Investigation of the subtype of α_2 -adrenoceptor mediating prejunctional inhibition of cardioacceleration in the pithed rat heart

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1 We have investigated the subtype of α_2 -adrenoceptor mediating prejunctional inhibition of cardioacceleration in the pithed rat heart in comparison with α_2 -adrenoceptor ligand binding sites.

2 In pithed rats, prejunctional α_2 -adrenoceptors were investigated in terms of the ability of α_2 -adrenoceptor antagonists to shift the inhibitory potency of the α_2 -adrenoceptor agonist, xylazine, against the tachycardia to a single electrical stimulus given via the pithing rod.

3 Antagonist potency at prejunctional α_2 -adrenoceptors in pithed rat heart was correlated with antagonist affinity at α_2 -adrenoceptor ligand binding sites in membranes of rat kidney and submandibular gland labelled with [³H]-yohimbine.

4 The correlation with the prejunctional α_2 -adrenoceptor in pithed rat heart was best for the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland ($r = 0.98$, $n = 10$, $P < 0.0001$), as compared to correlations with the α_{2A} -adrenoceptor ligand binding site of human platelet ($r = 0.90$, $n = 9$, $P < 0.001$), the α_{2B} -adrenoceptor ligand binding site of rat kidney ($r = 0.82$, $n = 10$, $P < 0.01$) and with published results for the α_{2C} -adrenoceptor ligand binding site ($r = 0.48$, $n = 6$, NS).

5 It is concluded that the functional prejunctional α_2 -adrenoceptor of pithed rat heart closely resembles the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland.

Keywords: Prejunctional α_2 -adrenoceptors; α_{2D} -adrenoceptors; pithed rat heart

Introduction

α_2 -Adrenoceptors have been subdivided into at least four subtypes, α_{2A} -, α_{2B} -, α_{2C} - and α_{2D} -adrenoceptors, based on ligand binding and molecular cloning studies (Lorenz *et al.*, 1990; Bylund, 1992), although the α_{2D} -adrenoceptor of rat submandibular gland may be a species homologue of the human α_{2A} -adrenoceptor, reducing the number of genes coding for α_2 -adrenoceptors to three (Lanier *et al.*, 1991; Harrison *et al.*, 1991; Smith & Docherty, 1992), so that the term $\alpha_{2A/D}$ -adrenoceptor could be used to describe these homologues. However, since the functional receptors differ pharmacologically, the term α_{2A} -adrenoceptor is used in this paper to describe the ligand binding site in human platelet and α_{2D} -adrenoceptor to describe the site on rat submandibular gland.

We have previously investigated functional prejunctional α_2 -adrenoceptors of adrenergic nerves in a number of tissues and suggested that the prejunctional α_2 -adrenoceptors in rat vas deferens and rat submandibular gland resemble the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland (Smith & Docherty, 1992), whereas the functional prejunctional α_2 -adrenoceptor of rat atrium differed (Smith *et al.*, 1992).

In this study, we have examined the functional prejunctional α_2 -adrenoceptor in pithed rat heart in relation to ligand binding sites in rat kidney (α_{2B} -adrenoceptor: see Michel *et al.*, 1989; Smith & Docherty, 1992), and rat submandibular gland (α_{2D} -adrenoceptor: see Bylund, 1992). For comparison, ligand binding data for human platelet membranes (α_{2A} -adrenoceptor: see Bylund, 1985; 1992) are included.

Methods

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

Pithed rat preparation

Rats were pithed by the method of Gillespie *et al.* (1970) and ventilated with 100% O₂ at a rate of 60 per min. Heart rate was derived from carotid arterial pressure and drugs were injected into the jugular vein. The pithing rod was used as an electrode positioned at T1 to stimulate the cardio-accelerator nerves with a single stimulus pulse (0.5 ms, supramaximal voltage) every 2 min (see Docherty & Hyland, 1986).

Dose-response curves to the α_2 -adrenoceptor agonists, xylazine, clonidine, UK 14,304 and oxymetazoline were constructed from the effects of increasing doses (10 fold increments) administered at 5 min intervals. Prejunctional effects of agonists were assessed as the inhibition of the cardio-acceleration to a single stimulus. Antagonist drugs, or vehicle, were administered intravenously 10 min before starting the agonist dose-response curve. The potency of xylazine was expressed as an ID₅₀ (dose causing 50% inhibition of the cardio-acceleration to a single stimulus), 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. 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.mean) were obtained from the effects of the antagonist in n experiments.

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 pA₂ ($-\log$ mol kg⁻¹) from the calculation: $-\log$ antagonist dose (mol kg⁻¹) + log(DR – 1). Expression of

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antagonist potency in this form allowed direct comparison with ligand binding data.

In some experiments, cardioaccelerator responses to isoprenaline ($1\text{--}10\text{ ng kg}^{-1}$) were examined before and following exposure to xylazine (1 mg kg^{-1}) or vehicle, to assess postjunctional actions of xylazine, if any.

Radioligand binding studies

Preparation of human platelet and rat kidney membranes was carried out as described in Connaughton & Docherty (1990), and preparation of submandibular gland membranes was as described for rat kidney. The resultant pellets were used immediately or stored at -20°C for later use. Pellets were reconstituted in 2.5 ml (platelet), 5 volumes (submandibular) or 10 volumes (kidney) of incubation buffer.

In saturation experiments, aliquots of membrane suspension were incubated with various concentrations of [^3H]-yohimbine (specific activity: 81 Ci mmol^{-1} , Amersham) at 37°C (human platelet: $0.3\text{--}20\text{ nM}$; incubation buffer: Tris-HCl 50 mM , MgCl_2 8 mM , EGTA 5 mM , pH 7.4 at 37°C) or at 25°C (rat kidney: $0.5\text{--}30\text{ nM}$; rat submandibular gland: $2.0\text{--}40\text{ nM}$; incubation buffer: Tris-HCl 50 mM , EDTA 5 mM , pH 7.4 at 25°C). In competition studies, [^3H]-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 ($10\text{ }\mu\text{M}$). Specific binding of [^3H]-yohimbine was $70\text{--}90\%$ of total binding. 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:

$$K_i = \text{IC}_{50}/(1 + [^3\text{H}]/k_D)$$

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 (human platelet: 5.50 nM , 95% confidence limits $3.80\text{--}7.94\text{ nM}$, $n = 6$; rat kidney: 8.71 nM , 95% confidence limits $7.41\text{--}10.2\text{ nM}$, $n = 7$; rat submandibular gland: 23.4 nM , 95% confidence limits $18.2\text{--}30.2\text{ nM}$, $n = 4$), and ^3H is the concentration of tritiated yohimbine employed (5 nM : platelet and kidney; 10 nM : submandibular gland). B_{max} values obtained in saturation experiments were 115 ± 10 ($n = 6$), 94 ± 13 ($n = 7$) and 146 ± 19 ($n = 4$) fmol mg^{-1} protein in human platelet, rat kidney and rat submandibular gland, respectively.

Statistical evaluation

In functional studies, values are expressed as agonist ID_{50} (dose producing 50% inhibition of the stimulation-evoked tachycardia), antagonist log (DR - 1) (where DR is the xylazine dose-ratio between potency in the presence of antagonist and presence of vehicle), and antagonist pA_2 . Antagonist pA_2 was calculated as the sum of antagonist dose ($-\log\text{ mol kg}^{-1}$) and $\log(\text{DR} - 1)$. Results are expressed as mean and 95% confidence limits or mean $\pm$ s.e. of mean. Effects of antagonist on xylazine ID_{50} were compared with effects of vehicle by Student's t test for unpaired data and Analysis of Variance. Correlation analysis was carried out on an Apple Macintosh using the Statworks and Cricketgraph programmes (Cricket software, Inc.).

Drugs

The following drugs were employed: abanoquil hydrochloride (gift: Pfizer, Sandwich, U.K.); ARC 239 (2-(2,4-(*o*-methoxyphenyl)-piperazin-1-yl)-ethyl-4,4 dimethyl-1,3-(2H,4H)-isoquinolindine chloride; gift: Karl Thomae, Biberach, Germany); BDF 8933 (4-fluoro-2-(imidazolin-2-ylamino)-isi-

indoline maleate, gift: Beiersdorf, Hamburg, Germany), benozathian hydrochloride (Research Biochemicals, Natick, U.S.A.); chlorpromazine hydrochloride (Sigma, Poole, U.K.), HV 723 (α -ethyl-3,4,5-trimethoxy- α -(3-((2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate; gift: Hokurika, Seiyaku, Katsuyama, U.K.); idazoxan hydrochloride (Research Biochemicals); isoprenaline hydrochloride (Sigma); oxymetazoline hydrochloride (Sigma); phentolamine hydrochloride (Sigma); prazosin hydrochloride (gift: Pfizer); spiroxatrine hydrochloride (gift: Janssen, Ireland); UK 14,304 (5-bromo-6-(2-imidazolin-2-yl amino)quinoxaline; gift: Pfizer); WB 4101 (2-(2',6'-dimethoxyphenoxyethyl)amino-methyl-1,4-benzodioxan; Research Biochemicals); xylazine hydrochloride (gift: Bayer, Ireland); yohimbine hydrochloride (Sigma).

Results

Pithed rat preparation

In pithed rat, resting blood pressure was $70.8 \pm 1.2/36.0 \pm 0.7\text{ mmHg}$ ($n = 81$), and resting heart rate was $256 \pm 3\text{ beats min}^{-1}$. Single pulse stimulation produced a cardioacceleration of $23.4 \pm 0.8\text{ beats min}^{-1}$ ($n = 81$). Most antagonist drugs caused a transient inhibition of the stimulation-evoked cardioacceleration but the response had recovered by 10 min (just before beginning the xylazine dose-response curve), except in the case of BDF 8933 (0.1 mg kg^{-1}) which significantly reduced the stimulation-evoked tachycardia to $65.1 \pm 8.4\%$ ($n = 5$) (vehicle: $96.6 \pm 2.8\%$, $n = 9$, $P < 0.01$). Chlorpromazine significantly increased the stimulated-evoked tachycardia to $239 \pm 26\%$ ($n = 6$) ($P < 0.001$), and the response following chlorpromazine was prolonged in a manner consistent with blockade of nor-adrenaline re-uptake (see Tuck *et al.*, 1972).

In vehicle experiments, xylazine, clonidine, UK 14,304 and oxymetazoline inhibited the stimulation-evoked tachycardia to a single stimulus with ID_{50} values of $3.80\text{ }\mu\text{g kg}^{-1}$ (95% confidence limits: $3.0\text{--}4.8\text{ }\mu\text{g kg}^{-1}$) ($n = 22$), $0.67\text{ }\mu\text{g kg}^{-1}$ ($0.37\text{--}0.98$) ($n = 6$), $0.57\text{ }\mu\text{g kg}^{-1}$ ($0.39\text{--}0.81$) ($n = 3$) and $0.47\text{ }\mu\text{g kg}^{-1}$ ($0.12\text{--}1.74$) ($n = 5$), respectively. These values are 15.1 , 2.51 , 1.29 and 1.58 nmol kg^{-1} , respectively. In vehicle experiments, in which saline replaced the test agonist, the response to a single stimulus was not significantly altered (control: $24.4 \pm 2.6\text{ beats min}^{-1}$; response following 5 saline injections over approximately 20 min : $25.9 \pm 2.0\text{ beats min}^{-1}$; $n = 8$).

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}), yohimbine (1 mg kg^{-1}) and BDF 8933 (0.1 mg kg^{-1}) (Figure 1). Antagonist doses employed and apparent pA_2 values obtained are shown in Table 1. All antagonists in the doses employed significantly altered the potency of xylazine (Student's t test, $P < 0.05$ or less; Analysis of Variance, $P < 0.0001$) (Table 1).

Xylazine (1 mg kg^{-1}) did not significantly reduce the cardioacceleration produced by isoprenaline. In vehicle experiments, the tachycardia to isoprenaline (3 ng kg^{-1}) was reduced from $36.6 \pm 8.1\text{ beats min}^{-1}$ to $31.6 \pm 6.8\text{ beats min}^{-1}$ ($n = 5$), whereas xylazine (1 mg kg^{-1}) reduced the tachycardia from $32.0 \pm 7.6\text{ beats min}^{-1}$ to $27.8 \pm 7.6\text{ beats min}^{-1}$ ($n = 4$) (no significant differences).

Radioligand binding studies

K_i values for the inhibition by ligands of [^3H]-yohimbine binding to human platelet, rat kidney 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.

Figure 1 Inhibition by the α_2 -adrenoceptor agonists, xylazine, of the tachycardia evoked by a single stimulus in the pithed rat preparation in the presence of antagonist or vehicle. Symbols: vehicle (○), ARC 239 (5 mg kg⁻¹) (●), HV 723 (2 mg kg⁻¹) (□), yohimbine (1 mg kg⁻¹) (▲). Values (expressed as % of control responses) are mean $\pm$ s.e. of mean from at least 4 experiments.

Figure 2 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 in the pithed rat heart. $r = 0.82$, $n = 10$, $P < 0.01$.

Correlation between ligand binding sites and the functional prejunctional α_2 -adrenoceptor in pithed rat heart

Correlations between α_{2B} - and α_{2D} -adrenoceptor ligand binding sites and the functional site in the pithed rat heart are shown in Figures 2 and 3. The correlation of the functional prejunctional α_2 -adrenoceptor of pithed rat heart was better with the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland ($r = 0.98$, $n = 10$, $P < 0.0001$) (Figure 3) than with the α_{2B} -adrenoceptor ligand binding site of rat kidney ($r = 0.82$, $n = 10$, $P < 0.01$) (Figure 2) or with the α_{2A} -adrenoceptor ligand binding site of human platelet ($r = 0.90$, $n = 9$, $P < 0.001$) (Figure 4).

The correlation between published ligand binding data for the α_{2C} -adrenoceptor ligand binding sites and functional site in pithed rat heart was also examined. The correlation between the α_{2C} -site (human α_2 -C4 gene expressed in COS-7 cells: Bylund *et al.*, 1992) and the functional receptor of the pithed rat heart was $r = 0.48$ (non-significant; $n = 6$ antagonists, excluding abanoquil, benoxathian, HV 723 and BDF 8933) (Figure 5).

Table 2 Affinities (pK_i , $-\log M$) of test agents at the α_2 -adrenoceptor ligand binding sites human platelet (α_{2A}), rat kidney (α_{2B}) and rat submandibular gland (α_{2D})

Test agent	Platelet	Kidney	Submand.
Chlorpromazine	6.48 $\pm$ 0.05	6.29 $\pm$ 0.03	5.26 $\pm$ 0.07
WB 4101	7.08 $\pm$ 0.22	6.94 $\pm$ 0.03	6.58 $\pm$ 0.10
BDF 8933	8.72 $\pm$ 0.06	8.91 $\pm$ 0.14	8.81 $\pm$ 0.20
Prazosin	5.62 $\pm$ 0.12	7.12 $\pm$ 0.04	6.24 $\pm$ 0.12
ARC 239	5.45 $\pm$ 0.02	7.06 $\pm$ 0.16	5.54 $\pm$ 0.13
Yohimbine	8.04 $\pm$ 0.10	7.93 $\pm$ 0.02	7.34 $\pm$ 0.06
Phentolamine	7.61 $\pm$ 0.12	7.24 $\pm$ 0.12	7.31 $\pm$ 0.17
Benoxathian	6.85 $\pm$ 0.05	5.82 $\pm$ 0.15	5.81 $\pm$ 0.05
Abanoquil	/	7.18 $\pm$ 0.14	5.98 $\pm$ 0.11
HV 723	6.58 $\pm$ 0.10	7.13 $\pm$ 0.20	6.24 $\pm$ 0.06

Values are mean and s.e.mean from at least 3 experiments.

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

Test agent	Xylazine ID_{50} ($\mu g kg^{-1}$)	$\log(DR - 1)$	$-\log$ antagonist pA_2 ($mol kg^{-1}$)
Vehicle ($n = 22$)	3.80 (3.0–4.8)	/	/
Yohimbine (1 mg kg ⁻¹) ($n = 4$)	302 (240–380)	1.89 $\pm$ 0.08	7.48
Prazosin (1 mg kg ⁻¹) ($n = 4$)	8.91 (6.61–12.0)	0.24 $\pm$ 0.11	5.86
BDF 8933 (0.1 mg kg ⁻¹) ($n = 5$)	589 (389–891)	2.19 $\pm$ 0.18	8.71
Phentolamine (1 mg kg ⁻¹) ($n = 5$)	417 (363–479)	2.04 $\pm$ 0.06	7.62
HV 723 (2 mg kg ⁻¹) ($n = 4$)	60.2 (40.7–89.1)	1.17 $\pm$ 0.18	6.66
Chlorpromazine (5 mg kg ⁻¹) ($n = 3$)	15.1 (12.6–18.2)	0.47 $\pm$ 0.11	5.32
WB 4101 (2 mg kg ⁻¹) ($n = 3$)	58.9 (40.7–85.1)	1.16 $\pm$ 0.17	6.44
Benoxathian (5 mg kg ⁻¹) ($n = 3$)	37.2 (21.4–64.6)	0.94 $\pm$ 0.27	5.84
ARC 239 (5 mg kg ⁻¹) ($n = 7$)	12.9 (9.55–17.4)	0.43 $\pm$ 0.15	5.38
Abanoquil (2 mg kg ⁻¹) ($n = 3$)	43.6 (27.5–69.2)	1.02 $\pm$ 0.06	6.01

Values are xylazine ID_{50} (dose producing 50% inhibition of the stimulation-evoked tachycardia) and 95% confidence limits, antagonist $\log(DR - 1)$ and s.e. of mean, 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. of mean as the $\log(DR - 1)$ column.

Figure 3 Correlation between antagonist K_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 heart. $r = 0.98$, $n = 10$, $P < 0.0001$.

Figure 4 Correlation between antagonist K_i ($-\log M$) obtained at α_{2A} -adrenoceptor ligand binding sites in human platelet membranes and the antagonist pA_2 obtained against xylazine in the pithed rat heart. $r = 0.90$, $n = 9$, $P < 0.001$.

Discussion

The major finding of this study is that the functional prejunctional α_2 -adrenoceptor of pithed rat heart closely resembles the α_{2D} -adrenoceptor ligand binding site in rat submandibular gland. This and other points of interest will be discussed.

The main objective of this study was to characterize the subtype of α_2 -adrenoceptor found prejunctionally in the pithed rat heart. Using the pithed rat preparation, the inhibition by the α_2 -adrenoceptor agonist xylazine of the tachycardia evoked by a single stimulus given via the pithing rod is a useful model of prejunctional α_2 -adrenoceptor function, and antagonist potency can be assessed in terms of the shift in agonist potency (see Docherty & McGrath, 1980). Ten antagonist drugs were employed in the present study, of which three (ARC 239, chlorpromazine, prazosin) showed marked selectivity between subtypes (> 10 fold differences in affinity), and doses were chosen which produced a shift in agonist potency. The correlation between the functional prejunctional receptor in the pithed rat heart and the ligand binding site of submandibular gland was so close ($r = 0.98$) and so significant ($P < 0.0001$) that it is virtually certain that these are the same receptor. The correlation between the functional prejunctional receptor in the pithed rat heart and the ligand binding site of rat kidney cortex was relatively poor ($r = 0.82$), ruling out identity with the α_{2B} -adrenoceptor

Figure 5 Correlation between antagonist K_i ($-\log M$) obtained at α_{2C} -adrenoceptor ligand binding sites (human α_2 -C4 gene expressed in COS-7 cells; Bylund *et al.*, 1992) and the antagonist pA_2 obtained against xylazine in the pithed rat heart. $r = 0.48$, $n = 6$, NS.

subtype. The correlation with the published data for the α_{2C} -adrenoceptor ligand binding site (Bylund *et al.*, 1992) was also relatively poor. Hence the functional prejunctional α_2 -adrenoceptor of pithed rat heart resembles the functional prejunctional α_2 -adrenoceptors of rat vas deferens and submandibular gland in that they all appear to resemble closely the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland (Smith *et al.*, 1992; Smith & Docherty, 1992).

Relative potencies of a series of agonists were also investigated in the pithed rat heart. Clonidine, UK 14,304 and oxymetazoline were approximately equipotent but 10 times more potent than xylazine. This order of potency is similar to that found for these agonists at the functional prejunctional α_2 -adrenoceptor of rat vas deferens, which may be of the α_{2D} -adrenoceptor subtype (Smith & Docherty, 1992).

Other authors have examined subtypes of functional prejunctional α_2 -adrenoceptors. Functional prejunctional α_2 -adrenoceptors in rat submandibular gland (Limberger *et al.*, 1992), rat cerebral cortex (Trendelenberg *et al.*, 1993) and rat kidney (Bohmann *et al.*, 1993) resemble the α_{2D} -adrenoceptor ligand binding site, whereas those in rabbit cerebral cortex (Trendelenberg *et al.*, 1993) and human cerebral cortex (Raiteiri *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.

However, the functional prejunctional α_2 -adrenoceptor in rat isolated atrium does not closely resemble the α_{2D} -adrenoceptor (Smith *et al.*, 1992; Limberger *et al.*, 1992), and this has led to the suggestion that the receptor may resemble the α_{2B} -adrenoceptor ligand binding site of rat kidney cortex (Smith *et al.*, 1992). Indeed, for the 10 antagonists employed in the current study, the correlation between prejunctional potency in rat atrium (authors, unpublished results, see Smith *et al.*, 1992) with the kidney α_{2B} -adrenoceptor ligand binding site ($r = 0.94$) was better than with the rat submandibular α_{2D} -adrenoceptor ligand binding site ($r = 0.89$). This leads to an apparent anomaly that the prejunctional receptor activated by xylazine in the pithed rat heart closely resembles the rat submandibular α_{2D} -adrenoceptor ligand binding site, whereas the prejunctional receptor activated by noradrenaline in rat isolated atrium does not. This may mean that the receptor activated by xylazine to inhibit tachycardia differs from the receptor activated by noradrenaline to inhibit release of transmitter in atria, suggesting two subtypes of prejunctional receptor in atria, although studies *in vitro* may

not examine exclusively nerves involved in control of heart rate. We are currently investigating this problem but have no answer at present.

In conclusion, the prejunctional α_2 -adrenoceptor in pithed rat heart mediating the cardioinhibitory actions of xylazine

closely resembles the α_{2D} -adrenoceptor ligand binding site of rat submandibular gland.

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