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Regional differences in the effects of capsaicin and tachykinins on motor activity and vascular permeability of the rat lower urinary tract

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Summary. 1. The effects of capsaicin, substance P (SP) and neurokinin A (NKA) on motor activity and vascular permeability was investigated in the rat lower urinary tract (bladder dome and neck, proximal urethra and ureters). 2. Capsaicin produced contractions of the rat bladder dome and neck and of the proximal urethra in vitro, which were unaffected by tetrodotoxin and abolished by ganglionectomy. SP and NKA were almost equipotent in producing a contraction of the rat isolated bladder dome or neck and urethra. However, the maximal response to NKA was about twice that of SP on the urethra and bladder neck. 3. Capsaicin did not affect motility of the unstimulated rat isolated ureter, while NKA or SP activated rhythmic contractions, NKA being about 850 times more potent than SP. Either capsaicin or field stimulation produced a transient inhibition of the NKA-activated rhythmic contractions of the rat isolated ureter which was prevented by capsaicin-desensitization. 4. The capsaicin-(1 μM) or field stimulation-induced inhibition of NKA-activated rhythmic contractions of the rat isolated ureter were unaffected by removal of pelvic ganglia but abolished by cold storage (72 h at 4° C). 5. Intravenous capsaicin induced an inflammatory response (Evans blue leakage) in the bladder, proximal urethra and ureters in vivo. Plasma extravasation was greater in the ureters, urethra and bladder neck than in the dome. SP, NKA and histamine produced a dose-dependent dye leakage in all segments of the rat urinary tract, the response being slightly greater in the bladder neck than in the dome. 6. The capsaicin-induced inflammatory response was abolished by systemic capsaicin-desensitization and reduced, to a variable extent, by pelvic ganglionectomy, in the various tissues examined. Topical application of tetrodotoxin on the bladder dome failed to affect the capsaicin-induced plasma extravasation in the urinary bladder. 7. These findings indicate that chemoceptive, capsaicin-sensitive nerves are present throughout the whole rat lower urinary tract and their activation determines a variety of visceromotor responses and an increase of vascular permeability. In various instances the response to capsaicin may be explained by the action of tachykinins but some effects may involve other sensory neuropeptides.

Key words: Capsaicin – Urinary tract – "Sensory efferent" function of capsaicin sensitive nerves – Neurogenic inflammation – Tachykinins

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Introduction

Recent findings indicate the presence, in the rat urinary bladder, of a capsaicin-sensitive innervation which regulates the micturition threshold by relaying information to the central nervous system on the degree of distension of the detrusor muscle (Maggi et al. 1984a, 1985a, b, 1986a, b, c; Holzer-Petsche and Lembeck 1984; Santicioli et al. 1985, 1986; Maggi and Meli 1986). According to Szolcsányi (1984) activation of cutaneous or visceral capsaicin-sensitive afferents may exert, in addition to the sensory function, a local "efferent" function produced through the release of neuropeptides from the peripheral terminal of sensory fibres.

Both biochemical (Holzer et al. 1982) anatomical (Sharkey et al. 1983; Yokokawa et al. 1985) and functional (Maggi et al. 1984a, 1985a, b; Maggi and Meli 1986) evidence indicates that substance P (SP) could be one of the transmitters released from the capsaicin-sensitive primary afferent fibres of the urinary bladder (Maggi et al. 1984a, 1985a). More recently, biochemical evidence indicated that neurokinin A (NKA) is present in capsaicin-sensitive structures of the urinary bladder (Hua et al. 1985a, b; Sundler et al. 1985). Since NKA coexists with SP in some sensory neurons; Dalsgaard et al. 1985; Saria et al. 1985; Sundler et al. 1985) it could be hypothesized that these tachykinins are co-released, both centrally and peripherally, in response to chemical or mechanical stimuli in the lower urinary tract.

Regional variations have been described in the density of cholinergic and sympathetic innervation of the urinary bladder which may have a functional counterpart in promoting urinary continence and/or bladder voiding (Elbadawi 1982; Santicioli et al. 1984). The hypothesis that the peptidergic innervation of the urinary bladder may not be homogenous was also suggested by anatomical (Alm et al. 1978) and biochemical (Terenghi et al. 1983) data indicating that in rats and guinea-pigs the density of SP fibres and/or concentration of SP-like immunoreactivity is greater in the bladder neck and trigonum than in the dome.

The aim of this study was to determine whether or not regional differences occur in the rat lower urinary tract (bladder, proximal urethra and ureters) with regard to the "efferent" component of responses mediated by the chemoceptive (capsaicin-sensitive) fibres, i.e.: a) the motor effects of capsaicin and b) the capsaicin-induced plasma extravasation (Evans blue leakage) (Saria et al. 1983; Lundberg et al. 1984). The effects of capsaicin were

compared to those of SP and NKA, two mammalian tachykinins present in the lower urinary tract (Holzer et al. 1982; Alm et al. 1978; Yokokawa et al. 1985; Theodorsson-Norheim et al. 1984; Hua et al. 1985a, b; Sundler et al. 1985).

Materials and methods

In vitro experiments. Male albino rats, Wistar Morini strain weighing 360–400 g were killed by cervical dislocation and exsanguinated. The whole urinary bladder, proximal urethra and ureters were rapidly removed and placed in a Krebs solution warmed to 37° C. The composition of Krebs solution was (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2 and glucose 11. The solution was continuously bubbled with a mixture of 96% O_2 and 4% CO_2.

After dissection, the preparations were transferred in a 5 ml organ bath and connected, under a constant load of 0.5 (ureters and proximal urethra) or 1 g (strips of bladder dome or neck) to an isometric strain gauge and contractile tone was recorded by means of a Basile 7050 Unirecord Poligraph. A strip of smooth muscle weighing about 10 mg was excised from the bladder dome or neck. The term bladder "neck" will be used hereafter to design a region of the bladder comprising the urethrovesical junction and an horizontal plane lying about 2–3 mm above the ureterovesical junction. The rationale for this division was that, in preliminary experiments, the inflammatory response to capsaicin exhibited a marked regional distribution, being much more intense in this part of the bladder than in the rest of the body or the dome. Thus the bladder neck region, referred to in this study is defined functionally and consists of the bladder "base" of classical neuroanatomy plus the most caudal portion of the bladder body. The ureters were taken just below the inferior renal pole to exclude the natural pacemaker region (cf. Maggi et al. 1986d) and were suspended in such a way to record tension from their longitudinal axis. A ring of proximal urethra weighing 4–10 mg was connected between two steel hooks to record tension of the circular muscle.

Field stimulation was carried out by means of two platinum wire electrodes placed at the top and the bottom of the organ bath and connected to a Grass S11 stimulator. Square wave pulses of supramaximal intensity (60 V) were delivered at a frequency of 0.1 (continuously) or 10 Hz (trains of 5 s every 60 s). Pulse width was 0.5 ms. The contractile effect of capsaicin was tested after a 60 min equilibration period only once in each preparation. Non-cumulative concentration-response curves (CRC) to tachykinins were recorded by adding increasing concentrations of these substances at 20 min intervals until maximal responses were obtained. Each concentration was left in contact with the tissue for 5 min. The contractile effect of the tachykinins was expressed as percent of the response to a maximally effective concentrations of acetylcholine (1 mM) or KCl (80 mM). KCl was used to compare changes in response between tissues from normal and ganglionectomized animals, in order to avoid the possible influence of changes in response to acetylcholine due to denervation supersensitivity.

Threshold concentration for eliciting rhythmic contractions of the ureters was determined by the non-cumulative addition of tachykinins at 20 min intervals.

The motor effects of capsaicin were investigated either in resting conditions or during the tachykinin-activated motor activity (ureters). Unless otherwise stated the effects of capsaicin were investigated only once in each preparation.

Plasma protein extravasation. Male albino rats Wistar-Morini strain, weighing 180–200 g were anaesthetized with subcutaneous urethane (1.2 g/kg) and artificially ventilated. Plasma protein extravasation was determined as described by Saria and Lundberg (1983). Test substances were injected at least 30 min after completion of surgical procedures. Briefly, Evans blue (20 mg/kg) was injected through a polyethylene tubing inserted into the left jugular vein 5 min before the intravenous administration of test substances (capsaicin, tachykinins, histamine). Five minutes after the i.v. administration of test substances, saline (50 ml in 30 s) was injected into the animals via the thoracic aorta. This was done to wash out from tissues the Evans blue present in the vascular system.

In experiments involving the topical application of capsaicin on the bladder dome (cf. Maggi et al. 1984a) a 10 min period was allowed to elapse before saline perfusion since preliminary experiments indicated that, in these preparations, the reaction developed more slowly than after i.v. capsaicin.

Evans blue content was determined by fluorimetry (Perkin-Elmer 512 Double Beam Spectrophotometer) as described by Saria and Lundberg (1983). The viscera from the rat urinary tract were gently blotted three times on filter paper and weighed. Evans blue content was expressed as ng/mg of wet weight.

Experiments in ganglionectomized animals. Some experiments were undertaken in preparations whose pelvic ganglia had been removed 72 h before the experiment by the method described previously (Santicioli et al. 1986; Maggi et al. 1986a). Results in ganglionectomized animals were compared to those obtained in sham-operated controls. The skin was closed with wound clips and the animals received benzathine-benzylpenicillin 200,000 U.I. The animals received s.c. bethanechol 1.25 mg/kg 12, 24 and 48 h before killing to allow a certain amount bladder voiding and prevent the adverse effect of overdistension.

Systemic capsaicin desensitization. Some experiments were performed in rats desensitized to capsaicin (160 μmol/kg s.c. 4 days before) as described previously (Maggi et al. 1984a). Control rats received the vehicle (Tween 80 10%, ethanol 10% and saline 80%).

Statistical analysis. All data in the text are mean ± SE. Statistical analysis of the data was performed by means of Student's *t*-test for paired or unpaired data or by means of the analysis of variance, when applicable.

For the contractile response to tachykinins in the bladder and urethra, the EC 50 and 95% confidence limits (c.l.) were calculated by means of regression analysis and the least squares method. The EC 50 were calculated as the concentrations of each tachykinin producing 50% of its own effect. Differences in effectiveness were evaluated by referring the maximal response to tachykinins to the response obtained in the same tissue to acetylcholine.

In the ureters, the relative abilities of the tachykinins to activate rhythmic contractions were evaluated by calculating the EC 50 for an all-or-none type of response, as described by Litchfield and Wilcoxon (1949).

Drugs. Drugs used were: capsaicin (Sigma, St. Louis, MO, USA), atropine HCl (Serva, Heidelberg, FRG), tetrodotoxin (TTX, Sankyo, Japan), hexamethonium bromide (Serva), acetylcholine HCl (Fluka, Neu-Ulm, FRG), SP (Peninsula, San Carlos, CA, USA), NKA (Peninsula), benzathine benzylpenicillin (Wyeth, Maidenhead, England), bethanechol HCl (Urecholine, Merck, Darmstadt, FRG), formamide (Sigma), Evans blue (Serva), histamine dihydrochloride (Fluka), diphenydramine hydrochloride (Sigma), cimetidine (SK&F, Philadelphia, PA, USA).

A stock solution of capsaicin (100 mM) was prepared in absolute ethanol and then diluted in Krebs solution.

To minimize binding of polypeptides to glassware both the organ bath and the microsyringe used for drug administration were treated with 2.5% dichloromethylsilane in benzene for 20 min and then rinsed in water before use.

Results

In vitro studies

Motor effects of capsaicin, SP or NKA on isolated muscle strips from the rat bladder dome or neck. Isolated preparations from the rat bladder dome and neck exhibited a low-amplitude myogenic contractile activity, as described previously (Maggi et al. 1984b). Both preparations responded to field stimulation (0.1 – 10 Hz) at low stimulation parameters (0.5 ms) with tetrodotoxin- (1 μM) sensitive phasic contractions.

Capsaicin (1 – 3 μM) produced, within a few seconds, a contraction in preparations from both bladder dome and neck which was qualitatively similar to those produced in the whole bladder (Maggi et al. 1985a; Santicioli et al. 1986). Capsaicin removal by washing out resulted in a prompt recovery of resting tone and spontaneous activity of the preparation. A second addition of capsaicin (up to 3 h from the first dose) had no further contractile effect. The response to capsaicin amounted to 22% ± 2% and 25% ± 3% of that produced by field stimulation (10 Hz, 60 V, 0.5 ms pulse width, trains of 5 s) in segments from the bladder dome and neck, respectively ($n = 10$ for each group, n.s.). Previous studies showed that the contractile response of the rat isolated bladder to capsaicin is almost abolished by ganglionectomy thus indicating their neurogenic origin (Santicioli et al. 1986; see also Maggi et al. 1986a).

Both SP and NKA produced a concentration-related (1 nM – 10 μM) contraction of muscle strips from the bladder dome or neck (Fig. 1) which was qualitatively similar to those observed in preparations of the whole bladder (Maggi et al. 1986a). NKA and SP were almost equipotent in producing a contraction of strips from the bladder dome (Fig. 1, left panel), their EC 50 and 95% c.l. (in brackets) being as follows: SP 104 nM (42 – 441), NKA 67 nM (31 – 229). The maximal effect of SP and NKA amounted to 80% ± 7% and 89% ± 6% of the response to acetylcholine (1 mM), respectively ($n = 10$ for each group, n.s.).

Likewise SP and NKA were almost equipotent in producing a contraction of strips from the bladder neck (Fig. 1, right panel), their EC 50 and 95% c.l. (in brackets) being as follows: SP 112 nM (67 – 213), NKA 107 nM (47 – 375). However, the maximal response to NKA, which amounted to 120% ± 9% of that to acetylcholine (1 mM) was about double as compared to the response to SP (61% ± 6%, $n = 10$ for each group, $p < 0.01$).

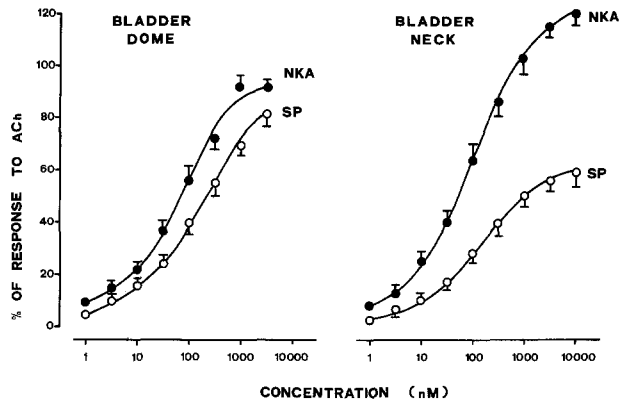

Fig. 1. Concentration-response curves for the motor effects of SP or NKA in muscle strips from the rat bladder dome and neck. Each point is mean ± SE of at least six experiments

Motor effects of capsaicin, SP or NKA in the rat isolated proximal urethra. The rat proximal urethra was almost quiescent. Phasic spontaneous activity in the form of slow oscillations (30 – 50 mg in amplitude) of the baseline could be observed in 15 – 30% of preparations (Fig. 2). Transient phasic contractions could be elicited by field stimulation (1 – 10 Hz). Contractile responses to field stimulation were observed with a low pulse width (0.5 ms) and were tetrodotoxin- (1 μM) sensitive, indicating their neurogenic origin. These contractions were reduced by atropine (1 μM) and unaffected by hexamethonium (10 μM) thus indicating their dependence upon a postganglionic innervation. At 10 Hz and using a train duration of 5 s, the response of the rat urethra to field stimulation was characterized by a phasic plus tonic contraction which returned to baseline upon cessation of the stimulus.

Capsaicin (1 – 3 μM) did not affect motility of the isolated urethra in 19 out of 32 preparations but produced a contraction in the remainder. The capsaicin-induced contraction of the rat isolated proximal urethra was characterized by a small "tonic" contraction (20 – 80 mg) on which a series of phasic contractions (30 – 150 mg) were superimposed (Fig. 2). These effects, which closely resembled those produced by a low concentration of SP, lasted for 3 – 10 min (Fig. 2). A second addition of capsaicin (3 μM) within 3 h from the first one did not alter the motor activity of the rat isolated urethra ($n = 6$).

Preparations excised from capsaicin-pretreated rats (160 μmol/kg s.c. 4 days before) were unresponsive to capsaicin (3 μM, $n = 8$). These preparations exhibited a strong contractile response to acetylcholine (1 mM), tachykinins (NKA or SP 1 μM) or field stimulation (10 Hz).

The response to field stimulation (10 Hz, 60 V) of urethral rings from capsaicin-pretreated animals (297 mg ± 32 mg, $n = 6$) was slightly but not significantly greater than that of controls (220 ± 41 mg, $n = 6$). Both SP and NKA produced a concentration-related "tonic" contraction (Figs. 2 and 3) of the rat proximal urethra which reached a maximum within 1 – 2 min from administration of each

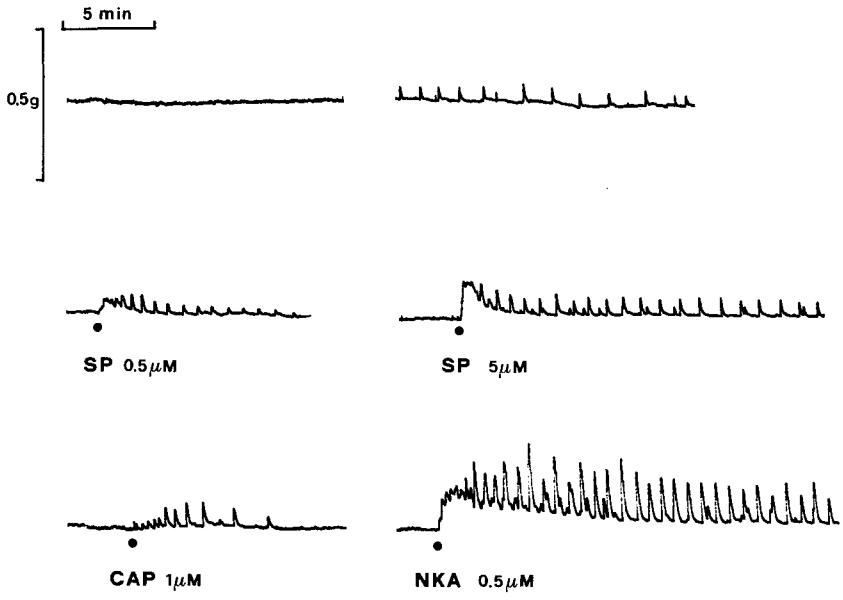

Fig. 2
Typical tracings illustrating the spontaneous mechanical activity of rings of the rat proximal urethra (*upper panels*) and the motor effect produced by tachykinins or capsaicin (*middle and lower panels*). Upper panels are from two different preparations. Middle and lower panels are from the same preparation

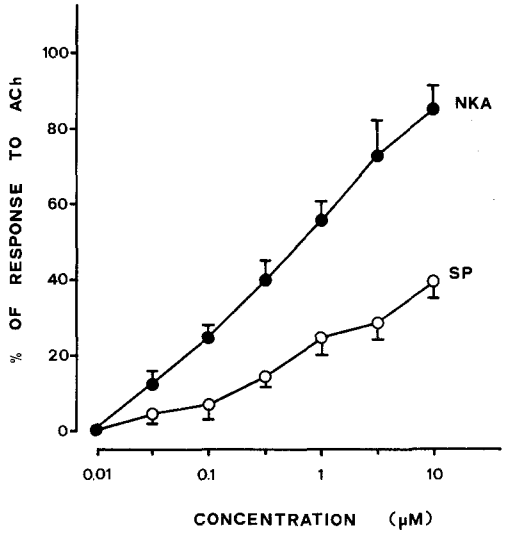

Fig. 3. Concentration-response curves for the motor effects of SP and NKA in isolated rings from the rat proximal urethra. For each concentration the maximal contractile response to tachykinins (either phasic or tonic) was used to construct the curve. Each point is mean ± SE of at least six experiments

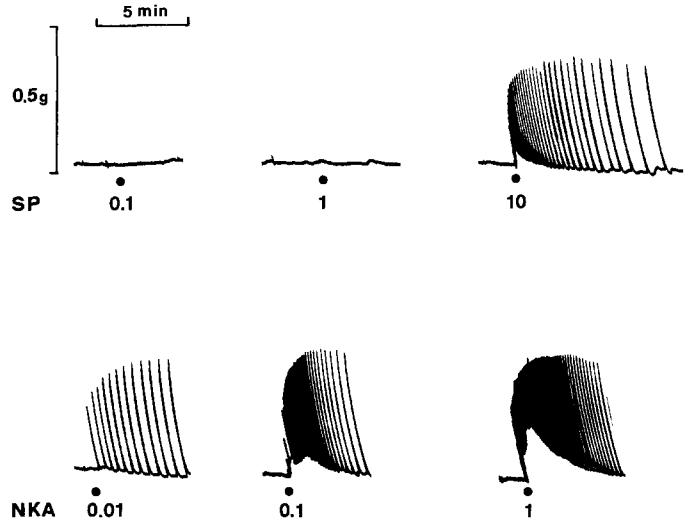

Fig. 4. Typical tracings illustrating the motor effect of SP or NKA (numbers are concentrations in μM) in the rat isolated ureters. These tracings were obtained from the same preparation on which increasing concentration of the two neurokinins were tested at 20 min intervals. Note that NKA is much more potent than SP in activating the rhythmic contractions of the rat isolated ureter

concentration and then slowly declined to baseline values. A series of "phasic" contractions superimposed on the "tonic" contraction and were still present even when tone had returned to baseline levels. Maximal contractions produced by NKA and SP amounted to 85% ± 6% and 40% ± 4% of that produced by acetylcholine (1 mM, $n = 6$ for each group, $p < 0.05$).

NKA and SP were almost equipotent in producing a contraction of the rat proximal urethra their EC 50 and 95% c.l. being 707 nM (188 – 6354) and 380 nM (219 – 747), respectively.

Effect of ganglionectomy on the response of the rat proximal urethra to field stimulation, capsaicin or tachykinins. Urethral rings from ganglionectomized animals responded to field stimulation (10 Hz, 0.5 ms, 60 V for 5 s) with contractions whose amplitude was, when expressed as percent of the response to KCl (80 mM) significantly reduced compared to the controls (36% ± 5% versus 80% ± 11%, respectively, $n = 6$ for each group, $p < 0.01$). The response to KCl 80 mM was not significantly different in ganglionectomized preparations (568 mg ± 82 mg, $n = 6$) when compared to controls (503 mg ± 51 mg, $n = 6$). The response to field stimulation in urethral preparations from ganglionectomized animals was abolished by tetrodotoxin (1 μM, $n = 4$) thus indicating its neural origin. In none of the preparations from ganglionectomized animals did capsaicin (3 μM) activate motility ($n = 11$, $p < 0.01$).

Motor effect of tachykinins on rat isolated ureters. Addition of NKA activated a series of rhythmic contractions of the

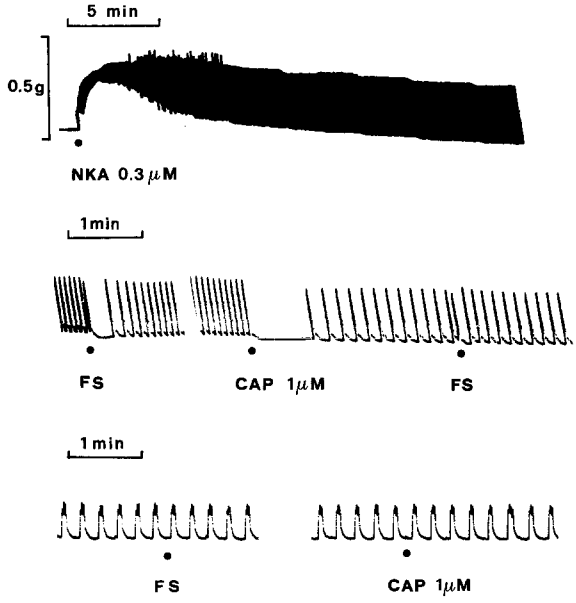

Fig. 5. Typical tracings illustrating the motor effect of NKA (0.3 μM) on the rat isolated ureter (*upper panel*) and the transient inhibitory effect produced by field stimulation (10 Hz, 60 V, 0.5 ms for 5 s) or capsaicin (*CAP*). Note that the inhibitory effect of field stimulation is abolished within a few minutes of capsaicin administration (*middle panels*). In the lower panels neither *FS* nor capsaicin are capable of inhibiting the NKA- (0.3 μM) induced rhythmic contractions of the ureter in a preparation stored for 72 h at 4°C. All tracings were obtained from different preparations with the exception of responses in the lower panels which were obtained in the same tissue

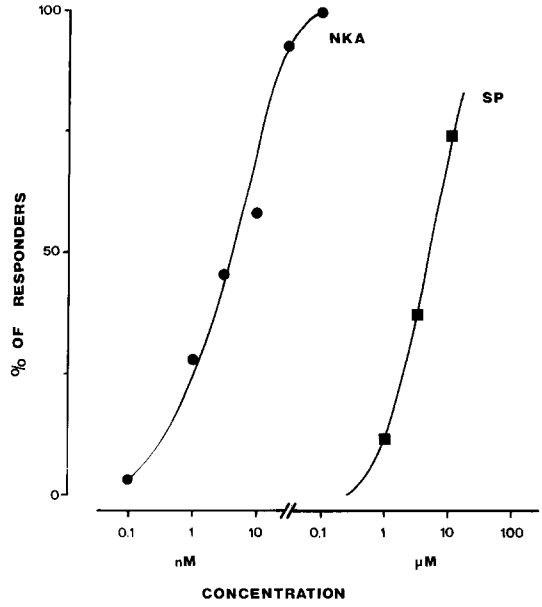

Fig. 6. Relative ability of SP and NKA to activate ureteral rhythmic contractions. Each point represents the percent of preparations responding to each tachykinin at that given concentration ($n = 12$ for each group). Responders were those preparations in which *SP* or *NKA* were able to activate at least three phasic contractions within 5 min from addition to the organ bath

rat isolated ureters (Figs. 4 and 5). Threshold concentration was 0.1–30 nM; the EC 50 for activation of rhythmic contractions was 4.7 nM (2.5–15.2; Fig. 6). Frequency of NKA-activated rhythmic contractions was concentration-related while amplitude was above 50% of maximal response at threshold concentrations (see Fig. 4). At high concentrations a transient "tonic" contraction was also observed on which the rhythmic contractions were superimposed (Figs. 4 and 5). This tonic contraction reached at 3 μM the 60–80% of the response to KCl (80 mM). The NKA- (0.1–0.3 μM) activated rhythmic contractions reached steady state values of frequency and amplitude after 10–15 min and then remained fairly constant for at least 15–30 min (see Fig. 5, upper tracing).

At high concentrations (> 1 μM Figs. 4 and 6) SP produced motor effects which were similar to those produced by NKA. SP was about 1/850 as potent as NKA in activating rhythmic contractions of the rat isolated ureter, its EC 50 being 4.0 μM (3.4–4.9).

The capsaicin-sensitive inhibitory innervation of the rat ureter. When capsaicin (1–3 μM) was added to the organ bath in preparations activated by NKA (0.3 μM) a transient inhibition of motility was observed (Fig. 5). A second addition of capsaicin (3 μM) within 1–3 h from the first one had no motor effect. Capsaicin did not affect the NKA-activated motor activity in ureters excised from capsaicin-pretreated rats (160 μmol/kg s.c. 4 days before). The capsaicin-induced inhibition of the NKA-activated urethral motility was unaffected by tetrodotoxin (1 μM) (Table 1).

Field stimulation (10 Hz, 0.5 ms, trains of 5 s at supramaximal voltage) produced a transient suppression of the NKA-activated ureteral motility similar to, but less intense than that produced by capsaicin. The inhibitory effect of field stimulation was prevented by tetrodotoxin (1 μM) (Table 1). The response to field stimulation could be consistently elicited at various times provided that fading of the NKA-activated motility was prevented by washing out and subsequent re-administration of the tachykinin.

Effect of capsaicin-desensitization on the field stimulation-induced inhibition of NKA-activated rhythmic contractions of the rat ureter. The inhibitory response to field stimulation was suppressed after acute desensitization to capsaicin in vitro (1 μM, Fig. 5) and could not be observed in preparations excised from capsaicin-desensitized animals (160–168 μmol/kg s.c. 4 days before). The blockade of the nerve-mediated responses in vitro could be observed as early as 1–4 min after capsaicin administration (Fig. 5), supporting the idea that the capsaicin-induced desensitization occurs, at the level of sensory terminals, shortly after the excitatory effect of this substance had subsided (see Szolcsányi 1985).

Effect of ganglionectomy or cold storage on capsaicin- or field stimulation-induced inhibition of the NKA-activated rhythmic contractions of the rat ureter. Rat ureters excised from animals whose pelvic ganglia were removed bilaterally 72 h before responded normally to NKA (0.1–0.3 μM). Likewise capsaicin (1 μM) or field stimulation produced a transient inhibition of the NKA-activated rhythmic contractions, as observed in controls (Table 1).

Rat ureters maintained for 72 h at 4°C responded normally to NKA (0.3 μM) but the inhibitory response to capsaicin (1 μM) or field stimulation was abolished (Fig. 5, Table 1).

Table 1. Effect of tetrodotoxin, systemic capsaicin-desensitization, ganglionectomy or cold storage on field stimulation- (10 Hz, 0.5 ms, 60 V for 5 s) or capsaicin- (1 μM) induced inhibition of the NKA- (0.3 μM) activated rhythmic contractions of the rat isolated ureter

	Dose	N	Intercontraction interval (s)			
			Field stimulation		Capsaicin	
			Resting value	Increase	Resting value	Increase
Controls	–	12	21 ± 2	56 ± 6	21 ± 2	135 ± 16
Tetrodotoxin	1 μM	9	20 ± 2	3 ± 1*	22 ± 2	126 ± 10
Capsaicin	160 μmol/kg	8	20 ± 2	1 ± 0.2*	21 ± 3	2 ± 0.5*
Ganglionectomy	–	8	21 ± 4	38 ± 4	18 ± 2	111 ± 19
Cold storage (72 h at 4° C)	–	8	18 ± 3	1 ± 0.4*	21 ± 3	7 ± 3*

Each value mean ± SE. Tetrodotoxin was added to the organ bath 10 min before. Systemic capsaicin desensitization was produced, in adult rats, by the s.c. administration of capsaicin, 4 days before the experiment. Bilateral removal of pelvic ganglia was performed 72 h before under ether anaesthesia

* Significantly different from controls $p < 0.05$

Table 2. Effect of capsaicin on Evans blue leakage from the rat lower urinary tract in control or capsaicin-pretreated animals

Treatment	Number of animals	Evans blue content (ng/mg)			
		Bladder dome	Bladder neck	Proximal urethra	Ureters
Controls	6	7 ± 2	7 ± 2	12 ± 2	17 ± 2
Capsaicin	6	22 ± 2*	61 ± 8*	111 ± 7*	75 ± 7*
Capsaicin in capsaicin-pretreated animals	5	10 ± 1**	10 ± 1**	23 ± 8**	25 ± 4**

Each value is mean ± SE. Capsaicin (5 μmol/kg) was administered intravenously. Systemic capsaicin desensitization was produced, in adult rats, by the s.c. administration of capsaicin 160 μmol/kg, 4 days before

* Significantly different from controls $p < 0.005$

** Significantly different from capsaicin group $p < 0.05$

In vivo studies

Effect of capsaicin, SP, NKA or histamine on Evans blue leakage in the rat lower urinary tract. Intravenous capsaicin (5 μmol/kg, 5 min before) produced a staining of various cutaneous and visceral areas whose distribution paralleled that described by Saria et al. (1984). In the lower urinary tract such a very intense staining was observed at ureteral and urethral level and in the bladder neck. In the bladder neck a very intense staining was observed also in correspondence of the ureteral orifices.

A larger dose of capsaicin (20 μmol/kg, $n = 5$) or a longer time lag (20 min, $n = 5$) between capsaicin administration and saline perfusion did not produce a greater dye leakage.

When capsaicin was applied topically on the bladder dome ($n = 6$) marked blue staining was observed in the dome and the neck but no leakage was observed in the proximal urethra and ureters ($n = 6$) and other cutaneous or visceral areas indicating that the reaction was entirely due to activation of intramural structures.

Quantitative analysis of Evans blue leakage confirmed the results of visual observation and demonstrated a significantly ($n = 5$, $p < 0.01$) greater (about three times) reaction in the bladder neck compared to the bladder dome after i.v. capsaicin (Table 2). An intense extravasation was also observed in the ureters but the most intense reaction was observed in the urethra (about five times greater than in the dome). Dye leakage in the bladder neck, proximal urethra and ureters was significantly ($p < 0.01$) greater than that observed in the bladder dome.

Effect of capsaicin-desensitization, tetrodotoxin or removal of pelvic ganglia on capsaicin-induced Evans blue leakage. The effect of i.v. capsaicin was almost absent (both on visual inspection and quantitative analysis) in rats systemically pretreated with a large dose of capsaicin (160 μmol/kg s.c. 4 days before, Table 2).

Pretreatment with topical tetrodotoxin at a dose (20 μg in 0.1 ml on the bladder, 3 min before capsaicin) previously shown to prevent the capsaicin- or tachykinins-induced activation of micturition reflex (Maggi et al. 1984a, b, 1985c, 1986e), failed to modify the Evans blue leakage produced by capsaicin (5 μmol/kg i.v.) both in the bladder dome and neck (23 ± 5 ng/mg and 52 ± 4 ng/mg, respectively, $n = 5$). An intense reaction was also observed in the proximal urethra and ureters (data not shown).

Following bilateral removal of pelvic ganglia (72 h before, $n = 10$) i.v. capsaicin (5 μmol/kg) did not produce any significant Evans blue extravasation in the bladder dome. In the bladder neck and proximal urethra intensity of Evans blue extravasation was reduced by about 80% ($p < 0.01$) and 70% ($p < 0.02$) as compared to sham-operated animals,

Table 3. Effect of capsaicin, substance P, neurokinin A or histamine on Evans blue content of various organs of the rat lower urinary tract

Treatment	Dose	Number of animals	Evans blue content (ng/mg)			
			Bladder dome	Bladder neck	Proximal urethra	Ureters
Controls	–	6	7 ± 2	7 ± 2	12 ± 2	17 ± 2
Substance P	0.37 nmol/kg	8	24 ± 3*	40 ± 6*	38 ± 8	54 ± 6*
	0.74 nmol/kg	12	34 ± 3*	64 ± 4*	81 ± 10*	75 ± 6*
	3.7 nmol/kg	8	64 ± 9*	109 ± 11*	106 ± 14*	77 ± 8*
Neurokinin A	0.37 nmol/kg	7	33 ± 3*	45 ± 4*	27 ± 2*	39 ± 2*
	0.74 nmol/kg	7	51 ± 9*	76 ± 13*	76 ± 13*	54 ± 6*
	3.7 nmol/kg	7	72 ± 9*	102 ± 6*	110 ± 13*	76 ± 2*
Histamine	0.9 μmol/kg	5	12 ± 3*	23 ± 7*	21 ± 2*	21 ± 4
	3.0 μmol/kg	5	26 ± 3*	37 ± 4*	106 ± 20*	51 ± 10*
	9.0 μmol/kg	5	30 ± 2*	52 ± 7*	99 ± 10*	78 ± 4*

Each value is mean ± SE capsaicin, substance P, neurokinin A or histamine were injected intravenously
* Significantly different from controls $p < 0.05$

respectively. In the ureters the Evans blue extravasation produced by i.v. capsaicin was almost unaffected by bilateral removal of the pelvic ganglia. This observation agrees well with functional in vitro experiments on ureteral motility relative to the effects of capsaicin on NKA-activated ureteral motility (see above).

Effect of tachykinins or histamine on dye leakage in the rat lower urinary tract. Intravenous SP or NKA (0.37–3.7 nmol/kg, 5 min before) produced a macroscopically evident dose-related blue staining of the rat lower urinary tract with regional variations similar to those described for i.v. capsaicin. Quantitative analysis confirmed the results of visual observation and indicated that Evans blue extravasation produced by tachykinins is greater in the bladder neck, proximal urethra and ureters compared to the bladder dome (Table 3). The regional variation, however in the response to SP or NKA was not as intense as observed for capsaicin: at all doses tested, the response in the neck was only 1.4–1.9 times greater than in the dome (Table 3). Likewise the response to SP or NKA in the proximal urethra was not significantly greater than in the bladder neck while, for capsaicin a significant difference (1.8 times) was observed. At a high dose (3.7 nmol/kg) both SP and NKA produced an almost uniform staining in the bladder dome and neck. However, quantitative analysis revealed that the response in the bladder neck was still significantly greater as compared to the bladder dome (Table 3).

Histamine (0.9–9 μmol/kg i.v.) produced a dose-related staining of the rat lower urinary tract qualitatively similar to that produced by SP or NKA (Table 3). The effect of histamine (9 μmol/kg) was almost suppressed by intravenous diphenhydramine (34 μmol/kg) plus cimetidine (40 μmol/kg, 20 min before) while that produced by capsaicin was unaffected ($n = 4$, data not shown).

In ganglionectomized preparations intravenous SP (3.7 nmol/kg) produced an Evans blue extravasation of the rat lower urinary tract which showed a regional variation similar to that seen in controls ($n = 4$).

Discussion

Acute effects of capsaicin involve motor and inflammatory responses whose *primum movens* is the release of neuropeptide(s) from peripheral nerve terminals of certain sensory neurons (Szolcsányi 1984; Saria et al. 1983; Lundberg et al. 1984; Maggi et al. 1984a, 1985a, b, 1986a, b, c, d; Santicioli et al. 1986) through a tetrodotoxin-resistant depolarization of the sensory receptor (Jancsó 1968; Szolcsányi 1984; Maggi et al. 1984a, 1986d). Present finding indicate that the responses to capsaicin in the rat lower urinary tract are most likely produced through a similar mechanism since they are tetrodotoxin-resistant while being abolished by tissue denervation (ganglionectomy or cold storage). Recent anatomical data provide further support to the hypothesis that neuropeptide(s) may be secreted from sensory nerve terminals in the rat lower urinary tract: Yokokawa et al. (1985) have described the presence, within SP-fibers of the rat urinary bladder, of synaptic vesicles, this supports the idea that a depolarization-induced secretion of neuropeptides may occur from the sensory receptor (Jancsó 1968; Szolcsányi 1984; Maggi and Meli 1986; Maggi et al. 1984a, 1986d).

Present findings indicate regional differences in the effects of capsaicin in the rat lower urinary tract which may be relevant to the pathophysiology of mechanisms regulating vesicourethral (Maggi et al. 1984a, 1985a, b, 1986a, b, c; Santicioli et al. 1985, 1986) and ureteral motility (Maggi et al. 1986d) and also to tissue trophism and/or inflammatory processes of these organs.

Responses to capsaicin in the urinary bladder

Previous findings (Alm et al. 1978; Terenghi et al. 1983) indicated that the content of SP-LI is greater in the neck than in the dome of the rat bladder. The density of SP-LI fibers of the muscular wall, however, shows no regional difference (Yokokawa et al. 1985) which agrees with the observation that the amplitude of the capsaicin-induced contraction, ascribable to neuropeptide release from sensory nerves (Maggi et al. 1984a, 1986d; Santicioli 1986), is similar in strips from the bladder neck and dome.

On the other hand, the observation that "the density of the SP fiber meshwork found in the submucosal layer is higher in the neck and the trigonum than in the fundic part of the bladder" (Yokokawa et al. 1985) provides an anatomical basis to our observation that the capsaicin-induced neurogenic plasma protein extravasation is greater in

the bladder neck than in the dome. Also exogenous tachykinins produced greater plasma extravasation in the neck than in the dome although these regional variations were not so intense as for capsaicin. Therefore, the greater ability of capsaicin to induce a neurogenic inflammatory response in the bladder neck or proximal urethra depends, at least in part, on the characteristics of vessels, i.e. is postjunctional in origin. This may involve a greater density of tachykinin receptors in blood vessels but also some regional variation in the stimulus-effect coupling.

The greater density of SP innervation (Alm et al. 1978; Yokokawa et al. 1985) and responsiveness to capsaicin (plasma extravasation) at bladder neck level suggests a precise functional significance: this may involve a defensive response towards irritants or foreign chemicals (such as products of bacterial metabolism) present in the urine. Accordingly, frequency of micturition, influenced by discharge of the capsaicin-sensitive chemo- and mechano-receptors, may be influenced by composition of the urine itself, by activating a "cleaning" mechanism which avoids a prolonged exposure to irritants.

The rat urinary bladder has been labelled as a SP-P tissue since all tachykinins are almost equipotent in producing a direct contraction of this tissue (Watson et al. 1983; Mathison and Solomos 1985; Maggi et al. 1986e). However, radioligand binding studies indicated the presence of both SP-P and SP-K sites on the muscle of the rat bladder (Buck and Burcher 1986).

In addition, the SP-P receptor (or NK-P according to Regoli et al. 1985) has now been re-defined as the one at which SP is distinctly more potent than the other mammalian tachykinins (Regoli et al. 1985, 1986). Likewise, the SP-K receptor (or NK-A) is the one for which NKA possesses the strongest affinity (Buck and Burcher 1986; Regoli et al. 1985, 1986).

Present findings suggest that the tetrodotoxin-resistant tachykinin-induced contraction of the rat isolated bladder or urethra may involve multiple receptors: in fact, NKA and SP were almost equipotent, which could be explained by the view that both SP-P and SP-K sites, detected by radioligand assay in the bladder (Burcher and Buck 1986) mediate the direct contractile effect of tachykinins.

However, in the bladder neck (as in the urethra) the maximal response to NKA was about twice as high as compared to that of SP, suggesting the predominance, at these levels, of an SP-K/NK-A receptor. Our data suggest that it would be interesting to compare the relative density of these receptors in the rat bladder dome and neck.

Responses to capsaicin in the proximal urethra

Present findings indicate the presence, in the rat proximal urethra, of a capsaicin-sensitive innervation which can produce motor effects and an intense inflammatory response. These fibres are of extrinsic origin since the effects of capsaicin are abolished or greatly reduced by removal of pelvic ganglia, as already described for the capsaicin-sensitive fibres which innervate the urinary bladder (Maggi et al. 1985b, 1986a; Santicioli et al. 1986).

It seems conceivable, as described for the urinary bladder, that the capsaicin-sensitive innervation of the rat proximal urethra subserves a sensory function which may involve the regulation of certain urethro-vesical and urethro-urethral reflexes regulating micturition (Rossier and Bors 1962; Rossier et al. 1979; Maggi et al. 1986f).

Unlike in the rat bladder (Santicioli et al. 1986), a consistent response to field stimulation was still observed in urethral rings excised from rats whose pelvic ganglia were removed 48 h before the experiments. This suggests that some part of the efferent innervation to the rat proximal urethra has its cell bodies at a site distal to the pelvic ganglia.

Responses to capsaicin in the ureters

Our results confirm previous observations indicating that capsaicin produces an intense plasma extravasation in the ureters, ascribable to tachykinin release from sensory nerve endings (Saria et al. 1983; Lundberg et al. 1984). In guinea-pigs, capsaicin-sensitive afferents reach the lower third of the ureters via the pelvic ganglia (Saria et al. 1983). On the other hand, in rats, neither the inflammatory nor the visceromotor response to capsaicin are affected by pelvic ganglionectomy. This may reflect species-related differences in the distribution of sensory nerves to target organs via the sympathetic and parasympathetic pathways.

Capsaicin produced a prompt inhibition of motility of the rat isolated ureter when a background activity was initiated by exogenous tachykinins (Maggi et al. 1986d). This effect undergoes prompt desensitization, a characteristic of the action of this substance on sensory nerves (Szolcsányi 1984; Maggi et al. 1987). Tachykinins are present in rather high concentrations in the ureters (Brodin and Nilsson 1981; Lundberg et al. 1984; Bucsics et al. 1983; Theodorsson-Norheim et al. 1984) and their release in response to capsaicin (Saria et al. 1983) produces consistent biological responses (inflammation) in this organ (Saria et al. 1983; Lundberg et al. 1984; present findings). Accordingly we hypothesized (Maggi et al. 1986d) that, in this preparation, some other substance (possibly a neuropeptide) is co-released together with SP/NKA and possesses a very potent inhibitory activity on ureters, i.e. exerts a *physiological antagonism* toward the excitatory effect of endogenous tachykinins, putatively co-stored in and co-released from the capsaicin-sensitive sensory nerves. Such a substance could co-operate with endogenous tachykinins to determine the inflammatory response in these organs.

Interestingly, similar conclusions were reached by Hua and coworkers (Hua 1986; Hua et al. 1986) about the visceromotor response to capsaicin in the guinea-pig ureter: the inhibitory effect of capsaicin was ascribed to release of calcitonin gene-related peptide from sensory nerve terminals (Hua 1986; Hua et al. 1986).

In a previous paper (Maggi et al. 1986d) we proposed, in accordance with Szolcsányi's hypothesis (1984, 1985) that the main site of capsaicin action is at the level of the sensory terminal itself and, particularly, that the desensitization occurs shortly after the excitatory phase. Such hypotheses are substantiated by the present results since, within minutes of capsaicin administration and shortly after the end of its visceromotor inhibitory effect, the response to field stimulation is abolished, as observed also in preparations excised from capsaicin-desensitized animals.

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