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Stretch-evoked inhibition of spontaneous migrating contractions in a whole mount preparation of the guinea-pig upper urinary tract

M.E. Teele & [1]R.J. Lang

Department of Physiology, Monash University, Clayton 3168, Victoria, Australia

1 The effects of circumferentially-applied stretch on the spontaneous contractility of a whole mount preparation of the guinea-pig upper urinary tract (UUT) (renal pelvis and ureter) were investigated by use of standard isometric tension recording techniques.

2 Simultaneous tension recordings of the proximal and distal portions of the renal pelvis (RP) and ureter revealed that spontaneous contractions, in 79% ($n=66$) of preparations, originated in the proximal RP (at a frequency of 4.5 min^{-1}) and propagated to the distal RP and ureter at a velocity of 1–3 cm s^{-1}. Pretreatment with tetrodotoxin (TTX) (3–10 μM) or N^G-nitro-L-arginine (100 μM) had little effect on the spontaneous contractility of the UUT, motility indexes (MIs) (contraction amplitude × contraction frequency) calculated after 20 min exposure were little affected by TTX or N^G-nitro-L-arginine (L-NOARG). ω-Conotoxin GVIA (100 nM) significantly reduced MI values in both the proximal RP and ureter.

3 Exposure of the spontaneously-active UUT to capsaicin (10 μM for 15 min) induced a transient increase in UUT contractility, followed by a prolonged negative inotropic effect. The MI values, calculated 60 min after the washout of capsaicin, for the proximal and distal RP and ureter were reduced to 56%, 53% ($n=18$) and 61% ($n=16$), respectively, of their control values. This capsaicin pretreatment blocked the positive inotropic effects of transmural electrical nerve stimulation on UUT contractility to reveal a small inhibitory effect which was readily blocked by tetrodotoxin (3 μM) ($n=3$). The excitatory and inhibitory actions of nerve stimulation were both blocked by TTX (3 μM)

4 A second exposure to capsaicin (10 μM for 15 min), further reduced the MI values (calculated 60 min after washout) in the proximal and distal RP to 41% and 31%, respectively ($n=6$; $P<0.05$), of the initial control values.

5 In 61% ($n=99$) of preparations, the application of stretch to the proximal RP (0.5 to 2 mm) evoked a decrease in the amplitude of the contractions recorded in the distal RP, but not in the ureter. Stretch applied to the distal RP or ureter had no effect on the contractions recorded in the other regions of the UUT.

6 In 5 out of 6 preparations, a single application of capsaicin (10 μM for 15 min) had little effect on the change in contractile force of the distal RP evoked upon stretch of the proximal RP.

7. The inhibition of the distal RP upon stretch of the proximal RP was partially reduced ($P<0.05$) when the UUT was pretreated with the calcitonin gene-related peptide (CGRP) receptor antagonist, hCGRP (8–37) (1 μM).

8. The application of the CGRP receptor agonist, hCGRP (100 nM) inhibited contractility in the UUT in a region dependent manner. The MI of the proximal RP was decreased 32% after 6 min; while the MIs of the distal RP and ureter were reduced 83% and 63%, respectively, within 5 min of the application of hCGRP.

9. Glibenclamide (1 μM) had little effect on the spontaneous contractility of the UUT, but significantly reduced the inhibition of the distal RP evoked upon stretch (0.5 to 2 mm) of the proximal RP. TTX (3–10 μM), L-NOARG (100 μM) or ω-conotoxin GVIA (100 nM) had little effect on the stretch-evoked inhibition of the distal RP.

10. It was concluded that circumferential stretch of the proximal RP inhibits the contractility of the distal RP and that a component of this inhibition involves the activation of a glibenclamide-sensitive mechanism via the release of endogenous CGRP, possibly from the varicosities of intramural sensory nerves.

Keywords: Capsaicin; CGRP; peristalsis; renal pelvis; stretch; upper urinary tract; ureter; sensory nerves

Introduction

In the mammalian urinary tract, capsaicin-sensitive sensory nerves have been demonstrated to be involved in a number of axonal reflexes. In the rat bladder, sensory nerves are thought to regulate the threshold for the initiation of the micturition reflex, as pretreatment of the animal *in vivo* with capsaicin, a blocker of sensory nerve function, results in an increase in the bladder volume and pressure threshold necessary to evoke the reflex response (Maggi *et al*., 1988). In the upper urinary tract (UUT) of the rat, distension of the ureter *in vivo* induces an increase in the activity of afferent renal nerves in the ipsilateral kidney. This distension also decreases the activity of the efferent renal nerves of the contralateral kidney which, in turn, increases contralateral urine flow rate and Na^+ excretion (Kopp & Smith, 1991a). This 'renorenal' reflex again involves capsaicin-sensitive sensory nerves, as the reflex was blocked by capsaicin pretreatment and mimicked by the application of substance P in the absence of ureteral distension (Kopp & Smith, 1991a). The synthesis and release of prostaglandins is thought to play a part in both of these reflexes as indomethacin

[1] Author for correspondence.

pretreatment mimics the effects of capsaicin (Maggi *et al*., 1988; Kopp & Smith, 1991b; Mapp *et al*., 1991; Hingtgen & Vasko, 1994).

Primary afferents have also been demonstrated to have an efferent role. Activation of sensory receptors on peripheral afferents are generally thought to induce action potential discharge which propagates to the central nervous system, as well as to collateral branches of the same sensory neurone (Maggi & Meli, 1988). This antidromic excitation of the sensory nerves releases neuropeptides from varicosities in the periphery in a manner sensitive to blockade by tetrodotoxin (TTX) or ω-conotoxin GVIA (Maggi *et al*., 1990). In the guinea-pig UUT, transmural electrical stimulation increases the contractile force of the spontaneously-active renal pelvis (RP), in a manner reduced by TTX or capsaicin pretreatment, but not by guanethedine or atropine (Maggi *et al*., 1992; Zhang & Lang, 1994). This positive inotropic effect was also blocked by the tachykinin antagonist, MEN 10,376, to reveal a negative inotropic effect, sensitive to blockade by the calcitonin gene-related peptide (CGRP) antagonist, hCGRP (8–37) (Maggi *et al*., 1992). In the ureter, electrical stimulation of sensory nerves transiently inhibits the spontaneous contractions induced upon exposure to a raised K saline or to neurokinin A. This inhibition was blocked by hCGRP (8–37), pretreatment with capsaicin and by ω-conotoxin GVIA in the guinea-pig ureter; in the rat ureter, ω-conotoxin GVIA had no effect (Maggi & Giuliani, 1991).

It is well established that the pacemaker cells necessary for the initiation of peristalsis in the UUT are likely to predominate in the proximal regions of the RP. Circumferentially-cut strips of the RP display a gradient in their frequency of contraction such that the frequency decreases as the strips are cut from more distal regions of the UUT (Golenhofen & Hannappel, 1973; Hannappel & Lutzeyer, 1978; Constantinou *et al*., 1978). This gradient has been correlated with a decreasing number of 'atypical' smooth muscle cells, histologically identified in the RP (Gosling & Dixon, 1974). The isolated ureter is generally quiescent and contains few 'atypical' smooth muscle cells, although spontaneous activity can be pharmacologically induced. In this study, we have developed a whole mount preparation of the UUT which displays propagating peristaltic contractions, originating mostly from the proximal RP. We have investigated the mechanisms which maintain these spontaneous contractions and whether the application of circumferentially-directed stretch to the proximal or distal regions of the UUT can alter the contractile properties of surrounding regions of the preparation. In addition, we have attempted to elucidate pharmacologically the nature of the mechanisms activated and neuropeptides released upon stretch. Some of these results have been presented previously in brief (Teele & Lang, 1996).

Methods

Guinea-pigs of either sex (200–400 g) were stunned and bled, and the upper urinary tract (UUT) (i.e. the kidney and 2–3 cm of the ureter) removed through an abdominal incision. The renal pelvis (RP) was first dissected free of the surrounding kidney, opened up along its longitudinal axis and pinned out in a dissecting dish. The RP was bisected into proximal and distal portions by a circumferentially-directed cut (applied approximately 5 mm from the proximal edge) to the midline of the UUT. The distal 0.5 mm of the ureter was also opened along its longitudinal axis, a circumferential strip of ureter was then created by two cuts to the midline of the ureter, applied approximately 1–2 cm distal of the pelviureteral junction. The intact half of the UUT was then pinned into an organ bath (1.3 ml) perfused with physiological saline (at 35°C) at 2–3 ml min^{-1}. The three areas of the UUT were attached, via thread, to Grass isometric force transducers (Grass FT03C, Grass Instruments, Quincy, Massachusetts) plugged into a MacLab 4s analogue-to-digital converter, driven by a Macintosh LC. A small tension (0.5–1 mN) was applied to the three sites of the UUT. The tissue was then left to equilibrate for a period of 30–60 min until all three regions of the UUT displayed contractions which were regular in both amplitude and frequency.

The resting length of the proximal RP was usually between 0.7 and 1.5 cm. Constant stretches (maintained until four spontaneous contractions were recorded; i.e. approx. 1 min) were applied to the proximal UUT by moving the attached force transducer, with a calibrated micrometer. Typically, a series of 4–6 stretches (between 0.5 to 2 mm) were applied before, during and one hour after a test procedure. At least five minutes were allowed between the application of each stretch. In some experiments, the proximal region of the renal pelvis was passed through two transmural platinum wire stimulating electrodes. Transmural electrical stimulation (0.2 ms duration, 60–90 V at 10 Hz for 10 s) was applied at 10 min intervals to the proximal RP by a Grass S4 stimulator (Grass Instruments, Quincy, Massachusetts).

In many experiments, the effects of a treatment on the spontaneous contractility of the UUT were quantified by use of a motility index (MI), calculated by multiplying the averaged amplitude of 5 contractions by the number of contractions min^{-1}. The effects of stretching the proximal RP on the amplitude of the contractions recorded in the distal RP were quantified as follows. First, the amplitudes of the four contractions recorded during the duration of an applied stretch were expressed as a percentage of the averaged amplitude of five control contractions recorded just before the period of stretch. These relative amplitudes (% of control) were averaged (n = the number of experiments as indicated) and then plotted against the amplitude of the stretch applied (0.5–2.0 mm); data are expressed as the mean ± s.e.mean. In some experiments, a three-way, repeated measures analysis of variance (ANOVA) was applied to test for significance. In these analyses, relative amplitudes were first transformed by use of an arcsine transformation; $P<0.05$ was considered statistically significant.

The physiological saline was of the following composition (in mM): NaCl 120, KCl 5.0, $CaCl_2$ 2.5, $MgCl_2$ 1.0, NaH_2PO_4 1.0, $NaHCO_3$ 25 and glucose 11. The pH of this solution was 7.3–7.4, after being bubbled with a O_2: CO_2 (95% O_2: 5% CO_2) gas mixture. The following drugs were used: tetrodotoxin (TTX), capsaicin, glibenclamide, thiorphan (all from Sigma, St. Louis, U.S.A.), calcitonin gene-related peptide (hCGRP 1–37), CGRP antagonist, hCGRP (8–37) (Auspep), ω-conotoxin GVIA (Peninsula), N^G-nitro-L-arginine (LNOARG) (NOVA Biochem). Glibenclamide was dissolved in dimethyl sulphate, while capsaicin was dissolved in ethanol. Thiorphan, CGRP, TTX and ω-conotoxin GVIA were dissolved in filtered distilled water and L-NOARG was dissolved directly in physiological saline. Drugs were diluted with physiological saline to their final concentrations as indicated. Before use, solutions were vigorously bubbled with 95% O_2: 5% CO_2 to restore any changes of pH.

Results

All three regions of the guinea-pig UUT developed spontaneous contractile activity after 30–60 min equilibration. These spontaneous contractions remained relatively consistent for 4–6 h, after which time contractile activity became increasingly irregular in both amplitude and frequency. In general, the more distal regions of the UUT were the first to develop irregular contractile responses with time. In 53 of 67 (79%) preparations, contractions were initiated in the proximal RP (Figure 1a(i)) and propagated along the UUT to the distal RP (Figure 1b(i)), and then the ureter (Figure 1c(i)). The velocity of these propagating contractions was approximately 1–3 cm s^{-1} ($n=7$). However, in three preparations, ureteral contractions preceded those in the distal RP. In comparison to the graded decrease in the frequency of contractions observed along the UUT when circular strips are taken from the more distal regions, the frequency of contractions in our whole-mount preparation was constant at all three regions of the UUT, at 4.53 ± 0.42 min^{-1} ($n=10$); similar to the contraction frequency observed in isolated strips of proximal RP (Constantinou *et al*., 1978; Potjer *et al*., 1992; Zhang & Lang, 1994; Lang & Zhang, 1996). However, there was a gradient in the circumferentially directed force developed at the three regions of the UUT. The averaged force developed by the proximal and distal RP was 2.6 ± 0.3 and 1.0 ± 0.2 mN, respectively; compared with 0.6 ± 0.1 mN ($n=10$) in the ureter (Figure 1(ii)). Orally-migrating contractions were recorded in only 11 preparations. In seven of these preparations, contractions were initiated in the distal RP (Figure 1b(ii)), followed by the proximal RP (Figure 1a(ii)), and then the ureter (Figure 1c(ii)). However, in the remaining four preparations the sequence of contractions was such that the ureter contracted first, followed by the distal RP, and then the proximal RP.

Effects of capsaicin

The application of capsaicin (10 μM) for 15 min evoked a transient increase in the amplitude of the contractions recorded in both the proximal and distal RP (Figure 2a(i)), as well as the ureter (data not shown) (Maggi & Giuliani, 1992; Maggi *et al*., 1992). This transient excitation was often followed by a long-lasting negative inotropic effect. The motility indexes (MI) for the proximal RP, distal RP and ureter, calculated 60 min after the washout of capsaicin, decreased to 56.2% ($n=18$; $P<0.05$), 53% ($n=18$; $P<0.05$), and 61% ($n=16$; $P<0.05$) of control values, respectively. This reduction of these MIs arose from a significant decrease in both the amplitude and frequency of the contractions in all three areas of the UUT (Table 1). Interestingly, exposures to capsaicin (10 μM) for a longer period of time (30 min) produced a transient increase in contractility in all three regions of the UUT, but did not significantly reduce the MI values measured 60 min later (Table 1). The transient increase in UUT contractility recorded in the presence of capsaicin (10 μM for either 15 or 30 min) was not recorded when tissues

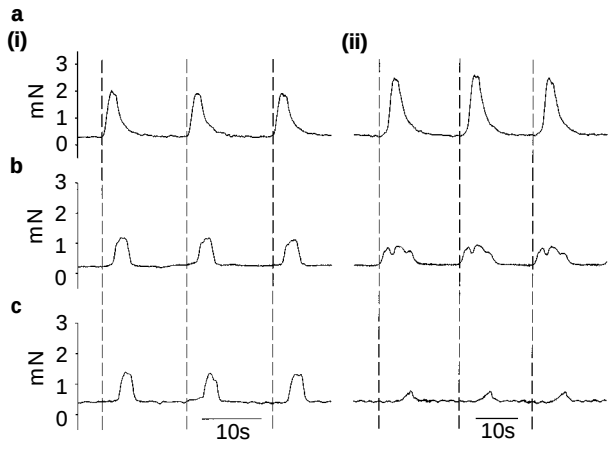

Figure 1 Spontaneous contractions were recorded simultaneously in the circumferential direction in the proximal renal pelvis (RP) ((i–ii) a), distal RP ((i–ii) b), and ureter ((i–ii) c) of the guinea-pig. (i) In most (79%) preparations of the upper urinary tract (UUT), contractions originated in the proximal RP and propagated in an aboral direction at a rate of 1–3 cm s^{-1}. (ii) In 17% of preparations, contractions were initiated in the distal RP which propagated to the proximal RP and then the ureter.

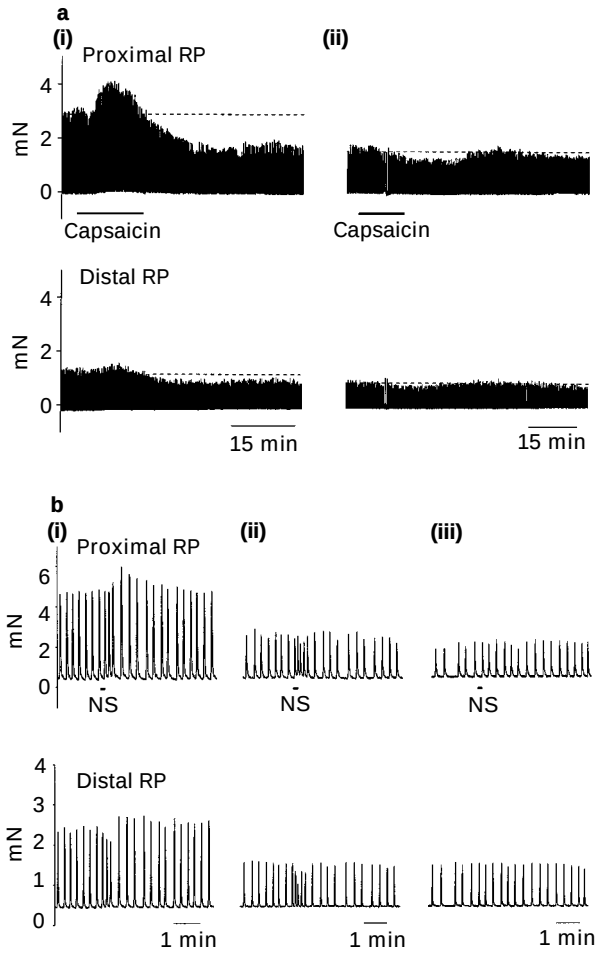

Figure 2 Treatment of preparations with capsaicin (10 μM) for 15 min evoked a transient increase in the amplitude of the contractions recorded in both the proximal (a(i) upper panel) and distal (a(i) lower panel) renal pelvis (RP). This transient excitation was often followed by a long-lasting negative inotropic effect in both the proximal and distal RP. (a(i)) This positive inotropic effect was not evident during a second exposure to capsaicin (10 μM for 15 min). However, the underlying long-lasting negative inotropic effect was still evident. (b) In most preparations, capsaicin pretreatment (10 μM for 15 min) reduced the positive inotropic effects of transmural electrical nerve stimulation (NS) (0.2 ms duration, 60–90 V at 10 Hz for 10 s) (b(i)) to reveal an underlying positive chronotropic effect associated with a decrease in contraction amplitude in both the proximal (b(ii) upper panel) and distal (b(ii) lower panel) RP. These residual effects of electrical nerve stimulation after capsaicin pretreatment were readily blocked by tetrodotoxin (TTX) (3 μM) (b(iii)).

Table 1 The effects of capsaicin on motility in the upper urinary tract of the guinea-pig

	Proximal renal pelvis			*Distal renal pelvis*			*Ureter*		
Drug	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)
Control	5.1±0.3	3.6±0.2	18.5±1.7	5.1±0.3	1.2±0.1	6.6±1.0	5.1±0.3	0.8±0.2	4.1±1.1
Capsaicin (10 μM – 15 min)	4.7±0.3*	2.3±0.3*	10.4±1.3* (*n* = 18)	4.5±0.4*	0.8±0.1*	3.5±0.7* (*n* = 18)	3.3±0.5*	0.6±0.2*	2.5±1.0* (*n* = 16)
Control	4.3±0.9	3.1±0.9	13.9±5.8	4.3±0.9	1.5±0.2	6.7±1.9	3.0±0.8	0.4±0.1	1.0±0.1
Capsaicin (10 μM – 30 min)	3.3±0.7	2.3±0.4	7.6±2.6 (*n* = 5)	3.3±0.7	1.2±0.2	3.9±0.8 (*n* = 5)	2.4±0.5	0.4±0.1	0.8±0.2 (*n* = 3)
Control	5.6±0.4	2.8±0.3	15.3±2.1	5.6±0.4	1.5±0.2	7.8±0.8	4.8±0.4	0.5±0.1	2.5±0.6
Capsaicin (×1)	5.0±0.6	1.8±0.4*	8.4±1.7*	4.4±0.6	0.9±0.2*	4.1±0.7*	2.5±1.1	0.2±0.1	0.7±0.3*
Capsaicin (×2)	4.0±0.7†	1.5±0.3†	6.3±1.7† (*n* = 6)	3.1±0.8	0.8±0.1†	2.4±0.6† (*n* = 6)	0.6±0.6†	0.04±0.04†	0.1±0.1† (*n* = 5)
Control	5.8±0.8	3.5±0.3	20.6±3.4	5.8±0.8	1.8±0.2	11.0±3.1	5.8±0.8	0.5±0.1	2.7±0.8
Capsaicin	4.2±0.5*	1.9±0.3*	8.0±1.9*	4.2±0.5*	0.9±0.2*	3.8±1.3*	2.4±1.2*	0.1±0.1	0.4±0.2
ω-Conotoxin GVIA	4.0±0.5	1.6±0.2◆	6.6±1.5 (*n* = 4)	3.9±0.5	0.7±0.2	2.9±0.8 (*n* = 4)	1.1±1.1	0.03±0.03	0.1±0.1 (*n* = 4)

*Denotes a significant difference between control saline and a single exposure to capsaicin. †Denotes a significant difference between control saline and a second exposure to capsaicin. ◆Denotes a significant difference between values recorded after capsaicin treatment and those in the presence of ω-conotoxin GVIA.

were exposed to a second application of capsaicin (10 μM for 15 min) ($n = 5$) (Figure 2a(ii)). However, the second application of capsaicin still evoked the long-lasting decrease in UUT contractility, the MIs in the proximal and distal RP and ureter (60 min after capsaicin washout) were further reduced to 41% ($n = 6$) and 31% ($n = 6$) and 4%($n = 5$), respectively, of the initial control values ($P < 0.05$) (Table 1).

To eliminate the possibility that capsaicin was having a direct inhibitory action on the smooth muscle contractility of the UUT, as has been previously described in the guinea-pig distal colon (Maggi *et al*., 1987), we examined the effects of capsaicin on the chronotropic and inotropic actions induced by electrically stimulating the intramural sensory nerves. In 17 of 20 preparations, transmural electrical stimulation (0.2 ms duration, 60–90 V at 10 Hz for 10 s) applied to the proximal RP produced a voltage-dependent positive chronotropic and inotropic effect on the contractility of the proximal RP (Figure 2b(i)). This increase in contraction frequency upon sensory nerve stimulation was also observed in the distal RP. However, there was a considerable tissue variation in whether the amplitude of these contractions increased or decreased upon nerve stimulation (Figure 2b(i lower panel)). In 13 of these 17 preparations, the positive inotropic response to nerve stimulation in the proximal RP was accompanied by a similar increase in contraction amplitude in the distal RP which was often preceded by a brief period of amplitude inhibition (Figure 2b(i lower panel)). All of these excitatory and inhibitory effects of electrical nerve stimulation were substantially reduced after 15–20 min of exposure to TTX (3 μM) ($n = 9$). Pretreatment of the tissue with capsaicin (10 μM for 15 min) blocked the increase in contraction amplitude upon nerve stimulation in all preparations ($n = 8$), to reveal that the nerve-evoked positive chronotropic effect was associated with an inhibition of the contraction amplitudes in both the proximal and distal RP (Figure 2b(ii)). This negative inotropic effect upon nerve stimulation after a single exposure to capsaicin was readily blocked by TTX (3 μM) ($n = 3$) (Figure 2b(iii)). Altogether, these data suggest that: (i) the net effect of sensory nerve stimulation applied to the proximal RP, whether excitatory or inhibitory, is variable between preparations, particularly in the distal RP; (ii) a single exposure to capsaicin (10 μM for 15 min) readily causes a complete release of tachykinins from the intramural sensory nerves, but only a partial release of any inhibitory neuropeptides present, and (iii) any inhibitory neuropeptides remaining can be released during a second exposure to capsaicin or during transmural electrical stimulation.

Effects of stretch

In most preparations, stretch (0.5–1.5 mm) applied to the proximal RP resulted in a stretch-dependent increase in the contractile force recorded in the proximal RP (Figures 3a(i) and 4Aa(i–iii)) (Potjer *et al*., 1992). Larger stretches (1.5–2 mm) often did not lead to any further increases in these contractions, presumably reflecting the length-tension characteristics of this preparation. There was little change in the frequency of the spontaneous contractions recorded in the UUT upon stretch of the proximal RP (Figures 3a(i) and 4Aa(i–iii)) (Hannappel & Lutzeyer, 1978; Potjer *et al*., 1992). However, in some preparations a premature contraction was triggered at the beginning of an applied stretch which reset the frequency of the subsequent contractions.

In 60 of 99 (61%) preparations, the application of stretch to the proximal RP induced a decrease in the amplitude of the contractions recorded in the distal RP throughout the period of stretch (Figures 3b(i), 4Ab(i–iii)). However, in most preparations contractions of the ureter were little affected upon stretch of the proximal RP (Figure 4Ac(i–ii)). The effects of stretch on the contractility of the UUT were also unidirectional. In 90% of the preparations which responded to stretch ($n = 60$), the application of stretch (0.5–2 mm) to the distal RP (Figure 3b(ii)) had no effect on the amplitude of the spontaneous contractions recorded in the proximal RP (Figure 3a(ii)), or ureter (data not shown), even though the inhibitory effects of stretching the proximal RP on the contractions recorded in the distal RP could be readily demonstrated (Figure 3(i)). Likewise, application of stretch to the ureter had little effect on the contractile activity in either the proximal or distal RP ($n = 8$) (data not shown).

In some preparations applications of stretch to the proximal RP were associated with small changes in the basal tone of the distal PR. This change in basal tone upon stretch of the proximal RP does not underlie the inhibition of the contraction amplitudes observed in the distal RP for the

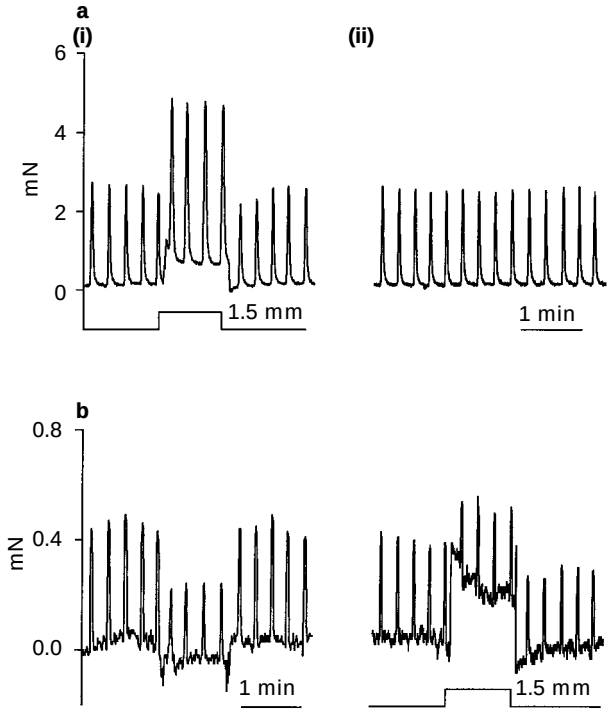

Figure 3 Application of circumferentially-directed stretch (1.5 mm for approximately 1 min) to the proximal RP ((i) a) evoked an inhibition in the contractile force of the distal RP ((i) b), but not in the ureter (data not shown). In contrast, the proximal RP ((ii) a) was not affected when a similar stretch was applied to the distal RP ((ii) b).

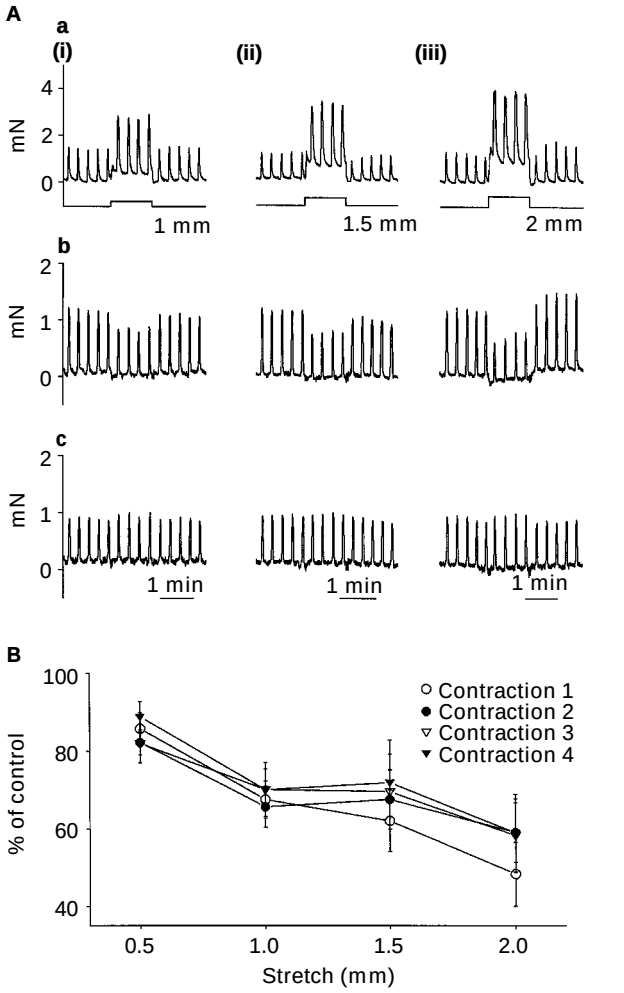

Figure 4 Inhibition of the distal RP, evoked upon stretch of the proximal RP, occurred in a stretch-dependent manner. Graded increases in stretch (1–2 mm) applied to the proximal RP (A(i–iii) a) induced an increasing inhibition of the contractile force in the distal RP (A(i–iii) b), without affecting the contractility of the ureter (A(i–iii) c). (B) The relative amplitude of contractions 1 to 4 measured in the distal RP during a stretch of the proximal RP were expressed as a percentage of the mean of the five preceding control contractions (% of control), averaged ($n=6$ preparations) and plotted against the degree of stretch (0.5–2 mm) applied to the proximal RP.

following reasons: (i) manually changing the basal tone of the distal RP, in the absence of any stretch applied to the proximal RP, had little effect on the amplitude of the contractions recorded in the distal RP; (ii) manually restoring the basal tone to control levels during a stretch of the proximal RP did not restore the amplitude of the contractions of the distal RP to control values, and (iii) similar changes in the basal tone were recorded in the distal RP, upon stretch of the proximal RP, when all spontaneous contractility was blocked in the presence of nifedipine (1 μM) ($n=2$).

Figure 4B summarizes the effects of stretch of the proximal RP on the four contractions recorded in the distal RP during the period of applied stretch. In this figure, the relative amplitude of contractions 1 to 4 have been averaged ($n=6$) and plotted against the increments of stretch (0.5–2 mm). It can be seen that the inhibitory effects of stretching the proximal RP on the contractile activity of the distal RP increased as the degree of stretch applied to the proximal RP was increased, and that this inhibition of the contractility of the distal RP lasted throughout the period of stretch. These effects of stretch were rapidly reversible, the contractile force of both the proximal and distal RP returning to control values upon release of the proximal RP (Figure 3(i), Figure 4A(i–iii)).

After a single exposure to capsaicin (10 μM for 15 min), the reduction of the contractile activity in the distal RP, induced by a stretch (0.5–2 mm) of the proximal RP, was little changed in 5 of 6 preparations (Figure 5). However, in the other preparation the effects of stretching the proximal RP on the distal RP were, in fact, enhanced by capsaicin pretreatment. A second exposure to capsaicin (10 μM for 15 min) also had little effect on the stretch-induced inhibition of the contraction amplitudes in the distal RP in 2 of 3 preparations. In contrast, the application of capsaicin (10 μM) for 30 min had variable effects on the stretch-induced inhibition of the distal RP; enhancing the stretch response in two preparations, reducing the stretch response in one preparation and having no effect in two other preparations. These data suggest that either the inhibitory mechanisms activated upon stretch of the proximal RP are predominantly resistant to desensitization by our capsaicin pretreatment, or the protocols/concentrations of capsaicin treatment in our experiments were not sufficient to deplete the sensory nerve varicosities of their inhibitory neuropeptides.

Effects of hCGRP and hCGRP (8–37)

The excitatory and inhibitory effects of sensory nerve stimulation on circumferentially-cut strips of the guinea-pig RP have been previously examined. The excitatory effects of nerve stimulation were blocked by the neurokinin antagonist, MEN 10,376, while the negative inotropic effects were blocked by the CGRP antagonist, hCGRP (8–37) (Maggi *et al*., 1992).

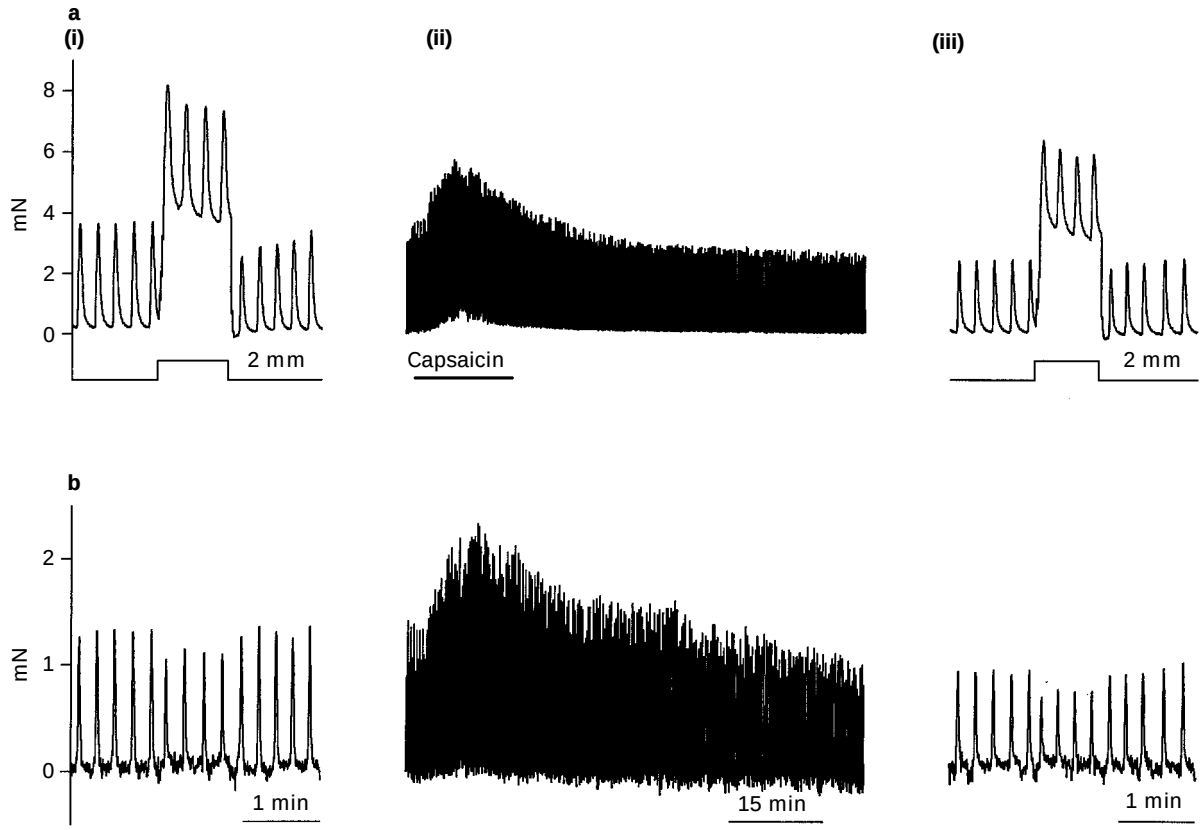

Figure 5 Effects of capsaicin on the spontaneous contractility of the UUT and the inhibition of the distal RP evoked upon stretch of the proximal RP. Capsaicin (10 μM) applied for 15 min and allowed to washout for 60 min, induced a transient increase in contractility, followed by a long-lasting inhibitory response in both the proximal ((ii) a) and distal ((ii) b) RP. The inhibition of the contractions in the distal RP ((iii) b) upon stretch (1.5 mm) of the proximal RP ((iii) a), was little affected ((i) a and b) after the pretreatment with capsaicin.

In the present experiments, the application of hCGRP (8–37) (1 μM) significantly reduced the inhibition of the four contractions recorded in the distal RP (Figure 6Ab(i–ii)) during a stretch of the proximal RP. For example, the averaged relative amplitude of contraction 1 (Figure 6B(i)) and contraction 4 (Figure 6B(ii)) after a 15 min exposure to hCGRP (8–37) was $86\pm5\%$ and $87\pm4\%$, respectively; significantly larger than those measured in control saline ($76\pm11\%$ and $80\pm12\%$, respectively); ($n=5$; $P<0.05$, 3-way repeated measures ANOVA) (Figure 6B(i–ii)). Higher concentrations of hCGRP (8–37) (3 μM), did not produce any further reductions of the effects of stretch of the proximal RP on the distal RP ($n=3$). These data suggest that a component (35–40%) of the inhibitory mechanism/s evoked upon stretch of the proximal RP may well involve the release of endogenous CGRP, which inhibits the contractility of the distal RP. However, we could not enhance this CGRP-dependent inhibitory component with thiorphan (1–10 μM), an inhibitor of neutral endopeptidase, which has previously been used to enhance the effects of sensory nerve-released CGRP in the guinea-pig RP and ureter (Maggi & Giuliani, 1994). In our experiments, thiorphan at concentrations of 1 μM ($n=6$) or 10 μM ($n=5$) had little effect on the stretch-induced decrease in the amplitude of the contractions recorded in distal RP ($P>0.05$; 3-way repeated measures ANOVA) (data not shown).

Application of the synthetic CGRP receptor agonist, hCGRP (100 nM), 60 min after pretreatment with capsaicin (10 μM for 15 min), inhibited the contractility of the UUT in a region-dependent manner. In the proximal RP, hCGRP (100 nM) caused a steady state decrease (of 24%) in the amplitude of the spontaneous contractions after 6 ± 0.7 min ($n=9$), with little change in the frequency of contraction (Figure 7a) (Maggi *et al*., 1995). At this time, the averaged MI of the proximal RP was reduced by 30% ($n=8$, $P<0.05$) (Table 2). The decrease in both the amplitude and frequency of contractions upon the addition of hCGRP (100 nM) occurred more rapidly in the distal RP. In 6 out of 8 preparations, there was a complete suppression of contractile activity in the distal RP after 4.9 ± 1 min exposure to hCGRP, thus the averaged MI value was reduced by 84% ($P<0.05$) (Figure 7b). The contractions of the ureter were completely suppressed in only 3 out of 5 experiments, leading to a 45% reduction in the MI values when compared to control values (Figure 7c). When contractions in both the distal RP and ureter were abolished, the rate at which these spontaneous contractions disappeared was similar. These inhibitory effects of hCGRP were found to be readily reversible in all three regions of the UUT upon washout of hCGRP (Figure 7c).

Effects of glibenclamide

Glibenclamide (1 μM), the blocker of ATP-dependent and/or cromakalim-activated potassium channels, has been previously demonstrated to block partially the effects of CGRP on the electrically-evoked contractions in the guinea-pig ureter (Maggi *et al*., 1994), but have little effect on the action of applied CGRP on the spontaneous contractions of the RP (Maggi *et al*., 1995). In the present study, the application of glibenclamide (1 μM) had no significant effect on the

contractility of the UUT such that the MIs for the proximal RP, distal RP and ureter, calculated after 30 min exposure to glibenclamide, were reduced by only 16.7% ($n=7$), 12.2% ($n=7$) and 0%, respectively ($n=5$; all $P>0.05$) (Figure 8a; Table 2).

The stretch-dependent inhibition of the distal RP, upon stretch (1.5 mm) of the proximal RP was significantly reduced in the presence of glibenclamide (1 μM) (Figure 8A(ii)) when compared to control ($n=4$) (Figure 8A(i)). In Figure 8B, the averaged relative amplitudes of contraction 1 (Figure 8B(i)) and contraction 4 (Figure 8b(ii)), in the absence and presence of glibenclamide (1 μM), have been plotted against stretch ($n=4$). It can be seen that the relative amplitude of contraction 1 and contraction 4 were both increased in the presence of glibenclamide. Statistical analysis of these data revealed that the effects of glibenclamide were significant at $P<0.05$ (3-way repeated measures ANOVA). Higher concentrations of glibenclamide (100 μM for >20 min), appeared to have a non-specific inhibitory action on the amplitudes of the contractions in the UUT, reducing the MI values in the proximal and distal RP and ureter by 71% ($n=4$), 79% ($n=4$) and 70% ($n=3$), respectively (Maggi *et al.*, 1995; Lang & Zhang, 1996) (Table 2). In 2 of these glibenclamide-affected preparations, the effects of stretch of the proximal RP on the distal RP were little affected; in one preparation, the effects of stretch were significantly enhanced, while in the other preparation the effects of stretch were significantly reduced.

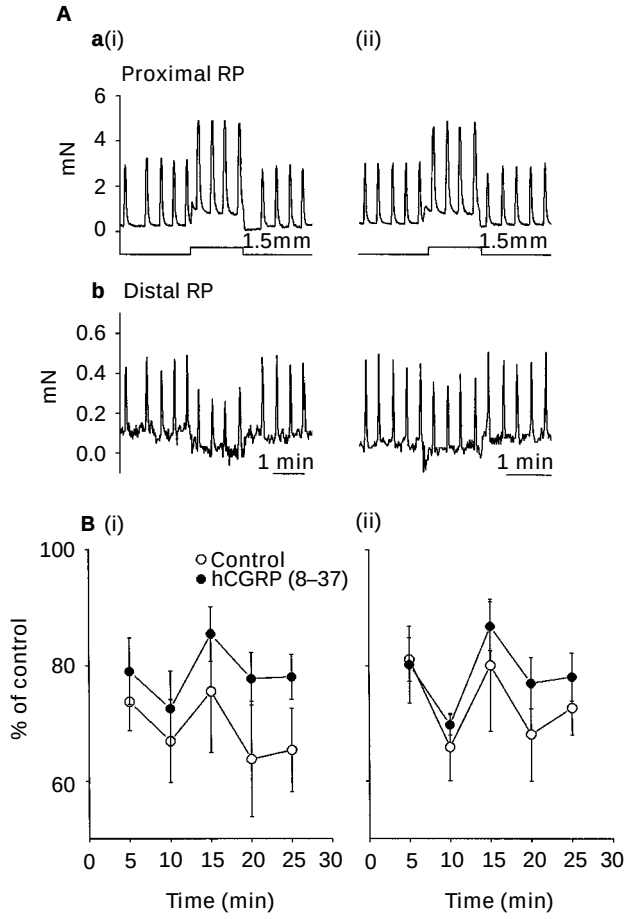

Figure 6 Example of the inhibition of the distal RP (Ab), evoked upon a 1.5 mm stretch of the proximal RP (Aa), in the absence (A(i)), and presence (A(ii)) of the CGRP receptor antagonist, hCGRP(8-37) (1 μM). (B) Averaged relative amplitudes (% of control) of contraction 1 (B(i)) and contraction 4 (B(ii)) ($n=5$) during an application of stretch were plotted against time. A 3-way repeated measures ANOVA of these data revealed that the effects of treatment, hCGRP(8-37), on the stretch-induced decrease of the distal RP were significantly different when compared to control.

Effects of TTX, ω-conotoxin GVIA and L-NOARG

The application of TTX (3 μM) had no significant effect on the spontaneous contractile activity of the UUT (Figure 9(ii)). The MIs for the proximal RP, distal RP and ureter, calculated 25 min after the addition of TTX (3 μM), were respectively, 89.6% ($n=7$; $P>0.05$), 84.0% ($n=7$; $P>0.05$) and 88.2% ($n=6$; $P>0.05$) of control values (Table 2). Tetrodotoxin (3–10 μM) also had little effect on the inhibition of the distal RP, evoked upon stretch of the proximal RP ($n=5$) (Figure 9(ii)). In only 2 of 6 preparations, the inhibitory effects of stretching the proximal RP on the contractility of the distal RP appeared to be reduced by TTX (3 μM) (data not shown). However, when the data in the presence of 3 μM TTX were pooled, the relative amplitudes of contraction 1 and contraction 4 in the distal RP (respectively $49.4\pm12.4\%$ and $80.7\pm5.7\%$) during a 1.5 mm stretch of the proximal RP were not significantly different from control ($55.6\pm14.8\%$ and $67.4\pm9.3\%$, respectively) ($P<0.05$). Similar results were obtained when 10 μM TTX was applied to the bath, only 2 out of 6 preparations showed an apparent block of the effects of stretch applied to the proximal RP.

Application of ω-conotoxin GVIA (100 nM), an agent which prevents influx of Ca^{2+} through 'N-type' Ca^{2+} channels, had a small inhibitory effect on the contractility of all three regions of the UUT. The MIs of the proximal and distal RP

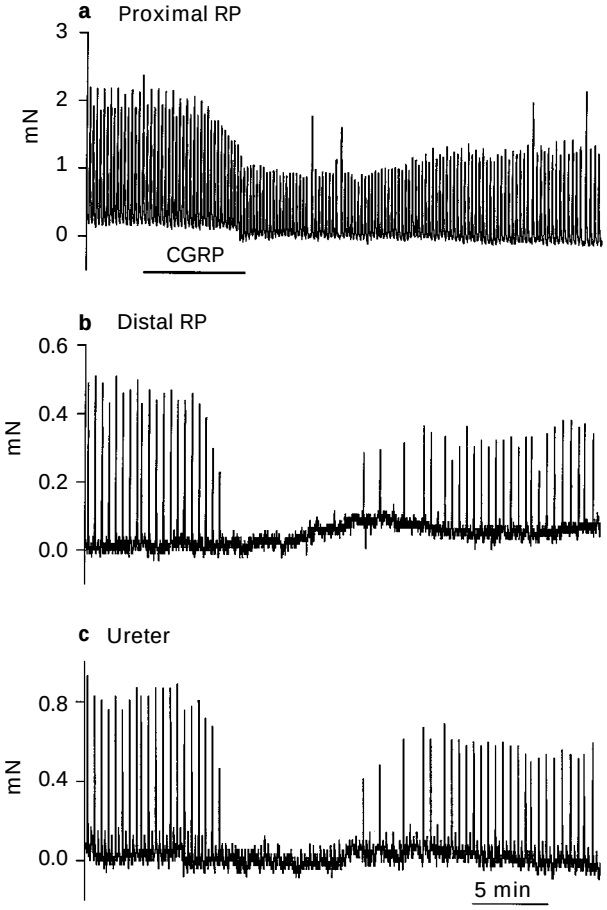

Figure 7 Typical example of the regional effects of hCGRP (100 nM) on the spontaneous activity of the UUT, illustrating the greater sensitivity of the distal RP (b) and ureter (c), when compared with the proximal RP (a).

Table 2 The effects of various agents on motility in the upper urinary tract of the guinea-pig

	Proximal renal pelvis			*Distal renal pelvis*			*Ureter*		
Drug	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)	*Frequency* (min^{-1})	*Amplitude* (mN)	*Motility index* (Freq × Amp)
Control	4.7±0.3	2.7±0.4	12.9±2.0	4.3±0.5	1.2±0.4	5.1±1.8	5.0±0.5	1.0±0.3	4.5±1.4
hCGRP (100 nM)	4.5±0.3	2.1±0.4*	9.0±1.7* ($n=8$)	0.8±0.6*	0.4±0.3*	0.8±0.6* ($n=8$)	4.6±0.7	0.5±0.2	2.6±1.2 ($n=5$)
Control	4.2±0.6	2.4±0.5	10.8±3.5	4.2±0.6	1.0±0.2	4.1±0.7	3.6±0.6	0.7±0.2	2.3±0.3
Glibenclamide (1 μM)	4.4±0.5	2.3±0.7	9.0±2.8 ($n=7$)	4.4±0.5	0.9±0.3	3.6±0.8 ($n=7$)	3.9±0.6	0.7±0.2	2.3±0.5 ($n=5$)
Control	4.1±1.0	2.6±0.3	10.7±3.5	4.1±1.0	0.8±0.3	4.1±2.6	3.1±0.5	0.3±0.1	0.9±0.3
Glibenclamide (100 μM)	3.8±1.3	1.1±0.3*	3.1±0.6 ($n=4$)	3.7±1.4	0.2±0.1	0.8±0.3 ($n=4$)	1.2±1.0	0.2±0.1*	0.3±0.2* ($n=3$)
Control	5.0±0.5	2.8±0.5	13.4±2.4	5.0±0.5	1.0±0.2	5.0±1.1	5.4±0.5	0.3±0.0	1.7±0.2
Tetrodotoxin (3 μM)	5.2±0.5	2.6±0.5	12.0±2.0 ($n=10$)	5.2±0.5	0.9±0.2	4.2±0.9 ($n=10$)	4.8±1.0	0.3±0.1	1.5±0.3 ($n=7$)
Control	3.5±0.8	1.3±0.4	3.7±0.6	3.5±0.8	1.2±0.2	3.9±0.5	2.9±0.8	1.0±0.1	2.3±0.2
ω-Conotoxin GVIA (100 nM)	3.0±0.7	0.8±0.2	2.2±0.5* ($n=4$)	3.0±0.7	1.2±0.2	3.1±0.3 ($n=4$)	2.6±0.9	0.9±0.2*	1.7±0.2* ($n=3$)
Control	4.1±0.3	1.4±0.3	5.8±1.8	4.1±0.3	0.6±0.2	2.4±0.6	3.7±0.2	0.4±0.1	1.7±0.5
N^G-nitro-L-arginine (100 μM)	4.1±0.3	1.3±0.5	5.6±2.3 ($n=4$)	4.1±0.3	0.5±0.2	1.9±0.5 ($n=4$)	3.1±0.5	0.3±0.1	1.1±0.4* ($n=4$)

*Denotes a significant difference between control saline and applied drug.

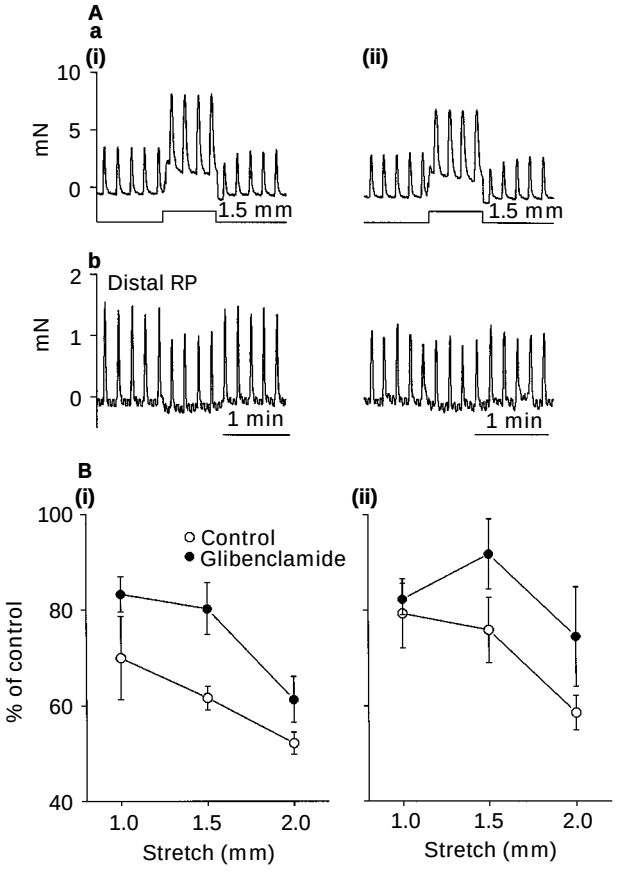

Figure 8 Glibenclamide (1 μM) significantly blocked the inhibition of the distal RP (A,b) evoked upon stretch of the proximal RP (A, a). (A) Typical example of the inhibition of the distal RP in the absence (A(i)) and presence (A(ii)) of glibenclamide (1 μM). (B) The averaged relative amplitudes of contraction 1 (B(i)) and contraction 4 (B(ii)) ($n=4$), plotted against increments of stretch (1–2 mm), were significantly greater in the presence of glibenclamide (1 μM) compared with control (3 way repeated measures ANOVA).

and ureter were 59.5% ($n=4$; $P<0.05$), 79.5% ($n=4$; $P>0.05$) and 73.9% ($n=3$; $P<0.05$) of control values, respectively (Table 2). The addition of ω-conotoxin GVIA, 60 min after the capsaicin pretreatment (10 μM for 15 min), had a significant effect on the amplitude of the contractions in the proximal RP, but the MIs in all three regions of the UUT were not significantly affected (Table 1), perhaps further supporting our suggestion that the capsaicin desensitization protocols used in the present experiments do not completely deplete the UUT of all sensory neuropeptides. The stretch (1.5 mm)-induced inhibition of the contractility of the distal RP was also not affected by ω-conotoxin GVIA (100 nM); the relative amplitudes of contraction 1 and contraction 4 (respectively, 46±16% and 69±6%) after 50 min exposure to ω-conotoxin GVIA (100 nM) were not significantly different from control (respective control relative amplitudes were 58±7% and 67±4%) ($n=4$; both $P>0.05$).

Except for the ureter, the nitric oxide synthase inhibitor, L-NOARG (100 μM applied for 30 min), had no effect on either the spontaneous contractility of the UUT, or on the inhibitory effects of stretching the proximal RP on the contractility of the distal RP. In four experiments, the MIs for the proximal and distal RP and ureter were 96.6%, 79.2% ($n=4$; both $P>0.05$) and 64.7% ($n=4$; $P<0.05$). The averaged curves of relative contraction amplitude against stretch (0.5–2 mm) in the absence and presence of L-NOARG were also superimposable (data not shown). These result are consistent with the previously demonstrated lack of effect of L-NOARG (30 μM) on the inhibitory action of applied CGRP in the RP and ureter (Maggi *et al*., 1994).

Discussion

Previous experiments on isolated circumferentially-cut strips of the UUT have led to the notion that ureteral peristalsis is generally myogenic in origin, and that peristaltic contractions

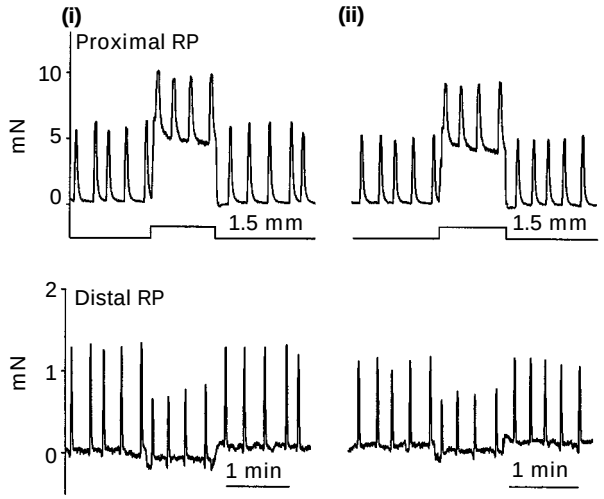

Figure 9 Exposure to tetrodotoxin (TTX, 3 μM) had little effect on the inhibition of the distal RP, evoked upon stretch of the proximal RP ((ii)) compared with control ((i)).

arise primarily from the intrinsic electrical activity of 'pacemaker' smooth muscle cells located in the proximal UUT, rather than through the activity of intrinsic motor nerves (Golenhofen & Hannappel, 1973; Hannappel & Lutzeyer, 1978; Constantinou *et al*., 1978; Potjer *et al*., 1992; Maggi & Giuliani, 1992). In this study, we have developed a whole mount preparation of the UUT which readily demonstrates these propagating spontaneous contractions. In most preparations (79%), propagating contractions originated in the proximal UUT at a frequency of 4.5 min^{-1} and travelled distally at a rate of 1–3 cm s^{-1}; although in some preparations propagating contractions appeared first in the distal RP. The intrinsic frequency of these spontaneous contractions in the UUT could be transiently increased upon electrical nerve stimulation or during exposure to capsaicin, but was not affected by pretreatment with TTX, ω-conotoxin GVIA or L-NOARG (Maggi *et al*., 1992), or upon circumferentially-applied stretch (Potjer *et al*., 1992). After washout of capsaicin (60 min), the frequency and amplitudes of the spontaneous contractions were reduced 8–23 and >30%, respectively, suggesting that the tonic release of tachykinins, from the varicosities of capsaicin-sensitive nerves maintains, the amplitude of contractions in the UUT, and, to a lesser extent, the mechanisms that trigger them. The release of these tachykinins is presumably spontaneous; requiring Ca^{2+} entry into sensory nerve varicosities, possibly through 'N-type' Ca^{2+} channels, but not the propagation of an action potential along the sensory nerves.

In the guinea-pig UUT, the effects of electrically stimulating the intramural sensory nerves are predominantly excitatory in the proximal UUT and inhibitory in the distal UUT (Maggi & Giuliani, 1991; Maggi *et al*., 1992; Zhang & Lang, 1994). To date, there is considerable evidence that tachykinins and CGRP are co-localized in sensory nerves within the smooth muscle layer and epithelial lining, and wrapped around blood vessels of the mammalian urinary tract (Sann *et al*., 1992). However, the ability of capsaicin to release these neuropeptides appears to vary between species and regions in a time- and concentration-dependent manner (Hua *et al*., 1987; Amann *et al*., 1988; Maggi *et al*., 1992). In the neonatal rat, the loss of extractable CGRP-like immunoreactivity was proportional to the number of capsaicin doses systemically injected. In the adult guinea-pig, systemic application of capsaicin, daily over 5 days, is necessary to produce a near complete (98%) disappearance of substance P- and CRGP-like extractable immunoreactivity from the ureter and a near disappearance of CGRP-like immunoreactive nerves in the RP (Su *et al*., 1986). In the guinea-pig gall bladder, capsaicin treatment reduces the tissue content of substance P- and CGRP-like immunoreactivity by only 50% (Maggi *et al*., 1989). In the present experiments, it is clear that, after a single exposure to capsaicin (10 μM for 15 min), a negative inotropic effect could still be evoked upon electrical nerve stimulation (sensitive to TTX blockade) or during a second exposure to capsaicin (Figure 2a). It is likely, therefore, that our capsaicin desensitization protocols did not completely deplete the UUT preparation of all of its sensory neuropeptides, particularly CGRP.

Movements of the micrometer attached to the tension transducer of 0.5 to 2 mm corresponded to a lengthening of the RP approximately 3 to 30%. These stretches of the proximal RP, in 60% of preparations, evoked an inhibition of the amplitude of the propagated contractions in the distal RP which remained throughout the period of stretch. This stretch-evoked response was region specific and unidirectional; occurring only upon stretch of the proximal RP, and affecting only the contractile force of the distal RP and not the ureter. In contrast, the application of stretch to the distal RP, or ureter, had little effect on the contractility of the other regions of the UUT, suggesting that this inhibition is not a non-specific artefact due to a geometric distortion of the preparation during an applied stretch.

In the present study, we have demonstrated that about 40% of the decrease in the amplitude of the contractions in the distal RP during a stretch of the proximal RP was blocked by hCGRP (8–37), suggesting the involvement of a stretch-evoked release of endogenous CGRP. The application of glibenclamide (1 μM) also partially, but significantly, reduced the inhibitory effects of stretch (1–2 mm) of the proximal RP to a similar extent (by 10–60%) as hCGRP (8–37) (Figures 6 and 8), perhaps suggesting that the CGRP component of the stretch-evoked inhibition involves the opening of glibenclamide-sensitive K^+ (K_{ATP}) channels. Previously, glibenclamide has been demonstrated to block the inhibitory effects of both nerve-released and directly-applied CGRP in the guinea-pig ureter (Maggi *et al*., 1994; Santicioli & Maggi, 1994), but to have little effect on the CGRP-evoked inhibition of the contraction amplitudes in the RP (Maggi *et al*., 1995). In contrast, the inhibitory actions of the K^+ channel opener, cromakalim, on the spontaneous contractions of the proximal and distal RP (Maggi *et al*., 1995) and the evoked contractions in the ureter (Meini *et al*., 1994) are readily reduced by glibenclamide. We have recently confirmed these observations in our spontaneously-active whole mount preparation. In our hands, lemakalim (1 μM) significantly reduced the MIs in the proximal and distal RP, and ureter to 13.7%, 6.6% and 60%, respectively of control MI values (all $P<0.05$; $n=4$). The addition of glibenclamide (10 μM) partially restored these MI values to 63.7%, 71.1% and 61% of control values respectively (B. Skelton & R.J. Lang unpublished data) (Maggi *et al*., 1994, 1995).

In the guinea-pig gall bladder, it has also been demonstrated that CGRP and lemakalim inhibit muscle contraction (Kline *et al*., 1991) upon the opening of glibenclamide-sensitive K_{ATP} channels which cause membrane hyperpolarization and inhibition of action potential discharge (Zhang *et al*., 1994b). Moreover, the activation of these glibenclamide-sensitive K_{ATP} channels by CGRP was directly demonstrated at the whole-cell current level and mimicked by the adenylate cyclase activator, forskolin, by membrane permeable analogues of adenosine 3′,5′-cyclic monophosphate and by the catalytic subunit of

protein kinase A (Zhang *et al*., 1994a). However, in our whole mount preparation forskolin (1 μM) partially inhibited contractility in all three regions of the UUT in a manner not reversed by glibenclamide. The calculated MIs for the proximal and distal RP and ureter in the presence of forskolin, were 81.7%, 48.8% and 22.2% ($n=5$), respectively, of control values. When glibenclamide was added in the presence of forskolin, these MIs were little affected (respectively being 82.9%, 47.5% and 27.7% of control) (all $P>0.05$; $n=5$) (B. Skelton & R.J. Lang, unpublished data). Thus, in the present experiments it appears that stretch of the proximal RP evokes the release of endogenous CGRP which activates glibenclamide-sensitive K^+ channels only in the distal regions of the UUT and that the transduction pathway between the activated CGRP receptor and K_{ATP} channel opening is unlikely to involve the adenylate cyclase system.

It is likely that stretch-evoked release of CGRP (Maggi *et al*., 1990) is acting either on the smooth muscle of the distal RP to reduce directly its contractility, or at the points where the proximal and distal RP are in syncytial contact to reduce the conductivity of the electrical signals responsible for the propagating contraction (Maggi *et al*., 1995). Such a CGRP-evoked inhibition of excitation conduction has recently been demonstrated in the guinea-pig ureter, where the direct application of human αCGRP or cromakalim to the middle region of the ureter prevented the propagation of contractions evoked in the proximal ureter (by electric field stimulation) to the distal ureter (Meini *et al*., 1994). The fact that stretch-evoked inhibition of the UUT is unidirectional may also arise, in part, from a regional distribution/sensitivities of CGRP receptors in the guinea-pig UUT. We have demonstrated that the proximal RP was significantly less affected by applied hCGRP than either the distal RP or ureter. Moreover, the distal RP appears to be more sensitive to hCGRP than the ureter (Figure 7). These data directly confirm the suggestion of such regional differences arising from the experiments of Maggi and colleagues, in which hCGRP was applied either to spontaneously-contracting strips of proximal and distal RP, or to electrically-driven strips of ureter, bathed in the Ca^{2+} channel agonist, Bay K 8644, (Maggi *et al*., 1994, 1995).

In the present study, the development of the stretch-evoked inhibition of the distal RP was considerably more rapid than the inhibitory effects of applied hCGRP. Moreover, only about 40% of the stretch-evoked inhibition of the distal RP was blocked by the CGRP antagonist, hCGRP (8–37) or glibenclamide. This stretch-evoked inhibition was also not significantly affected by TTX (3–10 μM) (Figure 9), ω-conotoxin GVIA (100 nM) (data not shown) or by capsaicin (10 μM for 15 min) (Figure 5), suggesting that additional mechanisms other than the release of endogenous inhibitory neuropeptides from nerves must be involved. Recently, it has been demonstrated that the spread of excitability in the sheep renal pelvis was invariably initiated from a single, often shifting, 'pacemaker' site at the pelvicalyceal border. Furthermore, the pathway of conduction from the proximal RP to the distal portions of the UUT was slow, inhomogeneous, such that a propagating electrical event could often meander throughout the pelvis before reaching the pelviureteric junction (Lammers *et al*., 1996). Conduction delay or blockade could also occur at any point within the renal pelvis, suggesting that the coupling between the smooth muscle bundles of the RP may not be permanent and therefore susceptible to modulation by endogenously-released factors or muscle stretch. It may well be that stretch of the proximal RP is influencing the conduction of excitability to the distal RP, which, in turn, contributes to the reduction of the contractions recorded in the distal RP. Such a mechanism could explain the rapid onset and offset of the inhibition of the distal RP seen with stretch of the proximal RP. The occurrence of the stretch-evoked inhibition in only 60% of preparations, and whether the net effects of electrical nerve stimulation were inhibitory or excitatory, could also be explained by the fact that the partial bisection of the RP into proximal and distal regions would be randomly interrupting these conduction pathways, as well as the intramural sensory nerves themselves. The elucidation of the inhibitory mechanism activated by stretch, but not blocked by hCGRP (8-37) or glibenclamide, requires further experimentation.

In summary, we have developed a whole mount preparation of the upper urinary tract of the guinea-pig that displays spontaneous propagating contractions which can be modulated by intrinsic sensory nerves, stimulated electrically or chemically with capsaicin. We have demonstrated that circumferentially-applied stretch of the proximal RP releases endogenous CGRP, presumably from capsaicin sensory nerves, in a manner independent of action potential discharge. Furthermore, this stretch-released CGRP contributes to the inhibition of the distal RP via the activation of a glibenclamide-sensitive mechanism, which is mostly absent in the proximal RP. Finally, the inhibition of the distal RP, evoked upon stretch of the proximal RP, appears to involve additional mechanisms, presumably within the smooth muscle layer of the RP.

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