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The adrenergic, cholinergic and NANC nerve-mediated contractions of the female rabbit bladder neck and proximal, medial and distal urethra

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1 The nerve-mediated contraction of the female rabbit bladder neck and different portions of the urethra (proximal, medial and distal) was studied *in vitro* by electrical stimulation (50 V, 30 Hz, 0.05 ms width, trains of 5 s every 5 min) by use of a superfusion system.

2 The amplitude (E_{max}) and the duration (D_{max}) of the stimulated contraction were studied in the four tissues. The E_{max} value was significantly higher in distal urethra (2.07 ± 0.15 g) compared to the bladder neck (1.08 ± 0.10 g), proximal urethra (0.73 ± 0.07 g) and medial urethra (0.87 ± 0.07 g). In contrast, the D_{max} value appeared slightly but significantly lower ($P < 0.05$) in distal urethra (68.5 ± 2.3 s) than in bladder neck (76.7 ± 6.0 s), proximal urethra (84.5 ± 5.0 s) and medial urethra (81.3 ± 3.5 s).

3 Cocaine (1 μM) significantly increased the basal E_{max} values in medial and distal urethra and the basal D_{max} values in the four tissues.

4 Prazosin (1 μM) significantly reduced E_{max} value in proximal, medial and distal urethra and D_{max} value in bladder neck and proximal urethra. Atropine (1 μM) also significantly reduced E_{max} values in bladder neck and proximal urethra and reduced D_{max} value in bladder neck, but not in other tissues. Yohimbine (0.1 μM) was devoid of effect in the four tissues.

5 The association of prazosin (1 μM) and atropine (1 μM) did not modify the E_{max} and the D_{max} values of the electrically-induced contractions, except in proximal urethra and in bladder neck where an additive inhibitory effect (on E_{max} only) was observed compared to prazosin and atropine alone.

6 The residual contractile response after combined treatment with prazosin and atropine was significantly diminished by tetrodotoxin (TTX; 1 μM) but not completely abolished. These NANC contractions were insensitive to P2X-purinoceptor desensitization by continuous tissue perfusion with α,β-methylene ATP (30 μM).

7 These results demonstrate that bladder neck and proximal urethra are mainly innervated by the parasympathetic nervous system, whereas medial and distal urethras are to a greater extent under the control of the sympathetic innervation. The residual responses, insensitive to prazosin and atropine, may indicate a NANC innervation in the four tissues. However, the nature of the NANC neurotransmitter remains to be identified.

Keywords: Muscarinic receptors; α-adrenoceptors; non-adrenergic, non-cholinergic transmission; bladder neck, rabbit; urethra, rabbit; electrical-field stimulation; cocaine

Introduction

It is widely accepted that the tone of rabbit urethral smooth muscles and intraurethral pressure are largely maintained by the activity of the sympathetic nervous system (Andersson *et al*., 1984). Although, by binding studies, the α-adrenoceptor population in the female rabbit urethra has been found to consist of 25% α_1- and 75% α_2-adrenoceptors (Andersson *et al*., 1984; Mattiasson *et al*., 1990), pharmacological studies have shown that the nerve-mediated contraction of the rabbit urethral smooth muscle is mainly mediated via α_1-adrenoceptors (Mattiasson *et al*., 1990; Chen & Brading, 1992). The sympathetic innervation of the urethra has also been demonstrated in dog (Hashimoto *et al*., 1992) and, extensively, in man (Caine *et al*., 1975; Ek *et al*., 1977; Furuya *et al*., 1982). In the last tissue, α_1-adrenoceptors are the main (Kondo *et al*., 1993) or the only (Kunizawa *et al*., 1985) α-adrenoceptor subtype mediating contraction of the prostatic urethral smooth muscle.

The parasympathetic innervation of the rabbit urethra has not been well documented, possibly because there are fewer muscarinic binding sites in the rabbit urethra than in the bladder (Johns, 1983). In man, studies have shown that muscarinic receptor stimulation or blockade do not influence the resting urethral pressure (Ek *et al*., 1978).

Mattiasson *et al*. (1990) studied the morphology and the nerve-mediated functions in the circular and longitudinal muscle layers of the female rabbit proximal urethra. They observed that smooth muscles dominated in the different layers of this tissue, but they proposed that striated muscles may also contribute to the nerve-mediated responses, since a fast non-adrenergic non-cholinergic (NANC) contraction correlated well with the presence of these muscles.

The NANC innervation of the rabbit urethra is now well documented. Various authors have demonstrated that nitric oxide (NO) is a mediator of the electrically-induced relaxation of the rabbit urethra (Andersson *et al*., 1991; 1992; Zygmunt *et al*., 1993; Lee *et al*., 1994). Regional differences in the nerve-mediated contraction of the rabbit urethra have not been studied in detail so far. Some investigations have been performed on the isolated submucosal urethra (Mattiasson *et al*., 1985b; Zygmunt *et al*., 1996), or on the circular (Hashitani *et al*., 1996) and the longitudinal smooth muscles (Sjögren *et al*., 1988; Mattiasson *et al*., 1990). The only comparisons

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performed in rabbit urethral tissues were between bladder neck and proximal urethra (Ito & Kimoto, 1985; Lee *et al*., 1994).

In contrast, regional variations of urethral nerve-mediated contractions in other animal species have been investigated. In the female pig it was demonstrated that, although there were no histological regional differences in the distribution of parasympathetic nerves, responses to nerve stimulation and to muscarinic agonists were more pronounced in the proximal urethra (Bridgewater *et al*., 1995). The same authors also observed a regional variation in the distribution of the sympathetic nerves within the urethra of the female pig. In the dog, studies on the neurogenic responses of the proximal, medial and distal urethra concluded that the circular muscle is reciprocally innervated by sympathetic adrenergic and NANC nerves (Hashimoto *et al*., 1992).

The purpose of the present study was, therefore, to characterize the neurogenic contraction of the rabbit bladder neck and of the proximal, medial and distal urethras with atropine, prazosin and yohimbine. Moreover, we investigated the nature of the NANC neurotransmitters mediating contraction and we studied the effects of cocaine on the amplitude and the duration of neurogenic contraction in the absence and presence of these antagonists.

Methods

Preparations

Female white rabbits (New-Zealand; ESD, France), approximately 18 weeks old, were killed by cervical dislocation and exsanguination. Circularly oriented rings were cut from the region of the bladder just below the trigone (bladder neck), and the entire urethra was dissected in three circular rings of $\sim$4 mm in width, namely proximal, medial and distal urethras, respectively.

Contractile experiments

Each ring was tied with two fine silk ligatures between two platinum electrodes, 0.2 mm apart tissue, in Coleman's superfusion organ baths (Type 840, Hugo Sacks Elektronik, D-79232, March-Hugstetten, Germany). One end of the ligature allowed the tissue to be fixed to the bottom of the organ bath. At the other end, tissues were attached to a F30 isometric transducer (Type 372, Hugo Sacks Elecktronik) and force was recorded on a Grass model 7D polygraph (AstroMed Inc., West Warwick, Rhode Island, U.S.A.). Tissues were continuously superfused with oxygenated (95% O_2 and 5% CO_2) warmed (between 32 and 33°C) Krebs solution of the following composition (in mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7 and ascorbic acid 1.1; pH 7.4, at a flow rate of 2 ml min^{-1} with a peristaltic multichannel pump (Watson-Marlow, model 501S). Bladder neck and urethras were allowed to equilibrate at a resting tension of 1 g for at least 1 h. This tension was maintained throughout the experiment. The viability of the preparations was confirmed by a supramaximal KCl (126 mM)-precontraction. KCl and all the other drugs studied during the experiment were added in Krebs solution.

After one hour of tissue equilibration, preparations were electrically-stimulated with the following parameters: 50 V, 0.05 ms width, 5 s train width every 5 min (Chen, 1990). The frequency of 30 Hz, corresponding to $\sim$85% of the maximal contractile response obtained at 50 Hz (determined after a preliminary study with different frequencies from 1 to 50 Hz), was kept constant during the experiment. Tissues were allowed to equilibrate with stimulation pulses for at least one hour before drug addition and amplitudes of basal contractile responses were measured. The effects of the drugs on the electrically-induced contractions were observed for 60 min (12 spikes named T5 to T60). Parallel experiments were performed in the absence of any compound in order to determine the reproducibility of control contractile responses.

The effects of prazosin (1 μM), atropine (1 μM) and yohimbine (0.1 μM) were evaluated in the presence of cocaine (1 μM), which was added in the Krebs solution at the same time as the antagonists alone or in combination. The NANC contractile response was studied in the presence of prazosin (1 μM), atropine (1 μM) and cocaine (1 μM). Tetrodotoxin (1 μM) and (+)-tubocurarine (1 μM) were added at the end of the experiments whatever the antagonist studied. In order to identify the phasic and tonic stimulated contractions, recorder chart speed was set to 50 mm min^{-1}. The maximal amplitude of the stimulated contraction (E_{max}) was expressed in g of tension and corresponded to the whole spike amplitude, since we could not differentiate between the phasic and the tonic response in medial and distal urethra. The duration (D_{max}) of the contraction was calculated as the time, expressed in s, elapsed between the start of the electrical stimuli and the return to the baseline value. E_{max} and D_{max} values are presented as % of the mean response of the three last contractions before drug addition.

Statistical analysis

Results are expressed as mean $\pm$ s.e.mean. Basal E_{max} and D_{max} values were compared between the four tissues by a Bonferroni one-way analysis of variances. Results obtained with cocaine were compared to the control value by unpaired Student's *t* test.

Statistical differences between responses obtained in the presence of cocaine (control) and of prazosin and atropine alone or in combination were performed by a Duncan one-way analysis of variance.

Drugs

Atropine sulphate, cocaine hydrochloride, α,β-methylene ATP, prazosin hydrochloride, propranonol hydrochloride, (+)-tubocurarine chloride and yohimbine were purchased from Sigma (France). Tetrodotoxin was from Research Biochemicals International (Illkirch, France). Hexamethonium bromide and physostigmine were synthesized by the Chemistry Department of Synthélabo Recherche (Rueil-Malmaison, France). Tetrodotoxin was dissolved in a buffer citrate obtained from Sigma (France). All the other compounds were dissolved in distilled water.

Results

Profiles of the electrically-induced contractile responses

When stimulated with constant pulses, basal contractile responses (before drug addition) of bladder neck, proximal and medial urethras were not significantly different from each other in terms of amplitude (E_{max}) and duration (D_{max}) (Table 1). In contrast, distal urethra displayed an approximately two fold higher E_{max} value ($P<0.01$), whereas D_{max} value was slightly but significantly lower ($P<0.05$) than values in the other three tissues (Table 1).

Table 1 Basal contractile amplitude (E_{max}) and duration (D_{max}) of the electrically-induced contractions of female rabbit bladder neck and proximal, medial and distal urethras

Parameters	n	*Bladder neck*	n	*Proximal urethra*	n	*Medial urethra*	n	*Distal urethra*
E_{max} (g)	48	1.08±0.10	47	0.73±0.07	48	0.87±0.07	47	2.07±0.15**
D_{max} (s)	44	76.7±6.0	42	84.5±5.0	44	81.3±3.6	43	68.5±2.3*

Data shown are mean±s.e.mean; n=number of experiments. E_{max} and D_{max}=maximum of the amplitude and the duration of the electrically-induced contraction, respectively, expressed as means of the three contractions just before drug addition. Tissues were stimulated at 30 Hz, 50 V, 0.05 ms pulse width, trains of 5 s every 5 min. *$P<0.05$; **$P<0.01$, when compared to other tissues (Bonferroni one way analysis of variance).

In the four tissues, the electrically-induced contraction always revealed two components. The phasic contraction appeared during the 5 s train of pulses and had a rapid onset, whereas the tonic contraction appeared after the end of the stimulation and developed slowly. However, in the distal urethra the tonic component of the contraction was relatively rapid and could hardly be differentiated from the phasic one.

The profiles of the contractile responses were always different between bladder neck, proximal, medial and distal urethras, but were reproducible for each given tissue. The amplitude of the phasic component of the electrically-induced contraction was markedly higher than that of the tonic contraction in bladder neck and slightly higher in proximal urethra, whereas the opposite was true for medial and distal urethras. The tonic component was less important in bladder neck and appeared to be of increasing amplitude up to distal urethra (Figure 2). Since, at the chart speed used (50 mm min^{-1}), it was difficult to separate visually the phasic from the tonic electrically-induced contraction, we were not able to perform a distinct pharmacological analysis of the two phases of the contractile response.

Amplitude and duration of electrically-induced contractions in control strips

During the 60 min period of electrical stimulation, E_{max} values in the absence of antagonists were stable for bladder neck and proximal urethra, whereas they regularly decreased for medial and distal urethras. At the 20th and the 60th min, E_{max} values (expressed as percentage of basal) were 97.3±3.8% and 91.2±7.7% ($n=4$, bladder neck), 100±0% and 97.6±2.4% ($n=3$, proximal), 88.0±8.0% and 71.7±10.4% ($P<0.05$, $n=3$, medial) and 93.0±3.0% and 78.2±4.4 ($P<0.01$, $n=4$, distal), respectively. The control D_{max} values of the electrically-induced contraction were not modified during the 60 min of the experiment in the four tissues.

Effects of cocaine on the amplitude of the electrically-induced contractions

Cocaine (1 μM), up to 60 min, did not modify E_{max} values of the stimulated-contractions of bladder neck ($n=5$) and proximal urethra ($n=5$) compared to the respective control values. In contrast, for medial and distal urethras, cocaine (1 μM) increased by approximately 46% ($n=5$, $P<0.05$) and 35% ($n=5$, $P<0.05$), respectively, the basal E_{max} value of the stimulated-contraction at T20, a time where the effect of cocaine was maximal (Figure 1a). Indeed, the effect of cocaine increased during the first 20 min of the experiment and then decreased to reach nearly the basal value at T60. Cocaine always increased the tonic component of the stimulated contraction but had no effect on the phasic component (Figure 2).

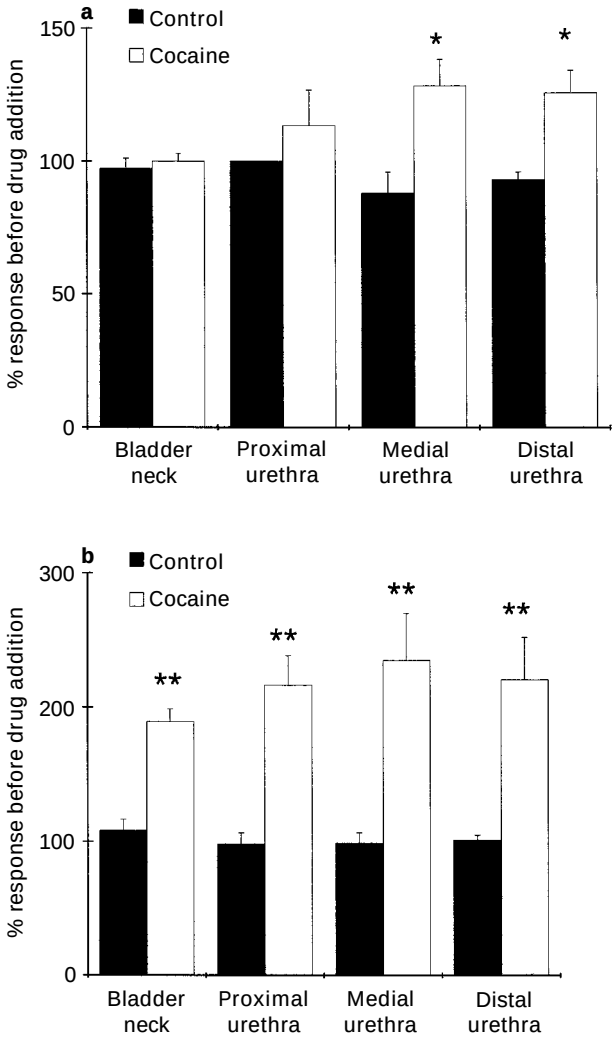

Figure 1 Amplitude (a) and duration (b) of the stimulated contraction of the female rabbit bladder neck and proximal, medial and distal urethra in the absence and presence of 1 μM cocaine. These results were taken at T20. Electrical field stimulation was performed with the following parameters: 50 V, 30 Hz, 0.05 ms, trains of 5 s every 5 min. *$P<0.05$, **$P<0.01$, when compared to the control value (unpaired Student's t test). Each column represents the mean±s.e.mean of 3 to 5 experiments.

Effects of cocaine on the duration of the electrically-induced contractions

Cocaine (1 μM) significantly increased the D_{max} value of the electrically-induced contractions from T5 to T60. The maximal potentiating effect was obtained at T20; at this time point, D_{max} value was increased by 75% in bladder neck, 121% in

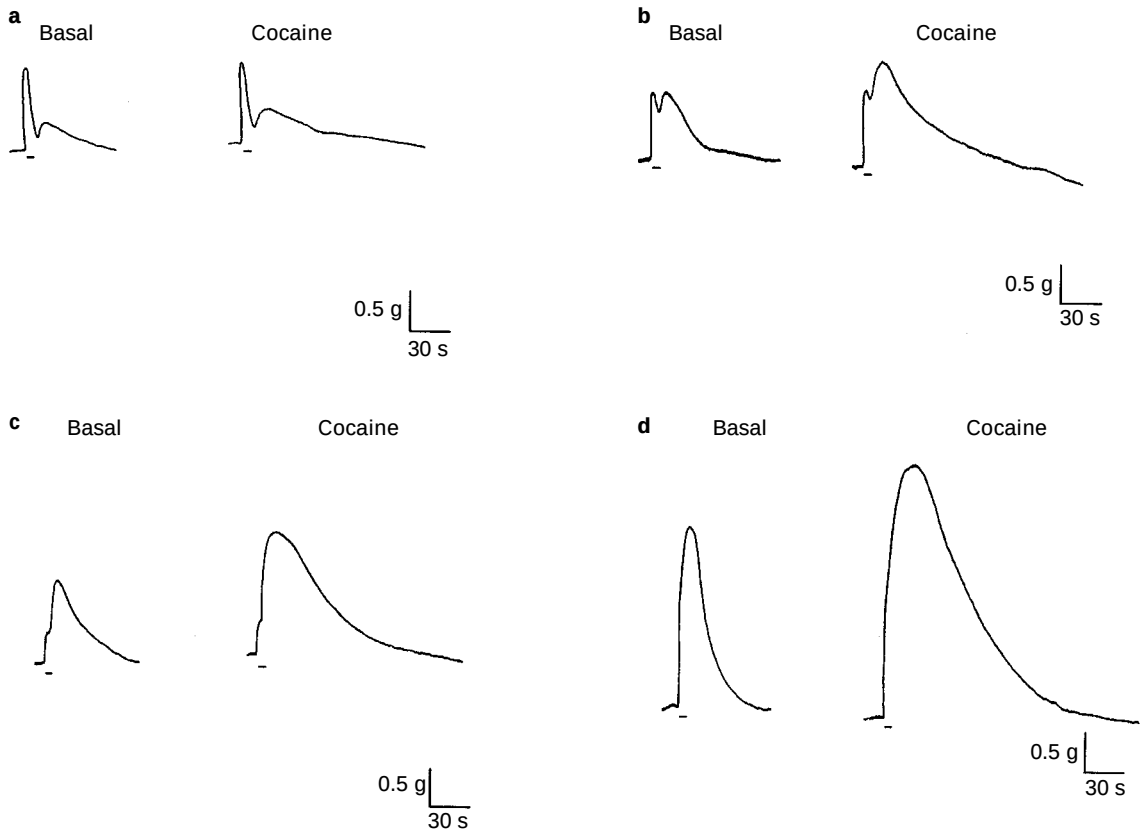

Figure 2 Profiles of the stimulated contractions of female rabbit bladder neck (a) and of proximal (b), medial (c) and distal urethra (d) before and after addition of cocaine (1 μM). Bar indicates the stimulation train. Electrical field stimulation was performed with the following parameters: 50 V, 30 Hz, 0.05 ms, trains of 5 s every 5 min.

proximal urethra, 138% in medial urethra and 119% in distal urethra ($P<0.01$ for all four tissues), compared to the control values (Figure 1b). Again, the effects of cocaine only appeared on the second component of the contractile response (Figure 2).

Effects of α-adrenoceptor and muscarinic antagonists on the amplitude of the electrically-induced contractions

Studies with prazosin, yohimbine and atropine were performed only in the presence of cocaine (1 μM). Results were compared to those obtained with cocaine alone. Furthermore, values were taken at T20 since the potentiating effects of cocaine were maximal at this time point. Yohimbine (0.1 μM) did not change the E_{max} value of the stimulated-contraction in bladder neck, proximal, medial and distal urethras ($n=4$ for each tissue).

Prazosin (1 μM) had no significant effect on the amplitude of the electrically-induced contractions in the bladder neck ($n=5$). However, in proximal, medial and distal urethras it significantly (by Duncan's multiple range test) reduced the E_{max} value of the contraction by approximately 30% ($n=4$, $P<0.05$), 55% ($n=4$, $P<0.05$) and 58% ($n=4$, $P<0.05$) respectively, from the respective control values (Figure 3a). Prazosin only decreased the second component of the stimulated contractions in proximal, medial and distal urethras and had no effect on the first component.

The effects of atropine (1 μM) were opposite to those of prazosin since it significantly reduced E_{max} value of the stimulated contraction by 51% ($n=5$, $P<0.05$) and 39% ($n=4$, $P<0.05$) of the control value in bladder neck and proximal urethra, respectively. However, atropine was devoid of effect in medial ($n=6$) and distal urethras ($n=5$) (Figure 3a). Moreover, in contrast to prazosin, atropine decreased the phasic component of the electrically-induced contraction (data not shown).

Effects of α-adrenoceptor and muscarinic antagonists on the duration of the electrically-induced contractions

The D_{max} value of the electrically-induced contractions was not modified by yohimbine (0.1 μM) in any of the four portions of the isolated urethra (Figure 3b). However, D_{max} was significantly reduced by prazosin (1 μM) in bladder neck by 56% ($P<0.05$) and in proximal urethra by 42% ($P<0.05$), but not in medial and distal urethra (Figure 3b). Atropine (1 μM) also significantly reduced the D_{max} value of the stimulated contraction in bladder neck by 52% ($P<0.01$), but had no effect in the other tissues (Figure 3b).

Effects of the association of prazosin and atropine on the amplitude of the electrically-induced contractions

In the bladder neck, at T20, prazosin (1 μM) was devoid of effect on the electrically-induced contraction, but the association with atropine (1 μM) significantly reduced by 33% the E_{max} value of the contractile response compared to the contraction obtained in the presence of atropine alone ($n=5$, $P<0.05$). The residual response with the association of prazosin and atropine was approximately 33% (Figure 4a). In proximal urethra, prazosin and atropine both reduced the E_{max} value of the contractile response by themselves, and their effects were potentiated when they were associated ($n=4$, $P<0.05$, compared to atropine alone and $P<0.05$, compared to prazosin alone). The residual

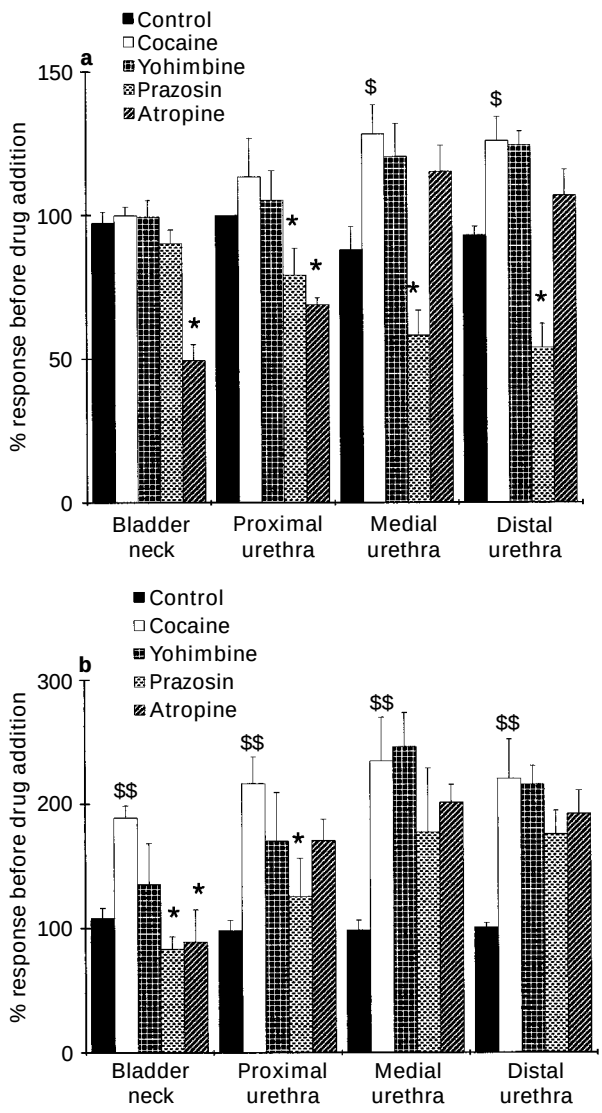

Figure 3 Amplitude (a) and duration (b) of the stimulated contraction of the female rabbit bladder neck and proximal, medial and distal urethra in the absence and presence of yohimbine 0.1 μM, prazosin 1 μM or atropine 1 μM. Experiments were performed in the presence of cocaine 1 μM except for controls. Results were taken at T20. Electrical field stimulation was performed with the following parameters: 50 V, 30 Hz, 0.05 ms, trains of 5 s every 5 min. $^{\$}P<0.05$, $^{\$\$}P<0.01$, cocaine effect compared to the control value (unpaired Student's t test). $^{*}P<0.05$, antagonist effects compared to cocaine-treated strips (Duncan one-way analysis) Each column represents the mean$\pm$ s.e.mean of 4 to 5 experiments.

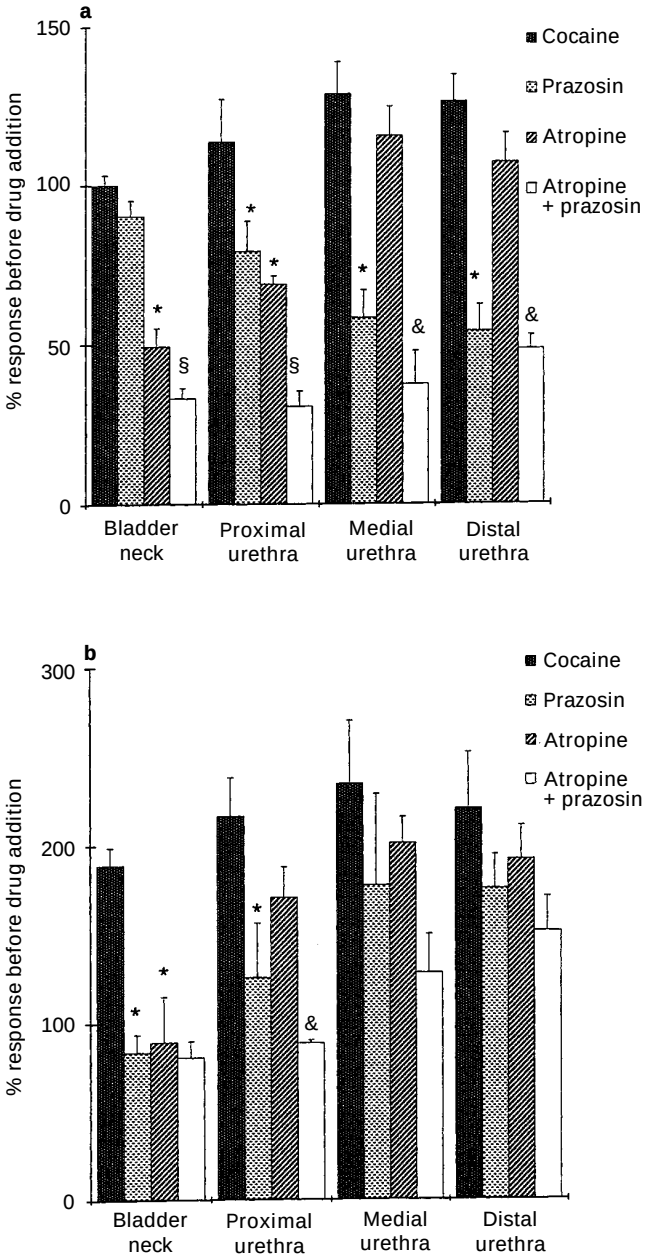

Figure 4 Amplitude (a) and duration (b) of the stimulated contraction of the female rabbit bladder neck and proximal, medial and distal urethra in the absence and presence of prazosin 1 μM, atropine 1 μM or prazosin 1 μM + atropine 1 μM. Experiments were performed in the presence of cocaine 1 μM. Results were taken at T20. Electrical field stimulation was performed with the following parameters: 50 V, 30 Hz, 0.05 ms, trains of 5 s every 5 min. $^{*}P<0.05$, when prazosin and atropine alone were compared to cocaine-treated strips (Duncan one-way analysis). $^{\$}P<0.05$, when the association of prazosin and atropine was significantly different from the effects of prazosin and atropine alone (Duncan one-way analysis). $^{\&}P<0.05$ when the association of prazosin and atropine was significantly different from the effect of atropine alone (Duncan one-way analysis). Each column represents the mean$\pm$s.e.mean of 4–5 experiments.

response to electrical stimulation was approximately 31% of the control response (Figure 4a). In medial and distal urethras, on which atropine had no significant inhibitory effect *per se*, we did not observe any potentiating effect of the combination of atropine and prazosin. In fact, residual responses in the presence of these two compounds at T20 were approximately 37% in medial urethra (vs 58% with prazosin alone) and 48% in distal urethra (vs 54% with prazosin alone). These differences were not statistically significant ($P<0.05$, by Duncan's one-way analysis) (Figure 4a).

Effects of the association of prazosin and atropine on the duration of the electrically-induced contractions

In bladder neck, atropine (1 μM) and prazosin (1 μM) significantly reduced the D_{max} value by themselves (Figure 3b). However, the association of the two drugs did not change the amplitude of the evoked contraction with respect to values obtained with atropine or prazosin alone (Figure 4b). In proximal urethra, prazosin induced a significant reduction of D_{max}, whereas atropine alone had no effect. Therefore, the association of atropine and prazosin did not modify the duration of the electrically-induced contraction since this effect was similar to that of prazosin alone (Figure 4b).

Neither prazosin nor atropine had significant effect on the D_{max} value of the stimulated contraction in medial and distal urethras. The association of the two drugs had no further inhibitory effect (Figure 4b).

Effects of tetrodotoxin on the amplitude of electrically-induced contractions

TTX (1 μM) did not completely abolish the contractile responses evoked by electrical field stimulation. If we considered that at the end of the experiment (where TTX was added whatever the antagonist studied) the response is equal to 100%, TTX reduced the residual responses by 75% in bladder neck, 60% in proximal urethra, 85% in medial urethra and 63% in distal urethra (data not shown).

Characterization of the residual contractile response in the presence of prazosin, atropine and cocaine

In order to desensitize putative postjunctional P2X-purinoceptors, superfusion of the four tissues with α,β-methylene ATP (30 μM) was performed for 20 min in the continuous presence of prazosin, atropine and cocaine. During the first minutes of superfusion, the P2X-purinoceptor agonist induced an initial tonic contraction in the four tissues with different amplitudes (between 20% of the basal response to electrically-induced stimulation in the medial urethra and 55% in the proximal urethra). Then the tension returned to baseline indicating complete desensitization of purinoceptors. This procedure had no effect on the amplitude of the residual contractile response to electrical field stimulation.

Nicotinic receptors were not activated in electrically-induced contractions since hexamethonium (10 μM) had no significant effect on the response. Furthermore, the evoked contractions were not affected by 1 μM (+)-tubocurarine in the four tissues (data not shown).

Discussion

It is widely accepted that the tone of the urethral smooth muscle and the intraurethral pressure are due to activation of the sympathetic nervous system, although it has been proposed that parasympathetic nerves may also contribute to the control of the intraurethral pressure in rabbit and man (Mattiasson *et al*., 1985a). For this reason, we have studied the involvement of sympathetic and parasympathetic nervous systems in the electrically-induced contraction of the bladder neck and three different portions of the urethra.

The contractile response induced by electrical field stimulation always revealed two components, a phasic one, which rapidly developed during the stimulation train and a tonic one which occurred after the end of the stimulation and developed more slowly. A similar pattern of contractile responses was obtained by Mattiasson *et al*. (1985a) in the female rabbit proximal urethra. The amplitude of the electrically-evoked contraction was significantly higher in distal urethra than in other tissues. This result could signify that a higher intraurethral pressure in this part of the urethra is generated *in vivo*. However, we observed that the amplitude of the phasic component, compared to the tonic one, was much greater in the bladder neck, slightly greater in proximal urethra and smaller in the other two portions of the urethra, suggesting an heterogeneity of neurotransmitters.

Cocaine had no significant effect on the amplitude of the stimulated contraction in bladder neck and proximal urethra, whereas it significantly increased it in medial and distal urethras. The effects of cocaine always appeared on the tonic phase of the response, so the overall maximal amplitude remained unchanged in bladder neck and proximal urethra. These results indicate that the contractile response is under the control of the sympathetic innervation in the four tissues, but to a greater extent in medial and distal urethra. This conclusion is supported by our findings that the effect of atropine and prazosin were different in the four tissues studied. Indeed, 1 μM prazosin had no effect on the amplitude of the electrically-induced contractions in bladder neck but significantly decreased the amplitude of the response, from a minimum of 30% in proximal to a maximum of 58% in distal urethra. The inhibition by prazosin always appeared on the tonic component of the evoked response. This may explain the lack of effect of this compound in bladder neck since, as discussed before, the phasic contraction was much higher than the tonic component in this tissue. On the other hand, E_{max} values in bladder neck and proximal urethra were reduced by atropine, but the muscarinic antagonist had no effect on E_{max} values in medial and distal urethras.

In female rabbit urethra, yohimbine discriminates between α_1- and α_2-adrenoceptors ($pA_2 = 5.93$ vs phenylephrine and 7.70 vs UK 14,304; Yoshida *et al*., 1991). At the concentration of 0.1 μM, which is probably selective for α_2-adrenoceptors, yohimbine had no significant effect on the amplitude of the stimulated contraction in all the tissues studied. We conclude that neurogenic contractions of the lower urinary tract are not mediated by α_2-adrenoceptors, confirming previous results (Chen & Brading, 1992). Taken together, these results indicate that tonic contractions induced by electrical stimulation are effectively under the control of the sympathetic innervation, via α_1- but not α_2-adrenoceptors, and that this innervation appears to increase from bladder neck to distal urethra, confirming previous results (Mattiasson *et al*., 1985a; Chen & Brading, 1992; Hashimoto *et al*., 1992).

The potentiating effect of cocaine on D_{max} of the tonic phase in all tissues suggests that noradrenaline uptake was efficiently blocked. Although prazosin was devoid of effect on E_{max} in bladder neck, it significantly reduced D_{max} in this tissue, namely it blocked the effect of cocaine, since the duration was not different from the control value. A similar effect was obtained in proximal urethra, but not in medial and distal urethra. A possible explanation for these results could be the presence, in bladder neck and proximal urethra, of prejunctional α_1-adrenoceptors devoted to facilitate cholinergic neurotransmission and blocked by 1 μM prazosin. Since yohimbine was ineffective on the D_{max} values in the four tissues studied, we can hypothesize that α_2-adrenoceptors are not involved in the control of acetylcholine release in rabbit lower urinary tract. Interestingly, atropine, like prazosin, blocked the potentiating effect of cocaine on D_{max} but in bladder neck only. This may indicate that atropine antagonized prejunctional muscarinic receptors on adrenergic nerves facilitating noradrenaline release.

The rabbit urethral musculature comprises an urothelial lining, a submucosal layer with longitudinal bundles of smooth muscles, a circular muscle layer with smooth and striated muscles and an outer longitudinal layer mainly of smooth muscles (Mattiasson *et al*., 1990). These authors observed that circular striated muscle is predominant in the distal part of the urethra, whereas the circular smooth muscle appears in the more proximal segments. In the present study, striated muscles do not seem to be involved in bladder neck and urethral

contractility observed *in vitro*, since (+)-tubocurarine was totaly ineffective in inhibiting the electrically-induced contractions.

Prazosin and atropine, when associated, inhibited in synergy E_{max} in bladder neck only, but had an additive action on proximal urethra. However, the two antagonists together had no effect on D_{max} values. These results confirm those of Ito & Kimoto (1985) showing adrenergic-cholinergic co-transmission in male rabbit smooth muscle cells isolated from proximal urethra. In additional experiments, we have observed that the cholinesterase inhibitor, physostigmine, increased E_{max} values in all tissues, after superfusion with 1 μM prazosin (data not shown). This could be related to the observation that physostigmine can potentiate both acetylcholine and noradrenaline release, as demonstrated in the rat isolated urinary bladder (Somogyi *et al*., 1996) and reinforces the hypothesis of co-transmission in the entire urethra. In this study, when prazosin and atropine were associated, the residual electrically-induced responses were 33% in bladder neck, 31% in proximal urethra, 29% in medial urethra and 38% in distal urethra. These residual responses were significantly but not completely inhibited by TTX, indicating that the majority of the electrically-induced contraction has a NANC origin. In order to investigate this excitatory NANC component, electrically-induced responses were studied in the presence of prazosin, atropine and cocaine. These contractions were not desensitized by prolonged perfusion with the selective P2X-purinoceptor agonist, α,β-methylene ATP, although this compound induced a consistent contraction in all tissues. These results indicate the presence of P2X-purinoceptors in the lower urinary tract of the female rabbit but these receptors do not seem to be involved in the genesis of the NANC response. Moreover, the implication of nicotinic receptors was discarded, since hexamethonium was devoid of effect. Mattiasson *et al*. (1984) also demonstrated the absence of these receptors in the urethra of rabbit and man. So, the excitatory NANC innervation of the rabbit lower urinary tract actually remains to be identified.

The persistence of a residual electrically-induced contraction after TTX treatment could indicate the presence of a calcium channel involved in neurotransmitter release. The existence of this type of channel on sympathetic nerves was originally demonstrated in superfused cat spleen slices (Kirpekar & Prat, 1978).

In conclusion, the present results show that the lower urinary tract of the female rabbit is mainly under the control of the sympathetic and parasympathetic nervous system. The nerve mediated contraction of the rabbit bladder neck and proximal urethra is essentially cholinergic, whereas neurogenic contractions in medial and distal urethras are mainly adrenergic and mediated by α_1- but not α_2-adrenoceptors. The residual response in the presence of atropine and prazosin could be due to a functional NANC innervation. Further studies are necessary to characterize the nature of the third neurotransmitter involved.

References

ANDERSSON, K.E., GARCIA-PASCUAL, A., FORMAN, A. & TOTTRUP, A. (1991). Non-adrenergic, non-cholinergic nerve mediated relaxation of rabbit urethra is caused by nitric oxide. *Acta Physiol. Scand.,* **141,** 133–134.

ANDERSSON, K.E., GARCIA-PASCUAL, A., PERSSON, K., FORMAN, A. & TOTTRUP, A. (1992). Electrically induced nerve-mediated relaxation of rabbit urethra involves nitric oxide. *J. Urol.,* **147,** 253–259.

ANDERSSON, K.E., LARSSON, B. & SJÖGREN, C. (1984). Characterization of the α-adrenoceptors in the female rabbit urethra. *Br. J. Pharmacol.,* **81,** 293–300.

BRIDGEWATER, M., DAVIES, J.R. & BRADING, A.F. (1995). Regional variations in the neural control of the female pig urethra. *Br. J. Urol.,* **76,** 730–740.

CAINE, M., RAZ, S. & ZEIGLER, M. (1975). Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. *Br. J. Urol.,* **47,** 193–202.

CHEN, H-I. (1990). Evidence for the presynaptic action of 5-hydroxytryptamine and the involvement of purinergic innervation in the rabbit lower urinary tract. *Br. J. Pharmacol.,* **101,** 212–216.

CHEN, H-I. & BRADING, A.F. (1992). The contribution of α-adrenoceptors to neurally-mediated contractions of the rabbit urethral smooth muscles. *Br. J. Pharmacol.,* **106,** 302–306.

EK,, A., ALM, P., ANDERSSON, K-E. & PERSSON, C.G.A. (1977). Adrenergic and cholinergic nerves of the human urethra and urinary bladder. A histochemical study. *Acta Physiol. Scand.,* **99,** 345–352.

EK, A., ANDERSSON, K-E. & ULMSTEN, U. (1978). The effects of norephedrine and bethanechol on the human urethral closure pressure profile. *Scand. J. Urol. Nephrol.,* **12,** 97–104.

FURUYA, S., KUMAMOTO, Y., YOKOYAMA, E., TSUKAMOTO, T., IZUMI, T. & ABIKO, Y. (1982). Alpha-adrenergic activity and urethral pressure in prostatic zone in benign prostatic hypertrophy. *J. Urol.,* **128,** 836–839.

HASHIMOTO, S., KIGOSHI, S. & MURAMATSU, I. (1992). Neurogenic responses of urethra from the dog. *Eur. J. Pharmacol.,* **213,** 117–123.

HASHITANI, H., VAN HELDEN, D.F. & SUZUKI, H. (1996). Properties of spontaneous depolarizations in circular smooth muscle cells of rabbit urethra. *Br. J. Pharmacol.,* **118,** 1627–1632.

ITO, Y. & KIMOTO, Y. (1985). The neural and non-neural mechanisms involved in urethral activity in rabbit. *J. Physiol.,* **367,** 57–72.

JOHNS, A. (1983). Alpha- and beta-adrenergic and muscarinic cholinergic binding sites in the bladder and urethra of rabbit. *Can. J. Physiol. Pharmacol.,* **61,** 61–66.

KIRPEKAR, S.M. & PRAT, J.C. (1978). Effect of tetraethylammonium on noradrenaline release from cat spleen treated with tetrodotoxin. *Nature,* **276,** 623–624.

KONDO, S., TASHIMA, Y. & MORITA, T. (1993). Quantitative analysis of adrenergic alpha-1 and alpha-2-receptors in human prostatic urethral tissue. *Br. J. Urol.,* **72,** 68–73.

KUNIZAWA, Y., KAWABE, K., NIJIMA, T., HONDA, K. & TAKENAKA, T. (1985). A pharmacological study of alpha adrenergic receptor subtypes in smooth muscle of human urinary bladder base and prostatic urethra. *J. Urol.,* **134,** 396–398.

LEE, J.G., WEIN, A.J. & LEVIN, R.M. (1994). Comparative pharmacology of the male and female rabbit bladder neck and urethra: involvement of nitric oxide. *Pharmacology,* **48,** 250–259.

MATTIASSON, A., ANDERSSON, K.E. & SJÖGREN, C. (1984). Adrenoceptor and cholinoceptors controlling noradrenaline release from adrenergic nerves in the urethra of rabbit and man. *J. Urol.,* **131,** 1190–1195.

MATTIASSON, A., ANDERSSON, K.E. & SJÖGREN, C. (1985a). Adrenergic and non-adrenergic contraction of isolated urethral muscle from rabbit and man. *J. Urol.,* **133,** 298–303.

MATTIASSON, A., ANDERSSON, K.E. & SJÖGREN, C. (1985b). Contractant and relaxant properties of the female rabbit urethral submucosa. *J. Urol.,* **133,** 304–310.

MATTIASSON, A., ANDERSSON, K.E., ANDERSSON, P.O., LARSSON, B., SJÖGREN, C. & UVELIUS, B. (1990). Nerve-mediated functions in the circular and longitudinal muscle layers on the proximal female rabbit urethra. *J. Urol.,* **143,** 155–160.

SJÖGREN, C., ANDERSSON, K.E., MATTIASSON, A. & UVELIUS, B. (1988). Different effects of neuropeptide Y on electrically induced contractions in the longitudinal and circular smooth muscle layers of the female rabbit urethra. *Acta Pysiol. Scand.,* **133,** 177–181.

SOMOGYI, G.T., TANOWITZ, M., ZERNOVA, G. & DEGROAT, W.C. (1996). M_1 muscarinic receptor-induced facilitation of Ach and noradrenaline release in the rat bladder is mediated by protein kinase C. *J. Physiol.,* **496,** 245–254.

YOSHIDA, M., LATIFPOUR, J., NISHIMOTO, T. & WEISS, R.M. (1991). Pharmacological characterization of alpha adrenergic receptors in the young and old female rabbit urethra. *J. Pharmacol. Exp. Ther.,* **257,** 1100–1108.

ZYGMUNT, P.M., ZYGMUNT, P.K.E., HÖGESTÄTT, E.D. & ANDERSSON, K.E. (1993). Effects of ω-conotoxin on adrenergic, cholinergic and NANC neurotransmission in the rabbit urethra and detrusor. *Br. J. Pharmacol.,* **110,** 1285–1290.

ZYGMUNT, P.K.E., HÖGESTÄTT, E.D. & ANDERSSON, K-E. (1996). Effects of potassium channel inhibitors on nitrergic and adrenergic neurotransmission in lamina propria of the female rabbit urethra. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **354,** 336–342.

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