Modulation of α_1 -adrenoceptors and functional consequences in the bisected rat vas deferens following chronic inhibition of neuronal noradrenaline uptake

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1 The adaptational changes induced after chronic inhibition of neuronal noradrenaline uptake on both functional responsiveness of α_1 -adrenoceptor activation and [³H]-prazosin binding were investigated in prostatic and epididymal portions of the rat vas deferens.

2 Contractile concentration-response curves to phenylephrine and saturation isotherms of [³H]-prazosin binding to homogenates of each of the portions of the bisected rat vas deferens were determined 48 h after the last injection of desipramine, nomifensine or nisoxetine (10 mg kg⁻¹; i.p. for 14 days).

3 Treatment with both nomifensine and nisoxetine decreased the potency (pD₂) of phenylephrine by about 10 and 8 fold respectively in the epididymal portion. However, administration of desipramine only reduced the potency of the α_1 -adrenoceptor agonist by about 1.8 fold. None of the treatments modified the maximal effect (E_{max}) elicited by phenylephrine in this portion of the vas deferens. In the prostatic portion only the treatment with nomifensine (1.4 fold) and nisoxetine (1.8 fold) decreased the potency of phenylephrine; the maximal contraction elicited by the agonist after the treatments was also reduced.

4 Chronic treatment with either nomifensine or nisoxetine did not change the K_D for [³H]-prazosin binding in either epididymal or prostatic membranes. However, these two treatments resulted in a significant decrease in the [³H]-prazosin B_{max} in membranes in both portions of rat vas deferens. The reduction in density of α_1 -adrenoceptors was higher in the epididymal than the prostatic half. Desipramine reduced the B_{max} only in the epididymal portion.

5 These results indicate that differential regulation of α_1 -adrenoceptors in either portion of the rat vas deferens could result from a greater degree of activation of these receptors in the epididymal half after chronic inhibition of neuronal noradrenaline uptake. The different functional consequences of the loss of α_1 -adrenoceptors in each portion seems to be explained on the basis of a different relationship between the occupancy of the receptor and the response elicited.

Keywords: α_1 -Adrenoceptors; [³H]-prazosin binding; inhibitors of neuronal noradrenaline uptake; rat vas deferens

Introduction

Several studies seem to indicate that the prostatic and epididymal portions of the rat vas deferens differ not only in their motor transmission (McGrath, 1978; Brown *et al.*, 1983) but in their postsynaptic responsiveness to α -adrenoceptor agonists (Vardolov & Pennefather, 1976; Kasuya & Suzuki, 1979; MacDonald & McGrath, 1980; Moore & Griffiths, 1982; Badia & Sallés, 1989; Sallés & Badia, 1991). These differences in sensitivity to α -adrenoceptor agonists are likely to involve more efficient α_1 -adrenoceptor coupling to stimulus-response mechanisms in the epididymal portion (Sallés & Badia, 1991). In addition, the prostatic portion of the rat vas deferens has a more dense noradrenergic innervation (Anton *et al.*, 1977) and a larger endogenous noradrenaline content (Zieher & Jaim-Etcheverri, 1971; Sjöstrand, 1981) compared to the epididymal half.

Drugs which inhibit neuronal noradrenaline uptake make more neurotransmitter available at postsynaptic α_1 -adrenoceptors and may therefore lead to compensatory down-regulation of these receptors. However, the loss of postsynaptic receptors, by such a mechanism, will depend, among other factors, on the density and symmetry of innervation and the width of the synaptic gap. Likewise, the functional consequences induced by such a compensatory mechanism rely on the efficiency of the stimulus-response coupling in the tissue under study. In order to gain more insight into the characteristics and regulation of postsynaptic α_1 -adrenoceptors of both portions of rat vas deferens, it was decided to study the influence of long-term inhibition of neuronal noradrenaline

uptake on these receptor sites. Three potent inhibitors of neuronal noradrenaline uptake, desipramine, nomifensine and nisoxetine were selected (Richelson & Pfenning, 1984). The effect of these chronic treatments on the number and functional responsiveness of α_1 -adrenoceptors in the epididymal and prostatic portions of rat vas deferens was analyzed. A preliminary account of this work has been presented to the XIth International Congress of Pharmacology, Amsterdam, June 1990, (Badia & Sallés, 1990).

Methods

Male Wistar rats (140–200 g) with free access to food and water and maintained on a 12 h light/dark schedule were injected i.p. daily for 14 days with either saline or 10 mg kg⁻¹ of nisoxetine, nomifensine or desipramine. Animals were killed by decapitation 48 h after 1 or 14 days of treatment. Both vasa deferentia were removed, carefully cleaned and bisected into prostatic and epididymal portions and used for functional or binding studies.

Functional studies

For the measurement of contractile responses induced by phenylephrine or noradrenaline, the tissues were suspended in isolated organ baths containing 20 ml of a physiological salt solution of the following 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. Propri-

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nolol (1 μM), desipramine (0.1 μM) and normetanephrine (1 μM) were present continuously in the medium throughout the experiments to block β -adrenoceptors, neuronal and extra-neuronal noradrenaline uptake respectively (Minneman *et al.*, 1983). The experimental conditions were thus carefully controlled to obtain accurate estimates of the potency of agonists on the α_1 -adrenoceptors. To assess the influence of neuronal noradrenaline uptake mechanisms on the concentration-response curves to noradrenaline and phenylephrine in each portion of the vas deferens of control animals, some experiments were carried out in the presence or absence of desipramine alone (0.1 μM) in the physiological salt solution.

At least 45 min were allowed to elapse before starting the experiment. After this equilibration period at a resting tension of 0.5 g, the tissues were exposed twice to a maximal concentration of noradrenaline and thoroughly washed. Contractions were registered by means of an isometric PIODEN (UF-1) transducer attached on a OmniScribe pen-recorder. Agonist-induced increases in isometric tension were determined with a non-cumulative dosage schedule where each concentration was washed out before the next concentration was added, using 6 min intervals between concentrations. 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 or phenylephrine. Data from concentration-response experiments for phenylephrine or noradrenaline were analyzed by nonlinear regression analysis, using the GraphPAD computer programme (ISI, Philadelphia, PA, USA), which gave estimates of the pD_2 ($-\log \text{EC}_{50}$, negative $\log_{10}$ of molar concentration of agonist producing 50% of its maximal effect), the maximal effect (E_{max}) and slope of the curve.

Radioligand binding studies

For binding assays, the epididymal and prostatic portions from several animals were pooled separately and 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 49,000 g for 20 min. The resultant pellet was washed twice by resuspension and centrifugation under the same conditions. Final pellets were resuspended in the same buffer at 2 mg protein ml^{-1} approximately. For saturation experiments aliquots (100 μg protein) of membrane suspensions were incubated in triplicate with seven concentrations (ranging from 0.05 to 4 nM) of [^3H]-prazosin in 0.25 ml (final volume) of 50 mM Tris/HCl buffer pH 7.5 at 25°C for 45 min. Incubations were stopped by rapid filtration through Whatman GF/C glass fibre filters that were washed 3 times with 5 ml of the same buffer. Radioactivity retained in the filters was determined at 48–50% efficiency. Nonspecific binding was defined as that insensitive to 10 μM phentolamine. Protein was estimated as described by Lowry *et al.* (1951) with bovine serum albumin used as standard.

Individual saturation isotherms for [^3H]-prazosin specific binding were analyzed by non-linear regression analysis, using the EBDA programme adapted for microcomputers as described by McPherson (1983). Parameters estimated were K_D , B_{max} and the Hill coefficient.

Statistical analysis

All the results are expressed as the means $\pm$ s.e.mean. Statistical significance of the observed differences was assessed by two tailed Student's *t* test. The statistical significance of the differences between the Hill coefficients and unity was tested with Student's *t* test, under a null hypothesis (Hill coefficient = 1).

Drugs

Drugs used and their sources were: noradrenaline bitartrate, phenylephrine hydrochloride, desipramine hydrochloride and propranolol hydrochloride (Sigma), normetanephrine hydrochloride (Calbiochem), phentolamine methansulphonate (Ciba-Geigy), nomifensine hydrogen tartrate (Hoechst), nioxetine hydrochloride (Lilly) and [^3H]-prazosin (82 Ci mmol^{-1} , New England Nuclear).

Results

Functional studies

In order to determine the influence of neuronal uptake mechanisms on the potency of α -adrenoceptor agonists in each portion of rat vas deferens, some experiments were designed to evaluate the influence of desipramine on the concentration-response curves to noradrenaline and phenylephrine. In the presence of 0.1 μM desipramine, only the maxima of the concentration-response curves to noradrenaline were markedly enhanced in both portions of rat vas deferens. This effect was pronounced in the epididymal than in the prostatic portions, with approximately 100% and 40% increases respectively (Table 1). An increase (5 fold) in the potency of noradrenaline was observed only in the epididymal half (Table 1).

Two days after the administration of a single dose (10 mg kg^{-1} ; i.p.) of nioxetine, nomifensine or desipramine neither the pD_2 nor the E_{max} values for phenylephrine were significantly modified in either half of the vas deferens. After 14 days of treatment with each compound the concentration-response curves to phenylephrine were shifted to the right in the epididymal portion (Figure 1b). The treatments with nioxetine and nomifensine reduced the potency of phenylephrine by about 8 fold ($P < 0.01$) and 10 fold ($P < 0.01$) respectively. However, desipramine treatment only reduced the potency of the agonist by about 1.8 fold ($P < 0.05$) (Table 2). It should be noted that the three treatments did not modify the maxima of the concentration-response curves to phenylephrine in the epididymal portion (Figure 1b and Table 2). In contrast, the administration of nioxetine and nomifensine for 14 days resulted in a marked reduction (32% and 45% respectively) of the maximal contraction elicited by phenylephrine in the prostatic half of rat vas deferens (Figure 1a and Table 2). In addition, these two treatments only induced small changes in the potency of phenylephrine in this portion (Table 2). The treatment with desipramine did not modify the concentration-response curve of phenylephrine in the prostatic half of the rat vas deferens (Table 2).

Binding studies

Specific binding of [^3H]-prazosin was saturable and Scatchard analysis of saturation isotherms resulted in straight lines in both control and treated groups (Figure 2). The Hill coefficients for the saturation isotherms were not significantly different from unity and the specific binding determined by 10 μM phentolamine was approximately 70% of the total binding at a concentration of [^3H]-prazosin close to the K_D value. As shown in Table 3, 2 weeks of treatment with nioxetine, nomifensine or desipramine did not affect the α_1 -adrenoceptor affinity for [^3H]-prazosin in either epididymal or prostatic membranes. However, the long-term treatment with nioxetine and nomifensine resulted in a significant decrease in the maximal number of α_1 -adrenoceptor binding sites (B_{max}) in both portions. In the epididymal half a 40% or 30% decrease was observed with nioxetine and nomifensine respectively. In the prostatic half the treatments with nioxetine and nomifensine resulted in smaller decreases in the density of α_1 -adrenoceptors of 23% and 15% respectively. In

Table 1 Responses of the bisected rat vas deferens to noradrenaline and phenylephrine in the absence and presence of $0.1 \mu\text{M}$ desipramine in the physiological salt solution

	Control		Desipramine ($0.1 \mu\text{M}$)	
	pD_2	E_{max}	pD_2	E_{max}
<i>Prostatic portion</i>				
Noradrenaline	5.30 ± 0.06	450 ± 35	5.31 ± 0.05	$630 \pm 25^{**}$
Phenylephrine	5.02 ± 0.04	420 ± 15	5.03 ± 0.02	413 ± 15
<i>Epididymal portion</i>				
Noradrenaline	5.60 ± 0.05	1162 ± 30	$6.31 \pm 0.04^{**}$	$2037 \pm 45^{**}$
Phenylephrine	5.84 ± 0.01	2063 ± 28	5.86 ± 0.02	2103 ± 40

Data are expressed as means $\pm$ s.e.mean of 10 experiments. E_{max} is the maximal muscle contraction to agonist expressed as mg of tension.

$^{**}P < 0.01$ when compared to the values obtained in the absence of $0.1 \mu\text{M}$ of desipramine.

Figure 1 Effects of chronic treatment with several inhibitors of neuronal noradrenaline uptake on the concentration-response curves to phenylephrine in the prostatic (a) and the epididymal (b) portions of rat vas deferens. Responses to phenylephrine from vehicle- (●), and desipramine- (○), nomifensine- (▲), and nisoxetine-treated animals (△), are calculated as a percentage of the averaged maximum response in control tissues. Each point represents the mean $\pm$ s.e.mean (vertical bars) of six experiments on separate preparations.

Figure 2 Scatchard plots for $[^3\text{H}]$ -prazosin binding to membranes from (a) prostatic and (b) epididymal portions of rat vas deferens. Vehicle- (●), desipramine- (○), nomifensine- (▲), and nisoxetine-treated animals (△). The values on the y-axis were determined by dividing the amount bound (B, fmol mg^{-1} protein) by the concentration of free radioligand (F, nM). The results shown are from a representative experiment carried out in triplicate.

Table 2 Effects of chronic treatment (10 mg kg⁻¹; i.p.; 14 days) with several inhibitors of neuronal noradrenaline uptake on the agonist activity of phenylephrine at post-synaptic α_1 -adrenoceptors in the bisected rat vas deferens

	pD ₂	Δ	E _{max}	%E _{max}
<i>Prostatic portion</i>				
Control	5.02 ± 0.04	—	400.0 ± 50.2	—
Nisoxetine	4.77 ± 0.03**	1.8	219.2 ± 49.3**	55
Nomifensine	4.89 ± 0.02*	1.4	272.5 ± 26.7*	68
Desipramine	4.99 ± 0.05	—	517.5 ± 51.2	—
<i>Epididymal portion</i>				
Control	5.86 ± 0.01	—	2012.8 ± 113.2	—
Nisoxetine	4.99 ± 0.06**	8	2004.5 ± 30.4	—
Nomifensine	4.89 ± 0.02**	10	1841.6 ± 40.7	—
Desipramine	5.59 ± 0.04*	1.8	1983.7 ± 69.8	—

Data are expressed as mean ± s.e.mean of 6 experiments. E_{max} is the maximal muscle contraction to phenylephrine expressed as mg of developed tension. %E_{max} is the percentage of the ratio E_{max} treated/E_{max} control and Δ indicates the potency changes calculated as antilog (pD₂ control - pD₂ treated) where these are significantly different from control.

**P < 0.01; *P < 0.05 vs control value.

contrast, treatment with desipramine only reduced the number of α_1 -adrenoceptors (by about 19%) in the epididymal half.

Discussion

It has been established that an increase in availability of noradrenaline in the synaptic cleft by long-term inhibition of its neuronal uptake results in a reduction in the number of central and peripheral α_2 -adrenoceptors and in a reduction in agonist potency (Svensson & Usdin, 1978; Smith *et al.*, 1982; García-Sevilla & Zubieta, 1986; Vila *et al.*, 1990). However, much less is known about the desensitization of responses mediated by α_1 -adrenoceptors following their chronic activation, or of the involvement of changes in receptor density in such modifications. The present study was designed to examine the regulation of both density and responsiveness of α_1 -adrenoceptors, in two tissue samples with a different efficiency of coupling between the occupancy of these receptors and the responses elicited (Sallés & Badia, 1991), after chronic stimulation resulting from the long-term inhibition of neuronal noradrenaline uptake by antidepressant drugs.

The results clearly show that the long-term treatment with inhibitors of neuronal noradrenaline uptake leads to α_1 -adrenoceptor down-regulation in both portions of rat vas deferens, probably due to an increased level of noradrenaline in the vicinity of its receptors. In this context, it should be borne in mind that the motor transmission in the rat vas deferens, independently of the adrenergic or non-adrenergic nature of the nerve terminals (Sneddon *et al.*, 1982), is modulated by presynaptic α_2 -adrenoceptors, stimulation of

which inhibits the release of the motor transmitter (Drew, 1977). Furthermore, these receptors become desensitized by chronic treatment with antidepressant drugs (García-Sevilla & Zubieta, 1986). Therefore, a reduced sensitivity of presynaptic α_2 -adrenoceptors to noradrenaline should result in an increased transmission in these nerve terminals, an effect that could also contribute to the development of postsynaptic α_1 -adrenoceptor down-regulation.

The long-term treatment with nomifensine and nisoxetine decreased the maximal number of binding sites labelled with [³H]-prazosin in both portions of rat vas deferens, but the decreases were higher in the epididymal than in the prostatic portions (Table 3). In contrast, long-term treatment with desipramine, that is also a potent inhibitor of noradrenaline uptake (Richelson & Pfenning, 1984), only decreased the number of [³H]-prazosin binding sites in the epididymal half. Desipramine, in contrast to the other two compounds, has a significant affinity for α_1 -adrenoceptors (Richelson & Nelson, 1984) and displays α_1 -adrenoceptor antagonist activity in the rat vas deferens (Bradley & Doggrell, 1985). Thus, α_1 -adrenoceptor antagonism by desipramine could oppose the effects due to the increase of synaptic noradrenaline that result from neuronal uptake inhibition.

The greater reduction in α_1 -adrenoceptor density achieved in the epididymal portion may be explained on the basis of the results shown in Table 1. The maximal contractile responses induced by noradrenaline in the epididymal half were potentiated to a greater extent than those in the prostatic half following inhibition of neuronal uptake by desipramine. Similar results have been reported with desipramine (Gonçalves & Guimaraes, 1991), or cocaine (Werneck de Avellar *et al.*, 1990). Pharmacologically, the magnitude of the potentiation can reflect the importance of the uptake process as a modulator of agonist concentrations in the receptor compartment and it has been shown that the epididymal portion has a greater capacity for noradrenaline accumulation than the prostatic portion (Werneck de Avellar *et al.*, 1990). Therefore, all these results could reflect a greater influence of the removal process, and consequently of its chronic inhibition, in the epididymal portion of rat vas deferens. The greater reduction in α_1 -adrenoceptor density in the epididymal versus the prostatic portion could result from a greater degree of receptor activation in this half after inhibition of neuronal noradrenaline uptake and it has been suggested that post-junctional α_1 -adrenoceptors are located closer to the varicosities in the epididymal than in the prostatic portion (Gonçalves & Guimaraes, 1991). Although a good correlation has been established between the potentiation of the responses to noradrenaline with the neuromuscular cleft width, but not with density of noradrenergic innervation (Verity, 1971; Trendelenburg, 1972), it should be noted that neuromuscular distance in some tissues may be exceeded in importance by asymmetry of innervation (Kenakin, 1984). Thus, the denser noradrenergic innervation in the prostatic portion (Anton *et al.*, 1977) does not necessarily imply that the α_1 -adrenoceptors are confined to the synaptic region and evidence for extrasynaptic localization of α_1 -adrenoceptors

Table 3 Effects of chronic treatment with several inhibitors of neuronal noradrenaline uptake on binding of [³H]-prazosin to α_1 -adrenoceptors of prostatic and epididymal portions of rat vas deferens

	<i>Prostatic portion</i>			<i>Epididymal portion</i>		
	K _D	B _{max}	Δ	K _D	B _{max}	Δ
Control	0.36 ± 0.03	137.5 ± 12.3	—	0.34 ± 0.03	129.4 ± 8.9	—
Nisoxetine	0.30 ± 0.02	105.1 ± 6.7*	23	0.29 ± 0.02	78.4 ± 1.1**	40
Nomifensine	0.42 ± 0.02	117.1 ± 4.1*	15	0.41 ± 0.05	88.2 ± 5.1**	30
Desipramine	0.38 ± 0.05	120.4 ± 10.1	—	0.41 ± 0.03	105.5 ± 2.1*	19

Data are expressed as means ± s.e.mean of 6 independent experiments. K_D is the affinity constant for [³H]-prazosin in nM, and B_{max} maximal number of binding sites in fmol mg⁻¹ protein. Δ % of decrease of B_{max} control where these are significantly different from control.

**P < 0.01; *P < 0.05 versus control value.

has been reported in the guinea-pig mesenteric and rat tail arteries (Itoh *et al.*, 1983).

Although a reduction in α_1 -adrenoceptor density has been obtained in the whole rat vas deferens after chronic treatment with desipramine (20% reduction, as determined by [3 H]-WB-4101 binding) (Wetzel *et al.*, 1981), this has not been consistently reproduced in other tissues, including the central nervous system (Vetulani *et al.*, 1984; Kendall & Nahorski, 1985; Newman & Lerer, 1988; Sallés *et al.*, 1989). Various factors affecting the concentration of noradrenaline in the synaptic cleft may account for these differences between tissues, and the smooth muscle of the rat vas deferens has the densest innervation and probably the narrower synaptic cleft (10–30 nm) (Furness & Iwajama, 1971), as compared to other noradrenergically innervated tissues. A high affinity binding site for [3 H]-desipramine, apparently associated with the neuronal noradrenaline uptake complex, is present in both peripheral and central tissues possessing noradrenergic innervation (Raisman *et al.*, 1982) and it has been demonstrated that there is a 16 fold higher binding capacity for this ligand in the rat vas deferens compared to the rat cerebral cortex (1680 versus 101 fmol mg⁻¹ protein), data that are in parallel with the endogenous noradrenaline content determined in both tissues (12756 versus 120 ng g⁻¹ tissue) (Langer *et al.*, 1984). Thus, the increase in noradrenaline concentration in the synaptic cleft following inhibition of neuronal noradrenaline uptake is likely to be greater in the rat vas deferens than in other tissues.

In the epididymal portion of the vas deferens, the three treatments decreased the potency of phenylephrine. In contrast, in the prostatic half nomifensine and nisoxetine not only reduced the potency but also the maximal contractions elicited by phenylephrine while treatment with desipramine did not modify the responses. It can be assumed that the α_1 -adrenoceptor subsensitivity observed after chronic treatment with the three compounds is not due to a direct antagonism of receptors by residual drug present in the

tissue, because of the relatively low affinity of these compounds for the α_1 -adrenoceptors (Richelson & Nelson, 1984).

Recently, it has been demonstrated that there is a non-linear relationship between response and occupancy of α_1 -adrenoceptors in both halves of rat vas deferens, the occupancy-response coupling being more efficient in the epididymal than in the prostatic portion (Sallés & Badia, 1991). In fact, in the epididymal half the maximal effect could be obtained when only 30% of the α_1 -adrenoceptors were occupied. An initial decrease in receptor density will increase the fraction of receptors that need to be occupied to produce a response, resulting in a parallel rightward shift of the concentration-response curve. In contrast, in the prostatic portion all the α_1 -adrenoceptors need to be occupied to induce a maximal effect and a decrease in receptor density will decrease the maximal response with only slight modification to the potency of the agonist. This pattern of functional changes with the loss of receptors agrees with the data obtained in our study in both portions of rat vas deferens.

In conclusion, our results indicate that the distinct regulation of α_1 -adrenoceptors in both portions of the rat vas deferens could result from a greater activation of these receptors in the epididymal as compared to the prostatic halves after chronic inhibition of neuronal noradrenaline uptake. Our results could support the hypothesis that, in the prostatic portion, postjunctional α_1 -adrenoceptors are less influenced by noradrenaline released from sympathetic nerve terminals, because they are probably more distant from the varicosities. The functional consequences observed in each portion of rat vas deferens after the decrease of the density of α_1 -adrenoceptors could be explained as a result of the existence of non-linearity of the relationship between agonist occupancy and response in these tissues.

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