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Species-related differences in the capsaicin-sensitive innervation of the rat and guinea-pig ureter

R. Amann[1], G. Skofitsch[2], and F. Lembeck[1]

[1] Department of Experimental and Clinical Pharmacology,
[2] Department of Zoology, Section on Wildlife Research and Parasitology, University of Graz, A-8010 Graz, Austria

Summary. 1. Comparison of the tissue content of calcitonin gene-related peptide (CGRP)-immunoreactivity (IR) and tachykinin (TK)-IR in the rat and guinea-pig ureter showed that in the rat tissue levels of CGRP-IR were 33-fold higher than those of TK-IR. In the guinea-pig ureter, both peptides were present in nearly the same concentration. 2. The in-vitro release of neuropeptides from guinea-pig and rat ureters was investigated using capsaicin as a stimulus for afferent neurons. Capsaicin induced the simultaneous release of CGRP-IR and TK-IR from the guinea-pig ureter while in the rat only the release of CGRP-IR was detectable. 3. It is known that TK potently stimulate and CGRP inhibits ureteric smooth muscle contractions. When the effect of capsaicin on ureteric motility was investigated in guinea-pig and rat, only in the guinea-pig ureter a stimulatory action ascribable to capsaicin-induced TK release was observed thus supplementing the results obtained by radioimmunoassay. 4. The results show that considerable species differences exist concerning the ratio of CGRP and TK which is stored and released from ureteric afferent nerve terminals. As a consequence, different functional responses are obtained in both species upon stimulation of these neurons by capsaicin. In the rat ureter, the capsaicin-sensitive innervation seems to be only inhibitory while in the guinea-pig stimulatory and inhibitory transmitters are released. The physiological significance of the simultaneous release of transmitters with opposing effects needs further investigation.

Key words: Ureter – Capsaicin – Tachykinins – Calcitonin generelated peptide

Introduction

In the rat and guinea-pig ureter there is a dense innervation with capsaicin-sensitive primary afferent neurones (Alm et al. 1978; Hoyes and Barber 1981; Sikri et al. 1981; Hua et al. 1987). Application of neuropeptides which are present in these afferents has a potent effect on ureteric motility: Tachykinins (TK) induce phasic contractions – neurokinin A (NKA) being about 10 times more potent than substance P (SP); calcitonin gene-related peptide (CGRP) inhibits smooth muscle contractions. Since the classical neurotransmitters like acetylcholine or noradrenaline have no or only a weak influence on ureteric motility, a physiological role of the release of neuropeptides from primary afferent terminals in the regulation of ureteric motility has been discussed (Hua and Lundberg 1986; Maggi et al. 1986, 1987).

Capsaicin selectively activates primary sensory neurons and induces transmitter release from their central and peripheral terminals and is therefore used as a tool to investigate the "local effector function" of these neurons (Holzer 1988).

Studies on the effect on motility of capsaicin in the guinea-pig ureter have shown that it induced contractions in 60% of the preparations but always inhibited spontaneous or TK-induced phasic activity. Both effects were mediated via the activation of primary afferent neurones in a tetrodotoxin insensitive manner. The involvement of TK and CGRP in the stimulatory and inhibitory action respectively has been suggested (Hua and Lundberg 1986). In the rat, however, no evidence of an excitatory influence of primary afferents on ureteric motility has been obtained: capsaicin inhibited contraction of ureteric smooth muscle presumably by releasing CGRP from afferent nerve terminals (Maggi et al. 1986, 1987). The reason for the variable results in the guinea-pig and for the lack of excitatory action of capsaicin in the rat has not been determined so far.

The aim of the present study therefore was to compare the tissue content of CGRP-IR and TK-IR in the rat and in the guinea-pig ureter and to correlate biochemical data of capsaicin-induced TK and CGRP release to capsaicin-induced changes of ureteric motility in both species:

In order to quantify the amount of stimulatory (TK) and inhibitory (CGRP) transmitter which is released by capsaicin, isolated ureters of rats or guinea-pigs were superfused and the respective immunoreactivities were determined in the outflow.

To investigate capsaicin-induced motility changes in the rat and guinea-pig ureter, we used an in-vitro model as described by Maggi et al. (1987). Since simultaneous capsaicin-induced release of CGRP and TK has been shown in other tissues (Saria et al. 1986) a similar effect can be expected in the ureter. TK and CGRP have opposing effects on ureteric motility. Therefore the capsaicin-induced motility changes are likely to be caused by a combined effect of the peptides on the smooth muscle (Hua and Lundberg 1986). In the rat, it has been demonstrated that preincubation with CGRP prevents further inhibitory effects of CGRP without influence on the inhibition caused by isoprenaline (Maggi et al. 1987). If under these conditions primary afferent neurons are stimulated with capsaicin, the excitatory action of released TK should be observable.

Send offprint requests to F. Lembeck at the above address

Methods

1. Animals and pretreatment. Sprague-Dawley rats (250–350 g b.w.) and guinea-pigs (300–350 g b.w.) of either sex were used. Capsaicin pretreatment of rats (50 mg kg^{-1}) was performed under ether anaesthesia on the second day of life according to Jancsó et al. (1977). Guinea-pigs were pretreated under ether anaesthesia with capsaicin 10 days before the experiment (2 × 50 mg kg^{-1} on 2 consecutive days) according to Hua et al. (1986).

2. Release experiments. The animals were killed by a blow to the head and both ureters removed. The ureters of three animals were sliced (1–2 mm) placed into a superfusion chamber (0.7 ml) and continuously perfused (0.5 ml min^{-1}) with gassed (95% O_2, 5% CO_2) physiological salt solution at 37° C (NaCl 118, KCl 4.6, $MgSO_4$ 1.17, $CaCl_2$ 2.5, NaH_2PO_4 1.17, $NaHCO_3$ 25, glucose 10 mM; bovine serum albumin 1 g/l). One milliliter fractions were collected every 2 min after an equilibration period of 15 min. The superfusates were collected in tubes containing acetic acid to give a final concentration of 2 M. After a control period of 4 fractions, capsaicin (10 μM) or potassium (60 mM) was added to the perfusate in fractions 5 to 7. The samples were lyophilized and redissolved for determination of TK-IR and CGRP-IR by radioimmunoassay (RIA).

For determination of the tissue content of TK-IR and CGRP-IR, samples were boiled for 10 min in 2 M acetic acid and homogenized. After centrifugation the supernatant was lyophilized and used for RIA.

3. Biochemical analysis. TK-IR was determined using antiserum RAS 7359 N (Peninsula) raised against NKA which crossreacts with the following peptides: kassinin: 100%, eledoisin: 80%, physalaemin: 6%, neuromedin K: 3%, substance P: 3%, substance P tripeptide: < 0.0001%, bombesin: < 0.0001%. [2-(I^{125}) iodohistidyl] NKA (Amersham, Amersham, UK) was used as radioligand and synthetic NKA (Peninsula, San Carlos, CA, USA) as standard.

CGRP-IR was determined using antiserum RAS 6009 N (Peninsula) raised against human CGRP. The crossreactivities are: rat-CGRP: 100%, rat calcitonin C-terminal adjacent peptide: < 0.001%, calcitonin < 0.001%, katacalcin: < 0.001%, Arg-vasopressin: 0%, oxytocin: 0%. [2-(I^{125}) iodohistidyl 10] CGRP, human (Amersham) was used as radioligand and synthetic human CGRP (Peninsula) as standard.

The identity of CGRP-IR was verified by reversed-phase high pressure liquid chromatography (HPLC) using a nucleosil C_{18} column (Waters) according to Franco-Cereceda et al. (1987). Elution was performed at 1.5 ml/min with a 40 min linear gradient from 20 to 60% acetonitrile. Fractions (1.5 ml) were collected, lyophilized, redissolved and assayed for CGRP-IR.

4. Ureteric motility in vitro. After sacrifice the middle part (1.5–2 cm) of one ureter was removed and placed in an organ bath (2 ml) containing gassed (95% O_2, 5% CO_2) physiological salt solution (NaCl 94.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.52, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.7 mM) at 37° C. Longitudinal contractions of the preparation were recorded isometrically under a constant load of 0.5 g using a HSE force displacement transducer. Capsaicin (30 mM) was dissolved in dimethylsulfoxide (DMSO) and diluted in buffer to the appropriate concentrations. Similar dilutions of DMSO were added to the organ bath and found to be without effect.

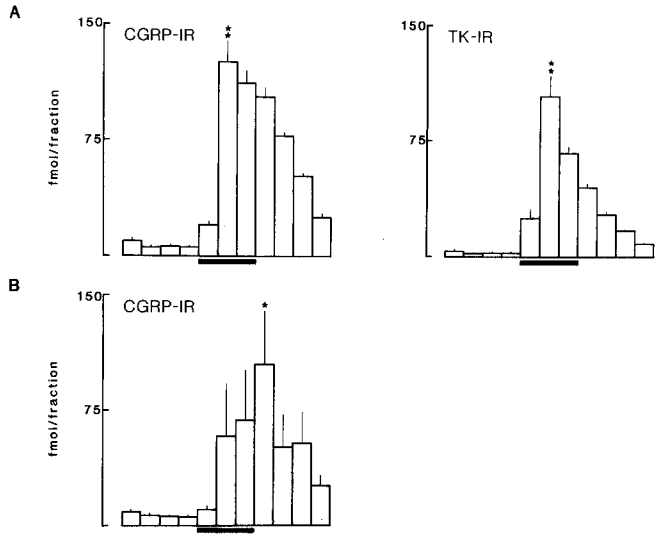

Fig. 1. In-vitro release of CGRP-IR and TK-IR from superfused ureters of guinea-pig (**A**) and rat (**B**). In the guinea-pig, 10 μM capsaicin caused a simultaneous increase of CGRP-IR and TK-IR in the outflow ($N = 6$). In the rat, however, only CGRP-IR but no TK-IR could be detected (detection limit of the assay: 0.6 fmol/tube) in the superfusates ($N = 6$). Stimulation periods are indicated by bars. Means ± SEM, ** $p < 0.001$, * $p < 0.05$ compared to the prestimulation period (*t*-test)

5. Drugs and statistics. Capsaicin, tetrodotoxin (Sigma, St. Louis, MO, USA), neurokinin A, calcitonin gene-related peptide (Peninsula). Values given are mean ± SEM, Student's *t*-test for unpaired data was used for statistical analysis.

Results

1. Tissue content of CGRP-IR and TK-IR

Determination of TK-IR and CGRP-IR in tissue extracts of guinea-pig and rat ureter showed that in the guinea-pig the tissue content of TK-IR (45.7 ± 5.5 fmol/mg) was slightly lower than the content of CGRP-IR (75.7 ± 5.7 fmol/mg; $N = 4$); in the rat ureter the concentration of TK-IR was found to be 33-fold lower (12.0 ± 0.8 fmol/mg) than the concentration of CGRP-IR (396.3 ± 32.4 fmol/mg; $N = 6$).

In ureters taken from capsaicin pretreated rats, TK-IR was reduced by 86% to 1.7 ± 0.2 fmol/mg ($N = 4$) which shows that TK-containing neurons of the rat ureter are affected by in-vivo treatment with capsaicin in a similar way as those containing CGRP (see Discussion).

2. Release of CGRP-IR and TK-IR

a) Guinea-pig. In the control period, the concentration of CGRP-IR in the outflow was 2.4 times higher than that of TK-IR. Capsaicin (10 μM) caused an 24-fold increase of CGRP-IR (from 5.7 ± 0.5 to 134.9 ± 13.9 fmol/fraction; $N = 6$) and simultaneously a 43-fold increase of TK-IR (from 2.4 ± 0.2 to 104.2 ± 14.1 fmol/fraction; $N = 6$) in the outflow (Fig. 1 A).

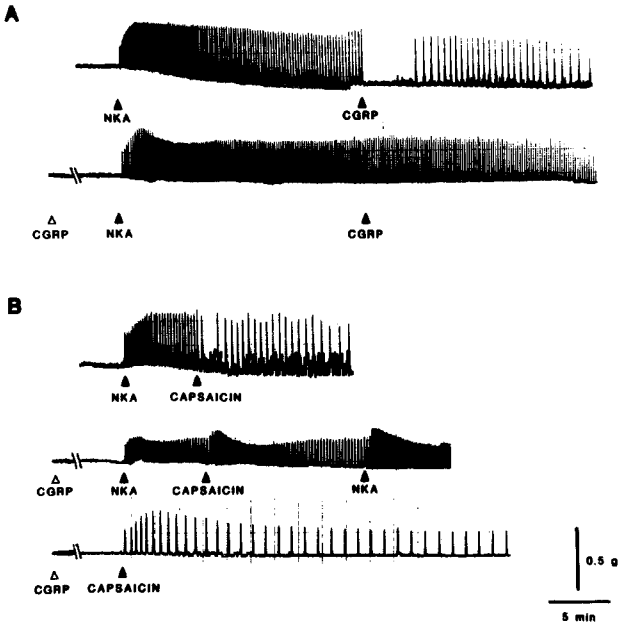

Fig. 2 A, B. Isometric recordings of longitudinal contractions of the isolated guinea-pig ureter. **A** Pre-incubation with CGRP prevents the further inhibitory action of this peptide. *Upper tracing:* NKA (300 nM) induces phasic contractions which are inhibited by 300 nM CGRP. *Lower tracing:* After pre-incubation with CGRP (300 nM) for 20 min NKA (300 nM) induced contractions are not influenced by 300 nM CGRP. **B** Pre-incubation with CGRP reverses the effect of capsaicin. *Upper tracing:* 1 μM capsaicin inhibits NKA (300 nM) induced phasic contractions. *Middle tracing:* After pre-incubation with 300 nM CGRP for 20 min, 1 μM capsaicin increases frequency and amplitude of NKA (300 nM) induced contractions. This effect can be mimicked by an additional application of 50 nM NKA. *Lower tracing:* Effect of 1 μM capsaicin in a preparation which has been pre-incubated with CGRP (300 nM) for 20 min. Calibration as indicated

b) Rat. The basal levels of TK-IR were below the detection limit of the assay (0.6 fmol/fraction). Capsaicin (10 μM) did not cause any detectable release of TK-IR ($N = 6$). In the same samples, however, capsaicin caused a significant increase of CGRP-IR (from 6.3 ± 0.8 to 105.4 ± 35.9 fmol/fraction) in the outflow (Fig. 1B).

To determine whether the lack of capsaicin-induced release of TK-IR was due to insensitivity of TK-containing neurons to capsaicin, another set of experiments ($N = 4$) was performed where capsaicin was replaced by 60 mM potassium. The results showed that while no release of TK-IR was detectable, potassium caused a significant increase of CGRP-IR in the outflow (from 11.7 ± 1.7 to 41.4 ± 9.9 fmol/fraction; $p < 0.05$).

3. Effect of capsaicin on isolated ureters in vitro

a) Guinea-pig. Capsaicin (1 μM) caused phasic contractions of the guinea-pig ureter in 3 out of 7 preparations. The contractions lasted for 2–5 min. Capsaicin (1 μM) inhibited NKA (300 nM) induced contractions in all preparations ($N = 12$; Fig. 2B). Neither the excitatory nor the inhibitory action of capsaicin was present when it was applied for a second time within 60 min or when the urether was taken from a capsaicin pretreated animal ($N = 4$).

In ureters which had been pre-incubated with 300 nM CGRP for 20 min, NKA-induced phasic contractions were not influenced by a second application of the same concentration of CGRP (Fig. 2A). In these preparations addition of capsaicin (1 μM) induced phasic contractions which lasted for more than 15 min ($N = 3$) or increased the frequency of the NKA-induced contractions ($N = 6$); a similar effect could be obtained by an additional application of NKA (Fig. 2B). The action of capsaicin was insensitive to tetrodotoxin (1 μM), exhibited complete desensitization – a second application within 60 min was ineffective – and was not seen in ureters dissected from animal which had been pretreated with capsaicin ($N = 4$).

b) Rat. Confirming previous studies (Maggi et al. 1986, 1987), our results show that preincubation with 300 nM CGRP for 20 min nearly abolishes the inhibitory effect of capsaicin on NKA-induced contractions. To exclude the possibility that the presence of exogenous NKA masks the effect of capsaicin-induced TK release, capsaicin (1 μM) was applied to unstimulated preparations but proved ineffective in all 8 ureters tested.

Discussion

Capsaicin-sensitive afferents can be divided into several subpopulations according to their neuropeptide content and it seems that the combination of neuropeptides which are stored in the afferents is related to the innervated tissue and to the species investigated (Gibbins et al. 1987; Su et al. 1986).

Acute application of capsaicin stimulates the orthodromic activation (i.e. sensory function) of primary afferents as well as the transmitter release from their peripheral terminals. The peripherally released neuropeptides exert their influence on blood vessels by inducing plasma extravasation (TK) and vasodilatation (CGRP and TK), and on smooth muscle by inhibiting or inducing contractions (TK and CGRP). This local effector function of sensory neurons is thought to play an important part in the reaction of various tissues to noxious stimuli (for references see Nagy 1982; Holzer 1988).

Pre-treatment with capsaicin results in a pronounced loss of the tissue content of CGRP and TK in the ureter of guinea-pigs (Hua et al. 1986, 1987; Gibbins et al. 1987) and rats (Su et al. 1986; and present results). These peptides are therefore thought to be stored nearly exclusively in primary sensory neurones.

We observed a 33-fold lower concentration of TK-IR than of CGRP-IR in the rat ureter while in the guinea-pig ureter both peptides seemed to be present to the same extent. Release experiments showed that in the rat ureter neither capsaicin nor potassium were able to stimulate a detectable TK-IR release. In the same samples, however, CGRP-IR was significantly increased. The lack of capsaicin-induced TK-IR release had not been observed in any other tissue so far. In the rat spinal cord, for example, TK-IR is simultaneously released with CGRP-IR by capsaicin (Saria et al. 1986).

It seems that the lack of detectable capsaicin-induced TK-IR release is due to the low amount of TK present in the rat ureter which is also indicated by the finding that a high concentration of potassium was equally ineffective. On

the other hand, a recent report (Maggi et al. 1988) shows that 10 μM capsaicin does not induce any detectable SP release from the rat urinary bladder up to an exposure time of 30 min while the presence of SP in primary afferents in this organ is confirmed. It may be possible therefore that the acute effects of capsaicin on afferents of the urogenital system of the rat does not necessarily include transmitter release from all subpopulations of neurons which are susceptible to the chronic effect of capsaicin pretreatment.

The finding that little if any TK-IR is released by capsaicin from the rat ureter may explain different results on motility effects of capsaicin on guinea-pig (Hua and Lundberg 1986) and rat ureter (Maggi et al. 1987). When the same experimental model was employed in both species, we confirmed the above cited results and showed that in the guinea-pig ureter a capsaicin-induced stimulatory motor response, which is most likely due to TK release from sensory nerve terminals (Hua and Lundberg 1986), is constantly observable if the effect of the simultaneously released CGRP is prevented by pre-incubation with a high concentration of this peptide.

Thus the peripheral effector function of primary afferents seems to be highly dependent on the characteristics of the afferent innervation of a given tissue. It is interesting to note in this respect that a recent paper (Koltzenburg and McMahon 1986) reports that distension of the rat urinary bladder – in contrast to mustard oil application – fails to induce plasma extravasation but activates the orthodromic function of capsaicin-sensitive afferents. It was concluded that distension does not activate the peripheral function of primary afferents. This conclusions seems justified if it is assumed that transmitter release from the peripheral terminals means TK release. On the other hand, bearing in mind the results of Maggi et al. (1988) and those presented in this study, it seems quite possible that TK release from the afferent innervation of the rat urogenital system is very moderate compared to the ability to release CGRP which does not cause plasma extravasation (Gamse and Saria 1985).

It remains to be investigated whether the large difference between the tissue content of CGRP and TK and the lack of detectable capsaicin-induced TK release is confined to the rat ureter or if a similar situation is also encountered in other organs.

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