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ATP and vasoactive intestinal polypeptide relaxant responses in hamster isolated proximal urethra

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1 Nitric oxide (NO) is known from previous studies to be the principle transmitter in NANC inhibitory nerves supplying the hamster urethra. However, the identity of the cotransmitter(s) responsible for the responses remaining following block with L-N^G-nitroarginine methyl ester (L-NAME) is not known.

2 Electrical field stimulation (EFS) of circular strips of hamster proximal urethra precontracted with arginine vasopressin (AVP 10^{-8} M), and in the presence of phentolamine (10^{-6} M), propranolol (10^{-6} M) and atropine (10^{-6} M), caused frequency-dependent relaxation, which was attenuated by suramin (10^{-4} M) and reactive blue 2 (RB2; 2×10^{-4} M), but not by pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 10^{-4} M), α-chymotrypsin (10–50 u ml^{-1}) or by the vasoactive intestinal polypeptide (VIP) antagonist, [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP, (5×10^{-7}–10^{-6} M). In the presence of indomethacin (10^{-6} M) frequency-dependent relaxations to EFS were enhanced, particularly at the lower frequencies of stimulation. EFS-induced relaxation was blocked by tetrodotoxin (10^{-6} M), indicating its neurogenic origin.

3 Exogenous ATP (10^{-7}–10^{-3} M) produced concentration-related relaxations which were attenuated by the P2-purinoceptor antagonists suramin (10^{-4} M) and RB2 (2×10^{-4} M) but not by PPADS (10^{-4} M). ATP-induced relaxations were also reduced significantly by indomethacin (10^{-6} M). The inhibitory responses to ATP were urothelium- and NO-independent, since they were not affected by either removal of urothelium or by L-NAME (10^{-4} M).

4 Exogenous VIP (10^{-9}–10^{-7} M) induced concentration-related relaxations which were not affected by urothelium removal, L-NAME (10^{-4} M), α-chymotrypsin (10–50 u ml^{-1}) or by [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP (3×10^{-7}–10^{-6} M). Nevertheless, suramin (10^{-4} M) and RB2 (2×10^{-4} M) but not PPADS (10^{-4} M) antagonized the VIP-induced relaxant responses. Calcitonin gene-related peptide (CGRP: 10^{-9}–10^{-7} M) was devoid of any effect or only elicited a small relaxant response in AVP-precontracted strips.

5 Exogenous prostaglandin E_2 (PGE_2; 10^{-9}–3×10^{-6} M) and the NO donor, sodium nitroprusside (SNP; 10^{-8}–3×10^{-5} M) elicited concentration-related relaxations on the hamster proximal urethra which were not attenuated by suramin (10^{-4} M), RB2 (2×10^{-4} M), or by PPADS (10^{-4} M), indicating a specific inhibitory effect of the antagonists used.

6 In summary, these results are consistent with the view that ATP is an inhibitory transmitter released from inhibitory nerves supplying the NANC relaxation of hamster proximal urethra. The relaxant effect of ATP is NO- and urothelium-independent. The present study did not demonstrate whether VIP is released from parasympathetic nerves during EFS, since both α-chymotrypsin and [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP were ineffective on neurogenic responses.

Keywords: Hamster urethra; ATP; VIP; NO

Introduction

Nitric oxide (NO) is the principal inhibitory neurotransmitter involved in the non-adrenergic non-cholinergic (NANC) relaxant response of the urethra, bladder neck and trigone from various species including man (Klarskov *et al*., 1983), pig (Klarskov, 1987; Persson & Andersson, 1992; Persson *et al*., 1993), rabbit (Dokita *et al*., 1994; Persson & Andersson, 1994, rat (Persson *et al*., 1992), sheep (Garcia-Pascual *et al*., 1991), cat (Levin *et al*., 1992) and dog (Hashimoto *et al*., 1993). In dog and pig urethra the neurogenic relaxation evoked at high frequencies (>5 Hz) appears to be produced by an unknown neurotransmitter in addition to NO (Bridgewater & Brading, 1993; Hashimoto *et al*., 1993; Werkström *et al*., 1995; 1997). In hamster proximal urethra too, NANC inhibitory responses are mainly due to NO, since the NO antagonist, L-N^G-nitroarginine methyl ester (L-NAME), greatly reduces the relaxations induced by electrical field stimulation (EFS) (Pinna *et al*., 1996). However, responses to EFS were not completely blocked by L-NAME; the responses were reduced by suramin, suggesting that ATP may be released as a cotransmitter. Other putative transmitters that could be involved in the NANC relaxation of the urethra during micturition include: calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP), both of which cause relaxation in the bladder neck and urethra when the tone is raised (Watts & Cohen, 1991; Hashimoto *et al*., 1993). These neurotransmitters are stored in the sensory-motor neurones and are released from central and peripheral nerve endings where they produce sensory and efferent effects, respectively (Maggi, 1993). VIP is also thought to play a role in parasympathetic neurotransmission in the urogenital tract, since postganglionic nerves from the pelvic ganglia containing VIP and acetylcholine project to the bladder (Keast *et al*., 1989).

The aim of the present work was to investigate the inhibitory response to ATP either in urothelium-free or in intact preparations and to study whether VIP and/or CGRP

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could be considered as cotransmitters together with NO and ATP in the EFS-induced relaxant responses in the hamster proximal urethra. Relaxant responses to prostaglandin E_2 (PGE_2) and sodium nitroprusside (SNP) were also studied in the absence and presence of suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and reactive blue 2 (RB2), to check whether the inhibition induced by these antagonists was specific.

Methods

Tissue preparations and recording of mechanical activity

Male Golden hamsters (120–140 g) were killed by asphyxiation with CO_2. The abdomen was opened and the bladder and the urethra were quickly removed and placed in cold, modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.4, $NaHCO_3$ 16.4, $MgSO_4$ 0.6 and glucose 7.7. The bladder and the proximal urethra were then separated by a transverse cut at the level of the bladder neck. The urethra was opened under a dissecting microscope and a circular smooth muscle strip (approximately 2 mm in length and 1 mm in width, 2.6 ± 0.3 mg wet weight) was prepared from each urethra. Urothelium was removed by scraping the lumen of the urethra with a needle (size: 0.5 mm) (Pinna *et al*., 1996). Each strip was threaded through a pair of platinum-ring electrodes (3 mm in diameter, 1 cm apart) connected to a Grass SD 9 stimulator, with one end attached to a tissue holder and the other to a Dynamometer UF1 isometric force transducer coupled to a four-channel Grass 79D ink-writing oscillograph. The strips were equilibrated for 1 h in 5 ml organ baths containing modified Krebs solution gassed with 95% O_2 and 5% CO_2 at 37 ± 0.5°C. The strips were initially loaded to a tension of 250 mg. A slight reduction in this value occurred after the equilibration period.

Experimental procedure

After the equilibration period, each preparation was exposed to noradrenaline (EC_{50}: 10^{-5} M) until two reproducible contractions were obtained. Relaxant responses to EFS and to the exogenous agonists ATP, CGRP, VIP, PGE_2 and SNP were studied in strips precontracted with arginine vasopressin (AVP; 10^{-8} M). In order to display NANC relaxant responses, the tissues were preincubated with phentolamine (10^{-6} M), propranolol (10^{-6} M) and atropine (10^{-6} M) for 30 min. The preparations were exposed to AVP (10^{-8} M), which produced a long-lasting and stable contraction. Frequency-response curves were then constructed: square wave pulses with a duration of 0.3 ms and a supramaximal voltage (80 V) were delivered for 30 s, at increasing frequencies (1–32 Hz), leaving a 4 min interval between each frequency step. After completion of the frequency curve, the tissues were washed 3 times with fresh Krebs solution and left in the bath until the resting tone had recovered. Either α-chymotrypsin (10–50 u ml^{-1}), [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP (5×10^{-7}–10^{-6} M), suramin (10^{-4} M), RB2 (2×10^{-4} M), PPADS (10^{-4} M) or indomethacin (10^{-6} M) were then added (together with phentolamine, propranol and atropine) 30 min before the strips were once again precontracted with AVP and subjected to electrical stimulation. In parallel with drug experiments, time controls were performed by repeating the frequency-response curve 30 min after the end of the first curve without addition of any drugs: no significant difference was observed between the first and the second frequency-response curves.

Drugs and solutions

Arginine vasopressin, ATP, atropine sulphate, calcitonin gene-related peptide, α-chymotrypsin (type II), Cibacron Blue 3GA (RB2), indomethacin, L-N^G-nitroarginine methyl ester, prostaglandin E_2, sodium nitroprusside, vasoactive intestinal polypeptide and [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-vasoactive intestinal polypeptide were all purchased from Sigma-Aldrich Company Ltd. (Poole, U.K.); phentolamine mesylate (Rogitine) was obtained from Novartis (formerly Ciba, Cambridge, U.K.), propranolol hydrochloride (Inderal) from Zeneca Pharmaceuticals (Cheshire, U.K.) and suramin (Germanin) from Bayer (Germany). Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) was a generous gift from Dr G. Lambrecht, University of Frankfurt, Germany. Stock solutions of the drugs were prepared in distilled water.

Data analysis

Relaxant responses are expressed as % inhibition of the AVP-induced contraction. All data in the text are expressed as mean ± s.e.mean of 5 to 6 experiments. Since only one strip was prepared from each urethra, the number of animals used for the experiments was the same as the number of experiments (n) performed, so only the value of n is given. All curves were prepared with the help of the computer programme Prism: for each curve, the programme calculates the lower and upper plateau, the slope, the EC_{50} and the pD_2 value ± s.e.mean. Antagonist potency of suramin against the responses to ATP and VIP calculated from single concentration was estimated by the equation: $pK_B = \log(\text{dose-ratio} - 1) - \log(\text{antagonist concentration})$, where dose-ratio was the difference between pD_2 values in the absence and presence of antagonist. Concentration-response curves were compared by a two-way analysis of variance (ANOVA), followed by the Tukey-Kramer *post hoc* test for the entire curve using the computer programme Minitab. A probability of $P < 0.05$ was considered significant.

Results

Neurogenic response in precontracted tissues

Circular smooth muscle strips of hamster proximal urethra, precontracted by AVP (10^{-8} M) and incubated with phentolamine (10^{-6} M), propranolol (10^{-6} M) and atropine (10^{-6} M), showed NANC frequency-dependent relaxations in response to EFS (Figure 1a). The neurogenic relaxation was significantly reduced by suramin (10^{-4} M) or RB2 (2×10^{-4} M) (ANOVA, $P < 0.05$; $n = 6$), but not by PPADS (10^{-4} M), α-chymotrypsin (10–50 u ml^{-1}) or by the VIP antagonist, [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP (3×10^{-7}–10^{-6} M). In the presence of indomethacin (10^{-6} M) a slight potentiation of AVP-induced contraction (20 ± 3%) was seen, and neurogenic relaxation to EFS was significantly enhanced (ANOVA, $P = 0.006$; $n = 5$), particularly at the lower frequencies (1 and 2 Hz) of stimulation. Furthermore, neurogenic relaxations were completely blocked by tetrodotoxin (10^{-6} M) (Figure 1b).

Effect of exogneous ATP

In circular smooth muscle strips of hamster proximal urethra, precontracted by AVP (10^{-8} M) and incubated with phentolamine (10^{-6} M), propranolol (10^{-6} M) and atropine (10^{-6} M), exogenous ATP (10^{-7}–10^{-3} M) provoked concentration-

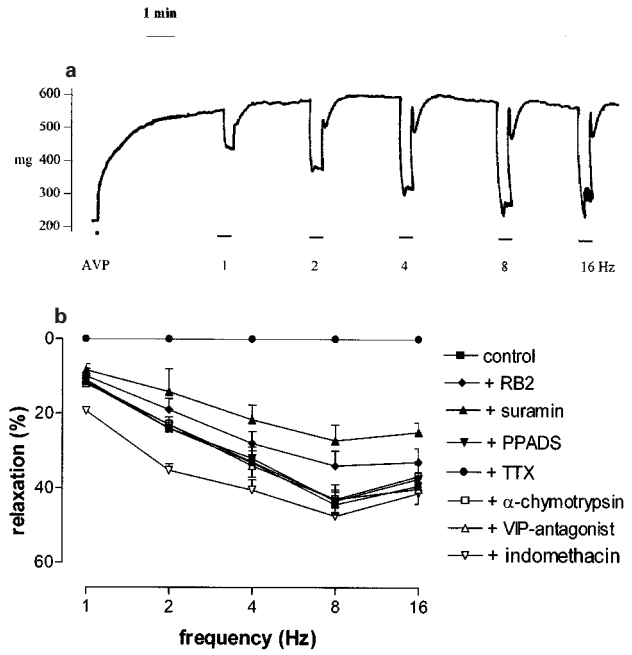

Figure 1 Responses of circular smooth muscle strips of hamster proximal urethra precontracted with arginine vasopressin (AVP, 10^{-8} M) in response to electrical field stimulation (80 V, 0.3 ms, 1–16 Hz). (a) Original tracing of frequency-induced relaxation. (b) Frequency-response curves before (control) and after incubation with tetrodotoxin (TTX: 10^{-6} M), suramin (10^{-4} M), reactive blue 2 (RB2: 2×10^{-4} M), pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS: 10^{-4} M), α-chymotrypsin (10 u ml^{-1}), [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]-VIP (VIP antagonist; 10^{-6} M) and indomethacin (10^{-6} M). Propranolol (10^{-6} M), phentolamine (10^{-6} M) and atropine (10^{-6} M) were present throughout the experiments. Points show the mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol. The curves in the presence of suramin and RB2 differ significantly from the control curve (ANOVA, $P<0.05$).

dependent relaxations (Figure 2a). The threshold concentration at which ATP induced a response was 10^{-6} M, the pD_2 value for ATP was 4.11 ± 0.05 and the maximal relaxation was 100% of the AVP-induced contraction and was displayed at 10^{-3} M. Removal of the urothelium or incubation with L-NAME (10^{-4} M) did not affect the concentration-dependent relaxations induced by ATP (Figure 2b), which were significantly reduced by indomethacin (10^{-6} M) (ANOVA, $P=0.002$; $n=5$), or by suramin (10^{-4} M) or RB2 (2×10^{-4} M) (ANOVA, $P<0.05$; $n=6$) but not by PPADS (10^{-4} M) (Figure 2c). The estimated pA_2 value for suramin against the action of ATP was 4.50 ± 0.07.

Effect of exogenous VIP and CGRP

Exogenous VIP (10^{-9}–10^{-7} M) evoked concentration-related relaxations on AVP-precontracted strips (Figure 3a). CGRP (10^{-9}–10^{-7} M) only elicited a small relaxant response in 4 out of 6 preparations at the maximal concentration tested (Figure 3b), but no response was induced in the remaining 2 strips. The threshold concentration at which VIP induced a response was 10^{-8} M, the estimated pD_2 value was 7.34 ± 0.04 and the relaxation induced by VIP at 10^{-7} M was 62.2 ± 2.5%. Neither removal of the urothelium nor incubation with L-NAME (10^{-4} M) affected the VIP-induced relaxant responses (Figure 3c). Likewise, α-chymotrypsin (10–50 u ml^{-1}) and the VIP antagonist (5×10^{-7}–10^{-6} M) were ineffective on VIP-induced relaxation (data not shown). In contrast, VIP-induced responses were inhibited by suramin (10^{-4} M) and by RB2 (2×10^{-4} M) but not by PPADS (10^{-4} M) (Figure 3d). The estimated pA_2 value for suramin against the action of VIP was 4.48 ± 0.13. There was no significant difference between the pA_2 values for suramin against VIP and ATP.

Effect of exogenous PGE_2 and sodium nitroprusside

PGE_2 (10^{-9}–3×10^{-6} M) and SNP (10^{-8}–3×10^{-5} M) provoked concentration-dependent relaxations of hamster proximal urethra (Figure 4a,b): maximal relaxations were 80 ± 7% and 96 ± 4%, respectively, of AVP-induced contraction and the pD_2 values were 6.34 ± 0.03 and 5.67 ± 0.07 for PGE_2 and SNP, respectively. Relaxant responses induced by PGE_2 or SNP were not inhibited by suramin (10^{-4} M), RB2 (2×10^{-4} M) or by PPADS (10^{-4} M) (Figure 4c,d).

Discussion

We previously demonstrated that, in the hamster proximal urethra, EFS-induced relaxant responses are largely but not completely inhibited by L-NAME and are restored by L-arginine (Pinna *et al*., 1996), suggesting (1) that NO is the principal inhibitory neurotransmitter in this tissue, and (2) that a cotransmitter(s) is involved during micturition in urethral relaxation. Since suramin was able to reduce the neurogenic response, we postulated that ATP was a cotransmitter released from NANC inhibitory nerves during EFS. This possibility was also supported by the fact that exogneous 2-methyl-thio-ATP, a more selective agonist than ATP on the P2Y receptors (Burnstock & Kennedy, 1985), produced a relaxation of about 25% at 3×10^{-6} M on AVP-precontracted strips, with a threshold concentration of 10^{-8} M. The results obtained in the present study confirm this hypothesis, since both suramin or RB2 were able to reduce NANC-relaxant responses to EFS. Moreover, exogenous ATP elicited a relaxation of about 25% at 3×10^{-5} M and up to 100% at 10^{-3} M on AVP-precontracted strips, with a threshold concentration of 10^{-6} M. Our results also show that neither removal of urothelium nor incubation with L-NAME affected relaxant responses to ATP, indicating that P2Y receptors are located on the smooth muscle layer and the synthesis of NO is not involved in this response.

Prostaglandins, particularly PGE_2 and $PGF_{2\alpha}$ are potent spasmogens of detrusor smooth muscle of urinary bladder (Hoyle, 1994). In hamster urethra PGE_2 caused concentration-related relaxations whereas $PGF_{2\alpha}$ evoked concentration-related contractions (Pinna *et al*., 1996). Indomethacin reduced significantly the relaxations induced by exogenous ATP in urethral strips. This result may indicate that, as in the urinary bladder (Hoyle *et al*., 1994), prostaglandins play an important role in urethral neurotransmission processes and that activation of the P2Y receptor by ATP can evoke synthesis and release of prostaglandins with relaxant effects. Indomethacin also increased neurogenic relaxations of urethral strips, particularly evident at lower stimulation frequencies. The synthesis of endogenous prostaglandins with contractile and/or relaxant effects, secondary to neural activity, could be important in maintaining the tone in the smooth muscle, as shown in the rabbit proximal urethra (Ito & Kimoto, 1985).

Exogenous VIP caused concentration-related relaxations in hamster urethra, which were not sensitive to either α-chymotrypsin or to [Lys^{1}, Pro^{2,5}, Arg^{3,4}, Tyr^{6}]-VIP, a reported competitive VIP antagonist thought to bind with equal affinities to all known VIP receptors (Gozes & Brenneman, 1989; Gozes *et al*., 1989). Both drugs were also ineffective on

EFS-induced relaxant responses. This is consistent with the pig urethra where relaxation induced by EFS was not affected by preincubation with α-chymotrypsin (Bridgewater & Brading, 1993) or by anti-VIP antisera, whereas responses to exogenous VIP were blocked. Similarly, [4-Cl-D-Phe[6],Leu[17]]-VIP, a competitive antagonist of VIP, failed to block either the

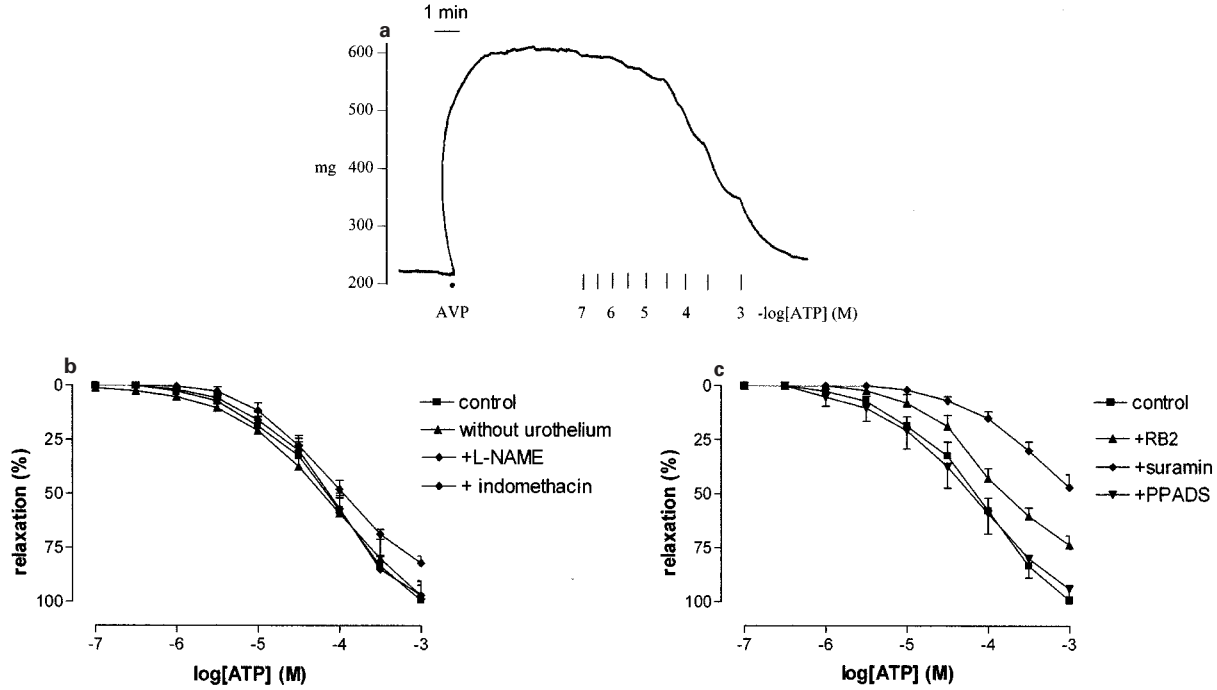

Figure 2 ATP responses of circular smooth muscle strips of hamster proximal urethra precontracted with arginine vasopressin (AVP, 10^{-8} M). (a) Original tracing showing the effect of ATP (10^{-7}–10^{-3} M). (b) Cumulative concentration-response curve to ATP before (control) and after incubation with L-NAME, in urothelium-free preparations and in the presence of indomethacin (10^{-6} M). (c) Cumulative concentration-response curve to ATP before (control) and after incubation with suramin (10^{-4} M), RB2 (2×10^{-4} M) and PPADS (10^{-4} M). Points show the mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol. The curves in the presence of suramin and RB2 differ significantly from the control curve (ANOVA, $P<0.05$).

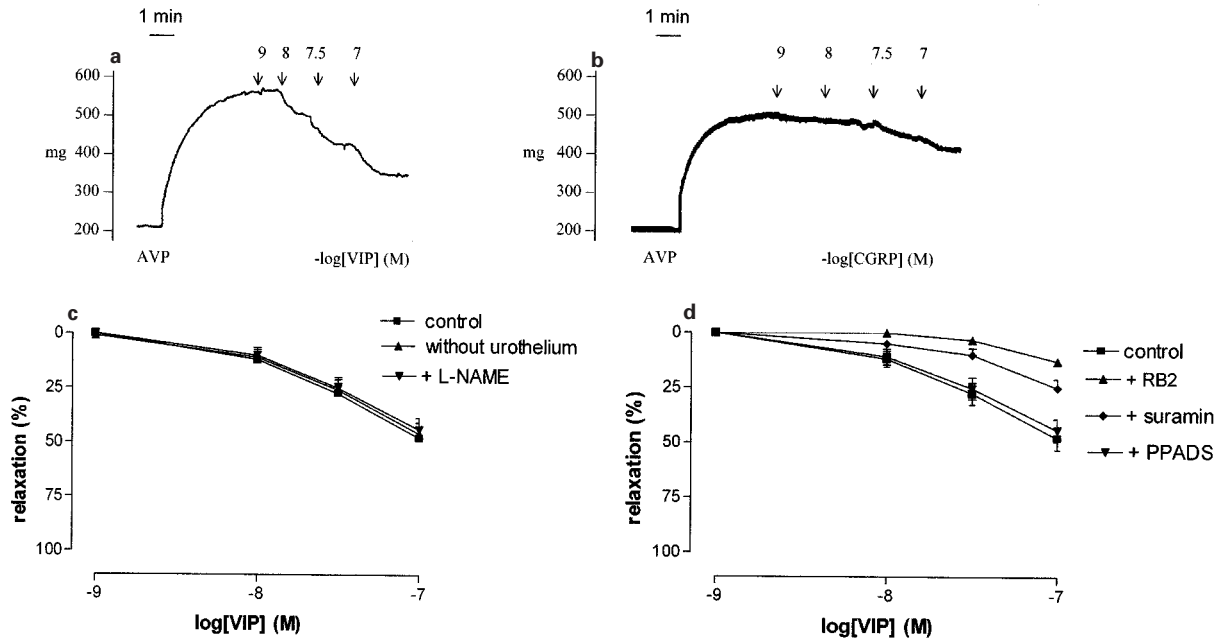

Figure 3 Vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) responses of circular smooth muscle strips of hamster proximal urethra precontracted with arginine vasopressin (AVP; 10^{-8} M). (a) Original tracing showing the effect of VIP (10^{-9}–10^{-7} M). (b) Original tracing showing the effect of CGRP (10^{-9} M–10^{-7} M). (c) Cumulative concentration-response curve to VIP before (control) and after incubation with L-NAME and in urothelium-free preparations. (d) Cumulative concentration-response curve to VIP before (control) and after incubation with suramin (10^{-4} M), RB2 (2×10^{-4} M) and PPADS (10^{-4} M). Points show the mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol. The curves in the presence of suramin and RB2 differ significantly from the control curve (ANOVA, $P<0.05$).

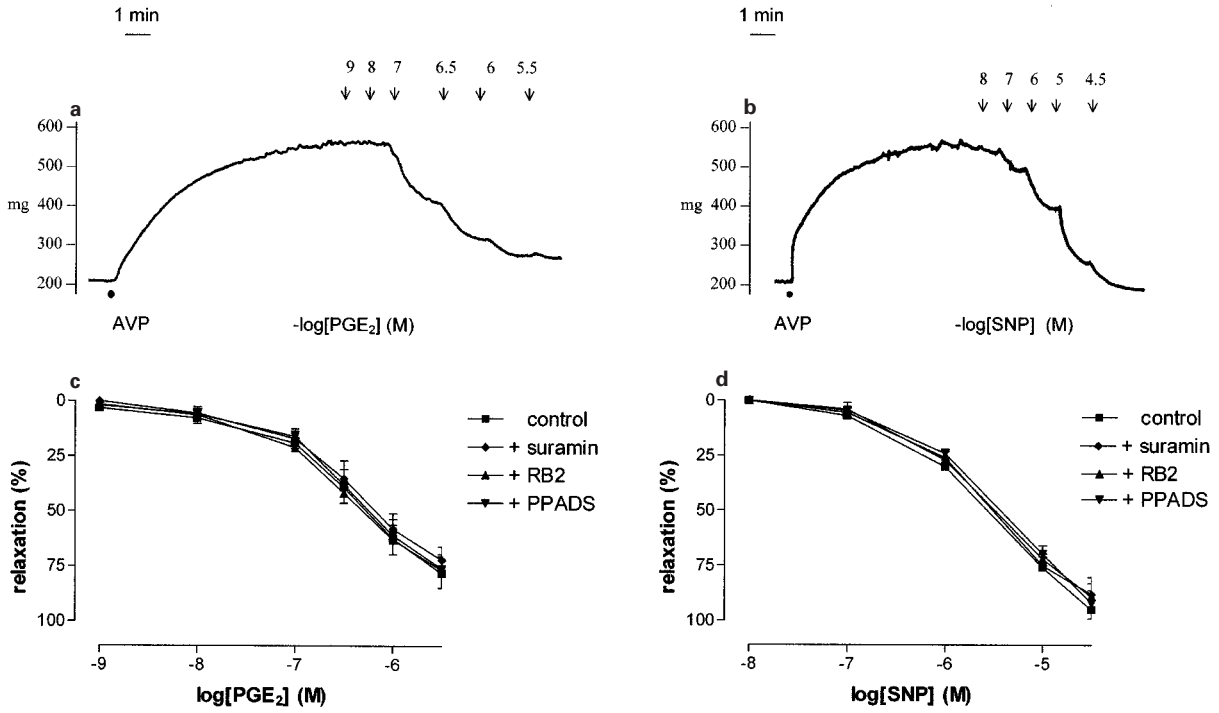

Figure 4 Prostaglandin E_2 (PGE_2) and sodium nitroprusside (SNP) responses of circular smooth muscle strips of hamster proximal urethra precontracted with arginine vasopressin (AVP; 10^{-8} M). (a) Original tracing showing the effect of PGE_2 ($10^{-9}-3\times10^{-6}$ M). (c) Cumulative concentration-response curves to PGE_2 and (d) SNP before (control) and after incubation with suramin (10^{-4} M), RB2 (2×10^{-4} M) and PPADS (10^{-4} M). Points show the mean and vertical lines s.e.mean of 6 experiments, unless occluded by symbol.

neurogenic relaxation or the response evoked by exogenous sensory peptides (Hashimoto *et al.*, 1993) in the dog urethra. A possible explanation for the lack of effect of α-chymotrypsin is that this compound is too large to enter into the synaptic space. This should not be the case for the VIP antagonist, [Lys[1], Pro[2,5], Arg[3,5], Tyr[6]]-VIP, composed of a portion of neurotensin (fragment 6–11) and a portion of VIP (fragment 7–28), or for other chimeric VIP analogues, which should be able to reach the receptors as easily as VIP can. It has been suggested that the usefulness of the VIP antagonists is limited because of their low potency, and furthermore in some systems some of them have agonist properties (Fishbein *et al.*, 1994). These authors found that neurotensin (6–11)-VIP(7–28), VIP(10–28) and [4-Cl-D-Phe[6],Leu[17]]-VIP as well, had low affinity for the VIP receptor: $K_d = 2.3$, 2.3 and 1.7 μM respectively, and that at a high concentration (3 μM) neurotensin (6–11)-VIP(7–28) also had agonist activity. In our experimental conditions [Lys[1], Pro[2,5], Arg[3,4], Tyr[6]]-VIP at the concentration used (1 μM), did not afffect the AVP-precontracted tone of urethral strips. Another point to be taken into account is the stability of this VIP antagonist: before carrying out the frequency-response curve or the concentration-response curve to VIP, strips were incubated with the VIP antagonist for 30 min, which is the same incubation time used by Gozes and co-workers (1989) in VIP binding experiments. Taken together these data suggest that the low potency of this VIP antagonist and not its instability under the conditions of the experiment, might explain its lack of effect on VIP- and EFS-induced responses. Whether VIP contributes to NANC-mediated responses of the proximal hamster urethra cannot be determined at this time, and conclusive evidence for its role will have to wait for the availability of specific and more potent VIP receptor antagonists.

In our experimental conditions, CGRP did not have a clear-cut effect on hamster urethra, suggesting that this peptide cannot be considered as a NANC inhibitory transmitter in this tissue.

An unexpected observation showed that ATP- and VIP-induced relaxations were both sensitive to suramin and RB2, but not to PPADS. These antagonists did not affect PGE_2- or SNP-induced relaxant responses, suggesting that in the hamster urethra, the inhibition induced by suramin or by RB2 (but not PPADS) was selective for relaxant responses involving P2Y purinoceptors. Moreover, both ATP and VIP responses were not NO- or urothelium-dependent since they were unaffected by L-NAME or by removal of urothelium. A possible explanation for this is that VIP can activate the prejunctional release of ATP from nerve endings which in turn produces a relaxant response, due to the activation of P2Y purinoceptors located on the smooth muscle layer of the proximal urethra. This hypothesis is supported by a similar pA_2 value for suramin on both ATP- and VIP-induced responses, suggesting that the relaxation elicited by both agonists involves the same receptors.

The normal process of micturition involves an initial urethral relaxation, followed by an increase of intravesical pressure, as well as a maintained relaxation of the urethra (Tanagho & Miller, 1970) and pharmacological evidence indicates that NO is the inhibitory NANC neurotransmitter in the urethra and bladder neck (Itoh *et al.*, 1995). In hamster proximal urethra, nitric oxide is involved in the initial phase of the relaxation and ATP is involved in the second phase of the response (Pinna *et al.*, 1996). The present data confirm ATP as a cotransmitter together with NO and also suggest that VIP is a prejunctional neuromodulator of the release of ATP on hamster proximal urethra.

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