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Investigation of the subtype of α_2-adrenoceptor mediating pressor responses in the pithed rat

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Abstract

We have investigated the subtype of α_2-adrenoceptor mediating postjunctional pressor responses in the pithed rat in comparison with α_2-adrenoceptor ligand binding sites. In pithed rats, postjunctional α_2-adrenoceptors were investigated in terms of the ability of α_2-adrenoceptor antagonists to shift the pressor potency of the α_2-adrenoceptor agonist xylazine. Antagonist potency at postjunctional α_2-adrenoceptors in the pithed rat was correlated with antagonist affinity at α_2-adrenoceptor ligand binding sites in membranes of rat kidney (α_{2B}), Sf9 cells expressing human recombinant receptors (α_{2C}) and rat submandibular gland (α_{2D}) labelled with [^{3}H]yohimbine. The correlation with the postjunctional α_2-adrenoceptor mediating pressor responses in the pithed rat was better for the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland ($r = 0.95$, $n = 9$, $P < 0.0001$) and the α_{2B}-adrenoceptor ligand binding site of rat kidney ($r = 0.90$, $n = 9$, $P < 0.001$) than with the human recombinant α_{2C}-adrenoceptor ligand binding site ($r = 0.81$, $n = 9$, $P < 0.01$). When the pressor potencies of three additional antagonists were included in the correlations for α_{2B}- and α_{2D}-sites only, the correlation with α_{2D}-adrenoceptor ligand binding site of rat submandibular gland ($r = 0.91$, $n = 12$, $P < 0.0001$) was much better than with the α_{2B}-adrenoceptor ligand binding site of rat kidney ($r = 0.77$, $n = 12$, $P < 0.01$). It is concluded that the functional postjunctional α_2-adrenoceptors mediating pressor responses in the pithed rat most closely resemble the α_{2D}-adrenoceptors subtype.

Keywords: α_2-Adrenoceptor, postjunctional; α_{2D}-Adrenoceptor; (Pithed rat)

1. Introduction

α_2-Adrenoceptors have been subdivided into three subtypes, α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors, based on ligand binding and molecular cloning studies (Lorenz et al., 1990;Bylund, 1992; Bylund et al., 1992). The α_{2D}-adrenoceptor is the rat homologue of the human α_{2A}-adrenoceptor (Lanier et al., 1991; Harrison et al., 1991; Smith and Docherty, 1992), so that the term $\alpha_{2A/D}$-adrenoceptor could be used to describe these homologues.

We have previously investigated functional prejunctional α_2-adrenoceptors of cardiac noradrenergic nerves in the pithed rat preparation and found that they resemble the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland (Smith et al., 1995). In this study, we have examined the functional postjunctional α_2-adrenoceptor mediating pressor responses in the pithed rat (see Drew and Whiting, 1979; Docherty et al., 1979) in relation to ligand binding sites in rat kidney (α_{2B}-adrenoceptor: see Michel et al., 1989; Smith et al., 1992a), Sf9 cells expressing human recombinant α_{2C}-adrenoceptors (see Eason and Liggett, 1993) and rat submandibular gland (α_{2D}-adrenoceptor: see Bylund, 1992).

2. Materials and methods

Male wistar rats (250–350 g) were obtained from Trinity College Dublin and employed in a number of studies, as outlined below.

2.1. Pithed rat preparation

Rats were pithed by the method of Gillespie et al. (1970) and ventilated with 100% O_2 at a rate of 60 per min. Heart rate was derived from carotid arterial pressure and drugs were injected into the jugular vein.

Dose-response curves to the α_2-adrenoceptor agonists xylazine and oxymetazoline were constructed from the effects of increasing doses (1 log unit increments) adminis-

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tered at 5 min intervals. Antagonist drugs, or vehicle, were administered intravenously 10 min before starting the agonist dose-response curve. Potency of xylazine was expressed as an ED_{50} (dose causing 50% of the maximum pressor response to xylazine: a 50% rise in diastolic blood pressure was approximately 50 mmHg) and the effects of antagonists were assessed from the difference between agonist potency in the individual antagonist experiment and the mean agonist potency in vehicle experiments. In many experiments, antagonists produced marked shifts in potency of xylazine so that a maximum response was not obtained: in these cases xylazine ED_{50} was taken as a rise in diastolic blood pressure of 50 mmHg. Shifts in agonist potency were expressed as log $(DR-1)$, where DR is the xylazine dose ratio (difference in potency of xylazine between vehicle experiments and experiments in the presence of antagonist), and mean values ($\pm$S.E.M.) were obtained from the effects of the antagonist in n experiments.

Potency of oxymetazoline was expressed as an ED_{50} (dose causing 50% of the maximum pressor response: a 50% rise in diastolic blood pressure was approximately 60 mmHg for oxymetazoline in vehicle experiments, but approximately 40 mmHg following α_1-adrenoceptor antagonism).

Antagonist dose administered was expressed in mol kg^{-1} to allow direct comparison between agents of widely differing molecular weights. Antagonist potency was expressed as an apparent pA_2 ($-\log$ mol kg^{-1}) from the calculation: $-\log$ antagonist dose (mol kg^{-1}) + log(DR − 1). Expression of antagonist potency in this form allowed direct comparison with ligand binding data.

2.2. Radioligand binding studies

Preparation of rat kidney membranes was carried out as described in Connaughton and Docherty (1990), and preparation of rat submandibular gland membranes were as described for rat kidney. Membranes of Sf9 cells expressing human recombinant α_{2C}-adrenoceptors were purchased from Research Biochemicals. The resultant pellets were used immediately or stored at −20°C for later use. Pellets were reconstituted in 5 volumes (submandibular), 10 volumes (kidney) or 25 volumes (Sf9 cells) of incubation buffer.

In saturation experiments, aliquots of membrane suspension were incubated with various concentrations of [^{3}H]yohimbine (specific activity: 81 Ci $mmol^{-1}$, Amersham) at 25°C (rat kidney: 0.5–30 nM; Sf9 cells: 0.2–20 nM; rat submandibular gland: 1.0–40 nM; incubation buffer: Tris-HCl 50 mM, EDTA 5 mM, pH 7.4 at 25°C). In competition studies, [^{3}H]yohimbine (5 or 10 nM) was incubated with competing ligands in concentrations from 0.1 nM to 1 mM in 0.5 log unit increments for 30 min. Non-specific binding was determined in the presence of phentolamine (10 μM). Specific binding of [^{3}H]yohimbine was 70–90% of total binding at the concentration used in displacement experiments. Assays were terminated by washing with ice-cold incubation buffer, followed by rapid vacuum filtration through Whatman GF/C filters, using a Brandel Cell Harvester. Radioactivity retained on filters was determined by liquid scintillation spectroscopy.

The inhibition constant (K_i) for inhibition of radiolabelled ligand binding was determined from the formula:

$$K_i = IC_{50}/\left(1 + [^3H]/K_d\right)$$

where IC_{50} is the concentration of competing ligand that inhibits radioligand specific binding by 50%, K_d is the dissociation constant for the radioligand (rat kidney: 8.80 $\pm$ 0.62 nM, $n = 7$; human recombinant α_{2C}-adrenoceptors: 8.7 $\pm$ 3.5 nM, $n = 3$; rat submandibular gland: 23.7 $\pm$ 2.0 nM, $n = 4$), and ^{3}H is the concentration of tritiated yohimbine employed (5 nM: rat kidney; 10 nM: Sf9 cells and rat submandibular gland).

2.3. Statistical evaluation

In functional studies, values are expressed as agonist ED_{50}, antagonist log $(DR-1)$ and antagonist pA_2. Results are expressed as means and 95% confidence limits or means $\pm$ S.E.M. Effects of antagonist on xylazine ED_{50} were compared with effects of vehicle by Student's t-test for unpaired data and Analysis of Variance (Instat program for MacIntosh, Graphpad Software). Correlation analysis was carried out on an Apple MacIntosh using the Statworks and Cricketgraph programs (Cricket Software). Ligand binding analysis and calculation of ED_{50} values was carried out using GraphPad Prism software for PC.

2.4. Drugs

The following drugs were employed: amidephrine mesylate (gift from Bristol Myers, USA); ARC 239 (2-(2,4-(*o*-methoxyphenyl)-piperazin-1-yl)-ethyl-4,4 dimethyl-1,3-(2*H*,4*H*)-isoquinolindine chloride; gift from Karl Thomae, Biberach, Germany); BDF 8933 (4-fluoro-2-(imidazolin-2-ylamino)-isindoline maleate, gift from Beiersdorf, Hamburg, Germany), benoxathian hydrochloride (Research Biochemicals, Natick, USA); BRL 44408 (2-((4,5-dihydro-1*H*-imidazol-2-yl) methyl)-2,3-dihydro-1-methyl-1*H*-isoindole; gift from Smith Kline Beecham, UK); chlorpromazine hydrochloride (Sigma, Poole, UK), HV 723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate; gift from Hokurika, Seiyaku, Katsuyama, UK); oxymetazoline hydrochloride (Sigma); phentolamine hydrochloride (Sigma); prazosin hydrochloride (gift from Pfizer); rauwolscine hydrochloride (Roth, Karlsruhe, Germany); SKF 104078 (6-chloro-9-[(3-methyl-2-butenyl)-oxyl]-3-methyl-1*H*-2,3,4,5-tetrahydro-3-benzazepine; gift from Smith Kline Beecham, USA); xylazine hydrochloride (gift from Bayer, Ireland);

WB 4101 hydrochloride (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxan hydrochloride: RBI); yohimbine hydrochloride (Sigma).

3. Results

3.1. Pithed rat preparation

In pithed rats, resting blood pressure was 68.5 ± 1.5/35.6 ± 0.9 mmHg ($n = 63$), and resting heart rate was 261 ± 4 beats min^{-1}. Most antagonist drugs caused only a transient effect on diastolic blood pressure. However, chlorpromazine (5 mg kg^{-1}), BDF 8933 (0.1 mg kg^{-1}) and SKF 104078 (5 mg kg^{-1}) raised diastolic blood pressure by 18.7 ± 3.2 mmHg ($n = 4$), 10.0 ± 0.4 mmHg ($n = 4$) and 7.6 ± 1.9 mmHg ($n = 8$), respectively (response measured 5 min after injection).

In vehicle experiments, xylazine produced pressor responses with a potency ($-\log ED_{50}$: mol kg^{-1}) of 6.11 ± 0.08 ($n = 16$) (200 μg kg^{-1}). Responses to xylazine obtained in vehicle experiments were compared with responses to xylazine obtained in experiments in the presence of antagonist, taking as examples ARC 239 (5 mg kg^{-1}), HV 723 (2 mg kg^{-1}) and BDF 8933 (0.1 mg kg^{-1}) (Fig. 1). Xylazine potency in the presence of antagonist dose, log (DR − 1) and apparent antagonist pA_2 values obtained are shown in Table 1. All antagonists in the doses employed, except for prazosin (1 mg kg^{-1}) and chlorpromazine (5 mg kg^{-1}), significantly altered the potency of xylazine (Student's *t*-test, $P < 0.05$; Analysis of Variance, $P < 0.0001$) (Table 1). Higher concentrations of prazosin (5 mg kg^{-1}) and chlorpromazine (10 mg kg^{-1}) proved difficult to dissolve except in dimethylsulphoxide (DMSO), and, when administered in DMSO, decreased resting blood pressure and virtually abolished pressor responses to xylazine. Hence, for prazosin (1 mg kg^{-1}) and chlorpromazine (5 mg kg^{-1}) the pA_2 values are shown as the concentration added and are therefore an overestimate of the potency of these agents.

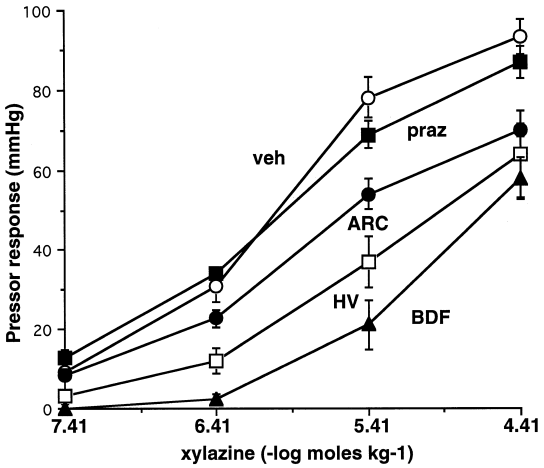

Fig. 1. Pressor responses (DBP) to the α_2-adrenoceptor agonist xylazine in the pithed rat preparation in the presence of antagonist or vehicle. Xylazine (10 μg kg^{-1}) equates with 7.41 ($-\log$ mol kg^{-1}). Symbols: vehicle (○), prazosin (1 mg kg^{-1}) (■), ARC 239 (5 mg kg^{-1}) (●), HV 723 (2 mg kg^{-1}) (□), BDF 8933 (0.1 mg kg^{-1}) (▲). Values (expressed as percentages of control response) are mean ± S.E.M. from at least 3 experiments.

3.1.1. Pressor responses to amidephrine, oxymetazoline and xylazine following α_1-adrenoceptor blockade

Prazosin and ARC 239 (both 100 μg kg^{-1}) produced a significant shift in the pressor potency of the α_1-adrenoceptor agonist amidephrine (Fig. 2), giving apparent pA_2 values for prazosin and ARC 239 at α_1-adrenoceptors of 8.53 ± 0.05 ($n = 4$) and 7.78 ± 0.25 ($n = 4$), respectively.

Potencies of xylazine and oxymetazoline following prazosin (1 mg kg^{-1}) and ARC 239 (5 mg kg^{-1}) (see Fig. 1 and Fig. 3) were used as estimates of pressor potency of

Table 1
Effects of vehicle and antagonists on the postjunctional potency of xylazine in the pithed rat preparation

Test agent	Xylazine ED_{50} $-\log$ (mol kg^{-1})	log (DR − 1)	Antagonist pA_2 $-\log$ (mol kg^{-1})
Vehicle ($n = 16$)	6.11 ± 0.17	–	–
Chlorpromazine (5 mg kg^{-1}) ($n = 4$)	5.9 ± 0.05	–	< 4.85
BDF 8933 (0.1 mg kg^{-1}) ($n = 7$)	4.84 ± 0.33 *	1.28 ± 0.14	7.80
Prazosin (1 mg kg^{-1}) ($n = 6$)	6.01 ± 0.09	–	< 5.62
ARC 239 (5 mg kg^{-1}) ($n = 6$)	5.66 ± 0.28 *	0.29 ± 0.14	5.24
Yohimbine (1 mg kg^{-1}) ($n = 5$)	5.20 ± 0.51 *	0.82 ± 0.22	6.41
Benoxathian (5 mg kg^{-1}) ($n = 4$)	5.63 ± 0.32 *	0.32 ± 0.12	5.22
HV 723 (2 mg kg^{-1}) ($n = 3$)	5.36 ± 0.31 *	0.66 ± 0.09	6.15
WB 4101 (2 mg kg^{-1}) ($n = 4$)	5.21 ± 0.44 *	0.72 ± 0.23	6.00
Rauwolscine (1 mg kg^{-1}) ($n = 3$)	5.12 ± 0.42 *	0.92 ± 0.15	6.51
Phentolamine (1 mg kg^{-1}) ($n = 5$)	4.76 ± 0.25 *	1.30 ± 0.09	6.88
SKF 104078 (5 mg kg^{-1}) ($n = 6$)	5.61 ± 0.25 *	0.33 ± 0.12	5.23
BRL 44408 (1 mg kg^{-1}) ($n = 3$)	5.13 ± 0.10 *	0.87 ± 0.07	6.26

Values are xylazine ED_{50} and 95% confidence limits, antagonist log (DR − 1) and S.E.M., and antagonist pA_2. Antagonist pA_2 was calculated as the sum of $-\log$ antagonist dose (mol kg^{-1}) and log (DR − 1), and so has the same S.E.M. as the log (DR − 1) column. Antagonist dose ($-\log$ mol kg^{-1}) can be obtained by subtracting log (DR − 1) from antagonist pA_2. Asterisks denote significant shifts in xylazine potency (* $P < 0.05$, Analysis of Variance and Dunnett's Multiple Comparison Test).

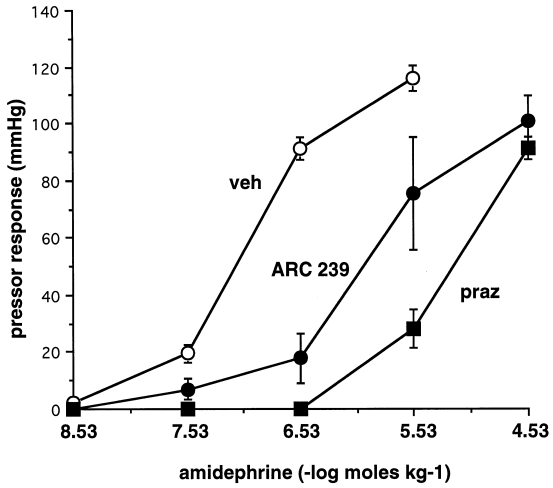

Fig. 2. Pressor responses (DBP) to the α_1-adrenoceptor agonist amidephrine in the pithed rat preparation in the presence of antagonist or vehicle. Amidephrine (1 μg kg^{-1}) equates with 8.53 (−log mol kg^{-1}). Symbols: vehicle (○), prazosin (0.1 mg kg^{-1}) (■), ARC 239 (0.1 mg kg^{-1}) (●). Values (expressed as percentages of control response) are mean ± S.E.M. from at least 3 experiments.

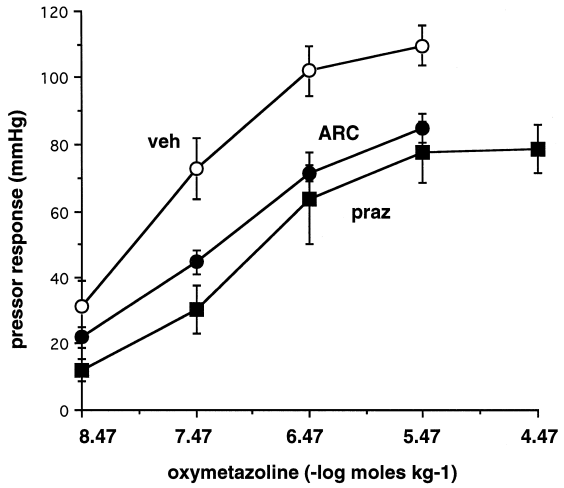

Fig. 3. Pressor responses (DBP) to the α_2-adrenoceptor agonist oxymetazoline in the pithed rat preparation in the presence of antagonist or vehicle. Oxymetazoline (1 μg kg^{-1}) equates with 8.47 (−log mol kg^{-1}). Symbols: vehicle (○), prazosin (1 mg kg^{-1}) (■), ARC 239 (5 mg kg^{-1}) (●). Values (expressed as percentages of control response) are mean ± S.E.M. from at least 3 experiments.

agonists at α_2-adrenoceptors without influence of α_1-adrenoceptors. Oxymetazoline was between 11 (following prazosin) and 87 (following ARC 239) times more potent than xylazine. However, since ARC 239, and perhaps to a lesser extent prazosin, also block α_2-adrenoceptors in the doses employed, the ED_{50} values obtained for oxymetazoline and xylazine following α_1-adrenoceptor blockade reflect relative rather than absolute potency at α_2-adrenoceptors.

3.2. Radioligand binding studies

K_i values for the inhibition by ligands of [^{3}H]yohimbine binding to rat kidney, Sf9 cells expressing human recombinant α_{2C}-adrenoceptors and rat submandibular gland membranes were obtained (see Table 2). Hill slopes for all ligands in all 3 tissues were close to unity, so that it was assumed that a single homogeneous population of ligand binding sites was present in all 3 tissues. Nine antagonists were examined at all 3 ligand binding sites, but a further 3 antagonists (phentolamine, SKF 104078 and BRL 44408) were not examined at human recombinant α_{2C}-adrenoceptors due to the cost of these membranes and to the lack of availability of the latter 2 antagonists. The affinities of the agonists xylazine and oxymetazoline are also shown (Table 2).

Table 2
Affinities (pK_i, −log M) of test agents at the α_2-adrenoceptor ligand binding sites in rat kidney (α_{2B}), human recombinant receptors (α_{2C}) and rat submandibular gland (α_{2D})

Test agent	Rat kidney 2B [+]	Human 2C	Rat submand 2D [+]
1. Chlorpromazine	6.29 ± 0.03 (3)	6.06 ± 0.13 (4)	5.26 ± 0.07 (4)
2. BDF 8933	8.91 ± 0.14 (3)	8.30 ± 0.12 (3)	8.81 ± 0.20 (3)
3. Prazosin	7.12 ± 0.04 (3)	6.92 ± 0.11 (3)	6.24 ± 0.12 (4)
4. ARC 239	7.06 ± 0.16 (7)	6.95 ± 0.10 (3)	5.54 ± 0.13 (4)
5. Yohimbine	7.93 ± 0.02 (4)	8.04 ± 0.17 (3)	7.34 ± 0.06 (5)
6. Benoxathian	5.82 ± 0.15 (4)	7.10 ± 0.12 (3)	5.81 ± 0.05 (4)
7. HV 723	7.13 ± 0.20 (4)	7.97 ± 0.17 (3)	6.24 ± 0.06 (4)
8. WB 4101	6.94 ± 0.03 (4)	8.39 ± 0.17 (3)	6.58 ± 0.10 (3)
9. Rauwolscine	8.39 ± 0.27 (3)	8.47 ± 0.18 (3)	8.04 ± 0.10 (4)
10. Phentolamine	7.24 ± 0.12 (3)	–	7.31 ± 0.18 (3)
11. SKF 104078	6.25 ± 0.06 (3)	–	6.62 ± 0.08 (3)
12. BRL 44408	6.11 ± 0.10 (4)	–	7.77 ± 0.16 (4)
Oxymetazoline	6.34 ± 0.09 (4)	6.77 ± 0.02 (3)	7.74 ± 0.14 (7)
Xylazine	5.98 ± 0.06 (3)	–	5.70 ± 0.09 (4)

Values are mean and S.E.M. with the number of experiments in parentheses. The numbers in the left hand column identify the test agents in Figs. 4–6.
[+] Values for ligand affinities at α_{2B} (except for rauwolscine, oxymetazoline and xylazine) and α_{2D}-sites (except for rauwolscine, phentolamine, SKF 104078, BRL 44408, oxymetazoline and xylazine) taken from Smith et al. (1992a,b, 1995).

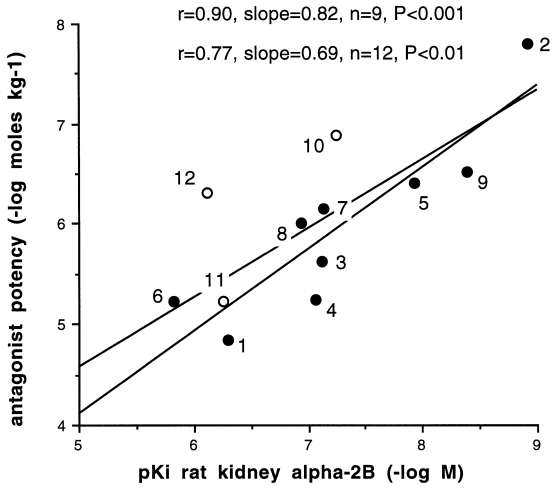

Fig. 4. Correlation between antagonist K_i ($-\log$ M) obtained at α_{2B}-adrenoceptor ligand binding sites in rat kidney membranes and the antagonist pA_2 obtained against xylazine for 9 antagonists in the pithed rat ($r = 0.90$, $n = 9$, $P < 0.001$). An additional 3 antagonists (phentolamine, SKF 104078 and BRL 44408) are indicated by the open circles ($r = 0.77$, $n = 12$, $P < 0.01$). For key to numbers, see Table 2.

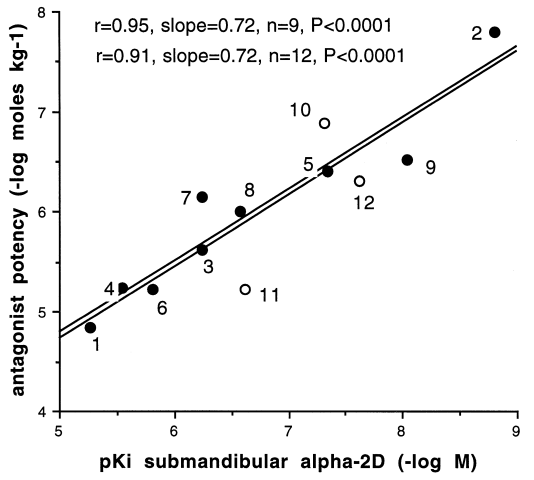

Fig. 6. Correlation between antagonist pK_i ($-\log$ M) obtained at α_{2D}-adrenoceptor ligand binding sites in rat submandibular gland membranes and the antagonist pA_2 obtained against xylazine in the pithed rat ($r = 0.95$, $n = 9$, $P < 0.0001$). An additional 3 antagonists (phentolamine, SKF 104078 and BRL 44408) are indicated by the open circles ($r = 0.91$, $n = 12$, $P < 0.0001$). For key to numbers, see Table 2.

3.3. Correlation between ligand binding sites and the functional postjunctional α_2-adrenoceptor in pithed rat

Correlations between α_{2B}-, α_{2C}- and α_{2D}-adrenoceptor ligand binding sites and the functional postjunctional site in the pithed rat are shown in Fig. 4 and Fig. 5 and Fig. 6. The correlation of the functional postjunctional α_2-adrenoceptor of pithed rat was better with the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland ($r = 0.95$, $n = 9$, $P < 0.0001$) (Fig. 6) and the α_{2B}-adrenoceptor ligand binding site of rat kidney ($r = 0.90$, $n = 9$, $P < 0.001$) (Fig. 4) than with the human recombinant α_{2C}-adrenoceptor ligand binding site ($r = 0.81$, $n = 9$, $P < 0.01$) (Fig. 5).

When correlations were carried out including the α_2-adrenoceptor antagonists phentolamine, SKF 104078 and BRL 44408 (data not available for the α_{2C}-adrenoceptor), the correlation with the the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland ($r = 0.91$, $n = 12$, $P < 0.0001$) was much better than the correlation with the α_{2B}-adrenoceptor ligand binding site of rat kidney ($r = 0.77$, $n = 12$, $P < 0.01$).

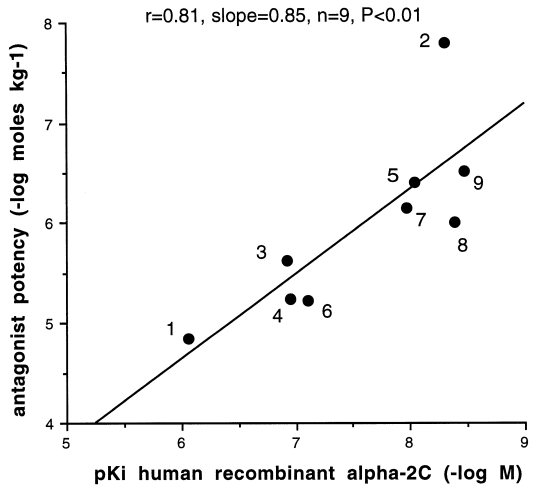

Fig. 5. Correlation between antagonist K_i ($-\log$ M) obtained at α_{2C}-adrenoceptor ligand binding sites in rat cerebral cortex membranes and the antagonist pA_2 obtained against xylazine in the pithed rat ($r = 0.81$, $n = 9$, $P < 0.01$). For key to numbers, see Table 2.

4. Discussion

The main objective of this study was to characterise the subtype of α_2-adrenoceptor found postjunctionally in the vasculature of the pithed rat. Using the pithed rat preparation, antagonist potency can be assessed in terms of the shift in agonist pressor potency (see Docherty and McGrath, 1980). Twelve antagonist drugs were employed in the present study, of which seven (BRL 44408, ARC 239, chlorpromazine, prazosin, benoxathian, HV 723, WB 4101) showed selectivity between subtypes (approximately 10-fold differences in affinity), and doses were chosen which produced a shift in agonist potency. Although xylazine showed selectivity for α_2-adrenoceptors (see Fig. 1), it cannot be ruled out that α_1-adrenoceptors play a role in the effects especially of high doses of xylazine. The correlation between the functional postjunctional receptor in the pithed rat and the ligand binding site of submandibular gland was so close for 9 antagonists ($r = 0.95$) and so significant ($P < 0.0001$) that it might have been taken as virtually certain that these are the same receptor. However, the correlation between the functional postjunctional recep-

tor in the pithed rat and the ligand binding site of rat kidney cortex was also very good for 9 antagonists ($r = 0.90$), leaving the possibility that the α_{2B}-adrenoceptor subtype, or both subtypes are present. The correlation with the α_{2C}-adrenoceptor ligand binding site ($r = 0.81$) was relatively poor. Hence, it was necessary to separate between rat submandibular gland α_{2D}-adrenoceptors and rat kidney α_{2B}-adrenoceptors. This was done by including the potency of the antagonists phentolamine, SKF 104078 and BRL 44408 (the latter showing selectivity for α_{2D}-ligand binding sites: see also Young et al., 1989) in relation to their ligand binding affinity at α_{2B}- and α_{2D}-ligand binding sites. The correlation was clearly better with the submandibular gland α_{2D}-adrenoceptor ($r = 0.91$, $n = 12$, $P < 0.0001$) than the rat kidney α_{2B}-adrenoceptor ($r = 0.77$, $n = 12$, $P < 0.01$). This suggests that the predominant receptor mediating pressor responses in the pithed rat is the α_{2D}-adrenoceptor, although we cannot rule out the possibility that other subtypes, especially the α_{2B}-adrenoceptor, are also involved.

Oxymetazoline has high affinity for α_{2D}-adrenoceptor ligand binding sites (see Table 2) and was 109 times more potent than xylazine at α_{2D}, but only 3 times more potent at α_{2B}. In functional studies, oxymetazoline was between 11 and 87 times more potent than xylazine at producing α_2-adrenoceptor-mediated pressor responses (following α_1-adrenoceptor blockade) in the pithed rat (see Table 2). Hence, the relatively high potency of oxymetazoline may also suggest an involvement of α_{2D}-adrenoceptors in pressor responses in the pithed rat preparation.

We have previously investigated the subtype of α_2-adrenoceptor involved in prejunctional inhibition of cardioacceleration in the pithed rat (Smith et al., 1995), and found that the receptor closely resembles the α_{2D}-adrenoceptor subtype. Hence, it is of interest to compare antagonist potencies at pre- and postjunctional α_2-adrenoceptors in the pithed rat preparation. The correlation between the two responses were $r = 0.97$, $n = 9$, $P < 0.0001$ (for the antagonists yohimbine, prazosin, BDF 8933, phentolamine, HV 723, chlorpromazine, WB 4101, benoxathian and ARC 239) (authors, unpublished observations). Hence, these pre- and postjunctional α_2-adrenoceptors resemble each other and resemble the α_{2D}-adrenoceptor subtype. Interestingly, the agonist xylazine and the nine antagonists tended to have greater absolute potency prejunctionally in the pithed rat heart than postjunctionally in terms of pressor responses: yohimbine, BDF 8933, benoxathian, WB 4101 and phentolamine were approximately 5–10 times more potent prejunctionally (compare present results with Smith et al., 1995). This may simply reflect differences in blood flow and non-equilibrium conditions pertaining in vivo, although we cannot rule out the possibility that these pre- and postjunctional α_{2D}-adrenoceptors differ.

Other authors have examined subtypes of functional pre- and postjunctional α_2-adrenoceptors. Functional prejunctional α_2-adrenoceptors in rat submandibular gland (Limberger et al., 1992), rat cerebral cortex (Trendelenburg et al., 1993) and rat kidney (Bohmann et al., 1993) resemble the α_{2D}-adrenoceptor ligand binding site, whereas those in rabbit cerebral cortex (Trendelenburg et al., 1993), dog mesenteric artery (Daniel et al., 1995) and human cerebral cortex (Raiteri et al., 1992) resemble the α_{2A}-adrenoceptor. Since the α_{2A}-adrenoceptor and α_{2D}-adrenoceptor may be species homologues (see Introduction), this would suggest that the $\alpha_{2A/D}$-adrenoceptor may be the predominant prejunctional α_2-adrenoceptor. In terms of postjunctional α_2-adrenoceptors, there is relatively little evidence available as to subtypes mediating vascular contractions, given the relatively few preparations in which these receptors can be demonstrated. We have previously investigated the postjunctional α_2-adrenoceptor mediating contraction of the human saphenous vein and found that it does not resemble the α_{2A}-adrenoceptor of human platelet, and may be an α_{2B}-adrenoceptor (Smith et al., 1992b), or more likely an α_{2C}-adrenoceptor (authors, unpublished). In the porcine palmer lateral vein and common digital artery a low potency of prazosin suggests the presence of an $\alpha_{2A/D}$-adrenoceptor (Blaylock and Wilson, 1995), and an $\alpha_{2A/D}$-adrenoceptor has also been reported in dog saphenous vein, although interpretation was complicated by the presence of α_1-adrenoceptors so that the presence of an α_{2B}-adrenoceptor could not be ruled out (Hicks et al., 1991). Other studies of α_2-adrenoceptors in the rat tail vasculature did not investigate the subtype involved (Redfern et al., 1995). $\alpha_{2A/D}$-Adrenoceptors have also been reported postjunctionally in rat panceatic islets where they mediate inhibition of insulin secretion (Niddam et al., 1990).

In conclusion, the postjunctional α_2-adrenoceptor in pithed rat mediating the pressor actions of xylazine closely resembles the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland.

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