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Excitatory motor and electrical effects produced by tachykinins in the human and guinea-pig isolated ureter and guinea-pig renal pelvis

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1 In isolated tissue experiments, neurokinin A (NKA) produced concentration-dependent contraction of human and guinea-pig ureter ($pD_2 = 6.7$ and 7.2, respectively); an effect greatly reduced (>80% inhibition) by the tachykinin NK_2 receptor-selective antagonist MEN 11420 (0.1 μM). The tachykinin NK_1 and NK_3 receptor agonists septide and senktide, respectively, were ineffective.

2 Electrical field stimulation (EFS) of the guinea-pig isolated renal pelvis produced an inotropic response blocked by MEN 11420 (0.01–1 μM). In the same preparation MEN 11420 (0.1 μM) blocked (apparent $pK_B = 8.2$) the potentiation of spontaneous motor activity produced by the NK_2 receptor-selective agonist [βAla^8]NKA(4–10).

3 In sucrose-gap experiments, EFS evoked action potentials (APs) accompanied by phasic contractions of human and guinea-pig ureter, which were unaffected by tetrodotoxin or MEN 11420 (3 μM), but were blocked by nifedipine (1–10 μM). NKA (1–3 μM) produced a slow membrane depolarization with superimposed APs and a tonic contraction with superimposed phasic contractions. NKA prolonged the duration of EFS-evoked APs and potentiated the accompanying contractions. MEN 11420 completely prevented the responses to NKA in both the human and guinea-pig ureter.

4 Nifedipine (1–10 μM) suppressed the NKA-evoked APs and phasic contractions in both human and guinea-pig ureter, and slightly reduced the membrane depolarization induced by NKA. A tonic-type contraction of the human ureter in response to NKA persisted in the presence of nifedipine.

5 In conclusion, tachykinins produce smooth muscle excitation in both human and guinea-pig ureter by stimulating receptors of the NK_2 type only. NK_2 receptor activation depolarizes the membrane to trigger the firing of APs from latent pacemakers.

Keywords: Tachykinins; tachykinin NK_2 receptor; tachykinin receptor antagonists; MEN 11420 (Nepadutant); human ureter; guinea-pig ureter and renal pelvis; urinary tract

Introduction

The mammalian tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), are widely distributed in the central and peripheral nervous system. The most widespread and extensively investigated biological effect exerted by tachykinins in the peripheral nervous system is smooth muscle contraction, produced through the activation of specific membrane receptors termed NK_1, NK_2 and NK_3 (Regoli *et al*., 1989; Guard & Watson, 1991; Maggi *et al*., 1993). Recently, evidence has been provided for the existence of a structurally distinct subtype of the NK_3 receptor, also termed NK_4 (Donaldson *et al*., 1996; Krause *et al*., 1997), but the relevance of this receptor in the peripheral effects of tachykinins is presently unsettled.

In the rat and guinea-pig renal pelvis and ureter, tachykinins (SP and NKA) are co-stored with calcitonin gene-related peptide (CGRP) in the peripheral endings of capsaicin-sensitive primary afferent neurons. SP and NKA are released from these sensory nerve endings to produce motor responses which are involved in the local regulation of pyeloureteral motility (see Maggi, 1995, for review). In particular, both NKA and SP produce contraction of the rat and guinea-pig, isolated ureter, characterized by the appearance of rhythmic and long-lasting phasic contractions (Hua & Lundberg, 1986; Maggi *et al*., 1986, 1987; Nagahisa *et al*., 1992). In the rat ureter, the contractile responses to tachykinins are mediated by NK_2 and NK_3 receptors (Maggi *et al*., 1998a), whereas the receptor(s) responsible for the contractile effect of tachykinins in the guinea-pig ureter has not been characterized yet. In the guinea-pig isolated renal pelvis tachykinins produce positive inotropic and chronotropic effects on spontaneous rhythmic contractions (Maggi *et al*., 1992a,b). Tachykinin receptors of the NK_2 and, at a lesser extent, of the NK_1 type mediate the effects of tachykinins in the guinea-pig isolated renal pelvis (Maggi *et al*., 1992a).

Nerve fibres containing tachykinin-like immunoreactive material (SP- and NKA-like immunoreactivity) are present in the human renal pelvis and ureter, showing a similar distribution to that observed in other species (Hua *et al*., 1987; Edyvane *et al*., 1992, 1994; Zheng and Lawson, 1997). Fewer information is available about the motor effect produced by tachykinins in the human ureter: to our knowledge the only report on this matter has been presented by Hua and coworkers (1987), who described an excitatory motor response produced by NKA. However, no attempt was made to characterize the receptor(s) involved in the above responses to NKA.

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In the present study we have investigated the motor and electrical activities exerted by natural and synthetic receptor-selective tachykinin agonists in the human isolated ureter, with organ bath and sucrose-gap electrophysiological techniques. Similar experiments have been performed on the guinea-pig isolated ureter, aiming to characterize the tachykinin receptors mediating ureteral contraction in this species. To assess the involvement of NK_2 receptors, we used the receptor-selective antagonist MEN 11420 (Nepadutant) (Catalioto *et al*., 1998), which was tested against exogenously-applied tachykinins in both the human and guinea-pig ureter. In addition, MEN 11420 was tested in the guinea-pig renal pelvis to block the excitatory motor effects produced by endogenous tachykinins released upon application of electrical field stimulation (EFS).

Methods

General

All the experiments performed on human preparations were conducted on longitudinal muscle strips excised from the middle region of the ureter. Ureteral specimens were obtained from five patients (three males and two females: age 60–83 years) undergoing cystectomy or nephrectomy because of tumours. No patient had received radio- or chemotherapy before surgery. In all patients pre-anaesthetic medication was intramuscular atropine (1 mg) and diazepam (10 mg). Anaesthesia was induced by sodium thiopenthal (500 mg i.v.) and maintained with N_2O/O_2 (1/2) and halothane (0.6–1%). The patients received pancuronium bromide (6 mg i.v.) during induction of anaesthesia.

All specimens appeared macroscopically normal without signs of tumour or inflammation. Small longitudinal strips of ureter were prepared immediately after surgical removal and placed in ice-cold, gassed (96% O_2 and 4% CO_2) Krebs-Henseleit solution having the following composition in mM: NaCl, 119; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.5; $CaCl_2$, 2.5; KCl, 4.7 and glucose 11. The strips were kept at 4°C overnight in oxygenated Krebs-Henseleit solution. The next day, 15–20 h from excision, the strips were used for functional (organ bath) or sucrose-gap experiments.

Male albino guinea-pigs (250–300 g) were stunned and bled. The whole kidney with ureter attached was rapidly removed and placed in oxygenated Krebs-Henseleit solution. The renal pelvis was carefully dissected from the renal parenchyma, separated from the ureter and cut in two halves, as described previously (Maggi *et al*., 1992a,b). Both ends of the guinea-pig ureter were cut, and the middle portion was used for both functional and sucrose-gap experiments.

Organ bath experiments

Human ureteral strips (10–15 mm long, 2–3 mm wide), guinea-pig ureters and circular strips of the renal pelvis were set up on 5 ml organ baths, containing warmed (37°C) and oxygenated (96% O_2 and 4% CO_2) Krebs-Henseleit solution and connected to isotonic force transducers against a load of ten (human ureter) or two (guinea-pig ureter or renal pelvis) mN. Indomethacin (3 μM) was added to Krebs-Henseleit solution for the experiments with the renal pelvis. All the experiments commenced after an equilibration period of 60–90 min. In both the human and guinea-pig ureteral preparations NKA elicited the start of rhythmic contractions (or increased the frequency of a pre-existing motor activity) and produced also a tonic contractile response. Owing to the nature of this response, cumulative concentration-response curves to NKA could not be constructed, and a consecutive procedure of agonist application was adopted, as follows: 10 fold-increasing concentrations of NKA were administered to human or guinea-pig ureteral preparations every 30 min, left in contact with the tissue until a maximal response had developed (at least 5 min) and then washed out. MEN 11420 was tested against the motor response produced by a single submaximal concentration of NKA in both the human and guinea-pig ureter. In these latter experiments NKA (0.1 μM, given every 30 min) was administered until a reproducible response was obtained. MEN 11420 (0.1 μM) was added to the bath 15 min before the next challenge with NKA.

A separate series of experiments was performed in the guinea-pig renal pelvis, to assess the potency of MEN 11420 in blocking the effect of endogenous tachykinins at this level. Cumulative concentration-response curves to the tachykinin NK_2 receptor selective agonist [βAla^8]NKA(4–10) were constructed: since a minor population of tachykinin NK_1 receptors exists in this preparation (Maggi *et al*., 1992a), all the experiments were performed in the presence of the tachykinin NK_1 receptor antagonist SR 140333 (0.1 μM, 30 min before) (Emonds-Alt *et al*., 1993) to prevent stimulation of tachykinin NK_1 receptors by [βAla^8]NKA(4–10) at high concentrations (see Patacchini *et al*., 1994).

MEN 11420 was tested on matched strips of renal pelvis obtained from the same animal: one strip received the vehicle and served as control, and the other was pretreated with MEN 11420 (0.1 μM; 15 min incubation period), before constructing a cumulative concentration-response curve to the agonist. In the guinea-pig renal pelvis MEN 11420 was also tested against the nonadrenergic noncholinergic (NANC) contraction elicited by EFS (trains of 100 V, 0.25 ms duration, 5 Hz, for 10 s given every 30 min) in matched preparations. The contractile response to KCl (80 mM) was used as the internal standard in all experiments.

Sucrose-gap

In sucrose-gap experiments, human ureteral strips (10–15 mm long, 0.5–0.8 mm wide) and guinea-pig ureteral segments (10–15 mm long) were prepared and placed in a 0.5 ml recording chamber. All preparations were continuously superfused (1 ml min^{-1}) with oxygenated (96% O_2 and 4% CO_2) and warmed (35$\pm$0.5°C) Krebs-Henseleit solution (composition as above) or, in the case of the guinea-pig ureter, with a modified Krebs' solution deprived of Mg^{2+} ions (composition in mM: NaCl, 120.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $CaCl_2$, 2.5; KCl, 4.7 and glucose 11). The latter solution was used because in preliminary experiments we found that removal of Mg^{2+} ions improves the reproducibility of action potentials evoked by EFS in the guinea-pig ureter. A single sucrose-gap, modified as described by Artemenko *et al*. (1982) and Hoyle (1987), was used to investigate simultaneously changes in membrane potential and contractile activity of the ureteral smooth muscles in response to chemical or electrical stimulation. All human and guinea-pig preparations were superfused with capsaicin (10 μM for 15 min), in order to block the efferent function of primary afferent neurons in the preparations. After a 60 min equilibration period, the preparations were stimulated by application of single electrical pulses (30–40 V, 1–5 ms pulse width) which were automatically delivered every 60 s. All drugs tested were administered by superfusion.

Concentrations of 1 and 3 μM of NKA, applied for 15 s, were selected for experiments in the guinea-pig and human

ureter, respectively: these concentrations were selected from the knowledge on the potency of NKA in the producing a contraction of the guinea-pig and human ureter in organ bath experiments. In particular, the concentration of NKA tested was about 10 fold larger than the estimated EC_{50} values (see results) in the two species. NKA was applied in superfusion every 45 min until reproducible responses were obtained.

All data were recorded and analysed by using a MacLab digital recorder system (AD Instruments).

Statistical analysis

The values in the text, tables or figures are expressed as means ± 95% confidence limits (95% c.l.), or ± s.e.mean. Statistical analysis was performed by means of Student's *t*-test for paired or unpaired data or by means of two-way analysis of variance (ANOVA), when applicable. Regression analysis of log concentration-effect curves was performed by the last squares method, considering the curves linear between 20 and 80% of the maximal response.

Evaluation of data

Agonist activity was expressed in terms of pD_2. Antagonist affinity of MEN 11420 tested at a single concentration was expressed as apparent pK_B, which was estimated as the mean of the individual values obtained with the Gaddum-Schild equation:

$$pK_B = \log [\text{dose ratio} - 1] - \log [\text{antagonist concentration}]$$

(see Kenakin, 1993; Jenkinson, 1991)

In sucrose-gap experiments the latency of action potentials (APs) evoked by EFS was measured as the time interval between stimulus artifact and onset of the change in membrane potential. The time to peak of APs was calculated from the onset to the maximal value in membrane potential. The duration of the APs and contraction was calculated from their onset to 90% recovery of repolarization or relaxation respectively.

Drugs

MEN 11420 (Nepadutant) (or: c{[(*β*-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2*β*-5*β*)}) and [*β*Ala^8]NKA (4–10) were synthesized at Menarini Laboratories, Florence, Italy, by conventional solid-phase methods. Nifedipine, indomethacin and capsaicin were from Sigma (St. Louis, MO, U.S.A.). NKA, senktide, septide and [Sar^9]substance P sulphone were from Peninsula Laboratories (St. Helens, England). Tetrodotoxin was from Sankyo (Japan). The nonpeptide antagonist SR 140333 [(S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-n-3yl]ethyl}-4-phenyl-1-

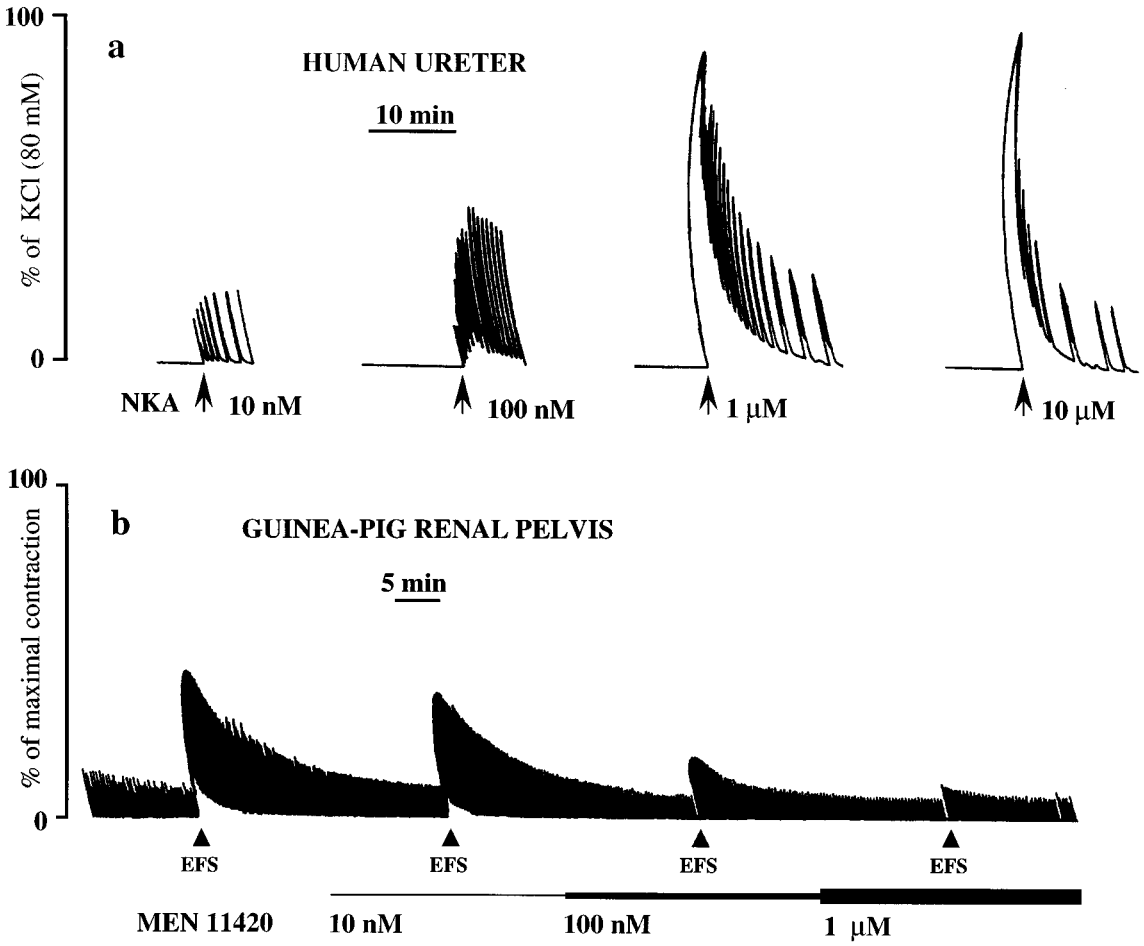

Figure 1 (a) Typical tracing showing the contractile responses produced by neurokinin A (NKA) in the human isolated ureter longitudinal strip preparation. The interval between two administrations of NKA is 30 min. (b) Typical tracing showing the inotropic response of the guinea-pig isolated renal pelvis to electrical field stimulation (EFS: trains of 100 V, 0.25 ms duration, 5 Hz, for 10 s, given every 30 min) before and after the administration of increasing concentrations of MEN 11420 (given 15 min before application of EFS).

azoniabi-cyclo[2,2,2]octane chloride] was kindly provided by Drs X. Emonds-Alt and G. Le Fur, Sanofi (Montpellier, France).

Results

Organ bath experiments

Human and guinea-pig ureter Most preparations of human ureter (about 70–80%) developed an irregular phasic contractile activity within 60–90 min from set-up. The amplitude of this spontaneous activity averaged 40±7% ($n=12$) of the maximal response to KCl 80 mM, at a frequency of 1.8±0.4 contractions min^{-1} ($n=12$) and declined or even disappeared following the elicitation of contractile responses to NKA or KCl. On the other hand, all guinea-pig preparations were quiescent.

The tachykinin NK_1 and NK_3 receptor-selective agonists septide and senktide (1 μM; $n=4$ each), respectively, failed to produce a contraction in either the human and guinea-pig isolated ureter. In contrast, NKA (10 nM–10 μM) evoked a concentration-dependent contraction in both the human ($pD_2=6.7\pm0.13$; $E_{max}=89\pm4\%$ of the response to KCl 80 mM, $n=4$) and guinea-pig ureter ($pD_2=7.2\pm0.07$; $E_{max}=67\pm13\%$ of the response to KCl 80 mM, $n=4$) (Figures 1a and 2a). In both cases the response to threshold concentrations of NKA consisted of a series of phasic contractions whereas at higher concentrations a tonic contraction developed onto which a series of phasic contractions superimposed (Figure 1a).

MEN 11420 (0.1 μM; 15 min before) almost abolished (96±2% inhibition, $n=4$) the contraction produced by a submaximal concentration of NKA (0.1 μM) in the human ureter and greatly reduced (81±7% inhibition, $n=4$) the corresponding response evoked by NKA in the guinea-pig ureter (Figure 2b).

Guinea-pig renal pelvis Circularly-oriented muscle strips from the guinea-pig renal pelvis showed a regular and long-lasting spontaneous phasic contractile activity, which persisted throughout the experimental period. Application of EFS produced a positive inotropic effect superimposed on the spontaneous activity (Figure 1b). MEN 11420 (10 nM–1 μM, 15 min before) inhibited in a concentration-dependent manner the response to EFS (Figure 1b). At the highest concentration tested MEN 11420 (1 μM) almost abolished the EFS-induced contraction (90±6% inhibition; $n=4$).

The tachykinin NK_2 receptor-selective agonist [βAla^8] NKA(4–10) (1 nM–3 μM), in the presence of SR 140333 (0.1 μM; 30 min before), produced a concentration-dependent contraction of the smooth muscle of the renal pelvis consisting of both a positivie chrono- and inotropic effect, as detailed previously (Maggi *et al*., 1992a,b). MEN 11420 (0.1 μM;

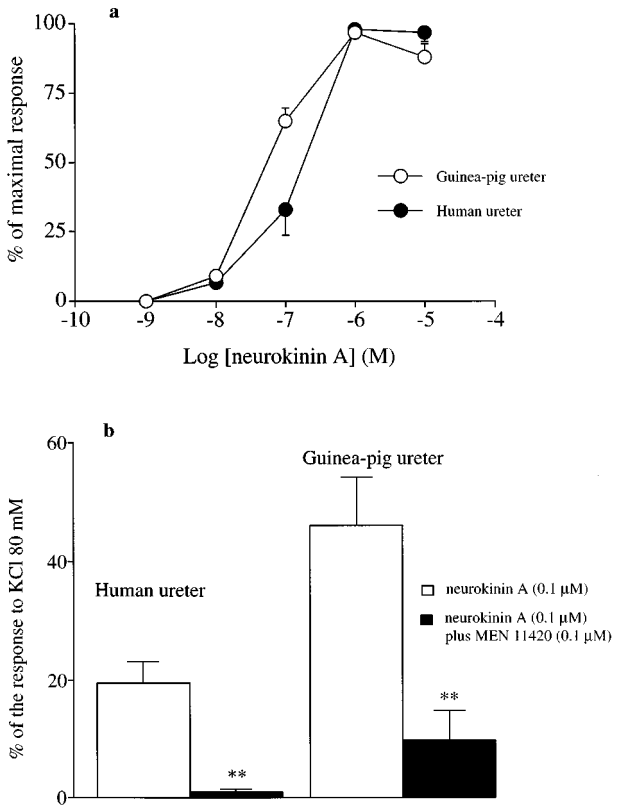

Figure 2 (a) Log concentration-response curves to neurokinin A in the human and guinea-pig isolated ureter. (b) Blockade by MEN 11420 (0.1 μM, 15 min before) of neurokinin A (0.1 μM)-induced contraction of the human or guinea-pig isolated ureter preparation. Each value in the figure is mean±s.e.mean of four experiments. **Significantly different from control response: $P<0.01$.

Table 1 Effect of neurokinin A (NKA), in the absence or in the presence of MEN 11420, on action potentials and contraction of the human and guinea-pig isolated ureter induced by electrical field stimulation (EFS)

	Control		*NKA*		*NKA + MEN 11420*	
	Human	*Guinea-pig*	*Human*	*Guinea-pig*	*Human*	*Guinea-pig*
Action potential						
Amplitude (mV)	12.5±1.5	18.7±1.1	10.9±1.5[b]	16.7±1.4[a]	11±0.3	19.2±1.8
Time to peak (ms)	261±33	132±11	285±88	145±15	288±81	97±13
Duration (ms)	1270±87	478±21	2156±361[a]	958±168[b]	1325±220	423±27
Latency (ms)	86±20	70±10	86±22	71±10	75±18	67±14
Spikes (number)		5.9±0.5		8.7±0.6[a]		5.2±0.7
Contraction						
Amplitude (mN)	5.9±1	3.2±0.2	7.4±1.4[a]	4.5±0.5[b]	5.5±1.6	3.1±0.7
Duration (ms)	3496±188	1530±62	5034±565[a]	2838±258[b]	3744±276	1775±244

Each value is mean±s.e.mean of four to six observations. NKA (1 or 3 μM for 15 s, in guinea-pig or human preparations, respectively) was administered 5 min before recording its effect on the EFS (single pulses of 30–40 V, 1–5 ms pulse width). The effect of MEN 11420 (3 μM) on the response to EFS was recorded after a superfusion period of 20 min. [a]Significantly different from the control value: $P<0.05$. [b]Significantly different from the control value: $P<0.01$.

15 min before) produced a rightward shift of the curve to the NK_2 receptor agonist without depressing E_{max} (not shown). An apparent pK_B of 8.2 ± 0.2 ($n=4$) was calculated from the agonist dose ratios.

Sucrose-gap

Human ureter All strips from the human ureter were mechanically and electrically quiescent. Application of EFS produced action potentials (APs) characterized by a rapidly rising depolarization, a sustained plateau and slow repolarization (Figure 3a; Table 1). Electrically-evoked APs were accompanied by phasic contractions (Table 1; Figure 3a): both responses were unaffected by tetrodotoxin (1 μM, $n=3$).

Superfusion with either senktide or [Sar^9]SP sulphone (3 μM for 15 s for each agonist, $n=4$) did not produce any effect, whereas NKA (3 μM for 15 s; $n=11$) (Figure 4a) produced a slow membrane depolarization (5.0 ± 0.7 mV), induced the appearance of Aps (amplitude 9.5 ± 1.4 mV; frequency 9.1 ± 1.6 cycles min^{-1}) and produced smooth muscle contraction characterized by a tonic response (3.4 ± 0.7 mN) with superimposed phasic activity (6.0 ± 1.4 mN). The phasic contractions developed in parallel with the NKA-induced Aps (Figure 4a). All the responses induced by 15 s superfusion of 3 μM NKA waned within 5 min from its application: at this time, the application of EFS elicited APs whose duration was significantly prolonged as compared to control (about 70% increase), whereas the amplitude of APs was slightly reduced (about 13% decrease) (Table 1; Figure 3a). The duration and amplitude of the accompanying phasic contraction were significantly increased (about 44 and 25% increase, respectively) as compared to controls (Table 1; Figure 3a).

Nifedipine (1 μM for 30 min, $n=6$) did not affect the resting membrane potential nor the tone of human ureter but completely blocked the APs and the phasic contraction induced by EFS (Figure 4b). Nifedipine also completely prevented both APs and the concomitant phasic contractile activity elicited by NKA, and reduced (74 ± 8% inhibition, $P<0.05$, $n=6$) the tonic component of NKA-induced smooth muscle contraction, while leaving unchanged (7 ± 13% reduction, n.s.) The amplitude of the slow membrane depolarization (Figure 4b).

MEN 11420 (3 μM for 20 min, $n=4$) did not affect the resting membrane potential nor the amplitude of EFS-evoked APs and contraction of the human ureter (Figure 3b). In contrast, MEN 11420 abolished both the electrical and motor responses produced by NKA ($n=4$, Figure 5a,b), and completely prevented the effects of NKA on the APs and contraction elicited by EFS (Table 1; Figure 3c).

Guinea-pig ureter All the preparations were mechanically and electrically quiescent. Application of EFS produced APs characterized by a rapidly rising depolarization followed by a plateau with superimposed spikes and repolarization (Table 1; Figure 4c). Electrically-evoked APs were accompanied by phasic contractions (Table 1; Figure 4c).

Likewise the effect observed in the human ureter, superfusion with NKA (1 μM for 15 s) produced a slow membrane depolarization with superimposed APs and smooth muscle contraction characterized by a tonic component and a phasic contractile activity: the latter developed concomitantly with the NKA-induced APs (Table 3 and Figure 4c). As also observed in the human ureter, the amplitude of the EFS-evoked APs, as measured 5 min after the administration of

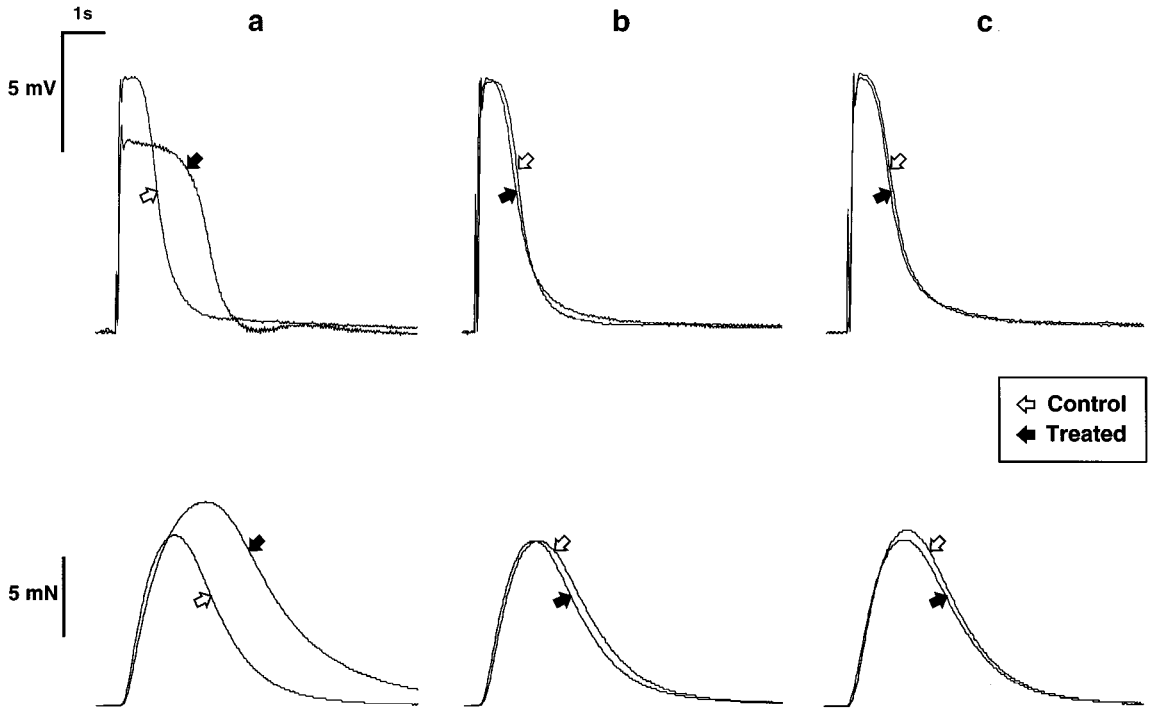

Figure 3 Superimposed typical tracings showing the action potentials (upper panel) and contractions (lower panel) induced by electrical field stimulation (EFS: single pulses of 30–40 V, 1–5 ms pulse width, given every min) in the human isolated ureter, in sucrose-gap experiments. (a) Electrical and mechanical responses to EFS obtained in preparations exposed to neurokinin A (3 μM for 15 s, given 5 min before EFS) vs control responses. (b) Electrical and mechanical responses to EFS obtained in the absence or in the presence of MEN 11420 (3 μM for 20 min). (c) Blockade by MEN 11420 (3 μM for 20 min) of the effects produced by neurokinin A (3 μM for 15 s, given 5 min before EFS) on electrical and mechanical responses to EFS.

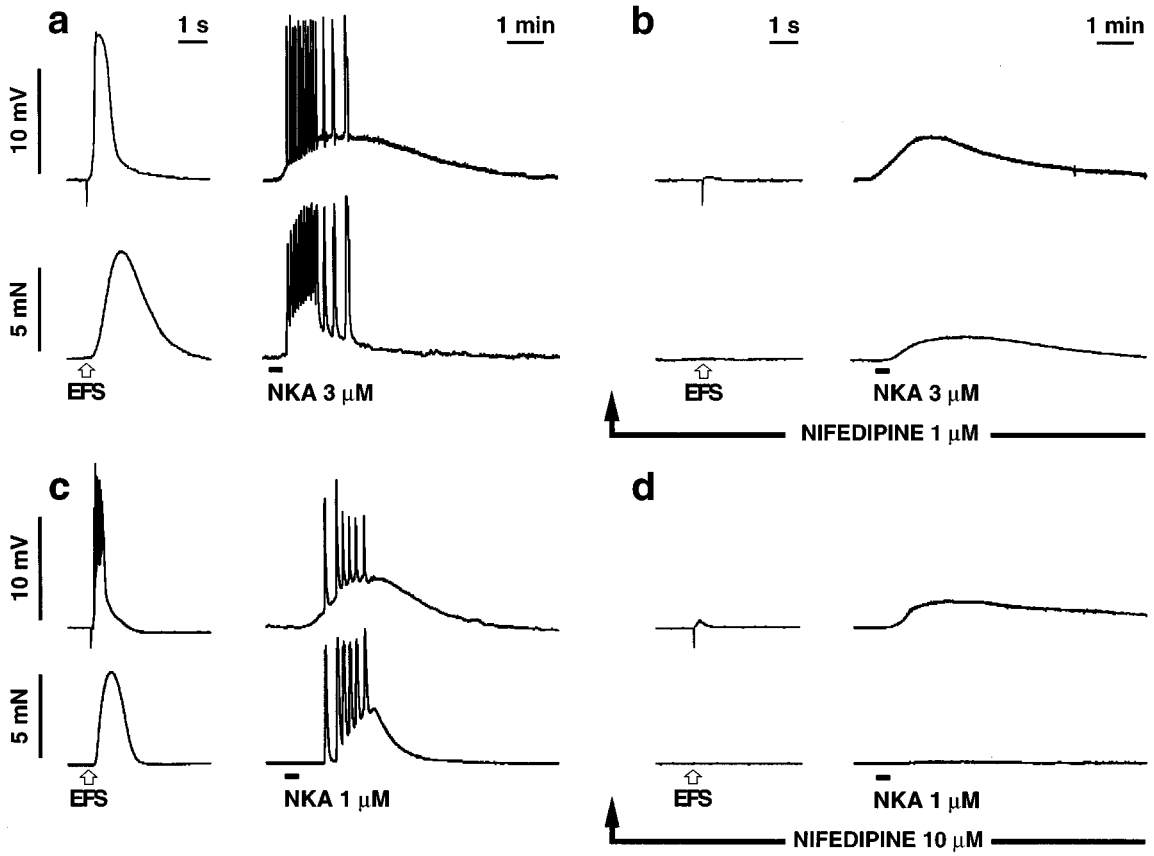

Figure 4 Typical tracings showing the effect of nifedipine on electrical and motor responses induced by EFS or neurokinin A (NKA) on human (a and b) and guinea-pig (c and d) ureter. (a) and (c) control responses to EFS (single pulses of 30–40 V, 1–5 ms pulse width, given every min) and to NKA (3 or 1 μM for 15 s, in human or guinea-pig preparations, respectively). (b) and (d) the same responses recorded after superfusion with nifedipine (1 or 10 μM for 30 min, in human or guinea-pig preparations, respectively. In each panel the upper tracing shows electrical responses (in mV) to stimuli while the lower one shows motor responses (in mN).

Table 2 Effect of nifedipine on action potentials and contraction induced by electrical field stimulation (EFS) in the guinea-pig isolated ureter

	Control ($n=10$)	*Nifedipine* 1 μM ($n=8$)	*Nifedipine* 3 μM ($n=7$)	*Nifedipine* 10 μM ($n=3$)
Action potential				
Responders	10/10	3/8	1/7	0/3
Amplitude (mV)	19.6±1.4	16.6±0.3	13.3	0±0
Time to peak (ms)	164±9	137±7	190	0±0
Duration (ms)	540±33	258±2	350	0±0
Latency (ms)	68±7	67±12	30	0±0
Spikes (number)	5.9±0.6	0±0	0±0	0±0
Contraction				
Amplitude (mN)	2.9±0.3	0±0	0±0	0±0
Duration (ms)	1450±29	0±0	0±0	0±0

Each value is mean±s.e.mean. Nifedipine was administered to preparations 30 min before recording its effect on the response to EFS (single pulses of 30–40 V, 1–5 ms pulse width, given every min). Responders: number of preparations responding to EFS in control experiments or after pretreatment with nifedipine. The mean values shown were calculated on the data obtained in the responder preparations only.

NKA, was reduced (about 11% inhibition) as compared to controls whereas their duration was significantly longer (about 100% increase), and the number of spikes superimposed onto the plateau phase of APs were increased by NKA by about 50% (Table 1). Also the duration and amplitude of the accompanying phasic contraction were increased by NKA (about 85 and 41% increase, respectively) (Table 1).

Nifedipine (1–10 μM, 30 min before) produced a concentration-dependent inhibition of both electrical and mechanical responses to EFS. It has to be noted that 1 μM nifedipine, which effectively suppressed the EFS-evoked APs in the human ureter, was only effective in three out of eight cases tested in the guinea-pig ureter (Table 2). In this preparation 10 μM nifedipine was required to suppress EFS-evoked APs in

Table 3 Effect of nifedipine on the electrical and mechanical responses produced by application of neurokinin A (NKA) in the guinea-pig ureter

	Control ($n=10$)	*Nifedipine* 1 μM ($n=8$)	*Nifedipine* 3 μM ($n=7$)	*Nifedipine* 10 μM ($n=3$)
Membrane potential				
Slow depolarization (mV)	5.8±0.8	4.5±0.7[a]	4.5±0.4[a]	4.4±0.9[a]
APs (number)	8.4±0.6	8.3±2.8	2.7±1.1[a]	0±0
APs amplitude (mV)	10.9±1.0	12.7±1.6	9.2±1.9	0±0
Contraction				
Phasic component (mN)	3.7±0.3	0.4±0.1[b]	0.2±0.1[b]	0±0
Tonic component (mN)	1.5±0.2	0±0	0±0	0±0

Each value is mean±s.e.mean. Nifedipine was administered to preparations 30 min before recording its effect on the response to NKA (1 μM for 15 s). APs (number) = number of action potentials elicited by NKA. [a]Significantly different from the control value: $P<0.05$. [b]Significantly different from the control value: $P<0.01$.

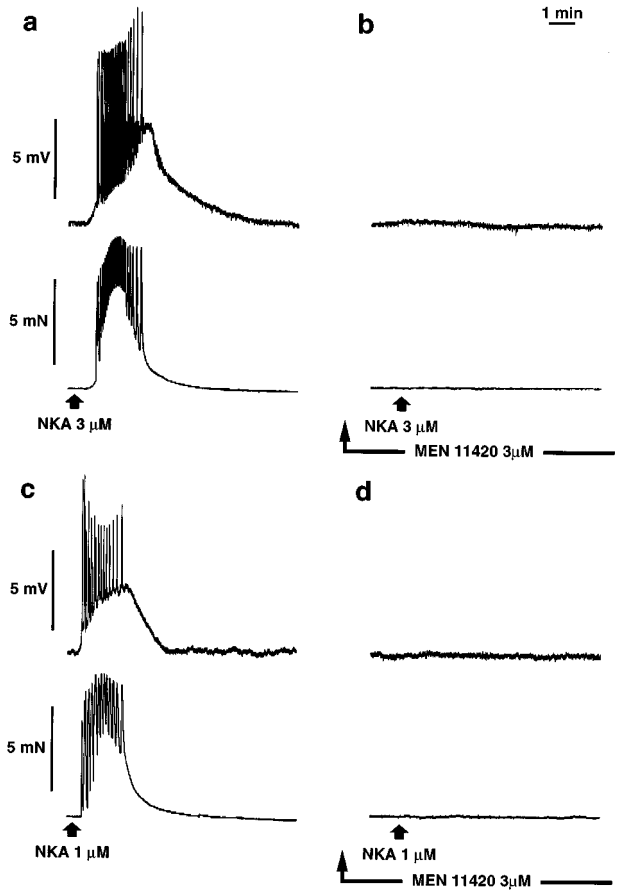

Figure 5 Typical tracings showing the effect of MEN 11420 on electrical and motor responses induced by neurokinin A (NKA) on human (a and b) and guinea-pig (c and d) ureter. (a) and (c) control responses to NKA (3 or 1 μM for 15 s, in human or guinea-pig preparations, respectively). (b and (d) the same responses recorded after superfusion with MEN 11420 (3 μM for 20 min). In each panel the upper tracing shows electrical responses (in mV) to NKA, while the lower one shows motor responses (in mN).

all cases tested, despite the fact that 1 μM nifedipine was sufficient to abolish the concomitant contraction in all preparations (Table 2; Figure 4d). The discrepancy is ascribable to a relative resistance of the first spike of the EFS-evoked AP to nifedipine which was however unable to sustain a contraction. Notably, in the three preparations in which an AP was evoked by EFS in the presence of 1 μM nifedipine, the latency of the first spike of the AP was not significantly different from control. In these latter preparations, no plateau phase was evident, nor the multiple spikes which superimpose on the plateau could be observed (Table 2)

Nifedipine (1–10 μM, 30 min before) concentration-dependently reduced both the electrical and mechanical responses to NKA: even in this case, the contractile response was more sensitive to inhibition by nifedipine than the electrical response (Table 3). In particular, the slow membrane depolarization induced by NKA was largely nifedipine-resistant (about 25% inhibition at 10 μM nifedipine). Moreover, the NKA-evoked APs were relatively resistant to nifedipine since a concentration of 10 μM was required to completely eliminate this component of response (Table 3). All components of the contractile response were abolished by 10 μM nifedipine (Table 3 and Figure 4d).

As also observed in the human ureter, MEN 11420 (3 μM for 20 min) did not affect the resting membrane potential and tone of the guinea-pig ureter, nor did it affect the electrical and mechanical responses to EFS. In contrast, MEN 11420 abolished all the electrical and motor responses produced by NKA (Figure 5c, d) and completely prevented the effects of NKA on APs and contraction elicited by EFS (Table 1).

Discussion

Our data indicate that, in both the human and guinea-pig ureter, tachykinins produce their excitatory effects by activating receptors of the NK_2 type only. This conclusion is based on the observation that synthetic receptor-selective agonists for the tachykinin NK_1 (septide and [Sar^9]SP sulphone) and tachykinin NK_3 receptors (senktide) (Wormser *et al*., 1986; Dion *et al*., 1987) have no effect, up to μmolar concentrations in both preparations. Moreover, all the excitatory responses produced by NKA were blocked by the NK_2 receptor-selective antagonist MEN 11420 (Catalioto *et al*., 1998). MEN 11420 is a newly developed antagonist possessing high affinity and selectivity for NK_2 receptor of several species, including human and guinea-pig (Santicioli *et al*., 1997; Patacchini *et al*., 1997; Catalioto *et al*., 1998). Up to 1 μM, MEN 11420 has low affinity for tachykinin NK_1/NK_3 receptors ($pK_B<6$; Santicioli *et al*., 1997; Catalioto *et al*., 1998). The specificity of MEN 11420 in both the human and guinea-pig ureter is documented by the ineffectiveness of this antagonist to reduce the response to EFS in sucrose-gap experiments, while it completely prevented the effects of NKA in the same preparations.

In the guinea-pig isolated renal pelvis MEN 11420 showed an affinity for the NK_2 receptor (apparent $pK_B = 8.2$) comparable to that measured against NK_2 receptor agonists in other guinea-pig preparations such as the circular muscle of proximal colon ($pK_B = 8.1$, Santicioli *et al*., 1997), main bronchus ($pK_B = 8.4$, Tramontana *et al*., 1998) and the common bile duct ($pK_B = 8.2$, Patacchini *et al*., 1997). Application of EFS to the guinea-pig renal pelvis produces a contraction mediated by endogenous tachykinins, released by capsaicin-sensitive primary afferent endings (Maggi *et al*., 1992b). Both NK_1 and NK_2 receptors are present in the renal pelvis but, based on the blocking action of antagonists, it was concluded that NK_2 receptor predominantly mediate the response to endogenous tachykinins unless degradation of endogenous peptides is reduced by peptidase inhibitors (Maggi *et al*., 1992a). In the present experiments the NK_1 receptor antagonist, SR 140333 (Emonds-Alt *et al*., 1993) was used to occlude NK_1 receptors: MEN 11420 almost totally abolished the NANC contraction to EFS of the renal pelvis, at a concentration (1 μM) at which it is selective for the NK_2 receptor. This result further reinforces the conclusion, drawn from results obtained with less potent and selective tachykinin antagonists (Maggi *et al*., 1992a), that NK_2 receptors provide the major contribution of the response to endogenous tachykinins and further validate the use of MEN 11420 as a tool to study tachykinin receptors in the pyeloureteral tract.

An important finding of the present study is that the NK_2 receptor is the predominant if not the exclusive mediator of the excitatory effect of tachykinins in the smooth muscle of the human ureter. This is in keeping with the results of previous studies, performed on smooth muscles from the human urinary bladder (Maggi *et al*., 1988b; Dion *et al*., 1990), gastrointestinal (ileum, colon and oesophagus) (Maggi *et al*., 1989; Giuliani *et al*., 1991; Huber *et al*., 1993) and respiratory tract (Naline *et al*., 1989; Astolfi *et al*., 1994; Dion *et al*., 1990), although excitatory responses mediated by tachykinin NK_1 receptors have also been described in selected cases as the circular muscle of the human ileum (Zagorodnyuk *et al*., 1997) and small bronchi (Naline *et al*., 1996).

The present findings demonstrate that NKA, by occupying tachykinin NK_2 receptors, produces a slowly developing depolarization of the membrane in both the human and guinea-pig ureter: in both species the depolarizing response to NKA is substantially resistant to blockade by nifedipine at concentrations which effectively block the APs produced by electrical stimuli which excite latent pacemakers in the smooth muscle. Several mechanisms have been proposed to account for NKA-induced depolarization of smooth muscles which include suppression of outward potassium current (Nakazawa *et al*., 1990; Nakajima *et al*., 1995), activation of calcium-dependent chloride current (Nakajima *et al*., 1995; Hazama *et al*., 1996) and activation of cation unselective channels (Lee *et al*., 1995). Each one of these mechanisms may, in theory, underlie the NKA-induced depolarization of ureter smooth muscle. It is important to note that the nifedipine-resistant slow depolarization is associated with a tonic type contraction in the human but not in the guinea-pig ureter. Release of Ca^{2+} ions from intracellular stores does not seem to contribute significantly to excitation-contraction coupling in the guinea-pig ureter (Maggi *et al*., 1994; 1995), while a significant contribution of a receptor-operated calcium store to excitation-contraction coupling has been reported in the rat ureter (Burdyga *et al*., 1995): a similar mechanism may contribute to the nifedipine-resistant tonic contraction of the human ureter to NKA.

In both the human and guinea-pig ureter, the depolarization induced by NKA triggers the appearance of a series of APs which are similar to those evoked by electrical stimuli: both the EFS- and the NKA-evoked APs were suppressed by nifedipine. In the guinea-pig, higher concentrations of nifedipine (up to 10 μM) were required to totally block APs and associated phasic contractions evoked by electrical stimuli (or NKA) than in the human ureter. This may be due to the fact that experiments in the guinea-pig ureter were performed in a nominally Mg^{2+}-free medium which we found useful to improve the achievement of long term recording of excitatory responses to electrical stimuli: removal of Mg^{2+} ions may have decreased the sensitivity of calcium channels to blockade by nifedipine. It is also worth noting that the relative resistance of the AP of the guinea-pig ureter to blockade by nifedipine is restricted to the first spike, as also observed in previous studies (for e.g. Brading *et al*., 1983; Burdyga & Magura, 1986) which was also observed in this study.

Nifedipine eliminated with similar effectiveness the APs and the associated phasic contractions induced by NKA in both species, supporting the interpretation that the phasic contractility evoked by NKA is produced by the excitation of latent pacemakers which, in turn, produce a propagated contraction. This mechanism may be important for producing antiperistaltic waves of contraction if NKA is released in the ureter wall in response to stimuli (chemical or mechanical) capable of exciting primary afferent nerves.

Upon disappearance of its direct depolarizing and contractile effects, NKA still affected the EFS-evoked AP and contraction: the effect of NKA, which was similar in the human and guinea-pig ureter, consisted in a slight depression of the amplitude of the initial spike of the AP, in a prolongation of AP duration and in a potentiation of the contractile response. Similar modulatory effects of noradrenaline and histamine on the AP of the guinea-pig ureter to electrical stimuli were reported previously by Shuba (1977). Shuba (1981) considered that a facilitation of the inward current could be held responsible for the prolongation of action potential induced by noradrenaline in the guinea-pig ureter. More recently Muraki *et al*. (1994) reported that noradrenaline actually inhibits the inward calcium current (I_{Ca}) in guinea-pig ureter smooth muscle cells and concomitantly inhibits the associated calcium-dependent potassium current $I_{K(Ca)}$. According to Muraki *et al*. (1994) the inhibition of $I_{K(Ca)}$ is stronger than the concomitant inhibition of I_{Ca} and this accounts for the net effect produced by noradrenaline on electromechanical coupling of the ureter. A similar mechansim may well account for the observed action of NKA on the AP of the guinea-pig and human ureter: in particular, the small but sizeable and statistically significant decrease in the amplitude of the first spike of the AP would be entirely consistent with a small inhibitory effect of NKA on I_{Ca}.

In conclusion, the present study shows that the tachykinin NK_2 receptor predominates in producing the excitatory effects of tachykinins in the smooth muscle of the human and guinea-pig ureter. We have shown that the administration of NKA leads to excitation of latent pacemaker cells, and that NKA itself also exerts a facilitatory effect on EFS-induced APs in this preparation. Considering that tachykinins are present in nerve fibres distributing to the human ureter and renal pelvis, it may be speculated that tachykinins released during pathological inflammatory conditions affect ureteral peristalsis: in particular tachykinins released in response to irritative stimuli could stimulate latent pacemakers and generate antiperistaltic waves of contractions. In this context, tachykinin NK_2 receptor antagonists, such as MEN 11420, may represent useful tools for assessing the pathophysiological role of these peptides in pyeloureteral tract.

References

ARTEMENKO, D.P., BURY, V.A., VLADIMIROVA, I.A. & SHUBA, M.F. (1982). Modification of the single sucrose-gap. *Physiol. Zhur.,* **28,** 374–380.

ASTOLFI, M., TREGGIARI, S., GIACHETTI, A., MEINI, S., MAGGI, C.A. & MANZINI, S. (1994). Characterization of the tachykinin NK_2 receptor in the human bronchus: influence of amastatin-sensitive metabolic pathways. *Br. J. Pharmacol.,* **111,** 570–574.

BRADING, A.F., BURDYGA, T.H.V. & SCRIPNYUK, Z.D. (1983). The effects of papaverine on the electrical and mechanical activity of the guinea-pig ureter. *J. Physiol.,* **334,** 79–89.

BURDYGA, T.H.V. & MAGURA, I.S. (1986). The effects of local anesthetics on the electrical and mechanical activity of the guinea-pig ureter. *Br. J. Pharmacol.,* **88,** 523–528.

BURDYGA, T.H.V., TAGGART, M.J. & WRAY, S. (1995). Major difference between rat and guinea-pig ureter in the ability of agonists and caffeine to release calcium and influence force. *J. Physiol.,* **489,** 327–335.

CATALIOTO, R.-M., CRISCUOLI, M., CUCCHI, P., GIACHETTI, A., GIANNOTTI, D., GIULIANI, S., LECCI, A., LIPPI, A., PATACCHINI, R., QUARTARA, L., RENZETTI, A.R., TRAMONTANA, M., ARCAMONE, F. & MAGGI, C.A. (1998). MEN 11420 (Nepadutant), a novel glycosylated bicyclic peptide tachykinin NK_2 receptor antagonist. *Br. J. Pharmacol.,* **123,** 81–91.

DION, S., D'ORLÉANS-JUSTE, P., DRAPEAU, G., RHALEB, N.E., ROUISSI, N., TOUSIGNANT, C. & REGOLI, D. (1987). Characterization of neurokinin receptors in various isolated organs by the use of selective agonists. *Life Sci.,* **41,** 2269–2278.

DION, S., ROUISSI, N., NANTEL, F., DRAPEAU, G., REGOLI, D., NALINE, E. & ADVENIER, C. (1990). Receptors for neurokinins in human bronchus and urinary bladder are of the NK-2 type. *Eur. J. Pharmacol.,* **178,** 215–219.

DONALDSON, L.F., HASKELL, C.A. & HANLEY, M.R. (1996). Functional characterization by heterologus expression of a novel cloned tachykinin peptide receptor. *Biochem. J.,* **320,** 1–5.

EDYVANE, K.A., SMET, P.J., TRUSSELL, D.C., JONAVICIUS, J. & MARSHALL, V.R. (1994). Patterns of neuronal colocalisation of tyrosine hydroxylase, neuropeptide Y, vasoactive intestinal polypeptide, calcitonin gene-related peptide and substance P in human ureter. *J. Autonom. Nerv. Syst.,* **48,** 241–255.

EDYVANE, K.A., TRUSSELL, D.C., JONAVICIUS, J., HENWOOD, A. & MARSHALL, V.R. (1992). Presence and regional variation in peptide-containing nerves in the human ureter. *J. Autonom. Nerv. Syst.,* **39,** 127–137.

EMONDS-ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.P., PROIETTO, E., VAN BROECK, D., SOUBRIÉ, P., LE FUR, G. & BRELIÉRE, J.C. (1993). In vitro and in vivo biological activities of SR 140,333, a novel potent nonpeptide tachykinin NK_1 receptor antagonist. *Eur. J. Pharmacol.,* **250,** 403–413.

GIULIANI, S., BARBANTI, G., TURINI, D., QUARTARA, L., ROVERO, P., GIACHETTI, A. & MAGGI, C.A. (1991). NK_2 tachykinin receptors and contraction of the circular muscle of the human colon: characterization of the NK_2 receptor subtype. *Eur. J. Pharmacol.,* **203,** 365–370.

GUARD, S. & WATSON, S.P. (1991). Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem. Int.,* **18,** 149–165.

HAZAMA, H., NAKAJIMA, T., HAMADA, E., OMATA, M. & KURACHI, Y. (1996). Neurokinin A and calcium current induce calcium-activated chloride currents in guinea-pig tracheal myocytes. *J. Physiol.,* **492,** 377–393.

HOYLE, C.H.V. (1987). A modified single sucrose-gap. Junction potential and electrotonic potentials in gastrointestinal smooth muscle. *J. Pharmacol. Methods,* **18,** 219–226.

HUA, X.-Y. & LUNDBERG, J.M. (1986). Dual capsaicin effects on ureteral motility: low dose inhibition mediated by calcitonin gene-related peptide and high dose stimulation by tachykinins? *Acta Physiol. Scand.,* **128,** 453–465.

HUA, X.-Y., THEODORSSON-NORHEIM, E., LUNDBERG, J.M., KINN, A.-C., HÖKFELT, T. & CUELLO, A.C. (1987). Co-localization of tachykinins and calcitonin gene-related peptide in capsaicin-sensitive afferents in relation to motility effects on the human ureter in vitro. *Neuroscience,* **23,** 693–703.

HUBER, O., BERTRAND, C., BUNNET, N.W., PELLEGRINI, C.A., NADEL, J.A., NAKAZATO, P., DEBAS, H.T. & GEPPETTI, P. (1993). Tachykinins mediate contraction of the human lower esophageal sphincter in vitro via activation of NK_2 receptors. *Eur. J. Pharmacol.,* **239,** 103–109.

JENKINSON, D.H. (1991). How we describe competitive antagonists: three questions of usage. *Trends Pharmacol. Sci.,* **12,** 53–56.

KENAKIN, T.P. (1993). Pharmacologic analysis of drug-receptor interaction. Second Ed. Raven Press New York, N.Y.

KRAUSE, J.E., STAVETEIG, P.T., MENTZER, J.N., SCHMIDT, S.K., TUCKER, J.B., BRODBECK, R.M., BU, J.K. & KARPITSKIY, V.V. (1997). Functional expression of a novel human neurokinin-3 receptor homolog that binds (3H)senktide and (125-I-MePhe-7) neurokinin B, and is responsive to tachykinin peptide agonsists. *Proceedings Natl. Acad. Sci. U.S.A.,* **94,** 310–315.

LEE, H.K., SHUTTLEWORTH, C.W. & SANDERS, K.M. (1995). Tachykinins activate nonselective cation currents in canine colonic myocytes. *Am. J. Physiol.,* **269,** C1394–C1401.

MAGGI, C.A. (1995). Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. *Progress in Neurobiol.,* **45,** 1–98.

MAGGI, C.A., GIULIANI, S. & SANTICIOLI, P. (1994). Effect of Bay K 8644 and ryanodine on the refractory period, potential and mechanical response of the guinea-pig ureter to electrical stimulation. *Naunyn Schmied. Archives Pharmacol.,* **349,** 510–522.

MAGGI, C.A., GIULIANI, S. & SANTICIOLI, P. (1995). Effect of the Ca^{2+} ATPase inhibitor, cyclopiazonic acid, on electromechanical coupling in the guinea-pig ureter. *Br. J. Pharmacol.,* **114,** 127–137.

MAGGI, C.A., PARLANI, M., ASTOLFI, M., SANTICIOLI, P., ROVERO, P., ABELLI, L., SOMMA, V., GIULIANI, S., REGOLI, D., PATACCHINI, R. & MELI, A. (1988a). Neurokinin receptors in the rat lower urinary tract. *J. Pharmacol. Exp. Ther.,* **246,** 308–315.

MAGGI, C.A., PATACCHINI, R., EGLEZOS, A., QUARTARA, L., GIULIANI, S. & GIACHETTI, A. (1992a). Tachykinin receptors in the guinea-pig renal pelvis: activation by exogenous and endogenous tachykinins. *Br. J. Pharmacol.,* **107,** 27–33.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993). Tachykinin receptors and tachykinin receptor subtypes. *J. Autonom. Pharmacol.,* **13,** 23–93.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., TURINI, D., BARABANTI, G., BENEFORTI, P., MISURI, D. & MELI, A. (1989). Human isolated small intestine: motor responses of the longitudinal muscle to field stimulation and exogenous neuropeptides. *Naunyn Schmied. Arch. Pharmacol.,* **339,** 415–423.

MAGGI, C.A., SANTICIOLI, P., ABELLI, L., PARLANI, M., CAPASSO, M., CONTE, B., GIULIANI, S. & MELI, A. (1987). Regional differences in the effects of capsaicin and tachykinins on motor activity and vascular permeability of the rat lower urinary tract. *Naunyn-Schmied. Arch. Pharmacol.,* **335,** 636–645.

MAGGI, C.A., SANTICIOLI, P., GIULIANI, S., ABELLI, L. & MELI, A. (1986). The motor effect of the capsaicin-sensitive inhibitory innervation of the rat ureter. *Eur. J. Pharmacol.,* **126,** 333–336.

MAGGI, C.A., SANTICIOLI, P., PATACCHINI, R., CELLERINI, M., TURINI, D., BARBANTI, G., BENEFORTI, P., ROVERO, P. & MELI, A. (1988b). Contractile response of the human isolated urinary bladder to neurokinins: involvement of NK_2 receptors. *Eur. J. Pharmacol.,* **145,** 335–340.

MAGGI, C.A., THEODORSSON, E., SANTICIOLI, P. & GIULIANI, S. (1992b). Tachykinins and calcitonin gene-related peptide as co-transmitters in local motor responses produced by sensory nerve activation in the guinea-pig isolated renal pelvis. *Neuroscience,* **46,** 549–559.

MURAKI, K., IMAIZUMI, Y. & WATANABE, M. (1994). Effects of noradrenaline on membrane currents and action potential shape in smooth muscle cells from guinea-pig ureter. *J. Physiol.,* **481,** 617–627.

NAGAHISA, A., KANAI, Y., SUGA, O., TANIGUCHI, K., TSUCHIYA, M., LOWE III, J.A. & HESS,H.J. (1992). Antiflammatory and analgesic activity of a nonpeptide SP receptor antagonist. *Eur. J. Pharmacol.,* **217,** 191–195.

NAKAJIMA, T., HAZAMA, H., HAMADA, E., OMATA, M. & KURACHI, Y. (1995). Ionic basis of neurokinin A-induced depolarization in single smooth muscle cells isolated from guinea-pig trachea. *Pflugers Archiv.,* **430,** 552–562.

NAKAZAWA, K., INOUE, K., FUJIMORI, K. & TAKANAKA, A. (1990). Neurokinin A suppresses a voltage-gated K current in smooth muscle cells from rat vas deferens. *Eur. J. Pharmacol.,* **182,** 189–192.

NALINE, E., DEVILLIER, P., DRAPEAU, G., TOTY, L., BAKDACH, H. & REGOLI, D. (1989). Characterization of neurokinin effects and receptor selectivity in human isolated bronchi. *Am. Rev. Resp. Dis.,* **140,** 679–686.

NALINE, E., MOLIMARD, M., REGOLI, D., EMONDS-ALT, X., BELLAMY, J.F. & ADVENIER, C. (1996). Evidence for functional tachykinin NK_1 receptors on human isolated small bronchi. *Am. J. Physiol.,* **271,** L763–L767.

PATACCHINI, R., BARTHÓ, L. & MAGGI, C.A. (1997). Characterization of receptors mediating contraction induced by tachykinins in the guinea-pig isolated common bile duct. *Br. J. Pharmacol.,* **122,** 1633–1638.

PATACCHINI, R., DE GIORGIO, R., GIACHETTI, A. & MAGGI, C.A. (1994). Different mechanism of tachykinin NK_2 receptor blockade by SR 48,968 and MEN 10,627 in the guinea-pig isolated gallbladder and colon. *Eur. J. Pharmacol.,* **271,** 111–119.

REGOLI, D., DRAPEAU, G., DION, S. & D'ORLEANS-JUSTE, P. (1989). Receptors for substance P and related neurokinins. *Pharmacology,* **38,** 1–15.

SANTICIOLI, P., GIULIANI, S., PATACCHINI, R., TRAMONTANA, M., CRISCUOLI, M. & MAGGI, C.A. (1997). MEN 11420, a potent and selective tachykinin NK_2 receptor antagonist in the guinea-pig and human colon. *Naunyn-Schmied. Arch. Pharmacol.,* **356,** 678–688.

SHUBA, M.F. (1977). The mechanisms of the excitatory action of catecholamines and histamine on the smooth muscle of guinea-pig ureter. *J. Physiol.,* **264,** 853–864.

SHUBA, M.F. (1981). Smooth muscle of the ureter: the nature of excitation and the mechanisms of action of catecholamines and histamine. In *Smooth muscle: an assessment of current knowledge*. ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 377–384. London: Edward Arnold.

TRAMONTANA, M., PATACCHINI, R., GIULIANI, S., LIPPI, A., LECCI, A., SANTICIOLI, P., CRISCUOLI, M. & MAGGI, C.A. (1998). Characterization of the antibronchoconstrictor activity of MEN 11420, a tachykinin NK_2 receptor antagonist, in guinea-pigs. *Eur. J. Pharmacol.,* **352,** 279–288.

WORMSER, U., LAUFER, R., HART, Y., CHOREV, M., GILON, C. & SELINGER, Z. (1986). Highly selective agonists for substance P receptor subtypes. *EMBO Journal,* **5,** 2805–2808.

ZAGORODNYUK, V., SANTICIOLI, P., TURINI, D. & MAGGI, C.A. (1997). Tachykinin NK_1 and NK_2 receptors mediated non-adrenergic non-cholinergic excitatory neuromuscular transmission in the human ileum. *Neuropeptides,* **31,** 265–271.

ZHENG, F. & LAWSON, S.N. (1997). Neurokinin A in rat renal afferent neurons and in nerve fibers within smooth muscle and epithelium of rat and guinea-pig renal pelvis. *Neuroscience,* **76,** 1245–1255.

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