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The contribution of α-adrenoceptors to neurally-mediated contractions of the rabbit urethral smooth muscle

[1]Hong-I Chen & *Alison F. Brading

Department of Urology/Surgery, 804 Army General Hospital, Taoyuan, Taiwan and *University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT

1 The nature of the nerve-mediated contractions in the urethral smooth muscle from the rabbit was studied *in vitro*. Field stimulation caused smaller contractile responses than in the detrusor of the rabbit.

2 There was no significant difference in response to field stimulation or exogenous agents acting on adrenoceptors between longitudinal and circular strips from the rabbit urethra. Histological studies showed that the urethral muscle is arranged in three layers, which run circularly and longitudinally.

3 Atropine had very little effect on the response to field stimulation, phentolamine almost abolished the contractile response to nerve stimulation and sometimes unmasked a relaxation.

4 The α_1-adrenoceptor blocking agent, prazosin, blocked both the contractile response to the α_1-receptor agonist phenylephrine and that to intrinsic nerve stimulation, with similar potencies. The α_2-blocking agent yohimbine shifted the dose-response curve of the contractile response to the α_2-agonist, clonidine, in a dose-dependent manner, 10^{-7} M causing a 10 fold shift. This concentration had no effect on the response to intrinsic nerve stimulation, suggesting that α_2-receptors are not involved in the response. Higher concentrations of yohimbine caused a suppression of the nerve-evoked response which is assumed to be non-specific.

5 Noradrenaline, phenylephrine, and clonidine caused dose-dependent contractile responses in the rabbit urethral strips. The contractions induced by clonidine developed more slowly than those induced by noradrenaline and phenylephrine.

6 These results demonstrate that the rabbit urethral smooth muscle contains both α_1- and α_2-adrenoceptors, and the nerve-mediated contraction of the rabbit urethra is adrenergic in nature and mediated mainly via α_1-adrenoceptors.

Keywords: α_1-Adrenoceptor; α_2-adrenoceptor; rabbit urethra; adrenergic innervation; field stimulation

Introduction

Adrenergic innervation of the bladder and urethra smooth musculature has been extensively demonstrated in animals by various investigators (Levin & Wein, 1979; Andersson *et al.*, 1984; Gosling, 1986). There is general agreement, however, that the smooth muscle of the bladder and proximal urethra in both a variety of animals and in humans contains both α- and β-adrenoceptors. These receptors play an important role in the filling/storage phase of the micturition cycle (Wein, 1987). In the urethra, smooth muscle tone is believed to be controlled by the sympathetic nervous system via α-adrenoceptors (Donker *et al.*, 1972; Awad & Downie, 1976). α-Adrenoceptor-mediated responses predominate in the bladder base and proximal urethra, whereas β-adrenoceptor-mediated responses predominate in the bladder body (Edvardsen & Setekleiv, 1968; Edvardsen, 1968; Anderson *et al.*, 1971; Elmer, 1974; Benson *et al.*, 1976; Levin *et al.*, 1980).

It is generally accepted that there are two major α-adrenoceptor subtypes, the α_1- and the α_2-adrenoceptors (Rang & Dale, 1987). By means of selective agonists and antagonists these α-adrenoceptor subtypes have been characterized in different tissues and species (Wikberg, 1979; Davey, 1986).

Andersson & Sjögren (1982) have established that both α-subtypes are present and 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.

The purpose of the present study was to examine the nature of the nerve-mediated contractions in the urethral smooth muscle from the rabbit *in vitro* by field stimulation of muscle strips, and recording the isometric tension generated. To investigate α-adrenoceptor subtypes in the urethra, the nature of the responses to the exogenously applied selective adrenoceptor agonists isoprenaline, phenylephrine and clonidine were examined. The inhibition of the responses to adrenoceptor agonists by their antagonists was also examined.

[1] Author for correspondence.

Methods

Preparation of specimens

Rabbit urethra were obtained from New Zealand White rabbits the organs of which were being used for experiments by other research groups in the University. They were of either sex, weighing from 600 g to 2500 g. The animals were stunned by a blow to the neck and exsanguinated. The specimens were placed in oxygenated Krebs solution. The mucosa was then dissected free from the urethral muscles. The urethral strips were made in either a longitudinal or transverse direction. An operating microscope was used to ensure that there was good longitudinal alignment of the muscle bundles within a strip. Strips of lower urinary tract smooth muscle measuring approximately 8 mm × 1 mm × 1 mm were prepared.

Tension recording and stimulation

Fine silk ligatures were tied to each end of a strip which was then mounted between platinum ring electrodes 1 cm apart in a specially constructed Perspex organ bath. The organ bath had a capacity of 0.2 ml and was continuously perfused with warmed (35–37°C) Krebs solution at a flow rate of 1 ml min^{-1} (Brading & Sibley, 1983). Initially, the strips were

allowed to equilibrate for at least 1 h, after a resting tension of 1 g had been applied. Tension was measured isometrically with Pioden UF1 transducers and recorded on a Watanabe multichannel pen recorder after amplification.

Activation of intrinsic nerves was achieved by electrical field stimulation by pulses with the following parameters: 50 V, 0.05 ms width, 5 s trains at varying frequency. Successive trains of stimuli were given at least 5 min after the previous contraction had returned to baseline. After each drug-induced response, recovery periods of 10–30 min were allowed before further drug application. Drugs and solutions were applied by dipping the ends of the feeder tubes for the perfusion system into the appropriate solutions. This allowed accurately timed exposure of the tissues to different solutions, and by following the bubbles introduced when the solution was changed the instant of tissue contract was recorded.

At the start of each experiment, the contractile response of the strips to a 2-min application of 126 mM KCl was obtained and subsequent responses were recorded as a percentage of the control response. This dose of KCl produced a near maximal contraction.

Drugs

The following drugs were used: hexamethonium bromide, tetrodotoxin (TTX), α,β-methylene adenosine 5′-triphosphate (α,β-methylene ATP), prazosin hydrochloride, clonidine hydrochloride, (−)-phenylephrine (all these drugs were obtained from Sigma); atropine sulphate and (−)-noradrenaline (obtained from B.D.H.); propranolol hydrochloride (I.C.I), isoprenaline sulphate (Burroughs Wellcome), phentolamine mesylate (Ciba). Drugs were, where possible, dissolved in distilled water to make a concentrated stock solution; these were refrigerated until needed. α,β-methylene ATP was stored in the deep freeze, and stock solution made up freshly for each experiment. Prazosin was dissolved in 1:9 of DMA (N N-dimethylacetamide, B.D.H.): distilled water, and subsequent dilution of the drug was made with Krebs solution. (−)-Phenylephrine or noradrenaline stock was prepared with added ascorbic acid (0.1 mg) to prevent oxidization. Drug concentrations are given as the final bath values. The vehicle was checked and had no effect. The Krebs solution used had the following composition (mM): NaCl 120.0, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, NaH_2PO_4 1.0 and glucose 11.5. All solutions were equilibrated with 97% O_2: 3% CO_2, pH 7.4 at 35–37°C. High K^+ solution (126 mM) was prepared by replacing NaCl with an equimolar amount of KCl in normal Krebs solution.

Statistical analysis

Student's *t* test and analysis of variance were used to compare differences in responses between the control and experimental curves. A probability level of $P < 0.05$ was accepted as significant. When appropriate, results are presented as means ± standard error of the mean (s.e.mean).

Results

The rabbit urethra is approximately 1 cm in length, in both male and female. Microscopically, the urethra comprises an outer muscle coat and an innner transitional epithelium. Transverse sections through the proximal and distal portion of the urethra and longitudinal sections through the whole urethra showed that muscle bundles are arranged in three ill-defined layers. Longitudinally-orientated muscle bundles predominate on the inner and outer aspects of a substantial middle layer composed of more circularly-disposed bundles.

Small contractile responses to field stimulation (approximately 30% of the response in the anterior detrusor) were obtained in urethral strips. Figure 1 shows the frequency-dependency of the response in comparison with the detrusor

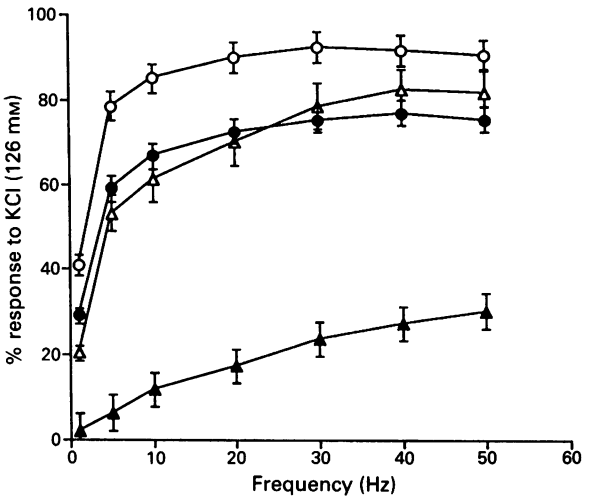

Figure 1 Frequency-response curves of the anterior detrusor (○, $n = 45$), posterior detrusor (●, $n = 48$), trigone (△, $n = 27$) and longitudinal urethral strips (▲, $n = 13$) from male rabbits. Stimulus parameters: 50 V, 0.05 ms width, 5 s trains. Vertical bars indicate s.e.mean.

and trigone. Occasionally, a slight relaxation followed by contraction was observed in the urethral strips. There were no significant differences in the response to field stimulation between longitudinal and circular strips from the rabbit urethra ($n = 8$).

Unlike rabbit urinary bladder, in the urethral strips 10^{-7} M atropine had very little effect on the response to field stimulation; the non-selective α-adrenoceptor blocker phentolamine (2×10^{-7} M), however, almost abolished the contractile response to nerve stimulation and sometimes unmasked a relaxation (Figure 2). To investigate the subtypes of the adrenergic nervous influence, selective α_1- and α_2-blockers were used. The results revealed that the α_2-blocker yohimbine (10^{-7} M) had no effect on the contractile responses to field stimulation ($n = 8$), and the α_1-blocker prazosin (10^{-7} M and 10^{-6} M) caused dose-dependent inhibition of the contractile response to field stimulation. The inhibition of field stimulation by the combination of prazosin and yohimbine (both 10^{-7} M, $n = 13$) was no different from that due to 10^{-7} M prazosin alone ($n = 7$). Interestingly, 10^{-6} M yohimbine produced a large reduction in the contractile response at all stimulation frequencies ($n = 6$). This reduction was similar to that caused by 10^{-6} M prazosin ($n = 6$) (Figure 3). All responses to transmural stimulation were blocked completely by TTX (0.1 μg ml^{-1}).

In experiments on strips from rabbit urethra, noradrenaline caused a dose-dependent contraction, the maximum response being reached after a 2 min application of 5×10^{-5} M noradrenaline. There was no difference in the contractile response to noradrenaline between circular and longitudinal muscle strips. This response could be inhibited by the α-blocking agent phentolamine (10^{-6} M), which unmasked a small relaxation response in some strips (Figure 4). To characterize the subtypes of α-receptor in the urethral strips, the α_1-receptor agonist phenylephrine, α_2-receptor agonist clonidine, selective α_1-blocking agent prazosin and α_2-blocking agent yohimbine were used. Dose-dependent contractile response to phenylephrine, noradrenaline and clonidine in the rabbit urethra strips were obtained (Figure 5), the maximum response induced by phenylephrine was reached with a 2 min application of 3×10^{-4} M, and for clonidine with a 10 s application in a concentration of 10^{-5} M. The contractions induced by clonidine developed more slowly than those induced by noradrenaline and phenylephrine, and the relaxation after wash-out of the agonist was also slower (Figure 4).

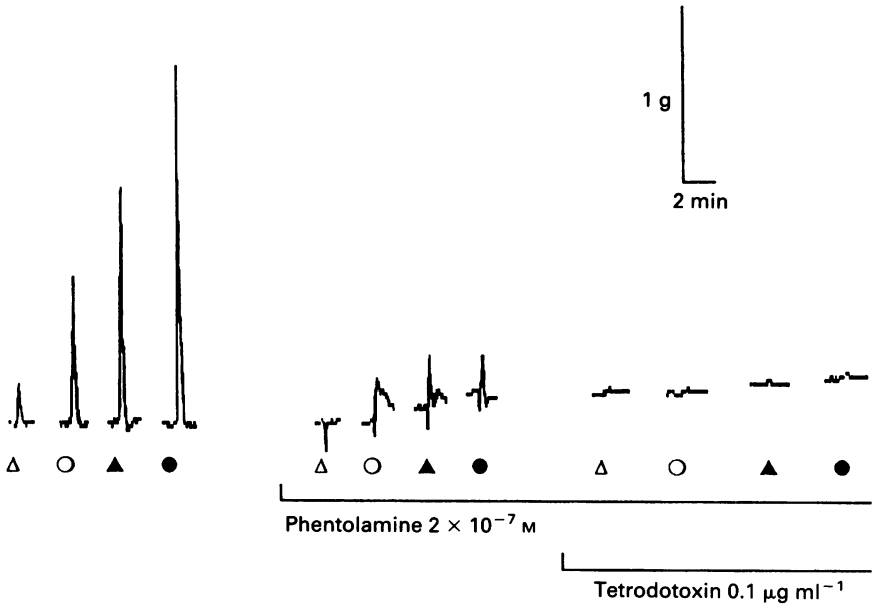

Figure 2 Field stimulation-induced contraction at 5 Hz (Δ), 10 Hz (○), 20 Hz (▲) and 40 Hz (●) in male rabbit urethra before and after exposure to phentolamine 2×10^{-7} M for 20 min. The effects of adding tetrodotoxin 0.1 μg ml^{-1} on the response is shown. Stimulus strength 50 V; stimulus duration 0.05 ms; stimulus train 5 s.

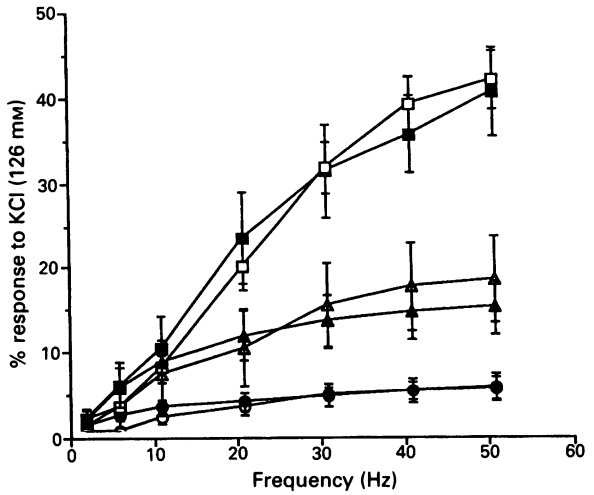

Figure 3 Effect of prazosin and yohimbine on the contractile responses to field stimulation in the male rabbit urethra. Yohimbine 10^{-7} M (■, $n = 8$) had no effect on the control (□) contractile responses to field stimulation; prazosin (10^{-7} M, Δ; 10^{-6}, ●) caused dose-dependent inhibition of the contractile response to field stimulation. The inhibition of field stimulation by the combination of prazosin and yohimbine (both 10^{-7} M, ▲, $n = 13$) was no different from that due to 10^{-7} M prazosin ($n = 7$) alone. Yohimbine 10^{-6} M (○, $n = 6$) produces a large reduction of contraction which is similar to that caused by 10^{-6} M prazosin ($n = 6$). Vertical bars represent s.e.mean.

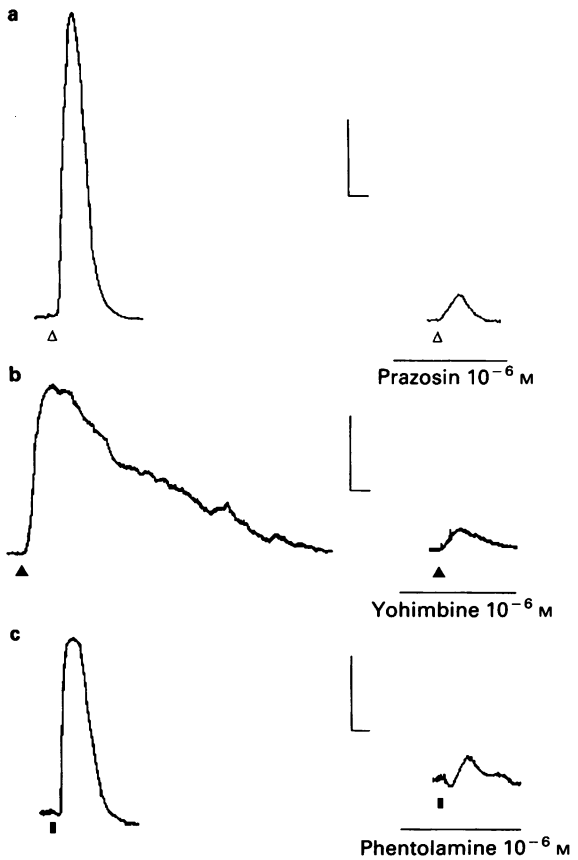

Figure 4 Contractile responses to noradrenaline (■, c), phenylephrine (Δ, a) and clonidine (▲, b) at the same concentration of 10^{-5} M in rabbit urethral strips. The contractions induced by clonidine (10 s application) developed more slowly than those induced by noradrenaline (2 min application) and phenylephrine (2 min application) and relaxation after contraction was also slower. The contraction was blocked after a 30 min exposure to the blockers. Horizontal bar represents 2 min. Vertical bar represents 0.5 g of tension.

The EC_{50} value for noradrenaline was 8.3×10^{-7} M ($n = 12$), for phenylephrine it was 4.5×10^{-6} M ($n = 16$) and for clonidine it was 3.0×10^{-6} M ($n = 6$). The contractile response to 3×10^{-4} M phenylephrine was larger than the contractile response due to 5×10^{-5} M noradrenaline (118.6 ± 2.2% of NA response; $n = 12$), whereas the contractile response to clonidine 5×10^{-5} M was smaller (60.2 ± 2.4% of NA response; $n = 9$) than that due to noradrenaline.

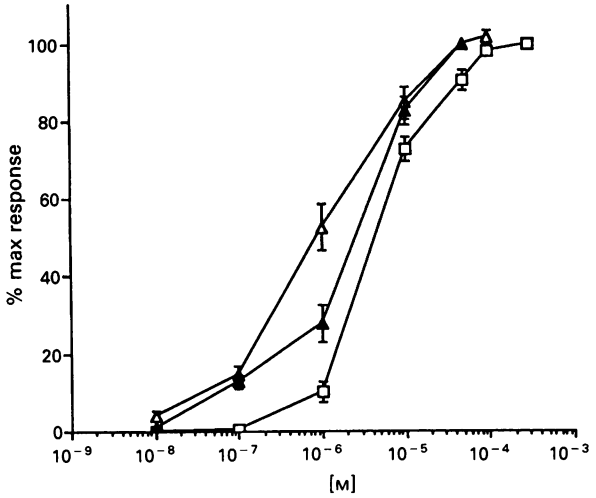

Figure 5 Dose-dependent contractile responses to noradrenaline (△, $n = 12$), clonidine (▲, $n = 6$) and phenylephrine (□, $n = 16$) in the rabbit urethral strips. The EC_{50} value for noradrenaline is $8.3 \pm 0.7 \times 10^{-7}$ M, for clonidine, $3.0 \pm 0.8 \times 10^{-6}$ M and for phenylephrine, $4.5 \pm 0.1 \times 10^{-6}$ M. 100% denotes the maximum response elicited by each agonist. Vertical bars indicate s.e.mean.

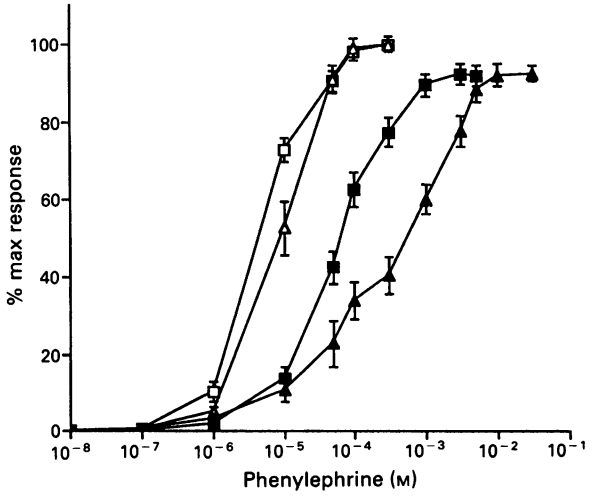

Figure 6 Effect of prazosin on the contractile response to phenylephrine in the rabbit urethra. Results showed that prazosin (10^{-8} M, △, $n = 10$; 10^{-7} M, ■, $n = 12$; 10^{-6} M, ▲, $n = 10$) cause dose-dependent inhibition, the response curves shifted to right of the control (□). The K_B value from the Schild plot was 7.2×10^{-9} M. Vertical bars show s.e.mean.

Prazosin caused a dose-dependent inhibition of the phenylephrine-induced contraction (Figure 6). Prazosin (10^{-6} M) inhibited the contractile response to phenylephrine (5×10^{-5} M) by $74.7 \pm 4.3\%$ and inhibited that to phenylephrine (3×10^{-4} M) by $60 \pm 5\%$ ($n = 10$). The drug shifted the dose-response curve to the right with little change in the maximum indicating a competitive antagonist ($K_B = 7.2 \times 10^{-9}$ M). The α_2-blocker yohimbine also caused a dose-dependent inhibition of the contractile response to clonidine (Figure 7). It was not possible from the results to tell if the antagonism was competitive, since high enough concentrations of clonidine were not used. Yohimbine (10^{-6} M) inhibited the contractile response to clonidine (5×10^{-5} M) by $91 \pm 2\%$ ($n = 6$). It was found that there was no significant effect of prazosin on the clonidine-induced contraction ($n = 4$), or yohimbine on the phenylephrine-induced contraction ($n = 4$) in the urethral strips.

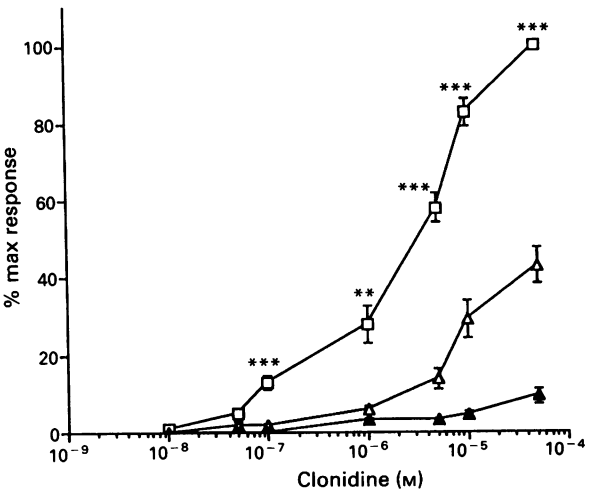

Figure 7 Effects of yohimbine 10^{-7} M (△, $n = 6$) and 10^{-6} M (▲, $n = 6$) on the contractile response to clonidine in the male rabbit urethral strips. It showed irreversible competitive antagonism on the clonidine concentration-effect curves. Control (□). Vertical bar represent s.e.mean. $^{**}P < 0.01$; $^{***}P < 0.001$.

Discussion

Studies of the effects of adrenergic nerves on the smooth muscle of rabbit urethra have been performed by isometric tension recording using electrical field stimulation and application of exogenous agents. Field stimulation caused smaller contractile responses than in the detrusor from the rabbit. There was no significant difference in response to field stimulation between longitudinal and circular strips from the rabbit urethra. This is in agreement with the findings of other studies (Mattiasson *et al.*, 1989). It is interesting to note that despite the histological evidence that the muscle is arranged in three layers, which run circularly and longitudinally, the pharmacological findings, such as responses to field stimulation, clonidine and phenylephrine showed no significant differences in behaviour between longitudinal and circular strips. This finding agrees with that of Andersson *et al.* (1984).

Phentolamine inhibited the nerve-mediated contraction in the majority of preparations and unmasked relaxation in some urethral strips, which was abolished by adding propranolol. However, atropine and α,β-methylene ATP had no effect on the contractile response to field stimulation in the rabbit urethra. This suggests that nerve-mediated contraction in the rabbit urethra is induced by α-adrenoceptor activation, but some effect via β-adrenoceptors may also be present in this tissue. It cannot be excluded that some other non-adrenergic non-cholinergic neurotransmitters are involved in the response of the urethra. Mattiasson *et al.* (1989) found that vasoactive intestinal polypeptide (VIP) completely relaxed both circular and longitudinal preparations from the female rabbit urethra which had been contracted by noradrenaline.

To study the postjunctional sub-types of α-adrenoceptors, we examined the responses of the rabbit urethral smooth muscle using selective α_1- and α_2-adrenoceptor agents and non-selective adrenoceptor agents. Noradrenaline, as well as the α_1-adrenoceptor selective agonist phenylephrine and the α_2-adrenoceptor selective agonist clonidine induced a dose-dependent contraction of the urethral smooth muscle. The α_1-blocker, prazosin, not only inhibited the contractile response to phenylephrine but also blocked nerve-mediated contraction in the urethra. A different result was noted with the

α_2-blocker, yohimbine. At 10^{-7} M, yohimbine had no effect on the contractile response to field stimulation, although at this concentration it markedly inhibited the contractile response to the α_2-agonist, clonidine. On the other hand, the inhibition of field stimulation by a combination of prazosin and yohimbine (both 10^{-7} M) was no different from that due to 10^{-7} M prazosin alone, suggesting the contractile response to nerve stimulation in the rabbit urethral preparations is mainly due to α_1-stimulation. Higher concentrations of yohimbine (10^{-6} M), which had no effect on the phenylephrine-induced contraction, produced clear inhibition of nerve-evoked contraction at all frequencies. This reduction was very similar to that caused by 10^{-6} M prazosin, suggesting that yohimbine has a non-selective effect on the response to intrinsic nerve stimulation at this concentration. However, these results demonstrated that the rabbit urethral smooth muscle contains both sub-types of α–adrenoceptors (Andersson *et al.*, 1984).

In common with cholinergic transmission, there is evidence for autoinhibition at adrenergic terminals, where NA acts on prejunctional α_2-adrenoceptors leading to negative feed back inhibition of the release of neurotransmitter (Langer, 1977; Burnstock, 1983). This is unlikely to be occurring in the present study because yohimbine had no specific effect on the contractile response to nerve stimulation as previously described. Nevertheless, the present result is in agreement with the finding of other studies that a significant part of the nerve-mediated contraction of full thickness preparations of the female rabbit urethra is adrenergic in nature and mediated mainly via α_1-adrenoceptors even if the muscle tissue contains a considerable number of α_2-adrenoceptors. The adrenoceptor population is reported to consist of 25% α_1- and 75% α_2-adrenoceptors (Andersson *et al.*, 1984; Mattiasson *et al.*, 1989).

In conclusion, the present results demonstrated that the rabbit urethral smooth muscle contains both sub-types of α-adrenoceptors, and the nerve-mediated contraction of the rabbit urethra is adrenergic nature and mediated mainly via α_1-adrenoceptors.

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