Mechanisms underlying the differential sensitivity to α_1 -adrenoceptor activation in the bisected rat vas deferens

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- 1 The factors underlying the different responsiveness of the prostatic and epididymal portions of rat vas deferens to α_1 -adrenoceptor stimulation were investigated.
- 2 The α_1 -adrenoceptors in membranes of both halves of rat vas deferens were labelled with [³H]-prazosin and the affinities of agonists and antagonists for these receptors were determined. In saturation studies, the $B_{\max}$ and K_D values for [³H]-prazosin in membranes of both portions were the same.
- 3 In competition studies, the inhibition curves for phentolamine were biphasic and consistent with the presence of both α_{1a} - and α_{1b} -adrenoceptor subtypes. The proportions of binding sites with high and low affinity for phentolamine in both halves of rat vas deferens were similar and in good agreement with the percentages of binding sites for WB-4101 and phentolamine previously reported in the whole rat vas deferens.
- 4 The phenylethylamines displaced [³H]-prazosin with a shallow inhibition curve. The data are compatible with the assumption of two affinity states for the binding sites. For the imidazoline compounds no such distinct affinity states could be demonstrated.
- 5 The affinity for, and the relative intrinsic efficacy on postsynaptic α_1 -adrenoceptors of both portions of rat vas deferens were studied for noradrenaline, phenylephrine and methoxamine by irreversible inactivation of the α_1 -adrenoceptors by phenoxybenzamine. The parameters for partial agonists were determined by comparing the responses to the partial agonist to those of a full agonist in the same tissue. Homogeneous estimates of the equilibrium dissociation constants (K_a) were obtained, indicating that these agonists bind to the receptors of both tissues in an identical manner. Further, estimation of the intrinsic efficacy of agonists relative to noradrenaline, indicated no differences between the two halves of rat vas deferens.
- 6 K_a values for agonist activation of the functional α_1 -adrenoceptors were compared with K_i values for agonist inhibition of specific [³H]-prazosin binding. The K_a values were well correlated with the low affinity K_i values for phenylethylamines in both portions of rat vas deferens, suggesting that the initial event in signal transduction by α_1 -adrenoceptors is the binding to the low affinity state of the receptor.
- 7 There was a non-linear relationship between response and receptor occupancy in both halves of rat vas deferens but the occupancy-response coupling was more efficient in the epididymal than in the prostatic portion. This fact may account for the differences observed in the functional responses.

Introduction

The rat vas deferens has been commonly considered as a suitable preparation for studying α -adrenoceptors. However, there is considerable evidence to substantiate that prostatic and epididymal portions of this tissue differ not only in their motor transmission (McGrath, 1978; Brown *et al.*, 1983) but in their postsynaptic sensitivity to α -adrenoceptor agonists (Vardolov & Pennefather, 1976; Kasuya & Suzuki, 1979; MacDonald & McGrath, 1980; Moore & Griffiths, 1982). In this regard it has been demonstrated that the imidazoline α_1 -adrenoceptor agonist St-587 behaves both as a partial agonist at α_1 -adrenoceptors in the epididymal half of the rat vas deferens and as an antagonist of prostatic α_1 -adrenoceptors, suggesting that a different efficiency of stimulus-response mechanism is operating along the tissue (Badia & Sallés, 1989). These differences in the sensitivity of each portion of rat vas deferens to α -adrenoceptor activation may therefore depend on factors such as variations in receptor density, changes in the affinity of agonists or to the presence or absence of spare receptors. In addition, recent binding studies reported the existence of two α_1 -adrenoceptor subtypes in the rat vas deferens (Han *et al.*, 1987; Hanft & Gross, 1989), so that heterogeneous distribution of these subtypes may also be involved in the different responsiveness of each portion of the tissue to agonists. In order to elucidate the factors responsible for these functional differences, the

binding parameters for α_1 -adrenoceptors and the contractile responses to different agonists in each half of rat vas deferens were determined. Thus, the equilibrium dissociation constants for agonists were calculated by two independent methods. In addition, it was possible to determine receptor occupancy and to analyze its relationship with response.

Methods

Animals

Adult male Sprague-Dawley rats weighing 250 to 350 g were used for the experiments. Both vasa deferentia were removed, carefully cleaned and bisected into prostatic and epididymal portions. These preparations were used for functional or radioligand binding assays.

Radioligand binding studies

For binding assays, the epididymal and prostatic portions from several animals were pooled separately, placed in 10 ml of ice cold 50 mM Tris/HCl buffer pH 7.5, and homogenized with a polytron type homogenizer (Rafer^{10/20}, setting 6, 2 × 15 s). Homogenates were filtered through a double layer of surgical gauze and centrifuged at 49,000g for 20 min. Pellets were washed twice by resuspension and centrifugation under the same conditions. Final pellets were resuspended in the same buffer at 2 mg protein per ml approximately. For

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saturation experiments aliquots (100 µg protein) of membrane suspensions were incubated in triplicate with increasing concentrations (0.05 to 4 nM) of [³H]-prazosin in 0.25 ml (final volume) of 50 mM Tris buffer pH 7.5 at 25°C for 45 min. The potencies of drugs in competing for the specific [³H]-prazosin binding was determined by incubation of a single concentration of [³H]-prazosin (0.3–0.4 nM) in the presence or absence of 15–20 concentrations of competing drug (range: 0.01 nM–1 mM). Incubations were stopped by rapid filtration through Whatman GF/C glass fibre filters which were subsequently washed 3 times with 5 ml of the same buffer. Radioactivity retained in the filters was determined at 48–50% efficiency by liquid scintillation spectrometry. Nonspecific binding was defined as that insensitive to 10 µM phentolamine. Protein was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Functional studies

For the measurement of contractile responses, prostatic and epididymal halves of rat vas deferens were set up in isolated organ baths containing 20 ml of Krebs bicarbonate solution composition (in mM): NaCl 112.0, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.1, MgSO₄ 1.2, NaHCO₃ 25.0 and glucose 11.1. The solution was maintained at 32 ± 0.5°C and gassed with 95% O₂ and 5% CO₂. Propranolol (1 µM), desmethylinipramine (0.1 µM) and normetanephrine (1 µM) were continuously present in the medium throughout the experiment to block β-adrenoceptors, and neuronal and extraneuronal uptake respectively. The preparations were equilibrated for at least 45 min at a resting tension of 0.5 g. Thereafter, in order to obtain a maximal response the tissues were exposed twice to 100 µM of noradrenaline and thoroughly washed. Contractions were registered by means of an isometric transducer (UF-1) on an OmniScribe pen-recorder.

Agonist-induced increases in isometric tension were determined by use of a non-cumulative dosage schedule with 6 min intervals between doses. Responses were measured as the peak tension developed while the preparation was exposed to the agonist and are expressed as a percentage of the maximal contraction induced by noradrenaline. Thus, noncumulative concentration-response curves of isometric contractions were obtained for the agonists in each tissue and the EC₅₀ (molar concentration of agonist inducing 50% of its maximal effect) was determined by linear regression of all points between 20–80% of the maximal response to that agonist.

To evaluate the antagonist potency of prazosin and phentolamine, cumulative agonist concentration-response curves were constructed in the absence and presence of the test antagonist. The maintained ('tonic') component of the contraction to the agonist was measured. Antagonists were allowed to equilibrate for 30 min before addition of the agonist. α₁-Adrenoceptor antagonist potency was expressed as a pA₂ value which was determined by the method of Arunlakshana & Schild (1959).

Exposure to phenoxybenzamine

After performing the initial non-cumulative concentration-response curve for agonist-induced contractions, preparations were washed three times. Fifteen minutes later, phenoxybenzamine at the selected concentration was added, and at the end of the incubation period (10 min) the tissues were washed three times successively and then six times over the following 60 min. Ten minutes later, agonist concentration-response curves were repeated. In some experiments the contralateral half was used as a control to correct the time-dependent changes in sensitivity of the tissue to agonist (Furchgott, 1972).

The equilibrium activation constants (K_A values) and relative efficacies of full agonists were determined by the

method developed by Furchgott (Furchgott, 1966; Furchgott & Bursztyn, 1967). This method employs the use of an irreversible antagonist to produce both a shift and a depression of the concentration-response curve of the agonist. To obtain K_A values in an experiment of the type outlined, the reciprocal of the concentrations of an agonist to produce given responses before phenoxybenzamine exposure (1/[A]) were plotted against the reciprocals of the corresponding equieffective concentrations after the treatment with the irreversible antagonist (1/[A']). The values for K_A and for the fraction of active receptors remaining (q) was calculated from the slope and intercept of the straight line fitting the points, on the basis of the equation (Furchgott, 1966):

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_A}$$

according to which K_A equals (slope-1)/intercept, and q equals 1/slope. For each experiment, the agonist concentration-response curves before and after phenoxybenzamine treatment were analyzed by linear regression of all points between 20% and 80% of the maximal response obtained in each case. Using the linear functions obtained in this manner, the concentration of agonist necessary to give 20, 30, 40, 50, 60% of the maximal response before and after phenoxybenzamine treatment was calculated. Linear regression of the reciprocals of these calculated values resulted in K_A and q values as described above.

The equilibrium activation constants of partial agonists (K_P) were also determined by a second method in which the responses to the partial agonist are compared to those of a full agonist in the same tissue (Waud, 1969). In this method the reciprocals of the concentrations of a full agonist to produce given responses (1/[A]) are plotted against the reciprocals of the concentrations of a partial agonist which produce the same responses (1/[P]). The K_P values of the partial agonists were calculated by the equation (Waud, 1969)

$$K_P = \frac{\text{slope}}{\text{intercept}}$$

Once the K_A and K_P values for full and partial agonists had been calculated by the methods mentioned above, the fractional receptor occupancy for a given response in each half of rat vas deferens could be determined from the equation:

$$[R]/[R_0] = [A]/(K_A + [A])$$

where [A] is the concentration of agonist producing a given response, [R]/[R₀] is the fractional receptor occupancy and K_A is the dissociation constant of the receptor-agonist complex.

Occupancy-response curves for agonists in each half of rat vas deferens were plotted on a logarithmic abscissa of % occupancy. Since absolute efficacy cannot be determined, it is usual to assign relative efficacies within a series of agonists, a full agonist being used as reference (Furchgott & Bursztyn, 1967). A relative efficacy of 1.0 was assigned for noradrenaline. The antilog of the distance between the two occupancy-response curves along the abscissa scale was taken as the ratio of the intrinsic efficacy of the second agonist to that of noradrenaline (Furchgott & Bursztyn, 1967), and thus it was a measure of the relative efficacy (e_r) of the second agonist as compared to noradrenaline.

Statistical analysis

The data were expressed as means ± s.e. of the mean. Student's two-tailed *t* test was used for statistical evaluations. Correlation coefficients were calculated by the least squares method. Significance level was set as *P* < 0.05. Ligand binding data were analyzed with computerized curve-fitting programs as described by McPherson (1983), *F*-test analysis was used to decide whether a model of one or two binding sites was more appropriate (*P* < 0.05).

Drugs

Drugs used and their sources were: noradrenaline bitartrate (Sigma), phenylephrine hydrochloride (Sigma), methoxamine hydrochloride (Gayoso-Wellcome), St-587 (2-(2-chloro-5-trifluoromethylphenylimino)imidazolidine nitrate) (Boehringer Ingelheim), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Fides), desmethylinipramine hydrochloride (Sigma), normetanephrine hydrochloride (Calbiochem), yohimbine hydrochloride (Sigma), phentolamine methansulphonate (Ciba-Geigy), phenoxybenzamine hydrochloride (Smith, Kline and French), tolazoline hydrochloride (Ciba-Geigy) and [^3H]-prazosin (76 Ci mmol $^{-1}$, New England Nuclear).

Results

Binding studies

Measurement of [^3H]-prazosin binding to α_1 -adrenoceptors in epididymal and prostatic halves of rat vas deferens The B_{max} and K_D values were not different in membranes of both portions (prostatic half: $K_D = 0.46 \pm 0.04$ nM and $B_{\text{max}} = 132.3 \pm 7.2$ fmol mg $^{-1}$ of protein, $n = 6$; epididymal half: $K_D = 0.44 \pm 0.04$ nM and $B_{\text{max}} = 130.2 \pm 9.4$ fmol mg $^{-1}$ of protein, $n = 6$). Specific binding was saturable and of high affinity. Scatchard analysis of saturation isotherms resulted in straight lines suggesting that the [^3H]-prazosin labels a homogeneous population of receptors in both portions of rat vas deferens. A Hill coefficient of 0.97 ± 0.03 and 0.95 ± 0.02 was obtained in the prostatic and epididymal portions respectively, indicating a lack of cooperativity and reinforcing that [^3H]-prazosin binds to a single population of receptors. Specific binding determined by $10 \mu\text{M}$ phentolamine was approximately 70% of the total binding at a concentration of [^3H]-prazosin close to the K_D value.

Displacement of specific [^3H]-prazosin binding by α -adrenoceptor antagonists and agonists Specific binding of [^3H]-prazosin to membranes of both halves of rat vas deferens was inhibited by several α -adrenoceptor antagonists (Table 1). The order of potency was prazosin > phentolamine $\gg$ yohimbine = tolazoline in each portion, a pattern similar to that expected for the interaction of these ligands with an α_1 -adrenoceptor. As shown in Table 1, prazosin, yohimbine and tolazoline inhibited the specific binding of [^3H]-prazosin monophasically with pseudo-Hill coefficients (n_H) close to unity and with similar K_1 values in both tissues. In contrast, phentolamine inhibited [^3H]-prazosin binding in both portions and resulted in shallow competition curves with pseudo-Hill coefficients significantly lower than unity. Iterative non-linear curve-fitting analysis (Munson & Rodbard, 1980) of competition curves obtained for phentolamine in both portions revealed two different components for the interaction of these agents at [^3H]-prazosin sites. The two populations of sites were present in a proportion approximately equal in both halves of rat vas deferens (Table 1).

As shown in Table 2, the parameters estimated by nonlinear regression of the competition curves of the agonists were similar in both halves of rat vas deferens. [^3H]-prazosin binding was inhibited monophasically with pseudo-Hill coefficients (n_H) close to unity by the imidazoline compounds St-587 and clonidine, and with similar K_1 values in both portions. In contrast, the phenylethylamine compounds, noradrenaline, phenylephrine and methoxamine inhibited [^3H]-prazosin binding in a biphasic way, resulting in shallow inhibition curves with pseudo-Hill coefficients significantly lower than unity in both portions. Two-site analysis of the data suggests the existence of two affinity states of α_1 -adrenoceptors for agonists in these tissues.

Functional studies

Concentration-response curves of agonists In the epididymal portion all the agonists studied were able to induce increases

Table 1 Inhibition of [^3H]-prazosin binding to α_1 -adrenoceptors of the bisected rat vas deferens by antagonists

Antagonist	n	n_H	K_{High}	K_{Low}	% R_{High}
<i>Prostatic half</i>					
Prazosin	5	0.93 ± 0.03	0.56 ± 0.03 nM		100
Phentolamine	4	0.64 ± 0.02	6.10 ± 0.15 nM	78.15 ± 3.30 nM	57.16 ± 3.40
Yohimbine	4	1.02 ± 0.03	2.86 ± 0.15 μM		100
Tolazoline	4	0.92 ± 0.03	7.08 ± 0.52 μM		100
<i>Epididymal half</i>					
Prazosin	5	0.99 ± 0.03	0.52 ± 0.02 nM		100
Phentolamine	6	0.66 ± 0.02	6.80 ± 0.50 nM	80.40 ± 3.28 nM	50.70 ± 3.20
Yohimbine	6	1.10 ± 0.11	3.15 ± 0.37 μM		100
Tolazoline	4	0.96 ± 0.04	8.05 ± 0.55 μM		100

Data are expressed as means $\pm$ s.e.mean of n experiments.

Table 2 Inhibition of [^3H]-prazosin binding to α_1 -adrenoceptors of the bisected rat vas deferens by agonists

Agonists	n	n_H	K_{High} (μM)	K_{Low} (μM)	% R_{High}
<i>Prostatic half</i>					
Noradrenaline	5	0.58 ± 0.02	0.19 ± 0.01	3.51 ± 0.15	45.30 ± 3.50
Phenylephrine	4	0.60 ± 0.03	1.47 ± 0.21	11.99 ± 0.82	50.15 ± 3.67
Methoxamine	3	0.62 ± 0.02	0.71 ± 0.05	36.58 ± 1.96	50.20 ± 3.76
St-587	4	0.99 ± 0.02	1.14 ± 0.06		100
Clonidine	4	1.06 ± 0.02	1.48 ± 0.05		100
<i>Epididymal half</i>					
Noradrenaline	5	0.59 ± 0.03	0.22 ± 0.03	5.68 ± 0.48	44.25 ± 6.03
Phenylephrine	4	0.71 ± 0.03	1.47 ± 0.03	11.03 ± 1.03	52.30 ± 2.60
Methoxamine	3	0.62 ± 0.03	0.72 ± 0.05	42.20 ± 2.36	53.20 ± 2.45
St-587	4	0.96 ± 0.02	1.26 ± 0.07		100
Clonidine	4	1.08 ± 0.02	1.41 ± 0.03		100

Data are expressed as means $\pm$ s.e.mean of n experiments.

in the tension in a concentration-dependent manner between 0.1 to 100 μM . However, only the three phenylethylamines were full agonists, while the imidazoline compounds behaved as partial agonists achieving 50% of the maximal effect of full agonists (Figure 1b). In the prostatic portion (Figure 1a) phenylephrine and methoxamine elicited a maximal response only $69.2 \pm 1.0\%$ and $60.3 \pm 2.0\%$ of that induced by noradrenaline respectively, and the two imidazoline compounds were only able to produce rhythmic contractions at high concentrations (100 nM) (Figure 1a). As shown in Figure 1, each agonist was more potent in the epididymal than in the prostatic portion.

To determine whether the actions of the agonists in each portion were mediated through activation of α_1 -adrenoceptors, the selective and nonselective

α_1 -adrenoceptor antagonists, prazosin and phentolamine were used. As shown in Table 3, the pA_2 values for each antagonist in both portions of rat vas deferens were virtually identical against the three phenylethylamines and St-587 in the epididymal portion. In all cases prazosin was thirty times more potent than phentolamine for each agonist. However the dissociation constant of prazosin was higher in functional studies compared with binding studies. Such differences may be due to the fact that incubation time was very short to establish equilibrium in the functional studies as has been previously reported (McGrath, 1984). The Schild regression analysis resulted in straight lines, their slopes being not significantly different from the theoretical value of 1. This indicates a reversible competition between the antagonist and the agonist for a single binding site which has the pharmacological characteristics of an α_1 -adrenoceptor.

Figure 1 Concentration-response curves of α -adrenoceptor agonists in (a) prostatic and (b) epididymal portions of rat vas deferens. Responses to noradrenaline ($\bullet$, $n = 10$), phenylephrine ($\blacktriangle$, $n = 6$), methoxamine ($\blacksquare$, $n = 6$), St-587 ($\triangle$, $n = 6$), clonidine ($\square$, $n = 6$) for each tissue. Vertical bars show s.e.mean.

Effect of phenoxybenzamine exposure on noradrenaline concentration-response curves in each portion of rat vas deferens In order to determine the contribution of spare receptors in the different sensitivity to the agonists in each portion of rat vas deferens, some experiments were designed to obtain a controlled partial alkylation of a certain proportion of receptors. Firstly, each portion was exposed to increasing concentrations of phenoxybenzamine (3 nM–1 μM) for 10 min. The analysis of concentration-response curve to noradrenaline after phenoxybenzamine incubation showed that low concentrations of the alkylating agent (3 and 10 nM) depressed by 40% and 80% the maximal contractile response to the agonist in the prostatic portion. In contrast, in the epididymal portion higher concentrations of phenoxybenzamine (0.03–0.3 μM) were required to obtain a similar decrease in the maximal contractile response to the agonist. In the receptor analysis the selected concentrations of phenoxybenzamine were 0.1 μM and 3 nM for the epididymal and prostatic portions respectively.

Determination of K_A values for full agonists (K_A) Table 4 shows the maximal responses ($E_{\max}$) and the EC_{50} values for the agonists after phenoxybenzamine incubation as compared with control experiments. The K_A and q determined for each agonist are also shown. Phenoxybenzamine (0.1 μM) after 10 min incubation caused a decrease in potency (17–19 times) and in maximal contractile response (30–40%) for the three agonists in the epididymal portion. In contrast, in the prostatic portion, phenoxybenzamine (3 nM) after 10 min of incubation induced only a moderate decrease in potency (1.4 times) and a marked depression of maximal response (40%) to noradrenaline.

Determination of K_A values for partial agonists (K_P) Since the $E_{\max}$ values induced by phenylephrine and methoxamine were smaller than those induced by noradrenaline in the prostatic half, the former compounds were considered as partial

Table 3 Mean pA_2 values of prazosin and phentolamine against α -adrenoceptor agonists in the bisected rat vas deferens

		Antagonist				
Agonist	n	Prazosin Slope	pA_2	n	Phentolamine Slope	pA_2
<i>Prostatic half</i>						
Noradrenaline	6	0.98 ± 0.03	8.47 ± 0.05	6	1.02 ± 0.03	7.65 ± 0.05
Phenylephrine	5	1.01 ± 0.02	8.43 ± 0.02	6	0.99 ± 0.05	7.61 ± 0.06
Methoxamine	6	0.95 ± 0.02	8.50 ± 0.05	6	1.04 ± 0.06	7.58 ± 0.05
<i>Epididymal half</i>						
Noradrenaline	6	0.95 ± 0.03	8.48 ± 0.02	6	0.92 ± 0.04	7.65 ± 0.03
Phenylephrine	6	1.08 ± 0.08	8.45 ± 0.03	6	1.01 ± 0.02	7.70 ± 0.04
Methoxamine	6	0.98 ± 0.03	8.50 ± 0.05	6	0.95 ± 0.02	7.54 ± 0.06
St-587	5	0.92 ± 0.05	8.52 ± 0.05			—

Data are expressed as means $\pm$ s.e.mean of n experiments.

Table 4 EC_{50}' and $\%E_{max}'$ values for agonists after phenoxybenzamine treatment and estimated values of K_A and q in the bisected rat vas deferens

Agonist	EC_{50}' (μM)	$\%E_{max}'$	EC_{50}'/EC_{50}	K_A (μM)	q	n
<i>Prostatic half</i>						
Noradrenaline	7.12 ± 0.52	60.04 ± 1.01	1.4	9.45 ± 0.30	40.6 ± 0.9	6
<i>Epididymal half</i>						
Noradrenaline	8.33 ± 0.32	70.03 ± 2.03	19	8.65 ± 0.2	4.5 ± 0.01	6
Phenylephrine	26.68 ± 1.43	64.94 ± 1.01	18	28.30 ± 0.3	6.8 ± 0.03	6
Methoxamine	161.21 ± 8.32	62.56 ± 1.27	17	118.36 ± 11.3	5.2 ± 0.04	6

Data are expressed as mean $\pm$ s.e.mean of n experiments.

agonists in this tissue. In the same way St-587 and clonidine were partial agonists in the epididymal half. The equilibrium activation constants of the partial agonists (K_p) in both halves were calculated as described by Waud (1969). The plot of the reciprocals of equieffective concentrations between the full and the partial agonist resulted in a straight line that allowed to estimate the K_p values for each partial agonist in the corresponding portion. The EC_{50} , E_{max} and K_p values are summarized in Table 5.

Relative efficacies of the agonists and relationships between occupancy and response The relative efficacies of the agonists were estimated by the method of Furchgott and Bursztyn (1967) in both portions of rat vas deferens. A plot of the relative responses to noradrenaline and the other agonists against the logarithm of the theoretical percentage of receptor occupancy yielded sigmoid curves. The antilog of the distance along the abscissa scale between each one of the agonists curves and the noradrenaline curve represents the relative efficacy (e_r) of each compound. As shown in Table 6, the e_r values for each compound were the same in the two portions. The plot of the fractional responses for noradrenaline against the theoretical percentage of receptor occupancy resulted in hyperbolic curves (Figure 2). As shown, noradrenaline induces the maximal effect in the epididymal half when only 50% of

the receptors were occupied, but in the prostatic portion 100% of the receptors were required to elicit the maximal response.

Comparison of K_I (K_{High} , K_{Low}) values for binding and the calculated K_a (K_A or K_p) values for α_1 -adrenoceptor agonists in both halves of rat vas deferens For the imidazoline compounds there was a good similarity between the K_p values obtained in functional studies and the K_I values for inhibiting [3H]-prazosin binding in the epididymal portion. In turn, the results obtained with the phenylethylamine compounds showed that the affinity rank order of these agonists (K_A , K_p) in functional studies in both portions of rat vas deferens, was

Figure 2 Curves of percentages of response of noradrenaline to maximum contractile effect against their percentage of receptor occupancy in the (■) prostatic and (●) epididymal portions of rat vas deferens.**Table 5** EC_{50} and $\%E_{max}$ values of partial agonists and estimated K_p values in the bisected rat vas deferens

Agonist	EC_{50} (μM)	$\%E_{max}$	K_p (μM)	n
<i>Prostatic half</i>				
Phenylephrine	7.68 ± 0.04	67.25 ± 1.65	22.64 ± 0.07	6
Methoxamine	25.72 ± 0.75	57.39 ± 3.30	100.80 ± 1.70	6
<i>Epididymal half</i>				
St-587	1.35 ± 0.03	51.49 ± 2.68	1.33 ± 0.02	6
Clonidine	2.88 ± 0.06	44.31 ± 1.70	1.53 ± 0.06	6

Data are expressed as mean $\pm$ s.e.mean of n experiments.

Table 6 Comparison of equilibrium dissociation constants calculated by different methods and relative efficacies of α -adrenoceptor agonists in the bisected rat vas deferens

Agonists	Prostatic		Epididymal	
	K_a (μM)	e_r	K_a (μM)	e_r
Noradrenaline	9.45 ± 0.30^a	1	8.65 ± 0.22^a	1
Phenylephrine	22.64 ± 1.32^b	0.67 ± 0.02	28.30 ± 1.61^a	0.66 ± 0.03
Methoxamine	100.80 ± 10.31^b	0.53 ± 0.03	118.23 ± 11.30^a	0.52 ± 0.01
St-587	1.31 ± 0.02^c	—	1.33 ± 0.05^b	0.032 ± 0.002
Clonidine	ND	—	1.53 ± 0.06^b	0.025 ± 0.002

Data are expressed as mean $\pm$ s.e.mean of at least 6 experiments. ND = not determined. ^aDetermined by the method of Furchgott.

^bDetermined with the method of Waud. ^cDetermined by the method of Schild (Badia & Sallés, 1989).

similar to the rank order of their dissociation constants (K_D) for the low-affinity state of the receptor (Tables 2 and 6). Moreover, an excellent correlation for prostatic ($r = 0.9931$, $P < 0.001$) and epididymal portions ($r = 0.9951$, $P < 0.001$) was obtained between the calculated dissociation constants from functional and binding studies.

Discussion

Several studies performed in the whole vas deferens have demonstrated that the increases in the isometric tension elicited by noradrenaline and other compounds are due to an interaction with α_1 -adrenoceptors and that this tissue lacks postsynaptic α_2 -adrenoceptors (Minneman *et al.*, 1983; Minneman & Abel, 1984). A functional reserve of α_1 -adrenoceptors for phenylethylamines, and the existence of a non-linear relationship between the fractional receptor occupancy and the fractional response elicited by these agonists (Minneman *et al.*, 1983; Minneman & Abel, 1984; Diaz-Toledo & Martí, 1988) has also been determined. On the other hand, accumulating evidence supports the idea that the prostatic and epididymal portions of rat vas deferens differ in their postsynaptic sensitivity to α -adrenoceptor agonists (Vardolov & Pennefather, 1976; Kasuya & Suzuki, 1979; Moore & Griffiths, 1982), the epididymal portion being more susceptible to the excitatory effects of imidazolines and phenylethylamines (MacDonald & McGrath, 1980; Badia & Sallés, 1989) than the prostatic portion.

Our results also confirm and extend the above mentioned findings; in the epididymal portion all the phenylethylamines tested behaved as full agonists and the imidazoline compounds showed only partial agonist activity. In contrast, in the prostatic portion only noradrenaline was a full agonist, while phenylephrine and methoxamine were partial agonists and the two imidazolines were unable to contract the tissue. The pA_2 values obtained for prazosin and phentolamine against all the agonists tested support the assumption that these compounds elicit the contractile responses by an interaction with α_1 -adrenoceptors in each portion. In addition, it has been previously demonstrated that St-587 is an α_1 -adrenoceptor antagonist in the prostatic portion with a pA_2 value similar to pEC_{50} value obtained in the epididymal half (Badia & Sallés, 1989), supporting an interaction of this compound with the same α_1 -adrenoceptor activated by phenylethylamines.

The existence of gradients in responsiveness to agonists which coincide with anatomical orientation has been extensively demonstrated in different tissues. For example, rabbit aorta is not uniformly sensitive to β -adrenoceptor agonists (Altura & Altura, 1970) and in the rabbit basilar artery a graded responsiveness to noradrenaline, increasing from the distal to the proximal portions has also been reported (Gintautas *et al.*, 1980). This variation in the response may be ascribed to the heterogeneous distribution of receptor types, as occurs in the urinary bladder of the rabbit: in the bladder body, α -adrenoceptors predominate over β -adrenoceptors whereas in the bladder base the opposite predominance occurs (Levin & Wein, 1979). Thus, the responses of both halves of rat vas deferens to selective α -adrenoceptor agonists may depend on a heterogeneous distribution of postsynaptic α_1 -adrenoceptors, as previously suggested (MacDonald & McGrath, 1980). Other possibilities include changes in affinity of the agonists for the receptors, a heterogeneous distribution of the recently proposed α_1 -adrenoceptor subtypes or differences in the efficiency of receptor coupling to response. All these possibilities were analyzed.

In binding studies, [3H]-prazosin labels an apparently homogeneous population of α_1 -adrenoceptors in each half of rat vas deferens. The maximal number of binding sites and the K_D values for [3H]-prazosin were not significantly different between the two tissues. The order of potency of

α_1 -adrenoceptor antagonists to displace [3H]-prazosin specific binding to membranes of both portions was similar to that expected for an interaction with an α_1 -adrenoceptor (Minneman *et al.*, 1983). However, the competition curves for phentolamine were biphasic, indicating two distinct binding sites. The percentages of high- and low-affinity sites in both portions as defined by the use of this antagonist were not different. Similar results have been previously reported in other tissues (including the rat vas deferens) with [3H]-prazosin used as radioligand. In these studies, on the basis of the affinities of several antagonists, the concept of α_1 -adrenoceptor subtypes was introduced (Morrow & Creese, 1986; Gröss *et al.*, 1988; Hanft & Gröss, 1989; Hanft *et al.*, 1989): the binding sites with high affinity for WB-4101 and phentolamine were designated as α_{1A} (or α_{1a}) and those with low affinity for these antagonists α_{1B} (or α_{1b}) (Morrow & Creese, 1986). On the basis of our present results it is very difficult to ascribe the differences in functional responsiveness between the two portions of rat vas deferens to a heterogeneous distribution of α_1 -adrenoceptor subtypes.

Analysis of competition experiments with imidazoline compounds yielded monophasic curves with pseudo-Hill coefficients (n_H) close to unity and with similar K_i values in both portions. In contrast, phenylethylamines inhibited [3H]-prazosin binding with pseudo-Hill coefficients significantly lower than unity in each portion of rat vas deferens. Two-site analysis of the data suggests the existence of two α_1 -adrenoceptor binding sites in these tissues. K_i values for the high (K_{High}) and for the low affinity sites (K_{Low}) for these drugs were in good agreement in both portions. However, several observations suggest that the two affinity states for agonists are distinct from the α_{1A}/α_{1B} binding sites above mentioned. It has been demonstrated that full agonists, like adrenaline and noradrenaline, can bind to α_1 -adrenoceptors with two affinity constants in various tissues (Ernsberger & U'Prichard, 1987; Gross *et al.*, 1988). Moreover, the addition of guanosine 5'-triphosphate (GTP) or its non hydrolyzable analogues can convert high affinity binding sites of α_1 -adrenoceptor for agonists into the low affinity state without affecting the antagonist binding (Gross *et al.*, 1988). In any case, the differences in responsiveness after α_1 -adrenoceptor activation observed in each portion of rat vas deferens are not associated with differences in the characteristics of binding.

In functional studies the equilibrium dissociation constants (K_A) for full agonists were determined by the method described by Furchgott (1966), and for partial agonists (K_P) by the method of Waud (1969). In our study the K_A obtained for the full agonists in both portions are similar to those previously reported in the whole vas deferens (Minneman & Abel, 1984; Diaz-Toledo & Martí, 1988). Similarly, the K_P values for the partial agonists phenylephrine and methoxamine in the prostatic portion were similar to the K_A obtained in the epididymal half. Moreover, the K_P for St-587 in the epididymal portion was coincident with the pA_2 previously reported in the prostatic portion (Badia & Sallés, 1989). In addition the dissociation constants of phenylethylamines for the low affinity state (K_{Low}) are well correlated with the K_A (K_A and K_P) for these agonists in both portions of rat vas deferens. Therefore, the initial step for the activation of the response in both portions might be the interaction of agonists with the low affinity state of the α_1 -adrenoceptor. Finally, another drug-related parameter considered was efficacy. In our studies no differences in the relative efficacies of agonists, as estimated by the method of Furchgott & Bursztyn (1967) were observed in the two portions of rat vas deferens.

The equilibrium dissociation constants K_A (K_A or K_P) for phenylethylamines were higher than the respective EC_{50} values in each portion (Table 6). These discrepancies could imply the existence of spare receptors in both halves. However, the assumption of the existence of spare receptors in a tissue based exclusively on the differences between K_A and EC_{50} for a certain agonist may lead to erroneous conclusions. A different non-linear relationship between occupancy and

response exists for noradrenaline in the two portions. In the epididymal half the maximal effect for noradrenaline could be obtained when only 50% of the α_1 -adrenoceptors were occupied. In contrast, in the prostatic portion 25% of the receptors were required to elicit half maximal responses but all the receptors need to be occupied to induce the maximal effects. Thus, in the prostatic portion there is no indication of spare receptors at maximal response of noradrenaline, though differences between K_A and EC_{50} seem to exist. Our results also demonstrate that the relationship between occupancy and response in the epididymal portion is more efficient than in the prostatic half. Thus, the greater amplification of stimuli in the epididymal portion, as compared with the prostatic

portion, is reflected in the steeper occupancy-response curve for the former tissue, as appears in the Figure 2.

In conclusion the differences observed in the sensitivity to α -adrenoceptor agonists along the rat vas deferens could not be explained by a heterogeneous distribution of receptors, changes in the affinity constants for agonists, or a different proportion of the postulated α_1 -adrenoceptor subtypes. The most suitable explanation of these differences may involve a tissue factor such as receptor coupling to stimulus-response mechanisms.

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