Subclassification of the presynaptic α_2 -autoreceptors in rabbit brain cortex

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1 α_2 -Adrenoceptor binding sites have been subclassified into α_{2A} sites of which a main characteristic is very low affinity for prazosin, and α_{2B} sites with relatively high affinity for prazosin. The presynaptic α_2 -autoreceptors in rabbit brain cortex were studied in order to classify them in terms of α_{2A} and α_{2B} . Release of [³H]-noradrenaline in cortical slices was elicited by trains of 4 pulses delivered at 100 Hz.

2 Clonidine caused concentration-dependent inhibition of the stimulation-evoked overflow of tritium, with an EC₅₀ of 7.5 nM and a maximal inhibition by 96%.

3 The following α -adrenoceptor antagonists shifted the concentration-response curve of clonidine to the right (antagonist-receptor dissociation constants K_D in brackets): yohimbine (14 nM), 2-[2H-(1-methyl-1,3-dihydroisindol-4-yl)-4,5-dihydroimidazole (BRL 44408; 15 nM) and 1,2-dimethyl-2,3,9,13b-tetrahydro-1H-dibenzo[c,f]imidazo[1,5-a]azepine (BRL 41992; 630 nM). Prazosin 1 μ M and 2-[2-[4-(o-methoxyphenyl)piperazine-1-yl]-ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (AR-C 239) 1 μ M failed to antagonize the effect of clonidine. Higher concentrations of prazosin and AR-C 239 greatly accelerated the basal efflux of tritium.

4 The method used permits the functional determination of antagonist affinities undistorted by endogenous α_2 -autoinhibition. A comparison with affinities derived from radioligand binding experiments indicates that the presynaptic α_2 -autoreceptors in rabbit brain cortex are markedly different from the α_{2B} -subtype and probably belong to the prazosin-insensitive α_{2A} -subtype.

Keywords: Rabbit brain cortex; noradrenaline release; presynaptic α_2 -autoreceptors; α_{2A} -adrenoceptors; α_{2B} -adrenoceptors; clonidine; yohimbine; prazosin

Introduction

Presynaptic α -autoreceptors of noradrenaline-releasing neurones were the prototype of α_2 -adrenoceptors. It was within this group that non-homogeneity of α_2 -adrenoceptors was first noticed (Doxey & Everitt, 1977; Dubocovich, 1979). Later subclassifications were mainly based on radioligand binding experiments (reviewed by Docherty, 1989; McGrath *et al.*, 1989). Extensive evidence now indicates that the α_2 -selective (Starke *et al.*, 1975; Weitzell *et al.*, 1979) ligands [³H]-yohimbine and [³H]-rauwolscine label at least two α_2 sites which differ, above all, in their affinity for prazosin. The prazosin-insensitive site was called α_{2A} (K_i of prazosin about 300 nM). The relatively prazosin-sensitive site was called α_{2B} (K_i of prazosin about 5 nM; Bylund, 1985; 1988; Nahorski *et al.*, 1985; Bylund *et al.*, 1988). The existence of further α_2 binding sites (see Discussion) is less well established.

It has been difficult to correlate the α_{2A} and α_{2B} binding sites with functioning receptors (see Docherty, 1989; McGrath *et al.*, 1989). From data in the literature, Bylund *et al.* (1988) suggested that 'it is possible that the α_2 receptors on noradrenergic axon terminals are of the B subtype'. This identification, however, is questionable. The presynaptic α_2 -autoreceptors of rat vas deferens, suggested to be α_{2B} by Bylund *et al.* (1988), are classically prazosin-insensitive, with functionally measured K_D values of 1 μ M or higher (Dubocovich, 1979; Doxey & Roach, 1980; Table 19 of Docherty, 1989); a recent functional study in fact indicates that the rat vas deferens autoreceptors are α_{2A} (Connaughton & Docherty, 1990). The same prazosin-insensitivity holds true for the presynaptic α_2 -autoreceptors of rat brain cortex (Dubocovich, 1979; Hedler *et al.*, 1981; Frankhuyzen & Mulder, 1982; the latter study on rat hippocampus), also suggested to be α_{2B} (Bylund *et al.*, 1988; see Gobbi *et al.*, 1990, for critical discussion of the suggestion); again a recent functional study classified the receptors as α_{2A} (Gobbi *et al.*, 1990).

One functionally observed heterogeneity of α_2 -adrenoceptors is a difference between presynaptic α_2 -autoreceptors in the rat and the rabbit (Starke, 1981; Reichenbacher *et al.*, 1982; Ennis, 1985; Lattimer & Rhodes, 1985; Alabaster *et al.*, 1986; Limberger *et al.*, 1989). Are the α_2 -autoreceptors of rabbit brain cortex α_{2B} , in contrast to the α_{2A} -autoreceptors of rat brain? We studied the question in brain slices, using brief trains of electric pulses to release noradrenaline, a method that avoids the development of autoinhibition which otherwise may falsify autoreceptor characterization (Limberger *et al.*, 1989; for review see Starke, 1987; Singer, 1988). With clonidine as agonist we determined the antagonist-autoreceptor dissociation constants K_D of five antagonists: yohimbine (non-selective in terms of α_{2A} and α_{2B}), prazosin, 2-[2-[4-(o-methoxyphenyl)piperazine-1-yl]-ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (AR-C 239; α_{2B} -selective; Bylund *et al.*, 1988), 1,2-dimethyl-2,3,9,13b-tetrahydro-1H-dibenzo[c,f]imidazo[1,5-a]azepine (BRL 41992; α_{2B} -selective; Young *et al.*, 1989) and 2-[2H-(1-methyl-1,3-dihydroisindol-4-yl)-4,5-dihydroimidazole (BRL 44408; α_{2A} -selective; Young *et al.*, 1989).

Methods

The experiments were carried out as described previously (Limberger *et al.*, 1989), with the differences mentioned below. Briefly, slices of the rabbit occipito-parietal cortex were preincubated with (–)-[³H]-noradrenaline 0.1 μ M for 30 min, then superfused with [³H]-noradrenaline-free medium containing desipramine 1 μ M, one slice per superfusion chamber, and stimulated by a train of 4 pulses, delivered at a frequency of 100 Hz, after 60, 100, 140 and 180 min of superfusion (S₁–S₄). Desipramine was always added throughout superfusion in order to block the re-uptake of [³H]-noradrenaline and thus increase the electrically evoked overflow of tritium. α -Adrenoceptor antagonists, when used, were also present throughout superfusion at a fixed concentration. Cumulative

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concentration-response curves of clonidine were determined by addition of increasing concentrations 20 min before S_2 , S_3 and S_4 . The superfusate was collected in 5 min periods. The outflow of tritium was expressed as fractional rate (min^{-1}). The overflow elicited by electrical stimulation was expressed as a percentage of the tritium content of the tissue at the time of stimulation. For further evaluation of basal tritium efflux, ratios were calculated of the fractional rate of outflow in the collection periods immediately before S_2 , S_3 and S_4 , and the outflow in the collection period immediately before S_1 (b_2/b_1 , b_3/b_1 and b_4/b_1). For further evaluation of the electrically evoked overflow, ratios were calculated of the overflow elicited by S_2 , S_3 and S_4 , and the overflow elicited by S_1 (S_2/S_1 , S_3/S_1 and S_4/S_1).

K_D values were determined as follows. Six slices were used in a single experiment. One or two slices were superfused with medium containing neither clonidine nor an antagonist (controls), or containing an antagonist only (at the highest concentration used). One or two other slices received only clonidine at three increasing concentrations before S_2 , S_3 and S_4 . The remaining slices were exposed to the antagonist as well as to clonidine. The percentage inhibition caused by clonidine in each single brain slice was calculated, taking the respective average control (no clonidine, no antagonist) S_2/S_1 , S_3/S_1 and S_4/S_1 ratio (line 1 of Table 2 below) as reference value. [One might object that the appropriate reference S_n/S_1 ratio in the presence of an antagonist would have to be obtained in experiments with the antagonist alone, given at the same concentration. This, however, was not considered necessary, because the S_n/S_1 ratios in controls (no clonidine, no antagonist) were very close to those obtained in the presence of the highest concentration of each antagonist used (no clonidine) and were all close to unity (see Table 2 below).] In contrast to our previous study, agonist concentrations causing less than 10 or more than 90% inhibition were included.

The K_D value of yohimbine was calculated as described by Waud (1975). Sigmoid curves were simultaneously fitted to all percentage inhibition values obtained in clonidine/yohimbine experiments (i.e., to all values on which Figure 1 below is based), using equation 52 of Waud (1975) which yields the EC_{50} of clonidine, its maximal effect, the K_D of yohimbine and a parameter Q corresponding to the slope of the Arunlakshana-Schild plot (Arunlakshana & Schild, 1959). Since Q was close to unity for yohimbine (0.87), it was constrained to 1 for the final calculation of K_D . The K_D of BRL 44408 was calculated in the same manner (i.e., sigmoid curves were fitted simultaneously to all percentage inhibition values

on which Figure 2 below is based; Q was 0.96). Prazosin, AR-C 239 and BRL 41992 caused no or only a small shift of the clonidine concentration-response curve, and in these cases the curve-fitting calculations did not converge. Therefore, one sigmoid curve was fitted to the percentage inhibition values for clonidine alone and, separately, one curve to the values obtained in the presence of each antagonist concentration (for instance, one curve was fitted to the 'clonidine alone' data and another curve was fitted separately to the 'clonidine in the presence of BRL 41992' data of Figure 3).

Results are expressed as arithmetic means $\pm$ s.e.mean except when derived from sigmoid curve fitting (EC_{50} value and maximal effect of clonidine and antagonist K_D values). Statistical comparisons of group means were done with the Mann-Whitney test if Kruskal-Wallis analysis indicated a significant difference. In the case of multiple comparisons, the significance levels to be exceeded were adjusted according to Bonferroni (Wallenstein *et al.*, 1980). In some cases, two concentration-response curves obtained by sigmoid curve fitting were tested for a significant difference using the procedure of Motulsky & Ransnas (1987; pp. 371–372). n is the number of brain slices.

(–)-[ring-2,5,6- ^3H]-noradrenaline, specific activity 41 Ci mmol^{-1} , was purchased from Du Pont (Dreieich, FRG). Yohimbine HCl was purchased from Roth (Karlsruhe, FRG). Clonidine HCl was a gift from Boehringer (Ingelheim, FRG), desipramine HCl from Ciba-Geigy (Basel, Switzerland), prazosin HCl from Pfizer (Karlsruhe, FRG), BRL 44408 and BRL 41992 from Dr M.A. Cawthorne, SmithKline Beecham (Great Burgh, Epsom, Surrey), and AR-C 239 HCl from Dr J.C.A. van Meel, Thomae (Biberach, FRG). Desipramine, clonidine, yohimbine, prazosin and AR-C 239 were dissolved in water. BRL 44408 and BRL 41992 were dissolved in 10 mM HCl. Control slices were always run with the respective solvent.

Results

The time course of the outflow of tritiated compounds has been shown previously (Mayer *et al.*, 1988; Limberger *et al.*, 1989). The basal outflow of tritium, expressed as fractional rate in the collection period before S_1 (b_1), was not greatly changed when α -adrenoceptor antagonists were present throughout superfusion, except for a 2.6 fold increase by prazosin 1 μM and a 1.7 fold increase by AR-C 239 1 μM (Table 1). Yohimbine and AR-C 239 did not change the overflow of tritium elicited by S_1 , whereas prazosin caused a 30% increase and both BRL substances caused a decrease (Table 1).

Table 1 Basal outflow and evoked overflow of tritium from slices of rabbit brain cortex

Drug present throughout superfusion (μM)	b_1 (min^{-1})	S_1 (% of tissue tritium)	n
Desipramine 1	0.00114 ± 0.00004	0.622 ± 0.030	53
Desipramine 1 + yohimbine 0.03	0.00097 ± 0.00007	0.546 ± 0.038	16
Desipramine 1 + yohimbine 0.1	$0.00085 \pm 0.00003^{**}$	0.559 ± 0.032	14
Desipramine 1 + yohimbine 0.3	0.00094 ± 0.00006	0.585 ± 0.049	15
Desipramine 1 + yohimbine 1	0.00114 ± 0.00007	0.653 ± 0.040	22
Desipramine 1 + BRL 44408 0.03	0.00122 ± 0.00005	$0.416 \pm 0.048^*$	12
Desipramine 1 + BRL 44408 0.1	0.00135 ± 0.00007	$0.371 \pm 0.031^{**}$	15
Desipramine 1 + BRL 44408 0.3	0.00125 ± 0.00003	$0.353 \pm 0.026^{**}$	15
Desipramine 1 + BRL 44408 1	0.00118 ± 0.00004	$0.334 \pm 0.045^{**}$	22
Desipramine 1 + BRL 41992 1	$0.00154 \pm 0.00008^{**}$	$0.384 \pm 0.022^{**}$	21
Desipramine 1 + prazosin 1	$0.00296 \pm 0.00013^{**}$	$0.807 \pm 0.045^*$	25
Desipramine 1 + AR-C 239 0.1	0.00125 ± 0.00006	0.548 ± 0.033	8
Desipramine 1 + AR-C 239 1	$0.00191 \pm 0.00008^{**}$	0.514 ± 0.030	26

Slices of rabbit brain cortex were preincubated with [^3H]-noradrenaline and then superfused with medium containing the drugs indicated. b_1 represents the outflow of tritium in the collection period immediately before the first period of electrical stimulation (55–60 min of superfusion) and is expressed as fractional rate (min^{-1}); the outflow in absolute terms, in slices superfused with desipramine only, was 0.86 ± 0.03 nCi per 5 min. S_1 represents the overflow of tritium elicited by the first period of electrical stimulation (4 pulses/100 Hz) and is expressed as a percentage of the tritium content of the tissue; the overflow in absolute terms, in slices superfused with desipramine only, was 0.88 ± 0.05 nCi. Means $\pm$ s.e.mean of n brain slices. Significant differences from desipramine only: * $P < 0.05$; ** $P < 0.01$.

Table 2 Stability of baseline conditions: S_n/S_1 ratios in the absence of clonidine

Drug present throughout superfusion (μM)	Stimulation-evoked overflow of tritium			
	S_2/S_1	S_3/S_1	S_4/S_1	n
Desipramine 1	1.09 ± 0.02	1.13 ± 0.02	1.16 ± 0.03	27
Desipramine 1 + yohimbine 1	1.10 ± 0.07	1.10 ± 0.07	1.12 ± 0.08	7
Desipramine 1 + BRL 44408 1	1.07 ± 0.04	1.08 ± 0.05	1.06 ± 0.04	9
Desipramine 1 + BRL 41992 1	1.10 ± 0.06	1.11 ± 0.06	1.04 ± 0.05	9
Desipramine 1 + prazosin 1	0.95 ± 0.08	1.07 ± 0.04	1.19 ± 0.05	14
Desipramine 1 + AR-C 239 1	1.11 ± 0.02	1.10 ± 0.04	1.14 ± 0.04	8

Slices of rabbit brain cortex were preincubated with [^3H]-noradrenaline and then superfused with medium containing the drugs indicated. They were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). S_n/S_1 values represent the ratios of the overflow elicited by S_2 , S_3 and S_4 , and the overflow elicited by S_1 . Means $\pm$ s.e.mean of n brain slices. There were no significant differences between S_n/S_1 ratios.

The electrically evoked overflow of tritium was reproducible in the absence of clonidine, independently of whether the superfusion medium contained only desipramine $1 \mu\text{M}$ or desipramine combined with the highest concentrations of the α -adrenoceptor antagonists tested (Table 2).

Clonidine, when added before S_2 , S_3 and S_4 at increasing concentrations, reduced the evoked overflow of tritium. Concentration-response curves are shown in Figures 1–5. The

EC_{50} in the absence of antagonists was 7.5 nM , and the maximal inhibition 96%.

Both yohimbine (Figure 1) and BRL 44408 (Figure 2), applied at concentrations of 30 nM to $1 \mu\text{M}$, shifted the clonidine concentration-response curve increasingly to the right. Sigmoid curve fitting yielded K_D values of $14.4 \pm 1.2 \text{ nM}$ ($pA_2 = 7.84$) and $15.1 \pm 1.5 \text{ nM}$ ($pA_2 = 7.82$) for yohimbine and BRL 44408, respectively.

The three other antagonists were very weak. BRL 41992 $1 \mu\text{M}$ caused only a small shift to the right (Figure 3), corresponding to a K_D value of 630 nM ($pA_2 = 6.2$) when calculated from equation 4 of Furchgott (1972). No shift at all was

Figure 1 Interaction between clonidine and yohimbine on the evoked overflow of tritium. Brain slices were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). Yohimbine, when used, was present throughout superfusion at a fixed concentration. Clonidine was added before S_2 , S_3 and S_4 at increasing concentrations. Abscissa scale, concentration of clonidine. Ordinate scale, percentage inhibition caused by clonidine, calculated from S_n/S_1 overflow ratios. Clonidine alone ($\circ$); in the presence of yohimbine 30 nM ($\blacktriangle$); 100 nM ($\square$); 300 nM ($\bullet$) and $1 \mu\text{M}$ (Δ). Means of 3–11 (clonidine alone 7–17) brain slices; vertical lines show s.e.mean. pA_2 of yohimbine: 7.84.

Figure 2 Interaction between clonidine and BRL 44408 on the evoked overflow of tritium. Brain slices were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). BRL 44408, when used, was present throughout superfusion at a fixed concentration. Clonidine was added before S_2 , S_3 and S_4 at increasing concentrations. Abscissa scale, concentration of clonidine. Ordinate scale, percentage inhibition caused by clonidine, calculated from S_n/S_1 overflow ratios. Clonidine alone ($\circ$); in the presence of BRL 44408 30 nM ($\blacktriangle$); 100 nM ($\square$); 300 nM ($\bullet$) and $1 \mu\text{M}$ (Δ). Means of 4–10 (clonidine alone 7–17) brain slices; vertical lines show s.e.mean. pA_2 of BRL 44408: 7.82.

Figure 3 Interaction between clonidine and BRL 41992 on the evoked overflow of tritium. Brain slices were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). BRL 41992, when used, was present throughout superfusion at a fixed concentration. Clonidine was added before S_2 , S_3 and S_4 at increasing concentrations. Abscissa scale, concentration of clonidine. Ordinate scale, percentage inhibition caused by clonidine, calculated from S_n/S_1 overflow ratios. Clonidine alone ($\circ$); in the presence of BRL 41992 $1 \mu\text{M}$ ($\square$). Means of 3–10 (clonidine alone 7–17) brain slices; vertical lines show s.e.mean. pA_2 for BRL 41992: 6.2.

Figure 4 Interaction between clonidine and prazosin on the evoked overflow of tritium. Brain slices were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). Prazosin, when used, was present throughout superfusion at a fixed concentration. Clonidine was added before S_2 , S_3 and S_4 at increasing concentrations. Abscissa scale, concentration of clonidine. Ordinate scale, percentage inhibition caused by clonidine, calculated from S_n/S_1 overflow ratios. Clonidine alone ($\circ$); in the presence of prazosin $1 \mu\text{M}$ ($\square$). Means of 3–8 (clonidine alone 7–17) brain slices; vertical lines show s.e.mean. pA_2 for prazosin: < 6 .

Figure 5 Interaction between clonidine and AR-C 239 on the evoked overflow of tritium. Brain slices were stimulated four times with 4 pulses/100 Hz (S_1 – S_4). AR-C 239, when used, was present throughout superfusion at a fixed concentration. Clonidine was added before S_2 , S_3 and S_4 at increasing concentrations. Abscissa scale, concentration of clonidine. Ordinate scale, percent inhibition caused by clonidine, calculated from S_n/S_1 overflow ratios. Clonidine alone (○); in the presence of AR-C 239 100 nM (△); 1 μ M (□). (○) and (□) groups are hard to distinguish because values were almost identical. Means of 4–15 (clonidine alone 7–17) brain slices; vertical lines show s.e.mean. pA_2 for AR-C 239: <6.

detectable in the presence of prazosin 1 μ M (Figure 4) and AR-C 239 0.1 and 1 μ M (Figure 5).

An evaluation of b_n/b_1 ratios indicated that the basal outflow of tritium was not changed when clonidine was added before S_2 , S_3 and S_4 (not shown).

Discussion

The pulse trains used in the present experiments are too short (30 ms train duration) for the development of autoinhibition and, hence, permit the determination of undistorted agonist EC_{50} and antagonist K_D (pA_2) values (see Starke, 1987; Singer, 1988). The lack of any release-enhancing effect of yohimbine in the present study (Table 1) confirms the absence of α_2 -adrenoceptor-mediated autoinhibition.

Prazosin, in contrast to yohimbine, increased the evoked overflow of tritium (Table 1), an increase that has been observed previously in rabbit brain cortex slices (Reichenbacher *et al.*, 1982). Since there was no autoinhibition, the effect of prazosin was not due to antagonism at autoreceptors. The observation emphasizes that caution is necessary when one interprets effects of prazosin on the release of noradrenaline. Prazosin possesses reserpine-like properties (Commarato *et al.*, 1978; Hedler *et al.*, 1981) which sometimes become manifest at low concentrations (10 nM; Bradley & Doggrell, 1983). A reserpine-like mechanism probably also underlies the acceleration of basal tritium efflux observed in the present experiments. It may be due to this property that prazosin enhances the electrically evoked release of noradrenaline (Cubeddu & Weiner, 1975), rather than to any blockade of prazosin-sensitive α_{2B} - (or α_1 -) adrenoceptors. We do not know why BRL 44408 and BRL 41992 reduced the evoked overflow of tritium. One possibility is a partial agonist action at α_2 -autoreceptors as has been observed for the presumed α -adrenoceptor antagonists phentolamine and idazoxan in several tissues (Limberger & Starke, 1983; Angus & Lew, 1984; Ennis & Lattimer, 1984) including rabbit brain cortex (Heepe & Starke, 1985).

Besides freedom from autoinhibition, our experimental model fulfils other prerequisites for the determination of K_D values (Limberger *et al.*, 1989): the baseline conditions are stable (see also Table 2) and the clonidine exposure time suffices for steady-state. Since the antagonism by yohimbine and BRL 44408 was surmountable (Figures 1 and 2) and the Q parameter (Waud, 1975), which corresponds to the slope of

the Schild plot, close to unity, our K_D values of these two drugs are correct estimates of the antagonist-autoreceptor dissociation constant. The K_D for BRL 41992, of which only one effective concentration could be tested, is less reliable. Pronounced increases in the basal efflux of tritium made higher concentrations of prazosin, AR-C 239 and BRL 41992 unsuitable for autoreceptor characterization.

In this and our previous study (Limberger *et al.*, 1989) we have measured the following antagonist K_D values at rabbit brain cortex α_2 -autoreceptors, with clonidine as agonist (in nM): rauwolscine 5, yohimbine 11 (mean of previous and present study), BRL 44408 15, idazoxan 51, BRL 41992 630, prazosin = AR-C 239 > 1,000. This order of affinities agrees with the order found at α_{2A} but not at α_{2B} binding sites (Bylund *et al.*, 1988; for the BRL compounds, Young *et al.*, 1989). For instance, at the autoreceptors, as at α_{2A} binding sites, prazosin and AR-C 239 possess much lower affinity than yohimbine and idazoxan, whereas at α_{2B} sites the affinity of prazosin is only 5 times lower than that of yohimbine and as high as that of idazoxan, and the affinity of AR-C 239 even surpasses that of idazoxan (Bylund *et al.*, 1988). Furthermore, at the autoreceptors the affinity ratio BRL 44408/BRL 41992 is 42 and at α_{2A} binding sites it is 61, whereas at α_{2B} sites it is 0.008 (Young *et al.*, 1989). Clearly, in terms of the proposed $\alpha_{2A/B}$ dichotomy, the presynaptic autoreceptors of rabbit brain cortex are α_{2A} . In this they agree with the α_{2A} -autoreceptors of rat brain cortex (Gobbi *et al.*, 1990), despite species differences between rabbit and rat α_2 -autoreceptors which are shown by other antagonists (see Introduction for references).

Additional α_2 binding sites have been suggested recently. For instance, in the rat submaxillary gland, [3H]-rauwolscine labelled a site distinct from α_{2A} and α_{2B} (Michel *et al.*, 1989). However, rauwolscine had 3 times less affinity for this site than idazoxan, whereas for the rabbit brain cortex autoreceptors it had a 10 times higher affinity than idazoxan. Both the ' α_{2C} ' site in an opossum kidney-derived cell line (Bylund, 1988) and the human ' α_2C2 ' site recently cloned (Lomasney *et al.*, 1990) are too sensitive to prazosin to correspond to the autoreceptors. The most likely binding equivalent of the autoreceptors is, in fact, the α_{2A} site.

There are several reasons for choosing clonidine as the agonist. First, in previous experiments we found no difference in the K_D values of antagonists when tested against clonidine, noradrenaline and α -methylnoradrenaline (Limberger *et al.*, 1989). Secondly, noradrenaline and other catecholamines displace [3H]-noradrenaline from the tissue despite the presence of desipramine, when high concentrations have to be tested against antagonists. Thirdly, clonidine has about equal α_{2A} and α_{2B} binding affinity or is slightly α_{2B} -selective (see Bylund *et al.*, 1988; Michel *et al.*, 1989) whereas 5-bromo-6-(2-imidazolyl-2-ylamino)-quinoxaline (UK 14304), which is more selective than clonidine for α_2 -receptors in general (as opposed to α_1), prefers the α_{2A} subtype. We were particularly interested in finding an α_{2B} -adrenoceptor.

There may be presynaptic α_{2B} -autoreceptors at the noradrenaline-releasing neurones of other tissues such as rat submandibular gland (see Bylund, 1988) and rat atria (Connaughton & Docherty, 1990). It should be noted, however, that rat atria were previously postulated to possess a mixture of presynaptic α_2 - and α_1 -autoreceptors (see Docherty, 1989), and the relationship between the two suggestions ($\alpha_2 + \alpha_1$ versus α_{2B}) has not been clarified.

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