0.10) 0.72 (0.02)	8.98 (0.14) 58.9 (3.20)	7.69 (0.17) 41.1 (3.20)
Prazosin	9.91 (0.14) 0.94 (0.05)		ND
Rauwolscine	6.29 (0.06) 0.90 (0.06)		ND
WB 4101	9.19 (0.17) 0.69 (0.07)	9.82 (0.16) 54.5 (3.80)	8.53 (0.09) 45.5 (3.80)

The values shown are the mean ($\pm$ s.e. mean; $n = 4-6$) for the negative logarithm of the K_i (pK_i) and the hill coefficient (nH). In rat cortex the data were better described by assuming [³H]-prazosin to label two populations of binding sites which amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 could differentiate. ND = 2 site fit not possible.

Discussion

The results of the present study add additional support to the concept of α_1 -adrenoceptor subtypes. The data obtained in the present study substantiate previous studies (Morrow & Creese, 1986; Han *et al.*, 1987) which have shown that benoxathian, phentolamine and WB 4101 differentiate two populations of binding sites in rat cortex. In the present study, the two populations of binding sites, which presumably represented the α_{1A} - and α_{1B} -adrenoceptors, were present in approximately equal proportions. Thus, it was difficult to determine unequivocally which of the sites, designated as site 1 and site 2, corresponded to the α_{1A} - and α_{1B} -adrenoceptor.

In addition to benoxathian, phentolamine and WB 4101, the agonist ligands amidephrine, oxymetazoline and xylometazoline also identified two populations of sites in rat cortex. As with the data obtained by use of the antagonists, the two sites discriminated by the agonists were present in equal proportions.

In order to determine unequivocally the selectivity of the agonist and antagonist compounds for the putative α_{1A} - and α_{1B} -adrenoceptor subtypes, they were further evaluated in a range of other tissues in

an attempt to determine if preparations which displayed homogeneous populations of the two putative binding sites could be identified. Of the tissues examined, the rat liver and rat submaxillary gland appeared to be the most interesting for detailed study.

Han *et al.* (1987) have previously shown that rat liver α_1 -adrenoceptors labelled with [¹²⁵I]-BE2254 are homogeneous and that they correspond to the α_{1B} -adrenoceptor subtype. The data obtained in the present study in which a range of 15 ligands was used support this contention since all compounds tested appeared to interact with only a single population of α_1 -adrenoceptors. Furthermore, affinity estimates for benoxathian, phentolamine and WB 4101 in rat liver were very similar to their affinity for the site 2 α_1 -adrenoceptors of rat cortex. Since benoxathian, phentolamine and WB 4101 display low affinity for the α_{1B} -adrenoceptor (Han *et al.*, 1987) it would appear that the low affinity component of binding in rat cortex, site 2, represented the α_{1B} -adrenoceptor. The data obtained with the agonists, amidephrine and oxymetazoline, also support this contention. By inference these results would indicate that site 1 α_1 -adrenoceptors of rat cortex correspond to α_{1A} -adrenoceptors.

Rat submaxillary gland α_1 -adrenoceptors have not been examined in detail before. Morrow & Creese (1986) have suggested that both α_{1A} - and α_{1B} -adrenoceptors are present in this tissue since the ratio of the affinity estimates for prazosin and phentolamine obtained in this tissue by Elverdin *et al.* (1984) is relatively high. The results of the present study would indicate, however, that rat submaxillary gland α_1 -adrenoceptors were homogeneous, and that these α_1 -adrenoceptors differed from those present in rat liver. The observation that affinity estimates for amidephrine, benoxathian, oxymetazoline, phentolamine and WB 4101 in rat submaxillary gland were almost identical to those obtained at site 1 in rat cortex (α_{1A}) would suggest that rat submaxillary gland α_1 -adrenoceptors were therefore of the α_{1A} -subtype.

In this respect it is interesting to note that in rat submandibular gland Bylund *et al.* (1982) have shown that phentolamine and WB 4101 display high affinity for [3 H]-prazosin binding sites. Based upon these findings Morrow & Creese (1986) have proposed that these receptors are of the α_{1A} -adrenoceptor subtype.

It is clear from the present study that while the sub-classification of α_1 -adrenoceptors into α_{1A} - and α_{1B} -adrenoceptor subtypes based upon their relative affinities for phentolamine and prazosin can be useful (Morrow & Creese, 1986), a more rigorous pharmacological classification would be desirable.

Such a classification requires the availability of selective ligands for the putative receptor subtypes. Previous studies have shown that benoxathian (an analogue of WB 4101), phentolamine and WB 4101 can discriminate between α_1 -adrenoceptor subtypes (Han *et al.*, 1987). In addition, 5 methyl-urapidil has also been reported as a selective α_{1A} -adrenoceptor ligand (Gross *et al.*, 1988). The results of the present study suggest that the α_1 -adrenoceptor agonists amidephrine, oxymetazoline and xylometazoline (a close analogue of oxymetazoline) can also discriminate α_{1A} - from α_{1B} -adrenoceptors. While interpretation of affinity estimates for these compounds is complicated by their agonist properties, the failure of

guanine-nucleotides or sodium ions to modulate either their affinity or their selectivity for submaxillary gland over liver α_1 -adrenoceptors (unpublished observations), would suggest that the selectivity of the agonists was not due to the presence of affinity states of the α_1 -adrenoceptor. In this respect it is also relevant to note that neither noradrenaline (Table 1) nor phenylephrine (unpublished observations) was able to discriminate between the rat liver and rat submaxillary gland α_1 -adrenoceptors.

With respect to the ability of oxymetazoline to discriminate between α_{1A} - and α_{1B} -adrenoceptors, this property may explain the previous finding that oxymetazoline revealed heterogeneity of [125 I]-BE 2254 binding to rat cortical membranes (Minneman, 1983). Indeed, Minneman (1988) has recently indicated that oxymetazoline is capable of differentiating between α_1 -adrenoceptor subtypes.

At present, we are not aware of any α_{1B} -adrenoceptor selective ligands. In the present study spiperone possessed 12.9 fold higher affinity for the rat liver α_{1B} -adrenoceptor than for the rat submaxillary gland α_{1A} -adrenoceptor and may therefore represent the first α_{1B} -selective ligand. It should be noted that this compound is not a specific α_1 -adrenoceptor ligand since it also displays high affinity for several other receptors, most notably the dopamine D_2 -receptor and the 5-HT $_2$ -receptors.

Specificity of action is also a problem with respect to the α_{1A} -adrenoceptor subtype selective ligands. Thus, WB 4101 is also a 5-HT $_{1A}$ ligand (Morrow & Creese, 1986) and possesses high affinity for α_{2A} -adrenoceptors (Cheung *et al.*, 1982). Phentolamine displays high affinity for α_2 -adrenoceptors of rat submaxillary gland (unpublished observations) while oxymetazoline is a potent ligand at α_2 -adrenoceptor subtypes (Bylund, 1985). The availability of a receptor binding assay for both the α_{1A} - and α_{1B} -adrenoceptor subtypes should aid in the development of the more selective and specific ligands which will be required for functional evaluation of the physiological role of the putative α_1 -adrenoceptor subtypes.

References

- BYLUND, D.B. (1985). Heterogeneity of alpha-2 adrenergic receptors. *Pharmacol. Biochem. Behav.*, **22**, 835-843.
- BYLUND, D.B., MARTINEZ, J.R. & PIERCE, D.L. (1982). Regulation of autonomic receptors in rat submandibular gland. *Mol. Pharmacol.*, **21**, 27-35.
- CHENG, Y.-C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099-3108.
- CHEUNG, Y.-D., BARNETT, D.B. & NAHORSKI, S.R. (1982). [3 H]-rauwolscine and [3 H]-yohimbine binding to rat cerebral and human platelet membranes: possible heterogeneity of alpha-2-adrenoceptors. *Eur. J. Pharmacol.*, **84**, 79-85.
- DOCHERTY, J. (1987). Alpha-1-adrenoceptor classification in vascular smooth muscle: a premature proposal? *Trends Pharmacol. Sci.*, **8**, 123.
- DREW, G.M. (1985). What do antagonists tell us about alpha-adrenoceptors? *Clin. Sci.*, **68**, Suppl. 15S-19S.

- ELVERDIN, J.C., LUCHELLIS-FORTIS, M.A., STEFANO, F.J.E. & PEREC, C.J. (1984). Alpha-1 adrenoceptors mediate secretory responses to norepinephrine in innervated and denervated rat submaxillary glands. *J. Pharmacol. Exp. Ther.*, **229**, 261–266.
- GROSS, G., HANFT, G. & RUGEVICS, C. (1988). 5-Methylurapidil discriminates between subtypes of the α_1 -adrenoceptor. *Eur. J. Pharmacol.*, **151**, 333–335.
- HAN, C., ABEL, P.W. & MINNEMAN, K.P. (1987). Alpha₁-adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. *Nature*, **329**, 333–335.
- McGRATH, J.C. (1982). Evidence for more than one type of post-junctional alpha-adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.
- McGRATH, J.C. & WILSON, V. (1988). Alpha-adrenoceptor subclassification by classical and response-related methods: same question, different answers. *Trends Pharmacol. Sci.*, **9**, 162–165.
- MICHEL, A.D. & WHITING, R.L. (1988). Methoctramine, a polymethylene tetraamine, differentiates three subtypes of muscarinic receptor in direct binding studies. *Eur. J. Pharmacol.*, **145**, 61–66.
- MINNEMAN, K.P. (1983). Binding properties of alpha-1 adrenergic receptors in rat cerebral cortex: Similarity to smooth muscle. *J. Pharmacol. Exp. Ther.*, **227**, 605–612.
- MINNEMAN, K.P. (1988). Alpha₁-adrenergic receptor subtypes, inositol phosphates and sources of cell Ca^{2+} . *Pharmacol. Rev.*, **40**, 87–121.
- MORROW, A.L. & CREESE, I. (1986). Characterization of alpha₁-adrenergic receptor subtypes in rat brain: A re-evaluation of [³H]WB 4101 and [³H]prazosin binding. *Mol. Pharmacol.*, **29**, 321–330.
- MORROW, A.L., BATTAGLIA, G., NORMAN, A.B. & CREESE, I. (1985). Identification of subtypes of [³H]prazosin labeled alpha₁-receptor binding sites in rat brain. *Eur. J. Pharmacol.*, **109**, 285–287.
- RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1982). Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J. Pharmacol. Exp. Ther.*, **221**, 309–314.

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