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Characterization of the α-adrenoceptors in the female rabbit urethra

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1 A radioligand binding technique was used to evaluate the proportions of α_1- and α_2-adrenoceptors in crude membrane preparations obtained from the female rabbit bladder base and urethra. In addition, urethral rings were studied *in vitro* in an attempt to determine if α_1- and/or α_2-adrenoceptors are located postjunctionally in the urethral smooth muscle.

2 Studies of the inhibition of [^{3}H]-dihydroergocryptine binding by the selective α_1-adrenoceptor antagonist prazosin or the selective α_2-adrenoceptor antagonist rauwolscine revealed the α-adrenoceptor population to consist of approximately 25% α_1-adrenoceptors and 75% α_2-adrenoceptors. These proportions were confirmed in saturation studies with [^{3}H]-prazosin and [^{3}H]-rauwolscine. The sum of α_1- and α_2-adrenoceptors labelled by these selective α_1- and α_2-adrenoceptor antagonists was about equal to the number labelled by the non-selective α-adrenoceptor antagonist [^{3}H]-dihydroergocryptine.

3 Noradrenaline, as well as the selective α_1-adrenoceptor agonist phenylephrine and the selective α_2-adrenoceptor agonist clonidine, induced contractions of urethral ring preparations. Prazosin blocked contractions induced by phenylephrine to a greater extent than contractions induced by clonidine. The opposite was true for the inhibitory effect of rauwolscine.

4 In addition to showing that both α_1- and α_2-adrenoceptor binding sites exist in membrane preparations of the rabbit bladder base and urethra, the results reveal the presence of both adrenoceptor subtypes postjunctionally in the rabbit urethra; and both mediate contraction of the smooth muscle.

Introduction

It is now generally accepted that there are two major α-adrenoceptor subtypes, the α_1- and the α_2-adrenoceptors. By means of selective agonists and antagonists these α-adrenoceptor subtypes have been characterized in different tissues and species (for reviews, see Langer, 1974; Berthelsen & Pettinger, 1977; Wikberg, 1979; Starke, 1981).

Studies of α-adrenoceptors with radioligand binding techniques have shown that various tissues may contain almost exclusively α_1- or α_2-adrenoceptors, or a mixture of these (Hoffman *et al.*, 1979). Functional studies on isolated vascular smooth muscle suggest the existence of postjunctional α_1-adrenoceptors in, e.g., the rat portal vein (Ruffolo *et al.*, 1981), postjunctional α_2-adrenoceptors in the feline middle cerebral artery (Skärby *et al.*, 1983), and both of these subtypes postjunctionally in canine saphenous vein (De Mey & Vanhoutte, 1981) and feline lingual arteries (Skärby *et al.*, 1983). Moreover, postjunctional α_1- and α_2-adrenoceptors have been suggested to be involved in the pressor responses to α-adrenoceptor agonists in the pithed rat (Timmermans & van Zwieten, 1980) and dog (Constantine *et al.*, 1980), and in the autoperfused hindlimb of the dog (Langer *et al.*, 1980).

In the urethra, smooth muscle tone (and intraurethral pressure) is believed to be controlled by the sympathetic nervous system via α-adrenoceptors (Donker *et al.*, 1972; Awad & Downie, 1976). It is well established that stimulation of these receptors increases and blockade decreases the intraurethral pressure, and that these effects can be used therapeutically in functional disorders of the lower urinary tract (see, Andersson & Sjögren, 1982). It has been suggested that drugs with selectivity for urethral α-adrenoceptors can be obtained (Gahlin & Sparf, 1978), which makes it of interest to study the α-adrenoceptor subtypes in this region. The aim of the present investigation was therefore to quantify the α_1- and α_2-adrenoceptor populations in the

female rabbit bladder base and urethra using a radioligand binding technique, and to study the functional role of postjunctionally located α_1- and/or α_2-adrenoceptors in contractions in the isolated urethra.

Methods

Dissection

Female rabbits of the Danish Land race (body weight approximately 3 kg) were killed and the bladder and urethra excised *en bloc*. Starting from the base of the bladder, the urethra was gently dissected free from the vaginal wall down to the urethrovaginal junction and removed.

Radioligand binding studies

Membrane preparation The rabbit bladder base and urethra were cut open and the mucosa, periurethral fat and connective tissue removed. The remaining tissue was placed in 0.9% w/v NaCl solution, frozen to −80°C, and stored for up to one month. For binding experiments, pooled tissues were carefully minced and homogenized with a Polytron PT 10/35 homogenizer (Kinematica) for 4 × 8 s (setting 7) in ice cold buffer containing 50 mM Tris-HCl (pH 7.5 at room temperature).

The homogenate was filtered through a 0.5 mm nylon mesh and centrifuged at 39,000 × *g* for 20 min at 4°C. The pellet obtained was suspended in fresh buffer and recentrifuged. The subsequent final pellet was resuspended in fresh buffer and filtered through a 0.118 mm nylon mesh and the filtrate was used in the binding experiments.

Binding assay Tritium labelled dihydro-α-ergocryptine ([^{3}H]-DHE), prazosin and rauwolscine were used.

[^{3}H]-DHE assay The [^{3}H]-DHE binding was performed as described previously (Larsson, 1983). In brief, membranes, [^{3}H]-DHE and solutions of drugs or their solvents were incubated in triplicate for saturation experiments and in duplicate for inhibition studies in a total volume of 0.5 ml at 25°C for 60 min. The incubation was terminated by diluting the samples with 1.5 ml of ice-cold buffer followed by rapid filtration under reduced pressure through Whatman GF/C glass fibre filters, pre-soaked with buffer containing 1 mg ml^{-1} bovine serum albumin. The filters were rapidly washed four times with 5 ml portions of ice cold buffer.

[^{3}H]-prazosin assay Membranes, [^{3}H]-prazosin and redistilled water or phentolamine (10^{-5} M) were incubated in triplicate in a total volume of 0.5 ml at 25°C for 15 min. The reaction was terminated as described for the [^{3}H]-DHE assay, followed by three 5 ml washes.

[^{3}H]-rauwolscine assay Membranes, [^{3}H]-rauwolscine and redistilled water or phentolamine (10^{-5} M) were incubated in triplicate in a total volume of 0.25 ml at 25°C for 20 min. The reaction was stopped as described for the [^{3}H]-DHE assay except that the samples were diluted with 1 ml of ice cold buffer, and the filters were pre-soaked in buffer without bovine serum albumin.

The filters were placed in scintillation vials and the tritium content determined in a liquid scintillation spectrometer (LKB RackBeta 1215). The counting efficiency was about 50% as determined by the method of external standard channels ratio.

Preliminary association experiments with the ^{3}H-ligands showed that steady state was reached within the incubation period for each respective ligand and that binding was stable for at least an additional 15 min period. All reactions were started by the addition of membranes and were continued for 60, 15, and 20 min for [^{3}H]-DHE, [^{3}H]-prazosin and [^{3}H]-rauwolscine, respectively. Thereafter the filtration procedure was started and was completed within 5–15 min.

Specific binding of each tritiated substance was defined as that which exceeded the binding in blanks containing 10^{-5} M phentolamine. To determine the concentration of radioactive ligand in the assay, an aliquot of 20 µl was taken from some tubes and counted for tritium activity. An estimation of free radioligand concentration in the saturation experiments was obtained from the difference between added and bound ^{3}H-ligand. Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard and 50 mM Tris-HCl buffer as a blank. Outliers were checked for by Dixon's gap test (Bliss, 1967). The mean values from duplicate or triplicate determinations were used for calculations. The linear least square fit of lines to the various plots was used where appropriate. K_i values were determined by the method of Cheng & Prusoff (1973).

Functional studies

The bladder was opened and the trigonal area and the internal urethral orifice identified. Two circular transverse sections, each 2–4 mm long, were taken from the middle and upper parts of the urethra. Each ring-formed preparation was transferred to a temperature controlled organ bath filled with Krebs solution (composition, see below) maintained at 37°C

and bubbled with a mixture of 95% O_2 and 5% CO_2 to give a pH of approximately 7.4. The preparation was mounted between two L-shaped steel hooks placed within the lumen of the urethral ring. One hook was connected to a force transducer (Statham FT 03) for registration of isometric tension. The other hook was fixed to a movable unit allowing adjustments of tension. The urethral rings were repeatedly stretched and were then allowed to relax until a stable tension level of approximately 6 mN was obtained.

After an equilibration period of 60 min, the preparations were exposed to 6.2×10^{-5} M noradrenaline (NA), added non-cumulatively, which in separate experiments was found to produce 90 ± 2% of the maximum response obtained when NA was added cumulatively. Concentration-response curves for NA, phenylephrine (PE), and clonidine were obtained by increasing the drug concentration cumulatively. The effects of α-adrenoceptor blockers were tested on a submaximal contractile response produced by a single concentration of agonist. Submaximal concentrations of agonists were used to maintain reproducibility. The blocking drug was added when two consecutive, reproducible (less than 10% variation) responses were achieved; the contact time for the antagonist was 10–20 min. The substances were washed out when a maximum response was reached.

Calculations The results are given as mean ± s.e.mean. IC_{50} and EC_{50} values were determined graphically from the curves. For binding studies, *n* denotes the number of experiments, whereas for functional studies *n* represents the number of urethral rings tested.

Solutions and drugs

Binding studies [^{3}H]-dihydro-α-ergocryptine was diluted in an aqueous solution containing 40% ethanol, giving a final concentration of approximately 1.6% ethanol in the assay tubes. [^{3}H]-rauwolscine was diluted in 1 mM HCl containing 20% ethanol (resulting in approximately 1.6% ethanol in the assay tubes). [^{3}H]-prazosin was diluted in 1 mM HCl.

Due to the difficulties in dissolving prazosin, two stock solutions containing 2.5×10^{-3} and 2.5×10^{-4} M prazosin were made by adding a few drops of methanol and then dissolving prazosin in 1 mM HCl, under gentle heat. In the highest concentration, prazosin had a tendency to precipitate at room temperature and was therefore gently heated just before being added to the assay tubes. In the lower concentration this was not observed. The high concentration stock solution was used for preparing the 2 highest concentrations of prazosin used experimentally; the low concentration stock solution was used for the rest. All dilutions from these stock solutions were made in redistilled water. The effect of NA on [^{3}H]-DHE binding was determined as previously described (Larsson, 1983). All other drugs were dissolved and serially diluted in redistilled water.

Functional studies The Krebs solution used in the functional studies had the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2 and glucose 11. Prazosin was dissolved in ethanol, whereas all other drugs were dissolved in 0.9% w/v NaCl solution (saline). Subsequent dilutions of the drugs (including prazosin) were made with saline.

Drugs and radiochemicals

[^{3}H]-dihydro-α-ergocryptine (21.9–30.5 Ci mmol^{-1}), and [^{3}H]-rauwolscine (84.4 Ci mmol^{-1}) were obtained from New England Nuclear, and [^{3}H]-prazosin (20.2 Ci mmol^{-1}) from Amersham. Other drugs used were phentolamine methanesulphonate (Ciba-Geigy), rauwolscine HCl (Roth), prazosin HCl (Pfizer), clonidine HCl (Boehringer Ingelheim), (−)-noradrenaline HCl (Aldrich), (±)-noradrenaline, (−)-phenylephrine (Sigma).

Chemicals used were of analytical grade. Redistilled water was used for preparing all solutions.

Results

Binding studies

When the selective α_1-adrenoceptor antagonist prazosin, and the selective α_2-adrenoceptor antagonist rauwolscine were used to inhibit specific [^{3}H]-DHE binding to membranes prepared from the female rabbit bladder base and urethra, biphasic displacement curves were obtained (Figure 1). The prazosin displacement curve reached a plateau between concentrations of 10^{-8} to 10^{-6} M. Using the mean values of the inhibition measured at these concentrations, the level of the plateau was estimated at 22 ± 1% inhibition. Similarly the rauwolscine inhibition curve reached a plateau between concentrations of 3×10^{-7} to 10^{-5} M, corresponding to an inhibition of 81 ± 2%.

A number of α-adrenoceptor agonists competed for specific [^{3}H]-DHE binding in the following order of potency; clonidine (log $K_i = -6.96 \pm 0.18$; $n = 5$) > (−)-noradrenaline (log $K_i = -6.36 \pm 0.20$; $n = 5$) > (−)-phenylephrine (log $K_i = -5.49 \pm 0.09$; $n = 5$) (Figure 2).

No additional inhibition of [^{3}H]-DHE binding was obtained when any of these agonists or antagonists were added in high concentrations together with

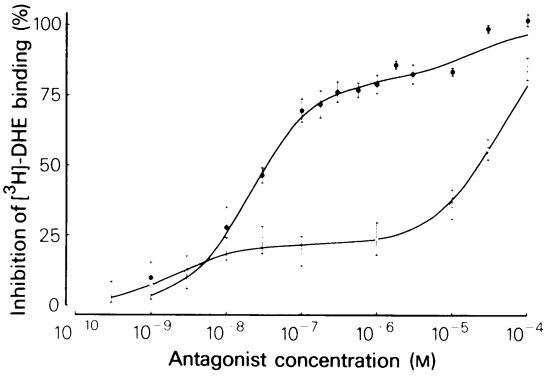

Figure 1 Inhibition of [^{3}H]-dihydro-α-ergocryptine ([^{3}H]-DHE) binding by prazosin (○) and rauwolscine (●). Membrane suspensions were incubated with 1 nM [^{3}H]-DHE in the presence of the indicated concentrations of the antagonists. The results are shown as % inhibition of specific binding ($n = 6$ for each antagonist). Assuming that binding occurred to two different binding sites, the lines were drawn according to the equation:

$$\% \text{ inhibition} = \frac{S_1 \times (L)}{K_1 + (L)} + \frac{S_2 \times (L)}{K_2 + (L)}$$

where S_1 and S_2 are the proportions of the respective binding sites, estimated from the plateau of the inhibition curves; K_1 and K_2 are the IC_{50} values for the inhibition to the respective binding sites; (L) is equal to the antagonist concentration used to inhibit [^{3}H]-DHE binding.

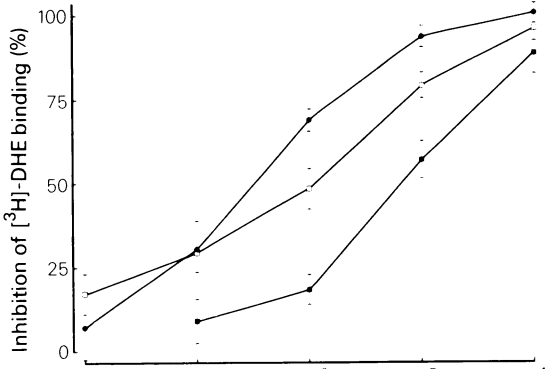

Figure 2 Inhibition of [^{3}H]-dihydro-α-ergocryptine ([^{3}H]-DHE) binding by α-adrenoceptor agonists, clonidine (●), (−)-noradrenaline (□) and (−)-phenylephrine (■). Membrane suspensions were incubated with 1 nM [^{3}H]-DHE in the presence of the indicated concentrations of the agonists. The results are shown as % inhibition of specific binding ($n = 5$ for each agonist).

10^{-5} M phentolamine, when compared to separate applications of 10^{-5} M phentolamine, suggesting that these substances compete for the same binding sites.

When the displacement of specific [^{3}H]-DHE binding was measured in the presence of 3×10^{-8} M prazosin, a 25 ± 4% ($n = 7$) inhibition of binding was found. The corresponding inhibition for rauwolscine, in a concentration of 3×10^{-7} M, was 75 ± 3% ($n = 7$). When these two substances were incubated together in the previously mentioned concentrations (in the same membrane preparation as that used for prazosin and rauwolscine displacement), the displacement of specific binding was 93 ± 3% ($n = 7$).

Saturation experiments with [^{3}H]-DHE, [^{3}H]-prazosin and [^{3}H]-rauwolscine were performed to determine the numbers of α-adrenoceptors and α_1- and α_2-adrenoceptors in the rabbit bladder base and urethra. [^{3}H]-DHE, a non-selective α-adrenoceptor blocker, was found to bind to a saturable number of binding sites amounting to 76 ± 7 fmol mg^{-1} protein (Table 1, Figure 3), as revealed by Scatchard plots. When the results from the saturation studies with [^{3}H]-prazosin and [^{3}H]-rauwolscine were treated in a similar manner, finite numbers of receptors were found for both of these substances with B_{max} values of 19 ± 4 and 51 ± 6 fmol bound/mg protein, respectively (Table 1, Figure 3). The Scatchard plots for the specific binding of [^{3}H]-DHE, [^{3}H]-prazosin and [^{3}H]-rauwolscine were all linear, suggesting binding to a single population of binding sites, i.e. the ligands had an equal affinity to the respective receptors they were bound to.

Functional studies

The non-selective α-adrenoceptor agonist NA as well as the selective α_1- and α_2-adrenoceptor agonists, PE and clonidine respectively, caused contractile responses of the rabbit urethra (Figures 4 and 5). The contractions induced by clonidine developed more slowly than those induced by NA and PE, and relaxation after wash-out of the agonist was also slower (Figure 4). The EC_{50} value for NA was 1.2×10^{-5} M ($n = 36$), for PE 4.3×10^{-6} M ($n = 6$) and for clonidine 2.3×10^{-7} M ($n = 6$). PE produced the same maximum contractile response as 6.2×10^{-5} M NA (105 ± 9%; $n = 6$), whereas the maximum response induced by clonidine was lower (73 ± 9%; $n = 6$) than that to NA.

The inhibitory effects of prazosin (10^{-6} M) and rauwolscine (1.5×10^{-6} M) were studied on contractions produced by a single concentration of NA (7.8×10^{-6} M). Prazosin depressed the NA-induced contractions by 84 ± 7% ($n = 6$), while rauwolscine inhibited these contractions by 52 ± 4% ($n = 7$).

The inhibitory effects of the same concentrations of rauwolscine and prazosin were also tested on

Table 1 Results from Scatchard plots obtained from saturation studies with the indicated tritiated ligands

Ligand	K_D (nM)	B_{max} (*fmol mg^{-1} protein*)	n
[^{3}H]-dihydro-α-ergocryptine ([^{3}H]-DHE)	0.8±0.1	76±7	6
[^{3}H]-rauwolscine	8.1±1.1	51±6	6
[^{3}H]-prazosin	0.4±0.1	19±4	5

Each experiment was performed in triplicate with 5–7 different concentrations of the ligand; n = number of determinations.

contractions induced by a single concentration of PE (7.9×10^{-6} M). It was found that these contractile responses to PE were inhibited by rauwolscine 34±5% ($n = 11$) and by prazosin 89±4% ($n = 6$). For clonidine (10^{-6} M) induced contractions, the corresponding values were 69±5% ($n = 5$), and 65±8% ($n = 5$), for prazosin and rauwolscine, respectively. However when a lower concentration of clonidine (10^{-7} M) was used to induce contractions, rauwolscine abolished the responses ($n = 6$), whereas in the presence of prazosin, only 13±4% of the contraction was blocked ($n = 6$).

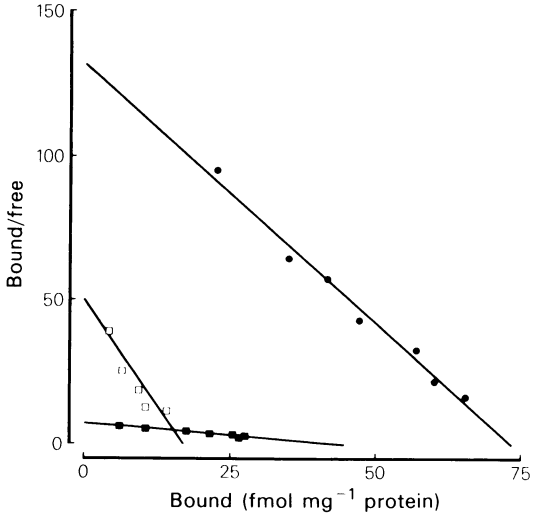

Figure 3 Scatchard plots for [^{3}H]-DHE (●), [^{3}H]-prazosin (□) and [^{3}H]-rauwolscine (■). Each plot represents one experiment performed in triplicate and replicated 6 times for [^{3}H]- DHE and [^{3}H]-rauwolscine, and 5 times for [^{3}H]-prazosin. The K_D and B_{max} values for these curves were 0.6, 0.4 and 6.3 nM and 71, 17, and 45 fmol mg^{-1} protein for [^{3}H]-DHE, [^{3}H]-prazosin and [^{3}H]-rauwolscine, respectively.

Discussion

It is well known that the highly selective α_1-adrenoceptor antagonist prazosin can give biphasic inhibition curves when various concentrations of this drug are used to inhibit the binding of a fixed concentration of [^{3}H]-DHE (Miach *et al.*, 1978; Greenslade *et al.*, 1979; Hoffman *et al.*, 1979; Hasegawa & Townley, 1982; Ito *et al.*, 1982). Since [^{3}H]-DHE is claimed to bind to α_1-and α_2-adrenoceptors with equal affinity (Hoffman *et al.*, 1979; Hoffman & Lefkowitz, 1980), the two phases of the inhibition curve may be assumed to represent displacement of [^{3}H]-DHE from the α_1- and α_2-adrenoceptors, respectively. The plateau of the inhibition curve will then represent the proportion of the subtype to which the ligand has selectivity, as compared to the total receptor population occupied by the ^{3}H-ligand. Hoffman *et al.*, (1979) concluded that the discriminating power of prazosin for the α_1- and α_2-

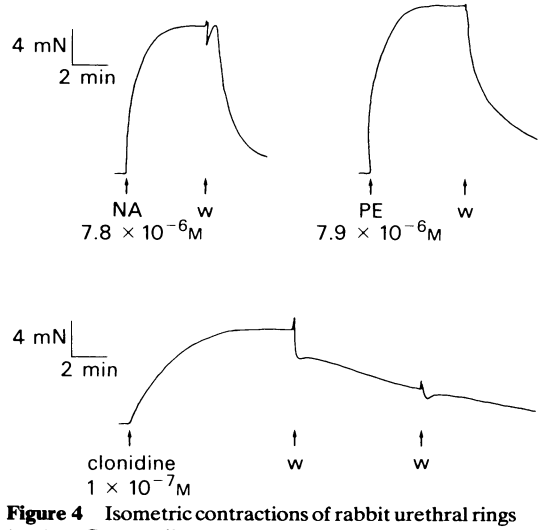

Figure 4 Isometric contractions of rabbit urethral rings *in vitro*. Contractile responses were induced by noradrenaline (NA), phenylephrine (PE) and clonidine in the concentrations shown at the arrows; the drugs were removed by washing at W. Note the long onset and offset time in the case of clonidine.

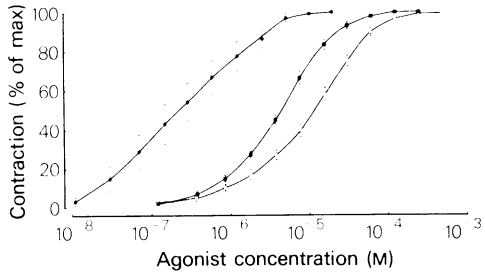

Figure 5 Concentration-response curves for clonidine (●) ($n = 6$), (−)-phenylephrine (■) ($n = 6$), and (±)-noradrenaline (□) ($n = 36$) added cumulatively to rabbit urethral ring preparations. 100% denotes the maximum response elicited by each agonist.

adrenoceptors in the rabbit uterus 'is of such magnitude that the proportion of the subtypes and the dissociation constants of the ligand for each subtype could be graphically estimated from the displacement curve itself. However visual, estimates from the yohimbine displacement curve would not provide reliable estimates because of the partial overlap of the two components'. Since rauwolscine has been reported to have a higher affinity for α_2-adrenoceptors than yohimbine (Weitzell *et al.*, 1979; Langer & Shepperson, 1981; Starke, 1981), we used rauwolscine as a selective α_2-adrenoceptor antagonist in our studies.

When prazosin was used to inhibit specific [^{3}H]-DHE binding to crude membrane preparations from the rabbit bladder base and urethra, well marked biphasic displacement curves were obtained. The level of the plateau indicated that the α-adrenoceptor population consisted of 22% α_1-adrenoceptors and accordingly 78% α_2-adrenoceptors. The IC_{50} value, estimated graphically from the mean curve for the first phase (α_1-adrenoceptors), was approximately 2.1×10^{-9} M, and the corresponding value for the second phase (α_2-adrenoceptors) approximately 3.9×10^{-5} M. Hence, prazosin appeared to have an 18,500-fold higher affinity for α_1- than α_2-adrenoceptors in these experiments. If the IC_{50} value for prazosin at the α_1-adrenoceptor is used in the equation described by Cheng & Prusoff (1973), a K_i value of approximately 0.9 nM is obtained, which is similar to the K_D value of 0.4 nM shown for [^{3}H]-prazosin in Table 1.

The plateau of the rauwolscine inhibition curve was not as marked as that for prazosin, indicating that rauwolscine is not as selective for α_2-adrenoceptors as prazosin is for α_1-adrenoceptors. The level of the plateau of the rauwolscine inhibition curve revealed the proportion of α_2-adrenoceptors to be 81% of the total α-adrenoceptor population and hence the proportion of α_1-adrenoceptors to be 19%. This is in good agreement with the values found for prazosin. The discriminating power for the α_1- and α_2-adrenoceptor subtypes by rauwolscine was graphically estimated to be approximately 900 times. When the K_i value for rauwolscine at α_2-adrenoceptors was calculated as above a value of 8 nM was obtained, which is equal to the K_D value of 8 nM shown for [^{3}H]-rauwolscine in Table 1.

It seems reasonable to assume that prazosin (3×10^{-8} M) and rauwolscine (3×10^{-7} M) displaced different subdivisions of the total α-adrenoceptor population labelled by [^{3}H]-DHE, since the inhibitory effect of these substances was nearly additive under the described assay conditions. Therefore we conclude that the two phases of the prazosin and rauwolscine inhibition curves represent inhibition of the respective α-adrenoceptor subtype.

Further evidence was obtained from saturation experiments with [^{3}H]-DHE, [^{3}H]-prazosin, and [^{3}H]-rauwolscine. From the sum of the binding sites, [^{3}H]-prazosin constitutes 27% and [^{3}H]-rauwolscine 73% of the total number of receptors labelled by these two drugs, that total closely agreeing with the number found for [^{3}H]-DHE. These proportions of α_1- and α_2-adrenoceptors parallel the previously estimated values from the displacement curves.

From radioligand binding studies, it is difficult to determine the cellular location of the labelled receptors. As α-adrenoceptors are known to exist on e.g. adrenergic and cholinergic nerve terminals and blood vessels, presumably some of these sites became labelled in these experiments, together with α-adrenoceptors on the smooth muscle. To study the postjunctionally located α-adrenoceptors we therefore examined some responses of the female rabbit urethral smooth muscle *in vitro*.

NA as well as the α_1-adrenoceptor selective agonist PE and the α_2-adrenoceptor selective agonist clonidine induced contractions of urethral smooth muscle. Although these data suggest that both α_1- and α_2-adrenoceptors are located postjunctionally and are involved in the contractile response, it is well known that PE and clonidine are not as selective for their respective α-adrenoceptor subtype as the antagonists prazosin and rauwolscine and so experiments were performed with the latter drugs. It was found that prazosin was almost able to abolish the submaximal contractions induced by PE, but influenced less the contractions induced by clonidine (10^{-6} M); against NA, the inhibitory effect of prazosin was intermediate between the former two values. However, when rauwolscine was used as the antagonist, the potency order was reversed. Furthermore, when a lower, and presumably 'more selective', concentration of clonidine (10^{-7} M) was used, rauwolscine abolished its contractile effect, whereas prazosin caused only about 10% blockade of these

contractions. Thus, both α_1- and α_2-adrenoceptors seem to be present postjunctionally and both mediate contraction.

A strict comparison between binding and functional studies is often difficult to make. In addition to different assay conditions agonists are frequently found to interact with [^{3}H]- DHE labelled α-adrenoceptors in binding experiments, in a way which results in concentration-inhibition curves with shallow slopes. In the present study the agonists NA, PE and clonidine were all found to interact with the α-adrenoceptors labelled by [^{3}H]-DHE, resulting in flat concentration-inhibition curves. This might be explained by, e.g., different affinities of the agonist for the different α-adrenoceptor subtypes, or possibly to a negatively cooperative interaction with the receptor. Alternatively the agonists might bind to a high and a low affinity binding site for one receptor subtype. This has been reported for the α_2-adrenoceptor (Hoffman *et al.*, 1980), but is questioned for the α_1-adrenoceptor (Stiles *et al.*, 1983). The interaction between an agonist and the α_2-adrenoceptor has been shown to be affected by guanine nucleotides (Hoffman *et al.*, 1980). Possibly an individual cell could regulate the concentration of these nucleotides, and therefore also its reactivity to α-adrenoceptor stimulation. The finding that our membrane homogenate contains about 75% α_2-adrenoceptors could well explain why we obtained shallow agonist inhibition curves. Furthermore, no β-adrenoceptor blockers or neuronal or extraneuronal NA uptake blockers were used in the functional studies, the presence of which would probably alter the EC_{50} values.

In conclusion α-adrenoceptors, consisting of approximately 25% α_1-adrenoceptors and 75% α_2-adrenoceptors, have been found in crude membrane preparations from the rabbit bladder base and urethra. It appears that some of both types of α-adrenoceptor are located postjunctionally on the urethral smooth muscle and that each type is able to induce contraction.

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