Noradrenaline modulates smooth muscle activity of the isolated intravesical ureter of the pig through different types of adrenoceptors

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1 We have studied the effects of α - and β -adrenoceptor agonists and antagonists on both phasic peristaltic activity and basal tone of the isolated intravesical ureter of the pig by means of isometric techniques *in vitro*.

2 Spontaneous phasic activity was exhibited by 21% of pig intravesical ureter preparations manifested as rhythmic contractions with average frequency and amplitude of $2.54 \pm 0.18 \text{ min}^{-1}$ and $1.48 \pm 0.16 \text{ g}$ ($n = 31$), respectively.

3 Adrenaline, noradrenaline and phenylephrine induced concentration-dependent increases in both phasic activity and basal tone of ureteral preparations, all three agonists being more potent in modifying ureteral phasic activity than baseline tone. B-HT 920, B-HT 933 and clonidine had no significant effect.

4 Phentolamine (10^{-9} – 10^{-7} M) and prazosin (3×10^{-11} – $3 \times 10^{-8} \text{ M}$) significantly inhibited increases in both frequency of phasic activity and baseline tone induced by a submaximal dose of noradrenaline. Rauwolscine (10^{-9} – 10^{-7} M) affected only the tone evoked by noradrenaline and higher concentrations of this antagonist were needed to block phasic activity.

5 Pretreatment of ureteral strips with the β -adrenoceptor antagonist, propranolol (10^{-6} M), significantly increased the maximum contraction evoked by noradrenaline. After incubation with phentolamine (10^{-6} M), noradrenaline (10^{-7} – 10^{-6} M) decreased phasic activity induced by prostaglandin $F_{2\alpha}$ (10^{-5} M). Isoprenaline and salbutamol also abolished $\text{PGF}_{2\alpha}$ -induced phasic activity. Pafenolol (10^{-6} M) and butoxamine (10^{-6} M) blocked the inhibitory effect of noradrenaline, isoprenaline, and salbutamol on $\text{PGF}_{2\alpha}$ -induced phasic activity.

6 These results suggest that noradrenaline may modulate both phasic peristaltic activity and basal tone of pig intravesical ureter through both α - and β -adrenoceptors.

Keywords: Porcine intravesical ureter; α -adrenoceptors; β -adrenoceptors; phasic activity; tone

Introduction

Transport of urine from pelvis to bladder is due to spontaneous peristaltic contractions of the ureter initiated by pacemaker activity at the renal pelvis and sustained, in part, by the local release of prostaglandins (Notley, 1970; Al-Ugaily *et al.*, 1986; Thulesius *et al.*, 1986). Peristalsis, which represents the phasic contraction of the ureteral smooth muscle is most likely myogenic, the autonomic nervous system playing a modulating role (Schulman, 1985).

Blok *et al.* (1985) observed that the ureterovesical junction develops a peristaltic activity which promotes active urine bolus transport from ureter to bladder and also reduces outflow resistance of this functional unit during the peristaltic contraction. Moreover, Morita *et al.* (1987) suggested that autonomic nerves may affect the rate of urine transport through the canine ureter by modulating not only peristaltic frequency but also urine bolus volume.

Morphological studies have demonstrated that the density of the nerve supply increases progressively from the top end of the ureter towards the bladder, the pelvic segment being more richly innervated than the lumbar, suggesting a higher nervous control of the intravesical ureter (Hannappel & Golenhofen, 1974). In addition, several studies have shown by means of histochemical techniques, that there is a rich network of adrenergic nerve fibres at the distal ureter and ureterovesical junction (El-Badawi & Schenk, 1971; Schulman *et al.*, 1973; Schulman, 1985; Prieto *et al.*, 1989; 1990) and numerous ganglion cells that constitute the ureterovesical ganglion complex which could play an important role in the coordination of the ureter and bladder activity at the ureterovesical junction (Schulman, 1985).

Since *in vitro* studies at the distal ureter (Labadía *et al.*, 1987) and ureterovesical junction (Benedito *et al.*, 1990; Rivera *et al.*, 1992a,b) are scarce, the aim of the present study was to characterize the postjunctional adrenoceptors involved in the responses of the porcine intravesical ureter to sympathetic neurotransmitters by use of selective α - and β -adrenoceptor agonists and antagonists.

Methods

Adult pigs of either sex with no lesions in their urinary tract were selected from the local slaughterhouse. Urinary bladders with attached ureters were removed immediately after the animals were killed and kept in chilled physiological saline solution (PSS) at 4°C. The adjacent connective and fatty tissues were removed with care and longitudinal preparations (4–6 mm long and 2–3 mm wide) of the intravesical ureter were isolated from the bladder by dissection. The strips were suspended vertically in 30 ml organ baths containing PSS maintained at 37°C and gassed with 95% oxygen and 5% carbon dioxide, pH = 7.4. The distal end of the preparations was attached to a metal hook and the other end connected to isometric transducers (Grass FT 03) with the signal continuously recorded on a polygraph (Grass 79E). Passive tension of 2 g was applied to the preparations and they were allowed to equilibrate for 60 min.

Experimental procedure

The contractile capacity of the preparations was challenged by exposing the preparations to 120 mM potassium-rich physiological saline solution (K^+PSS). Induced phasic

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activity described by frequency (number of contractions min^{-1}) and amplitude (g) of rhythmic contractions, and increases in basal tone (g) were examined by addition of isolated increasing concentrations of noradrenaline, adrenaline, the α_1 -selective agonist, phenylephrine and the α_2 -selective agonists, B-HT 920, B-HT 933 and clonidine. Noradrenaline concentration-response curves were generated in the presence of cocaine (10^{-6} M), corticosterone (10^{-6} M) to block neuronal and extraneuronal uptake, respectively, and propranolol (10^{-6} M) to block the β -adrenoceptors.

Due to the development of a strong tachyphylaxis of the tissue to the agonists, two consecutive concentration-response curves could not be constructed in the same preparation. Since the response to single submaximal concentrations of noradrenaline (10^{-5} M) was reproducible after repetitive exposures, it was used to determine the effect of the non-selective α -adrenoceptor antagonist, phentolamine, the α_1 -adrenoceptor antagonist, prazosin and the α_2 -adrenoceptor antagonist, rauwolscine. The preparations were incubated with the antagonist for 20 min before noradrenaline was added. Control preparations without antagonist incubation were run parallel to correct for tissue fatigue and time-induced changes. When studying relaxation, the preparations were exposed to a single dose (10^{-5} M) of prostaglandin $F_{2\alpha}$ and after obtaining rhythmic phasic activity, the relaxing action of the non-selective β -adrenoceptor agonist, isoprenaline, or the β_2 -adrenoceptor agonist, salbutamol, was determined. Finally, experiments were performed with preparations incubated either with the non-selective β -adrenoceptor antagonist, propranolol, the β_1 -adrenoceptor antagonist, pafenolol, or the β_2 -adrenoceptor antagonist, butoxamine, and the protocol for the study of relaxation responses as described above was repeated.

Drugs and solutions

The composition of physiological saline solution (PSS) was (mM): NaCl 119, KCl 4.6, CaCl_2 1.5, MgCl_2 1.2, NaHCO_3 24.9, glucose 11, KH_2PO_4 1.2, EDTA (ethylene diamine tetraacetic acid) 0.027. The K^+ PSS was PSS with KCl exchanged for NaCl on equimolar basis. Stock solutions were prepared daily in distilled water.

The following drugs were used: (–)-adrenaline HCl, ($\pm$)-noradrenaline HCl (Serva, Germany), B-HT 920 (5-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazol-(4,5-d)-azepin-dihydrochloride), B-HT 933 (2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazol-(4,5-d)-azepin-dihydrochloride), isoprenaline HCl (Boehringer Ingelheim, Germany), butoxamine HCl (Burroughs Wellcome, U.K.), clonidine HCl, cocaine HCl, corticosterone, pafenolol HCl, phentolamine HCl, phenylephrine HCl and propranolol HCl (Sigma, U.S.A.), prazosin HCl (Pfizer, U.K.), prostaglandin $F_{2\alpha}$ (Upjohn, U.S.A.), rauwolscine (Roth, Germany) and salbutamol (Glaxo, U.S.A.).

Adrenaline was prepared in 0.25 N HCl and further diluted in twice distilled water. Prazosin was dissolved in warm water (50°C) at pH 4–5 with constant agitation. Prostaglandin $F_{2\alpha}$ was dissolved in benzylic alcohol. The other drugs were dissolved in twice distilled water containing ascorbic acid (10^{-3} M). Previous experiments showed that the solvents had no effect on the preparations.

Calculations

For each concentration-response curve, the concentration required to give half-maximal response (EC_{50}) was determined by computerized iteration, fitting the responses and logarithmic concentrations to the Hill equation (Graph Pad software 3.0, San Diego, Calif., U.S.A.). Sensitivities to drugs are expressed in terms of pD_2 values, where $\text{pD}_2 = -\log \text{EC}_{50}$, the EC_{50} being the agonist concentration needed to produce 50% of the maximal response. pIC_{50} values for antagonists were calculated as the negative logarithm of the antagonist concentration required to inhibit 50% of the res-

ponse induced by a single dose (10^{-5} M) of the agonist (Skärby & Larsson, 1987).

Statistics

The results are expressed as mean $\pm$ s.e.mean. Statistical differences were calculated by Student's *t* test and one-way analysis of variance (ANOVA) with an *a posteriori* Bonferroni test (Wallestein *et al.*, 1980). Differences were considered significant with a probability level of $P < 0.05$.

Results

Spontaneous activity

Of 148 isolated intravesical ureteral preparations of the pig, 31 (21%) showed spontaneous phasic contractions with a frequency of $2.54 \pm 0.18 \text{ min}^{-1}$ ($n = 31$) and amplitude of $1.48 \pm 0.16 \text{ g}$ ($n = 31$) (Figure 1). The spontaneous contractions persisted on average for 5 h after mounting the preparations.

Responses to α -adrenoceptor agonists and antagonists

Adrenaline (Ad), noradrenaline (NA), and phenylephrine (Phe) induced concentration-dependent increases in both frequency of phasic activity and basal tone of porcine intravesical ureter preparations (Figures 2 and 3). However, B-HT 920, B-HT 933 and clonidine had only sporadic or no effect even after precontraction with either 40–80 mM potassium, 10^{-5} M $\text{PGF}_{2\alpha}$ or 10^{-5} M phenylephrine.

Concentration-response curves constructed by adding individual doses of noradrenaline could not be repeated for the same preparations due to the development of strong tachyphylaxis to the agonists. Thus, in the first concentration-response curve, the pD_2 and E_{max} values were 5.22 ± 0.04 and 1.33 ± 0.08 ($n = 8$) respectively, while in a second curve the pD_2 and E_{max} values were 5.08 ± 0.03 and 0.97 ± 0.06 ($P < 0.05$ and $P < 0.01$, respectively, paired *t* test). However, both contractions and increases in frequency induced by a single submaximal dose (10^{-5} M) of noradrenaline were reproducible after repetitive exposures. The frequency and tone developed by a single dose (10^{-5} M) of noradrenaline in a first exposure were 21.25 ± 1.72 con-

Figure 1 Typical isometric recordings of isolated intravesical ureter of the pig without (a) and with (b) spontaneous phasic activity, but both responding to K^+ -PSS.

Figure 2 Traces showing the response of intravesical ureteral preparations of the pig to 120 mM K^+ -PSS and increasing concentrations of noradrenaline (NA, 10^{-7} – 10^{-4} M) added in single doses with washout (W) between each response. Numbers indicate molar concentration in the bath.

tractions min^{-1} and 0.92 ± 0.07 g respectively, compared to 20.56 ± 1.64 contractions min^{-1} and 0.94 ± 0.08 g, respectively, in a fifth exposure ($n = 16$).

Table 1 shows pD_2 and E_{max} values for the different adrenoceptor agonists, for both phasic activity and tone. The agonists were equipotent in inducing tone and phasic activity since there was no significant difference amongst the pD_2 values: Ad = NA = Phe. However, the maximal response

(E_{max}) in ureteral basal tone evoked by adrenaline was significantly larger than E_{max} to noradrenaline or phenylephrine: Ad > NA = Phe. The maximum effect, E_{max} , in phasic activity to adrenaline was larger than that to phenylephrine, while E_{max} induced by noradrenaline was not significantly different from those induced by adrenaline or phenylephrine (Table 1). All three agonists were more effective in inducing phasic activity than in increasing basal tone of porcine intravesical ureter preparations (Table 1, Figure 3).

Pretreatment of ureteral strips with the non-selective β -adrenoceptor antagonist, propranolol (10^{-6} M), significantly enhanced the maximum increase in basal tone induced by noradrenaline ($P < 0.05$) without affecting sensitivity. Moreover, cocaine (10^{-6} M) significantly ($P < 0.01$) increased the pD_2 value for phasic activity of the noradrenaline concentration-response curve, but did not affect the E_{max} (Table 2, Figure 4). Incubation with corticosterone (10^{-6} M), which blocks noradrenaline extraneuronal uptake, did not alter the response to 10^{-5} M noradrenaline; the tone was 1.03 ± 0.34 g and the phasic activity 13.25 ± 1.57 contractions min^{-1} before, and tone 1.23 ± 0.37 g and phasic activity 12.88 ± 1.63 contractions min^{-1} after incubation with corticosterone ($n = 6$).

Phentolamine (10^{-9} – 10^{-7} M) significantly inhibited both tone ($P < 0.001$) and phasic activity ($P < 0.001$) induced by a submaximal dose of noradrenaline (Table 3, Figure 5). The α_1 -adrenoceptor antagonist, prazosin (3×10^{-11} – 3×10^{-8} M), caused a significant inhibition ($P < 0.001$) of increases in tone induced by noradrenaline, whereas higher concentrations of antagonist were needed to block the noradrenaline-induced phasic activity (Table 3, Figure 6). Likewise, prazosin significantly ($P < 0.001$) affected both tone ($pIC_{50} = 9.72 \pm 0.09$) and phasic activity ($pIC_{50} = 9.87 \pm 0.06$) evoked by phenylephrine (10^{-5} M) (Figure 7).

The α_2 -selective adrenoceptor antagonist, rauwolscine, inhibited increments in basal tone evoked by noradrenaline at concentrations as small as 3×10^{-9} M ($P < 0.01$), but had no effect on phasic activity (Table 3, Figure 8). Tonic contractions to noradrenaline of the porcine intravesical ureter were invariably blocked by α -adrenoceptor antagonists, whereas higher concentrations of antagonists were needed to block the noradrenaline-induced phasic activity (Table 3).

Responses to β -adrenoceptor agonists and antagonists

Figure 9 shows the effects of β -adrenoceptor agonists and antagonists on intravesical ureteral preparations of the pig: 10^{-5} M $\text{PGF}_{2\alpha}$ induced phasic activity with a frequency of 3.12 ± 0.31 contractions min^{-1} and an amplitude of 1.69 ± 0.24 g ($n = 12$). Cumulative additions of isoprenaline did not affect the phasic activity induced by $\text{PGF}_{2\alpha}$ until reaching concentrations of 10^{-8} M, which totally abolished the phasic activity. The effect of isoprenaline was reversed by 10^{-6} M propranolol. Both pafenolol (10^{-6} M) and butoxamine (10^{-6} M) reversed the inhibitory effect of 10^{-8} M isoprenaline on the $\text{PGF}_{2\alpha}$ -induced phasic activity, although higher con-

Figure 3 Concentration-response curves of porcine intravesical ureteral preparations to increasing single doses of noradrenaline (O), adrenaline (□) and phenylephrine (Δ). (a) Phasic activity, and (b) tone of the preparations. Each point represents mean ($\pm$ s.e.mean, vertical bar) of 8 preparations. Results are expressed as absolute values.

Table 1 Effect of α -adrenoceptor agonists on porcine intravesical ureter

Agonist	n	Tone		Phasic activity	
		pD ₂	E _{max}	pD ₂	E _{max}
Noradrenaline	8	5.22 $\pm$ 0.04	1.33 $\pm$ 0.08	5.82 $\pm$ 0.06*	23.6 $\pm$ 0.17
Adrenaline	8	5.05 $\pm$ 0.05	1.91 $\pm$ 0.17 ^a	5.81 $\pm$ 0.05*	26.0 $\pm$ 0.6
Phenylephrine	8	5.20 $\pm$ 0.05	1.13 $\pm$ 0.06 ^b	5.77 $\pm$ 0.07*	19.5 $\pm$ 0.7 ^b

n, number of ureters. pD₂ = -log EC₅₀; EC₅₀ is the effective concentration which induces 50% of the maximal response. E_{max} is the maximum effect in case of ureteral tonus (g) or phasic activity (number contractions min⁻¹). Results are expressed in absolute values as means $\pm$ s.e.mean. ^aSignificantly different parameter compared to noradrenaline ($P < 0.05$, *a posteriori*, Bonferroni). ^bSignificantly different parameter compared to adrenaline ($P < 0.05$, *a posteriori*, Bonferroni). *Significantly different comparing pD₂ of phasic activity to tone of the agonist response in the same preparation ($P < 0.05$, paired *t* test).

Table 2 Effects of cocaine and propranolol on the noradrenaline-induced responses in the porcine intravesical ureter

Antagonist	n	Tone		Phasic activity	
		pD ₂	E _{max}	pD ₂	E _{max}
Control	8	5.31 $\pm$ 0.03	1.40 $\pm$ 0.08	5.91 $\pm$ 0.06	23.3 $\pm$ 1.8
Cocaine	6	5.50 $\pm$ 0.09	1.50 $\pm$ 0.15	6.52 $\pm$ 0.15 ^a	23.8 $\pm$ 2.0
Propranolol	6	5.18 $\pm$ 0.10	2.40 $\pm$ 0.25 ^{a,b}	5.93 $\pm$ 0.06 ^b	30.6 $\pm$ 1.6
Cocaine + propranolol	6	5.37 $\pm$ 0.10	2.06 $\pm$ 0.08	5.95 $\pm$ 0.02 ^b	27.0 $\pm$ 1.3

n, number of ureters. pD₂ = -log EC₅₀; EC₅₀ being effective concentration to produce 50% of the maximal response. E_{max} is the maximal effect of noradrenaline on basal ureteral tonus (g) or phasic activity (number contractions min⁻¹). Results are expressed in absolute values as means $\pm$ s.e.mean. ^aSignificantly different parameter compared to control ($P < 0.05$, *a posteriori*, Bonferroni). ^bSignificantly different parameter compared to cocaine ($P < 0.05$, *a posteriori*, Bonferroni).

Table 3 Effects of α -adrenoceptor antagonists on tone and phasic activity induced by noradrenaline (10^{-5} M) in porcine intravesical ureter

Antagonist	n	Tone pIC ₅₀	Phasic activity pIC ₅₀
Phentolamine	6	8.43 $\pm$ 0.08	7.65 $\pm$ 0.57
Prazosin	6	9.57 $\pm$ 0.08	8.17 $\pm$ 0.06
Rauwolscine	6	8.22 $\pm$ 0.20	—

n, number of ureters. pIC₅₀ defined as the negative logarithm of the antagonist concentration that causes a 50% inhibition of the contraction induced by noradrenaline (10^{-5} M). Results are expressed in absolute values as means $\pm$ s.e.mean.

centrations of isoprenaline were needed to inhibit the phasic activity induced by PGF_{2 α} when incubating with pafenolol than when incubating with butoxamine. Moreover, the selective β_2 -adrenoceptor agonist, salbutamol (10^{-7} M) abolished the phasic activity induced by PGF_{2 α} and this effect was reversed by prior incubation with butoxamine (10^{-6} M).

Discussion

The present *in vitro* study indicates low spontaneous activity in the intravesical ureter and suggests that adrenergic neurotransmitters play an important role in the regulation of ureteral smooth muscle activity. These findings are supported

Figure 4 Concentration-response curves of intravesical ureter of the pig to noradrenaline alone (○) and in the presence of cocaine (■), propranolol (▲), and cocaine and propranolol (●). (a) Phasic activity, and (b) tone of the preparations. Results are means ($\pm$ s.e.mean, vertical bar) of 6–8 preparations and expressed as absolute values.

Figure 5 Effects of phentolamine on phasic activity (open columns) and tone (solid columns) induced by noradrenaline (10^{-5} M). Each column represents mean ($\pm$ s.e.mean, vertical bars) of 6 strips. Results show the relative response to noradrenaline after incubation with increasing concentrations of phentolamine. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (indicate the response is significant compared to control in absolute values, paired t test).

Figure 7 Effect of prazosin on phasic activity (open columns) and tone (solid columns) induced by phenylephrine (10^{-5} M). Each column represents mean ($\pm$ s.e.mean, vertical bars) of 8 tissues. Results show the relative response to phenylephrine after incubation with increasing concentrations of prazosin. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (indicate the response is significant compared to control in absolute values, paired t test).

Figure 6 Effects of prazosin on phasic activity (open columns) and tone (solid columns) induced by noradrenaline (10^{-5} M). Each column represents mean ($\pm$ s.e.mean, vertical bars) of 6 tissues. Results show the relative response to noradrenaline after incubation with increasing concentrations of prazosin. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (indicate the response is significant compared to control in absolute values, paired t test).

Figure 8 Effects of rauwolscine on phasic activity (open columns) and tone (solid columns) induced by noradrenaline (10^{-5} M). Each column represents mean ($\pm$ s.e.mean, vertical bars) of 6 experiments. Results show the relative response to noradrenaline after incubation with increasing concentrations of rauwolscine. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (indicate the response is significant compared to control in absolute values, paired t test).

by the presence of several subpopulations of adrenoceptors probably with different functions. α_1 -Adrenoceptors increase both phasic activity and basal tone of the intravesical ureter, while the α_2 -adrenoceptors mainly affect the tonus of the ureteral wall. Furthermore, our results show that induced phasic contractile activity is abolished by β -adrenoceptor agonists acting through both subtypes of β -adrenoceptors.

The ureterovesical junction (UVJ) plays an important role in both the active transport of urine bolus from ureter to bladder and the prevention of vesicoureteral reflux during bladder filling (El-Badawi & Schenk, 1971; Blok *et al.*, 1985). Two types of pressure waves in the transport of urine

through the ureterovesical junction have been described by use of urodynamic techniques. Firstly, fast pressure waves, which represent ureter peristaltic activity and discharge urine bolus into the bladder and secondly, slow pressure waves due to detrusor activity upon urine transport through the UVJ (Blok *et al.*, 1985). The activity of UVJ as a whole preparation *in vitro* has been characterized (Benedito *et al.*, 1991; Rivera *et al.*, 1992a,b), but to our knowledge this is the first *in vitro* study describing the activity and influence of autonomic drugs on the isolated ureteral component of the UVJ.

Ureteral peristalsis is responsible for transport of the urine

Figure 9 Effect of noradrenaline (NA, 10^{-6} M) in absence (a) and presence (b) of phentolamine (10^{-6} M) (PHT) on phasic activity induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-5} M). The inhibitory effect of isoprenaline (Isop) on phasic activity induced by prostaglandin $F_{2\alpha}$ (10^{-5} M) (c). Effects of propranolol (Prop) (d), pafenolol (Paf) (e) and butoxamine (But) (f) on relaxant response of isoprenaline in porcine intravesical ureter. Action of salbutamol (Salb) in absence (g) and presence (h) of butoxamine on pig isolated intravesical ureteral preparations.

bolus from kidney to bladder. This peristalsis is controlled by a pacemaker located in the intrarenal extension of the ureter, calix (Thulesius *et al.*, 1987). In our study, 21% of the intravesical ureteral preparations shows spontaneous phasic contractions with an intermittent motility pattern. Phasic activity might be the consequence of the electrical spikes generated by the renal pacemaker (Mostwing, 1986; Thulesius *et al.*, 1986). Thus, Thulesius *et al.* (1986) found spontaneous contractions in all preparations from intrarenal extension of the ureter, while the spontaneous activity in segments of the dog middle ureter was 49% (Yano *et al.*, 1984). Therefore, the rare spontaneous activity found in our preparations might be ascribed to the distance from the intravesical ureteral segments to the renal pacemaker. Furthermore, there is a higher autonomic innervation with a marked influence at the distal parts of the ureter (Schulman, 1985; Prieto *et al.*, 1989; 1990).

Cocaine caused a significant leftwards shift of the frequency concentration-response curve for noradrenaline in the pig intravesical ureter, inducing earlier appearance of phasic activity, results which agree with those found in the mesenteric artery, where cocaine increased the sensitivity to noradrenaline without affecting the maximum contractile response (Högstätt & Andersson, 1984). According to Langer

(1981), our observations therefore suggest that, after cocaine incubation, a higher fraction of the agonist becomes available for the activation of α -adrenoceptors. Since the main role of cocaine is to inhibit the neuronal uptake of noradrenaline, these results reinforce the role of sympathetic nerves in the modulation of ureteral smooth muscle activity.

Several pressure studies in the canine ureter have shown that autonomic nerves affect the rate of urine transport through the ureter by modulating not only ureteral peristaltic frequency but also urine bolus volume. Noradrenaline causes an increase in ureteral peristalsis, an elevation in intraureteral baseline and contractile pressure, and a decrease in a bolus volume, with a resulting decrease in the rate of fluid transport. Moreover, noradrenaline produced a marked increase in ureteral resistance at both low and high flow rates (Morita *et al.*, 1986; 1987).

In our study, adrenaline and noradrenaline evoked dose-dependent contractions of pig intravesical ureter, which suggests that α -predominate over β -adrenoceptor. This is consistent with previous investigations in horse ureter (Labadia *et al.*, 1987) and sheep ureterovesical junction (Rivera *et al.*, 1992a). However, Yano *et al.* (1984) found that noradrenaline and adrenaline produced biphasic changes in the spontaneous contractions of dog isolated middle ureters. At low concentrations, they induced increments in frequency and tone, while at high concentrations, they attenuated both parameters. Thus, they considered that at lower concentrations both noradrenaline and adrenaline would stimulate contractile properties of ureteral smooth muscle through activation of α -adrenoceptors and at higher concentrations they would attenuate them through activation of β -adrenoceptors. These discrepancies between the latter and our results could be due to interspecies differences and, in particular, to regional heterogeneity in the reactivity of ureteral segments to adrenergic stimuli.

In our work, the fact that propranolol increased the maximum effect on tone induced by noradrenaline but not the frequency of phasic activity, could indicate a possible modulatory role of the β -adrenoceptors in the regulation of the ureteral wall tonus.

Due to the development of strong tachyphylaxis to the adrenoceptor agonists and the impossibility of constructing two consecutive concentration-response curves with single doses in the same preparation of porcine ureteral tissue, the α -adrenoceptor characterization in the present study has been based on a comparison of the potency and maximum effects of both agonists and antagonists, following the theoretical model proposed by Skärby & Larsson (1987) for single concentrations of agonists.

Phenylephrine was equipotent with noradrenaline, whereas the selective α_2 -agonist, B-HT 920 failed to elicit contractile activity, which suggests a predominance of α_1 -subtype adrenoceptors, results similar to those obtained by García-Pascual *et al.* (1991) in the lamb preprostatic urethra.

Phentolamine inhibited the contractile effects on both frequency and tone induced by noradrenaline, which is consistent with functional studies in the dog ureter (Morita *et al.*, 1987) where phentolamine produced a 67% prolongation of the ureteral discharge interval, an 84% increase in bolus volume and an 18% increase in the rate of fluid transport. Moreover, Yano *et al.* (1984) showed that phentolamine reversed the increased responses induced by noradrenaline to the inhibitory ones in dog isolated middle ureters.

The selective block of α_1 -adrenoceptors with prazosin produced inhibition of the stimulatory effects of noradrenaline and phenylephrine. The pIC_{50} value (8.2) for prazosin on the noradrenaline-induced phasic activity could be compared to the pA_2 values for the antagonist found in other tissues of the lower urinary tract, such as rabbit trigone, urethral and prostate smooth muscle (Honda *et al.*, 1985), and lamb urethra (García-Pascual *et al.*, 1991). On the other hand, we had earlier reported a lower pA_2 value for prazosin in the sheep ureterovesical junction (Rivera *et al.*, 1992a), which

could be ascribed either to the presence of detrusor muscle in this preparation or to species differences.

Rauwolscine showed less potent inhibitory action than prazosin. In fact, this α_2 -selective antagonist only blocked the tone without affecting the phasic activity induced by a submaximal dose of noradrenaline. However, B-HT 920, B-HT 933 and clonidine failed to elicit contractions, despite the initial depolarization induced by low concentrations of potassium, prostaglandin $F_{2\alpha}$ and phenylephrine, which have been found to induce or enhance B-HT 920 contractions in human saphenous vein (Harker *et al.*, 1990) and in cow oviductal artery (Costa *et al.*, 1992). Since the range of concentrations of rauwolscine used in our study is supposed to be selective for α_2 -adrenoceptors (Andersson *et al.*, 1984), we cannot discard the presence of α_2 -adrenoceptors in porcine ureteral tissue which could be possibly involved in the maintenance of ureteral wall tonus as proposed by Ford *et al.* (1989) in vascular smooth muscle.

On the other hand, noradrenaline had an inhibitory effect on the phasic activity induced by prostaglandin $F_{2\alpha}$ after previous blockade of α -adrenoceptors with phentolamine, which suggests the presence of a population of β -adrenoceptors, which had already been manifested by the increased noradrenaline contractile response after blockade with propranolol. Moreover, isoprenaline caused a total inhibition of phasic rhythmic activity induced by prostaglandin $F_{2\alpha}$ and by prior administration of propranolol, the relaxant response of isoprenaline was abolished. This is consistent with functional *in vivo* studies in the canine ureter, where isoprenaline caused a decrease in ureteral peristalsis frequency and a fall in ureteral wall tonus and contractile pressure or it completely abolished peristalsis and bolus formation. Moreover isoprenaline significantly decreased ureteral resistance at both low and high flow rates. These changes were accompanied by an increase in the rate of fluid transport (Kondo *et al.*, 1985; Morita *et al.*, 1987). Furthermore, Yano *et al.* (1984) confirmed this relaxant response of

isoprenaline on canine isolated ureteral preparations. These data agree with those found in other structures of urogenital tract such as oviduct, where the stimulation of β -adrenoceptors facilitates the transport of the ovum or embryo (Isla *et al.*, 1989). However, Bloch *et al.* (1984) showed that isoprenaline had no effect on guinea-pig isolated ureter previously contracted with BaCl_2 . They concluded that the ureter may have β -adrenoceptors but they probably have little physiological significance. This variability could be due to species differences or the use of different agonists for precontraction of the preparations. The inhibitory effect of isoprenaline on ureteral function and studies of the cyclase-phosphodiesterase system provide strong evidence in favour of a β -adrenoceptor inhibitory mechanism in the ureter.

The inhibitory effect of noradrenaline on phasic activity of pig intravesical ureter could be considered to be mediated by both β_1 - and β_2 -adrenoceptors, because of the inhibition by pafenolol and butoxamine of the isoprenaline relaxant responses, results similar to those found in horse ureter (Labadía *et al.*, 1987) and sheep ureterovesical junction (Rivera *et al.*, 1992a).

Our data lead us to conclude that noradrenaline increases both phasic activity and basal tone of pig intravesical through a population of α_1 -adrenoceptors, although the presence of α_2 -adrenoceptors possibly involved in the maintenance of ureteral wall tonus cannot be excluded. Moreover, noradrenaline contractile responses seem to be modulated through a population of β -adrenoceptors which also cause inhibition of ureteral phasic activity through both β_1 - and β_2 -adrenoceptors.

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References

- AL-UGAILY, L., THULESIUS, O. & ANGELO-KHATTAR, M. (1986). New evidence for prostaglandin induced motility of the ureter. *Scand. J. Urol. Nephrol.*, **20**, 225–229.
- ANDERSSON, K.-E., LARSSON, B. & SJOGREN, C. (1984). Characterization of the α -adrenoceptors in the female rabbit urethra. *Br. J. Pharmacol.*, **81**, 293–300.
- BENEDITO, S., PRIETO, D., RIVERA, L., COSTA, G. & GARCIA-SACRISTAN, A. (1991). Mechanisms implicated in the histamine response of the sheep ureterovesical junction. *J. Urol.*, **146**, 184–187.
- BLOCH, R., DECKER, N. & KOSTAKOPOULOS, A. (1984). Effects of theophylline and isoproterenol on the activity of the isolated guinea-pig ureter. *Urol. Int.*, **39**, 308–311.
- BLOK, C., VENROOIJ, G.E.P.M. VAN & COOLSAET, B.L.R.A. (1985). Dynamics of the ureterovesical junction: a qualitative analysis of the ureterovesical pressure profile in the pig. *J. Urol.*, **134**, 818–824.
- COSTA, G., ISLA, M., GARCIA-PASCUAL, A., JIMENEZ, E., RECIO, P., LABADIA, A. & GARCIA-SACRISTAN, A. (1992). Characterization of postsynaptic α -adrenoceptors in the arteries supplying the oviduct. *Br. J. Pharmacol.*, **105**, 381–387.
- EL-BADAWI, A. & SCHENK, E.A. (1971). A new theory of the innervation of the urinary bladder musculature. II The innervation of the ureterovesical junction. *J. Urol. (Balt.)*, **105**, 368–371.
- FORD, S.P., FARLEY, D.B. & VAN ORDEN, D.E. (1989). Role of postsynaptic α_2 -adrenergic receptors (AR) in mediating uterine arterial tone in pigs. *Society for Gynecology Investigation, San Diego C.A.*, Abstr. 127.
- GARCIA-PASCUAL, A., COSTA, G., ISLA, M. & GARCIA-SACRISTAN, A. (1991). Characterization of α -adrenoceptors in the preprostatic urethra of sexually immature male lambs. *Eur. J. Pharmacol.*, **203**, 259–265.
- HANNAPPEL, J. & GOLENHOFEN, K. (1974). The effect of catecholamines on ureteral peristalsis in different species (dog, guinea-pig and rat). *Pflügers Arch.*, **350**, 55–68.
- HARKER, C.T., OUSLEY, P.J., HARRIS, E.J., EDWARDS, J.M., TAYLOR, L.L.M. & PORTER, J.M. (1990). The effects of cooling on human saphenous vein reactivity to adrenergic agonists. *J. Vasc. Surg.*, **12**, 45–49.
- HOGESTATT, E.D. & ANDERSSON, K.-E. (1984). On the postjunctional α -adrenoceptors in rat cerebral and mesenteric arteries. *J. Auton. Pharmacol.*, **4**, 161–173.
- HONDA, K., MIYATA-OSAWA, A. & TAKENAKA, T. (1985). α_1 -Adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **330**, 16–21.
- ISLA, M., COSTA, G., GARCIA-PASCUAL, A., TRIGUERO, D. & GARCIA-SACRISTAN, A. (1989). Intrinsic spontaneous activity and β -adrenoceptor-mediated tubal dilatation affect ovum transport in the oviduct of the cow. *J. Reprod. Fert.*, **85**, 79–87.
- KONDO, S., MORITA, T., SAEKI, H. & TSUCHIDA, S. (1985). Effects of autonomic drugs on *in vivo* recording of electromyograms of canine renal pelvis and ureter. *Urol. Int.*, **40**, 260–266.
- LABADIA, A., RIVERA, L., COSTA, G. & GARCIA-SACRISTAN, A. (1987). Alpha and beta-adrenergic receptors in the horse ureter. *Rev. Esp. Fisiol.*, **43**, 421–426.
- LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmacol. Rev.*, **32**, 337–362.
- MORITA, T., WADA, I., SAEKI, H., TSUCHIDA, S. & WEISS, R.M. (1987). Ureteral urine transport: changes in bolus volume, peristaltic frequency, intraluminal pressure and volume of flow resulting from autonomic drugs. *J. Urol.*, **137**, 132–135.
- MORITA, T., WHEELER, M.A. & WEISS, R.M. (1986). Effects of noradrenaline, isoproterenol and acetylcholine on ureteral resistance. *J. Urol.*, **135**, 1296–1298.
- MOSTWING, J.L. (1986). Electrical and mechanical aspects of bladder contractility. *D. Phil. Thesis, Oxford*.
- NOTLEY, R.G. (1970). The musculature of the human ureter. *Br. J. Urol.*, **42**, 724–727.

- PRIETO, D., BENEDITO, S., RIVERA, L., HERNANDEZ, M. & GARCIA-SACRISTAN, A. (1990). Autonomic innervation of the equine urinary bladder. *Anat. Histol. Embryol.*, **19**, 276–285.
- PRIETO, D., BENEDITO, S., RODRIGO, J., MARTINEZ-MURILLO, R. & GARCIA-SACRISTAN, A. (1989). Distribution and density of neuropeptide Y-immunoreactive nerve fibers and cells in the horse urinary bladder. *J. Auton. Nerv. Syst.*, **27**, 173–180.
- RIVERA, L., HERNANDEZ, M., BENEDITO, S., PRIETO, D. & GARCIA-SACRISTAN, A. (1992a). Mediation of contraction and relaxation by alpha- and beta-adrenoceptors in the ureterovesical junction of the sheep. *Res. Vet. Sci.*, **52**, 57–61.
- RIVERA, L., HERNANDEZ, M., BENEDITO, S., PRIETO, D. & GARCIA-SACRISTAN, A. (1992b). Mediation of contraction by cholinergic muscarinic receptors in the ureterovesical junction. *J. Auton. Pharmacol.*, **12**, 175–182.
- SCHULMAN, C.C. (1985). Innervation of the ureter: a histochemical and ultrastructural study. In *Urodynamics, Upper and Lower Urinary Tract*, II. ed. Lutzeyer, W. & Hannappel, J. pp. 292–361. Berlin: Springer-Verlag.
- SCHULMAN, C.C., DUARTE-ESCALANTE, O., BOYARSKY, S. & GREGOIR, W. (1973). New concepts of ureterovesical innervation. *J. Urol. (Baltimore)*, **109**, 381–384.
- SKARBY, T.V. & LARSSON, B. (1987). Theoretical and functional studies on α_1 and α_2 -adrenoceptors: and examination using the Schild plot. *J. Auton. Pharmacol.*, **7**, 185–198.
- THULESIUS, O., ANGELO-KHATTAR, M. & ALI, M. (1987). The effect of prostaglandin synthesis inhibition on motility of the sheep ureter. *Acta Physiol. Scand.*, **131**, 51–54.
- THULESIUS, O., UGAILY-THULESIUS, L. & ANGELO-KHATTAR, M. (1986). Generation and transmission of ovine ureteral contractions with special reference to prostaglandins. *Acta Physiol. Scand.*, **127**, 485–490.
- WALLESTEIN, S., ZUCKER, G.L. & FLEISS, J.L. (1980). Some statistical methods useful in circulation research. *Circ. Res.*, **47**, 1–9.
- YANO, S., UEDA, S., IKEGAMI, K., MUTOH, S. & SAKANASHI, M. (1984). Actions of some autonomic drugs on spontaneous contractions of isolated dog ureter. *Urol. Int.*, **39**, 100–104.

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