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Effects of capsaicin in rat and pigeon on peripheral nerves containing substance P and calcitonin gene-related peptide*

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Summary. Substance P and calcitonin gene-related peptide were immunohistochemically identified in axons innervating the cornea and the ureter of adult rats and pigeons. The two neuropeptides were similarly distributed in both species. Capsaicin pretreatment induced depletion of the immunoreactivity; this was quantitatively and qualitatively different in rats and pigeons. Topical application of capsaicin (1%) reduced the immunoreactivity in the cornea in both species by 50%. Systemic capsaicin treatment completely depleted both peptides from the corneal innervation of rats but reduced the peptide content only by 50% in the cornea of pigeons. In the ureter of rats, capsaicin pretreatment completely depleted the peptide immunoreactivity. In pigeons the peptide depletion was only complete in the outer longitudinal muscle layer. Whereas only a few immunoreactive fibres were observed in the circular muscle layer, about 50% of the peptide remained in the inner longitudinal muscle layer. The results demonstrate that peptidergic afferents in the cornea and ureter of pigeons are sensitive to capsaicin, although birds do not show nociceptive responses to local administration of the drug. The long-term depletion of substance P and calcitonin gene-related peptide by capsaicin is discussed with regard to the possibility that functionally capsaicin receptors may exist in the axon but not at nerve endings.

Key words: Substance P – Calcitonin gene-related peptide – Sensory axons – Capsaicin – Domestic pigeon – Rat (Wistar)

In adult mammals administration of capsaicin causes excitation followed by desensitization, and in some cases, degeneration of a population of primary afferent neurones (Nagy 1982; Szolcsányi 1982; Fitzgerald 1983; Jancsó et al. 1985; Buck and Burks 1986). When applied to cutaneously derived tissues (e.g. skin, cornea and lips) it produces an intense burning sensation in man and elicits a series of protective reactions in other mammals (Jancsó et al. 1967; Jancsó 1968; Szolcsányi et al. 1975). In addition, it causes local vasodilation and extravasation of plasma proteins at the site of application (Lembeck and Holzer 1979; Saria et al. 1983b). Electrophysiological studies have shown that capsaicin excites a population of polymodal nociceptors with unmyelinated or thinly myelinated axons (Handwerker et al. 1984; Szolcsányi et al. 1988).

In mammals, many of the actions of capsaicin have been attributed to the release and/or depletion of neuropeptides from primary neurones (Nagy 1982; Fitzgerald 1983; Buck and Burks 1986). A number of neuropeptides have been observed in cell bodies of primary afferent neurones. These peptides include substance P, neurokinin A and B, somatostatin, vasoactive intestinal polypeptide, cholecystokinin, galanin and dynorphin (Hökfelt et al. 1975, 1976; Dalsgaard et al. 1982; Kawatani et al. 1983; Ch'ng et al. 1985; Hua et al. 1985; Kuo et al. 1985; Tuchscherer and Seybold 1985; Gibbins et al. 1987). Recently, a newly discovered peptide, calcitonin gene-related peptide (CGRP; Amara et al. 1982; Rosenfeld et al. 1983), has been localized in most, and possibly all, primary afferent neurones containing substance P; it is also present in a separate population that does not contain substance P (Gibson et al. 1984; Wiesenfeld-Hallin et al. 1984; Gibbins et al. 1985; Lee et al. 1985; Skofitsch and Jakobowitz 1985; Gulbenkian et al. 1986).

There is evidence to suggest that substance P and CGRP are substantially depleted from dorsal root ganglia and the peripheral and central processes of primary afferents after systemic capsaicin treatment (Nagy et al. 1982; Fitzgerald 1983; Gibbins et al. 1985; Buck and Burks 1986; Franco-Cereceda et al. 1987). The actions of capsaicin on other peptides in afferent neurones are less certain (Buck and Burks 1986).

In contrast to mammals, capsaicin evokes only weak behavioural responses in birds and amphibians (Mason and Maruniak 1983; Szolcsányi et al. 1986; Sann et al. 1987). Capsaicin has virtually no effect on thermoregulation or on chemo-nociception in birds (Mason and Maruniak 1983; Geisthövel et al. 1986; Sann et al. 1987). In the best-studied avian species, the pigeon *Columba livia*, capsaicin fails to elicit a nocifensive response following instillation into the eye (Szolcsányi et al. 1986). It is not known whether the ineffectiveness of capsaicin in the bird is a result of the lack of the peptidergic afferent innervation of peripheral tissues. However, it has been shown that the pigeon contains substance P and CGRP in primary afferent neurones

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and also in the dorsal horn of the spinal cord (Katz and Karten 1980; Pierau et al. 1987; Rössler et al. 1987). Two tissues that in mammals are highly sensitive to the effects of capsaicin are the cornea and ureter. Substance P and CGRP are both found in capsaicin-sensitive corneal afferents in mammals (Miller et al. 1981; Tervo et al. 1981; Colin and Kruger 1986; Stone et al. 1986), but it is not known whether substance P and CGRP are present in the cornea of the pigeon. In the ureter of the rat and guinea pig, the vast majority of the substance P and CGRP innervation is sensitive to capsaicin (Wharton et al. 1981; Hua et al. 1985; Su et al. 1986); it is not known whether the pigeon ureter has a substance P or CGRP innervation, and if so, whether it is sensitive to capsaicin.

Hence, in the present study, we examined the substance P- and CGRP-containing nerves of the cornea and the ureter in the pigeon and compared these findings with those in the rat. Having established a peptidergic innervation of the cornea and the ureter, we then examined the action of capsaicin on these tissues. A preliminary account of this work has been published (Sharkey et al. 1988).

Materials and methods

(1) Animals

Experiments were performed on 34 adult male Wistar rats (250–320 g; Zentralinstitut für Versuchstierzucht, Hannover) and 35 adult pigeons (*Columba livia*, 310–520 g; Wagner, Alten Buseck).

The animals were used as follows:

(a) Normal animals. This group was made up of 8 normal rats and 8 normal pigeons.

(b) Topical capsaicin treatment. Under halothane (Fluothane, ICI) anaesthesia 12 rats and 12 pigeons had a solution of 1% capsaicin (Sigma; dissolved in 10% ethanol, 10% TWEEN 80, 80% physiological saline) instilled into one eye (40–50 µl) and the solvent instilled into the other on five consecutive days. At the end of this protocol, the rats were tested for insensitivity to capsaicin using the wiping test (Jancsó 1968; Szolcsányi et al. 1975). The animals were killed 3 days later and the tissues removed (see below).

(c) Systemic capsaicin treatment. Fourteen rats were anaesthetized with halothane and were injected subcutaneously with 50 mg/kg capsaicin ($n=8$) or an equal volume of solvent ($n=6$). The rats were treated on the 4 following consecutive days with increasing doses of 100, 200, 200 and 400 mg/kg capsaicin giving a total dose of 950 mg/kg, or with the appropriate volume of the solvent, according to Jessel et al. (1978). Three days after the last injection, the rats were tested for insensitivity to capsaicin using the wiping test and then killed and the tissues removed (see below).

Fifteen pigeons were anaesthetized with halothane and given an intraperitoneal injection of capsaicin (50 mg/kg; $n=8$) or an equal dose of solvent ($n=7$). Intraperitoneal injection was used, since the injected solution easily leaks from subcutaneous depots in pigeons unlike rats. On the following 4 consecutive days, the pigeons were treated with increasing doses as were the rats and killed 3 days after the last injection.

(2) Tissue preparation

Animals were killed with an overdose of sodium pentobarbitone (Nembutal, Ceva) and both corneas and ureters were removed, washed in phosphate-buffered saline (PBS, pH = 7.4) and pinned out onto balsa wood. The tissues were fixed by immersion in Zamboni's fixative (Stefanini et al. 1967) for 48 h at 4° C. They were then dehydrated in an alcohol series (2 × 50%, 80%, 95%, 100%), cleared in xylene and rehydrated to PBS. Corneas were then prepared for whole mounts according to the method of Miller et al. (1981) and processed for immunocytochemistry. The ureters were washed overnight at 4° C in PBS containing 20% sucrose. Longitudinal frozen sections (32 µm) were cut on a cryostat (Reichert-Jung), thaw-mounted onto chrome-alum gelatine-coated slides and processed for immunohistochemistry. Two animals (one rat and one pigeon) were fixed by perfusion with ice-cold Zamboni's fixative. Lumbal spinal cord (L3–L6) was dissected and immersed in the same fixative for 2 h at 4° C. The tissues were washed in phosphate-buffered sucrose (20%) at 4° C overnight. Transverse frozen sections (10 µm) were cut on a cryostat, thaw-mounted onto chrome-alum gelatine-coated slides and processed for immunohistochemistry.

(3) Immunohistochemistry

Tissues were incubated for indirect immunofluorescence as follows: The tissues were pre-incubated in wells (whole mounts) or in a moist chamber (ureter) with 10% foetal calf serum in PBS for 1 h at room temperature. They were then incubated at 4° C for 48 h with either an anti-substance P antiserum (1:2000, antiserum P6/5) or an anti-CGRP antiserum (1:2000, Cambridge Research Biochemicals: AB 08, Lot No. 1209). The antiserum P6/5 was raised by immunization of a rabbit with synthetic substance P (Sigma) conjugated to thyroglobulin (Sigma) with carbodiimide (Merck). The sections were then washed 3 times for 10 min in PBS containing 1% Triton X-100 (Sigma) and incubated with a fluorescein isothiocyanate conjugated goat-anti-rabbit IgG (ICN Biomedicals) diluted 1:40 for 1 h at room temperature. Finally they were washed 3 times for 10 min in PBS containing 1% Triton X-100 and mounted in phosphate-buffered glycerol (1+9; pH=7.4). All preparations were examined using a Zeiss Universal epi-fluorescence microscope with an excitation wavelength of 450–490 nm and an emission wavelength of 500–560 nm. Black and white photographs were taken with Agfachrome 200RS and Ilford FP4 (125 ASA) film, respectively.

The effects of the various capsaicin treatments on the immunoreactive nerve fibres innervating the cornea were estimated by counting the major nerve branches. The ratio of fibers between untreated and capsaicin-treated animals was used to approximate the effect of the capsaicin treatments.

The immunohistochemical localization of the antisera was examined on spinal cord sections after preabsorption with the original haptens (synthetic substance P, Sigma; synthetic rat CGRP, Cambridge Research Biochemicals) at 100, 10 and 1 nmol/ml diluted antiserum. Cross-reactivity was tested by preabsorption with 100 and 10 nmol/ml diluted antiserum of neurokinin A, neurokinin B, physalaemin, eledoisin, kassinin, somatostatin, dynorphin, CGRP, CCK-8, bombesin (all peptides from Cambridge Research Biochemicals) and substance P (Sigma).

In order to characterize the anti-substance P antiserum

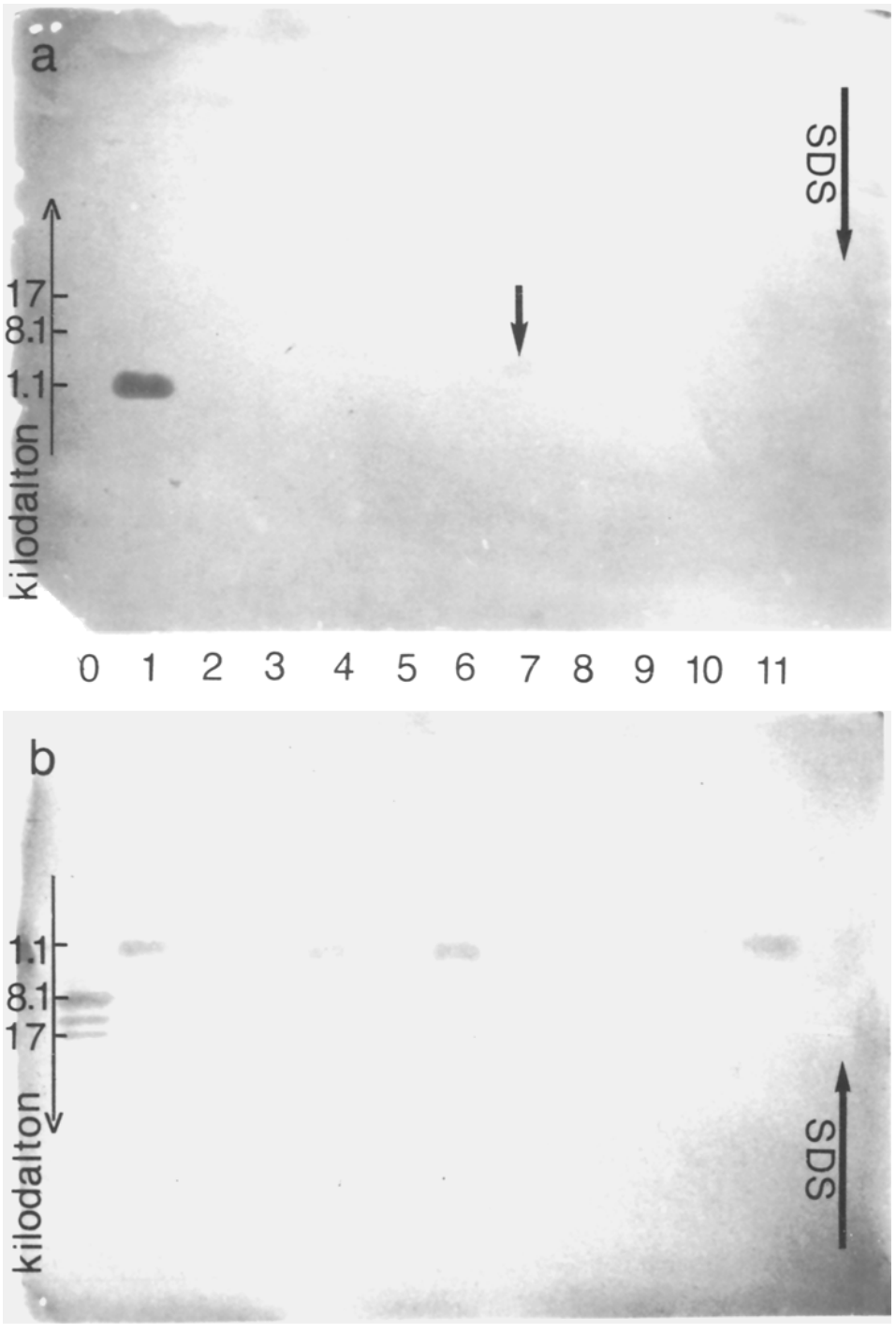

Fig. 1. a Nitrocellulose-immunoblot of anti-substance P serum P6/5. The intense-labelled band corresponds to SP and the weak one to kassinin (*short arrow*). **b** Western blot after Fast-Green protein staining. Fast-Green only weakly labels proteins lighter than 5 kilodalton; *0* molecular weight standard (Sigma MW-SDS-17); *1* substance P; *2* neurokinin A; *3* neurokinin B; *4* somatostatin; *5* CGRP; *6* dynorphin; *7* kassinin; *8* physalaemin; *9* eledoisin; *10* cholecystokinin; *11* bombesin; *long arrows* direction of SDS-electrophoresis

(P6/5) further Western blots of the peptides (10 µg) were made from gradient SDS-polyacrylamide gels (10–20%) using conventional methods (Laemmli 1970). The peptides were bidirectionally transferred from the gels onto three layers of nitrocellulose paper (0.45 µm mesh) either side of the gel (Bowen et al. 1980). The transferred peptides were visualized by Fast-Green staining on one set of nitrocellulose blots (Potter 1974). The other set of blots were then processed with the anti-substance P antiserum as follows (Avrameas and Guilbert 1971): the nitrocellulose sheet was incubated with anti-substance P antiserum (P6/5; 1:100) for 48 h at 4° C. The sheets were then washed 5 times for 5 min in PBS containing 0.5% Triton X-100, 0.5% BSA (bovine serum albumin) and 0.1% SDS. They were then incubated with goat-anti-rabbit IgG conjugated with horseradish peroxidase (HRP) for 2 h at room temperature. After this, they were washed 5 times for 5 min in PBS containing 0.5% Triton X-100, 0.5% BSA and 0.1% SDS and the HRP was visualized by incubation in tris-buffered methanol (20% v/v; pH = 7.6) containing chloronaphthol (0.06%; Sigma) and hydrogen peroxide (0.003%) for 5 min at 0° C. The blots were dried and then photographed.

Results

Immunohistochemical controls and antibody specificity

The immunohistochemical localization of substance P and CGRP was tested by liquid-phase pre-absorption of the diluted antisera on frozen sections of the lumbar spinal cord of rats and pigeons with neurokinin A, neurokinin B, physalaemin, eledoisin, kassinin, somatostatin, dynorphin, CCK-8 and bombesin. In all cases, no apparent change was observed in the quality of the staining pattern for either of the two antisera. There was no observable effect on the staining pattern when the substance P antiserum was preabsorbed with CGRP or when the CGRP antiserum was preabsorbed with substance P. Preabsorption of the antisera with their respective haptens (synthetic substance P and synthetic rat CGRP) completely abolished the staining at all dilutions tested (100, 10 and 1 nmol/ml diluted antisera).

The substance P antiserum (P6/5) was characterized further by testing cross-reactivity of the structurally related and unrelated peptides mentioned above on Western blots. Two bands of immunoreactive staining were seen on these blots, a heavily stained band which corresponded to reactivity with substance P and a lightly stained band, which corresponded to cross-reactivity with the non-mammalian tachykinin kassinin (Fig. 1).

Although these tests show that both antisera are relatively specific for their haptens, cross-reactivity with peptides not tested could contribute to staining and the terms "substance P-/calcitonin gene-related peptide-like immunoreactivity" (SP-LI/CGRP-LI) will be used throughout.

Behavioural controls

In order to test whether rats react to capsaicin after topical or systemic pretreatment with the drug, a drop of 1% capsaicin was instilled into one eye. No wiping reactions were observed after these treatments in any of the rats, indicating complete desensitization. The control eyes of the topically treated group were not tested with capsaicin to avoid histochemical changes of the corneal afferents.

Immunohistochemical localization of substance P-LI and CGRP-LI in the cornea and the effects of capsaicin treatment

Substance P-like immunoreactivity (SP-LI) and CGRP-like immunoreactivity (CGRP-LI) were found in a sparse plexus of nerves innervating the cornea of rat and pigeon (Fig. 2a, b). It was apparent that the pigeon contained fewer immunoreactive fibres for both peptides (see Table 1). In both rat and pigeon, the distribution of immunoreactive fibres was similar, with the majority of fibres localized in the subepithelial layers underneath the outer stratified epithelium. These fibres radiated in from the edge of the tissue towards the center where they split up. Occasionally, some branches were seen in the outer stratified epithelium.

Topical application of capsaicin into the eye reduced the number of SP-LI and CGRP-LI nerve fibres by about 50% as compared with the solvent treated contralateral eye in rats and pigeons (Fig. 2c, d; see Table 1). After systemic treatment, no SP-LI or CGRP-LI was observed in the cornea in rats, whereas in pigeons the numbers of substance P- and CGRP-immunoreactive fibres were only reduced by about 50% (Fig. 2e, f; Table 1).

Table 1. Numbers of immunoreactive fibres of the main nerve branches innervating the cornea of rats and pigeons in untreated, solvent-treated and capsaicin-treated animals

	Rat		Pigeon	
	Number of immunoreactive fibres (mean + SEM)			
	SP	CGRP	SP	CGRP
Untreated ($n=6$)	17.8 + 0.7	14.5 + 1.1	10.8 + 0.6	8.8 + 0.4
Solvent-treated ($n=6$)	18.8 + 1.0	15.5 + 1.0	10.5 + 0.6	8.3 + 0.4
Systemic treated ($n=6$)	0	0	5.3 + 0.4	4.8 + 0.4
Topical treated ($n=5$)	16.4 + 0.8	13.8 + 0.6	10.4 + 0.6	8.6 + 0.6
Topical capsaicin ($n=5$)	7.2 + 0.6	6.2 + 0.7	5.4 + 0.4	4.6 + 0.3

Localization of substance P-LI and CGRP-LI in the ureter and the effects of capsaicin treatment

Substance P-LI and CGRP-LI nerves were observed throughout all three muscle layers of the ureter. It appeared that substance P-LI was denser than CGRP-LI in both species. In longitudinal sections, immunoreactive nerve fibres ran parallel to the muscle fibres of the outer and inner longitudinal muscle layers. The nerve fibres visualized in the outer longitudinal muscle layer appeared slightly larger than those innervating the inner longitudinal muscle layer. In the circular muscle layer, where the muscle fibres were cut transversely, immunoreactive dots and occasionally short sections of immunoreactive nerve fibres were observed (Fig. 3a–d). Immunoreactive fibers were never seen in the submucosal connective tissue (Su et al. 1986).

No SP-LI and CGRP-LI was observed in any of the three muscle layers of the ureter in rats after systemic capsaicin treatment. In pigeons, systemic capsaicin treatment completely depleted SP-LI (Fig. 3e) and CGRP-LI (Fig. 3f) in the outer longitudinal muscle layer (ol). A few immunoreactive fibres were usually still detectable in the circular muscle layer (cm). In the inner longitudinal muscle layer (il), the immunoreactivity to both neuropeptides was reduced by about 50% (Fig. 3).

Discussion

Using immunohistochemical techniques, we have shown that SP-LI and CGRP-LI are present in the cornea and the ureter of the pigeon. Furthermore, this immunoreactivity is sensitive to the actions of capsaicin but the resulting depletion differs in some respects from the effect of capsaicin upon the immunoreactivity localized in the same tissues in the rat. These observations extend previous immunohistochemical studies on the distribution of these peptides in avian species (Katz and Karten 1980; Pierau et al. 1985; Harti et al. 1987; Rössler et al. 1987).

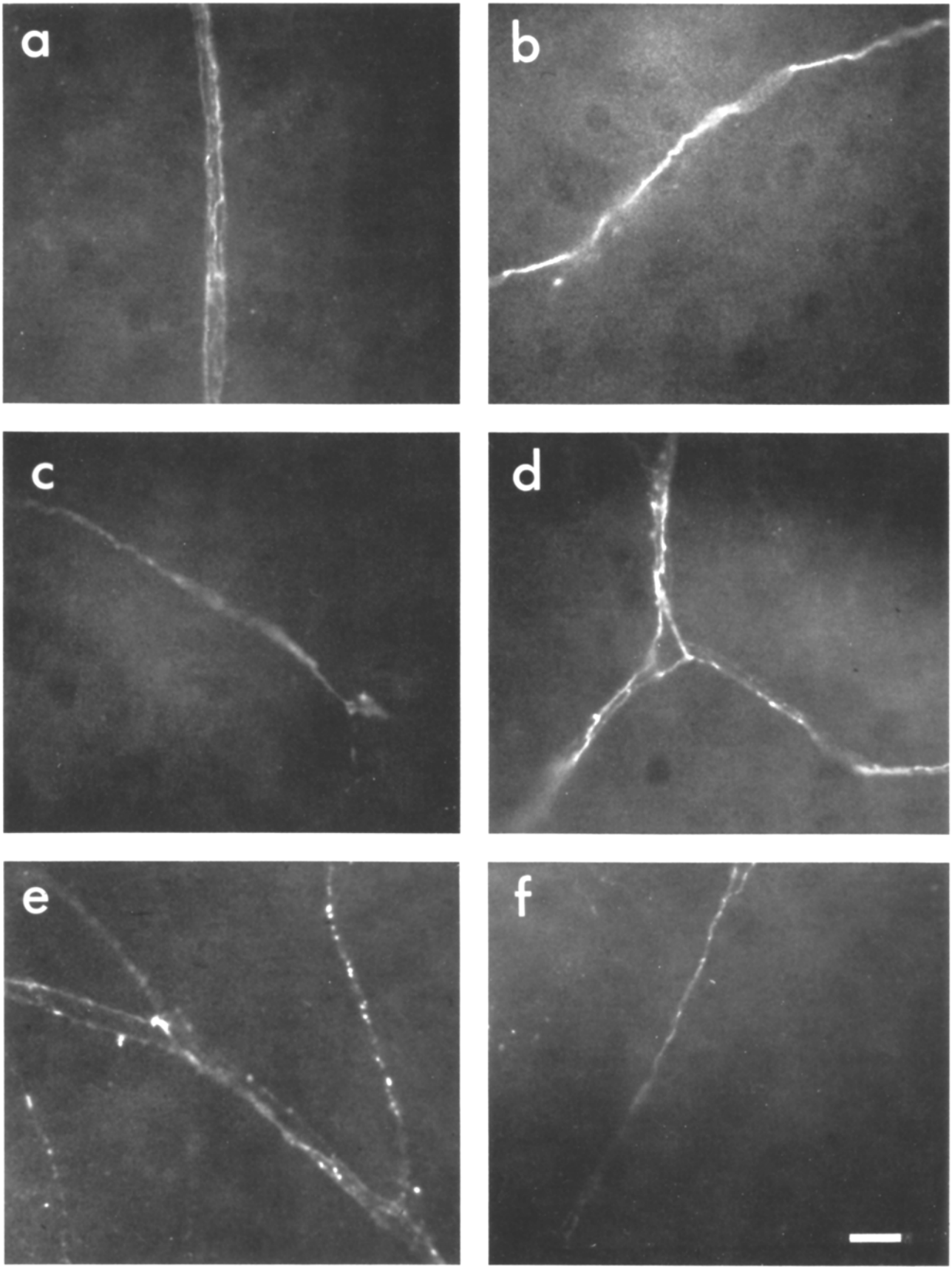

Fig. 2a–f. Nerve fibres in the cornea of pigeons (**a–c, e, f**) and rats (**d**) containing SP-LI (**a, c, e**) or CGRP-LI (**b, d, f**). Controls before capsaicin treatment (950 mg/kg; **e, f**). Branching axons are frequently observed in the outer portion of the cornea (**d, e**). *Bar* = 20 μm; × 325

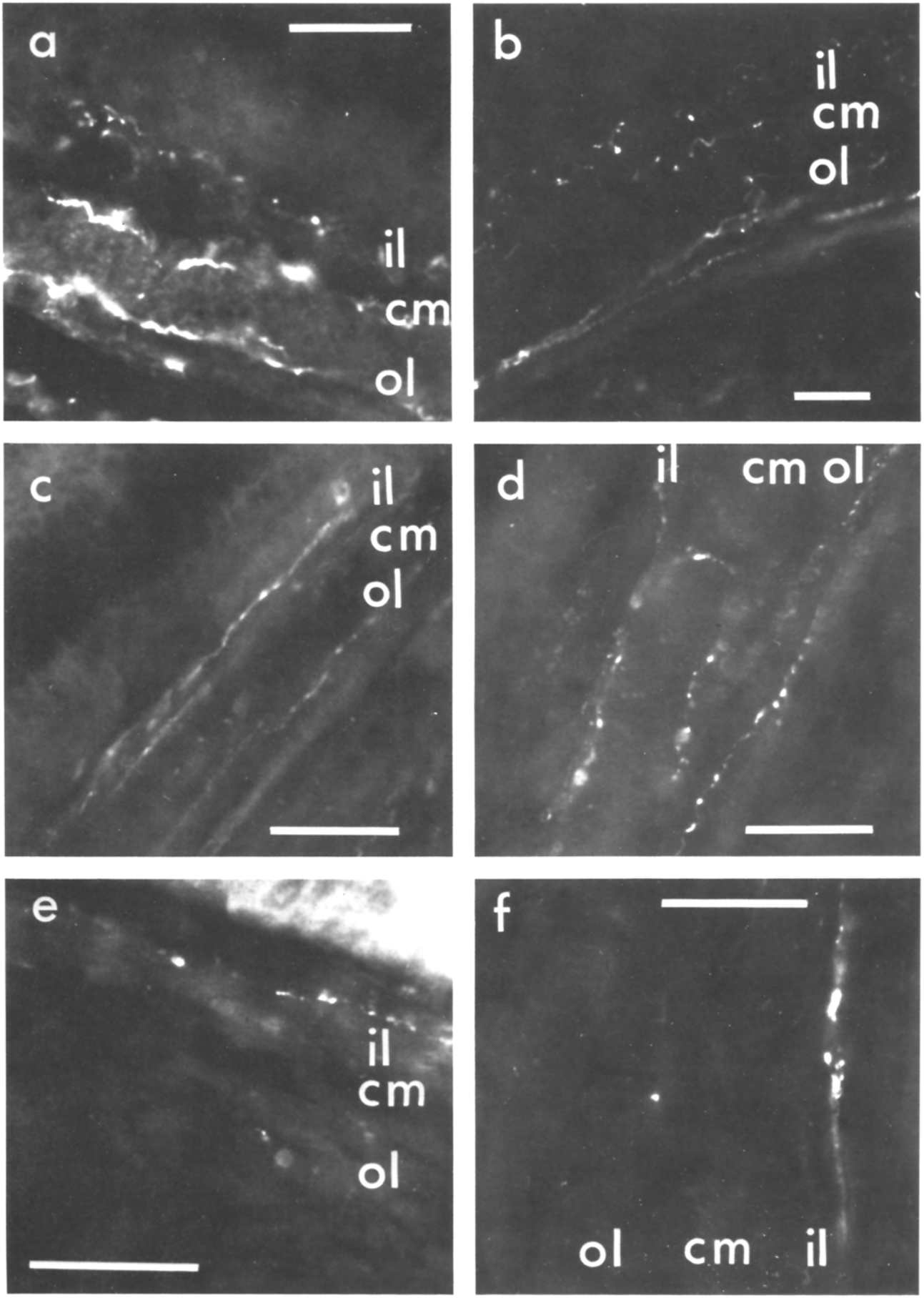
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Table 2. Amino-acid sequences of tachykinins that were tested for cross-reactivity

Number of identical amino acids	Peptide	Amino-acid sequence
	Substance P	ARG – PRO – LYS – PRO – GLN – GLN – PHE – PHE – GLY – LEU – MET-NH_2
7	Kassinin	ASP – VAL – PRO – LYS – SER – ASP – GLN – PHE – VAL – GLY – LEU – MET-NH_2
5	Neurokinin A	HIS – LYS – THE – ASP – SER – PHE – VAL – GLY – LEU – MET-NH_2
4	Neurokinin B	ASP – MET – HIS – ASP – PHE – PHE – VAL – GLY – LEU – MET-NH_2
5	Eledoisin	GLU – PRO – SER – LYS – ASP – ALA – PHE – ILE – GLY – LEU – MET-NH_2
5	Physalaemin	GLU – ALA – ASP – PRO – ASN – LYS – PHE – TYR – GLY – LEU – MET-NH_2

Antibody specificity

In this study, we used antisera of relatively high specificity for the peptides we examined. On tissue sections, no apparent cross-reactivity between the substance P antiserum and any of the tested peptides was observed; however, at low dilutions, we observed a small degree of cross-reactivity with kassinin on immuno-blots. This suggests that the antiserum P6/5 recognizes a long C-terminal fragment of the substance P molecule since kassinin and substance P show a higher degree of homology in their C-terminal hexapeptide sequence than any of the other tachykinins (see Table 2). The CGRP antiserum was bought from a commercial supplier and showed no cross-reactivity with the tachykinins or other structurally unrelated peptides.

Peptidergic innervation of the cornea

It is now well established that substance P is present in the mammalian cornea and that it originates from primary afferent neurones in the trigeminal ganglion (Miller et al. 1981; Tervo et al. 1981; Keen et al. 1982; Lethosalo 1984; Lee et al. 1985; Colin and Kruger 1986). Recently, CGRP-LI nerves have been demonstrated in the mammalian cornea; these also originate from the trigeminal ganglion (Lee et al. 1985; Terenghi et al. 1985; Colin and Kruger 1986; Gulbenkian et al. 1986). By analogy with other sytems it is likely that some trigeminal ganglion neurones innervating the cornea contain both substance P and CGRP (Lee et al. 1985; Gulbenkian et al. 1986). We have shown that the pigeon cornea contains substance P-LI and CGRP-LI nerves; qualitatively this appears similar to the situation found in the rat, although there are apparently fewer immunoreactive fibres than in the rat cornea.

The effect of capsaicin, in the cornea of rats, was to cause a substantial reduction in substance P- and CGRP-immunoreactivity upon topical treatment and a complete depletion of the peptides after systemical treatment. In the pigeon, substance P- and CGRP-immunoreactivity was reduced by capsaicin treatment; however, no complete depletion was observed after systemic application.

Fig. 3a–f. Nerve fibres in the ureter of rats (**a, b**) and pigeons (**c–f**), containing SP-LJ (**a, c, e**) or CGRP-2J (**b, d, f**). Controls before capsaicin treatment (**a–d**); after systemic application of capsaicin (950 mg/kg; **e, f**). In pigeons capsaicin depleted both peptides in the outer longitudinal muscle layer (*ol*); only a few IR-spots were left in the circular muscle (*cm*); about 50% of the immunoreactivity remained in the inner longitudinal muscle layer (*il*). *Bar* = 200 μm; × 325

The functions of substance P and CGRP in the cornea are not clear. The substance P content of the tissue is not correlated with corneal sensitivity (Keen et al. 1982; Lembeck and Donnerer 1981), neither are animals pretreated with capsaicin insensitive to mechanical stimuli (Camras and Bito 1980; Bynke et al. 1984). However, the neurogenic component of chemical injury to the cornea induced by nitrogen mustard or capsaicin is reduced by pretreatment with capsaicin (Szolcsányi et al. 1975; Camras and Bito 1980; Lembeck and Donnerer 1981).

Ocular injury is characterized by (i) miosis, (ii) vasodilation, (iii) increased intraocular pressure, (iv) a breakdown of the blood-aqueous barrier resulting in leakage of protein into the anterior chamber, and (v) lachrymation. Substance P of primary afferent origin has been strongly implicated in the miosis produced by injury (Stjernschantz 1985; Hakanson et al. 1985). However, substance P has no effect on intraocular pressure or on blood flow, and causes only a small disruption of the blood-aqueous barrier (Stjernschantz 1985). In contrast, CGRP has no effect on pupillary muscle in vitro or in vivo but causes an intense aqueous flare reaction (breakdown of the blood-aqueous barrier) and an increase in intra-ocular pressure (Unger et al. 1985; Wahlestedt 1985). It remains to be established whether CGRP increases blood flow in the eye, although it is a potent vasodilator (Brain et al. 1985).

Peptidergic innervation of the ureter

In mammals, substance P- and CGRP-immunoreactivity has been described in the ureter (Alm et al. 1978; Sikri et al. 1981; Wharton et al. 1981; Gibbins et al. 1985; Hua et al. 1985; Su et al. 1986). Recently, it has been demonstrated that these peptides occur in the same nerve endings (Franco-Cereceda et al. 1987). In the mammalian ureter, both peptides are localized in capsaicin-sensitive primary afferent fibres (Hoyes and Barber 1981; Saria et al. 1983a; Gibbins et al. 1985; Su et al. 1986; Franco-Cereceda et al. 1987). In the present study, we have found substance P- and CGRP-immunoreactivity in capsaicin-sensitive nerves of the ureter in rat and pigeon. In the pigeon ureter, it appears that capsaicin is less effective in depleting substance P- and CGRP-immunoreactivity from the inner longitudinal muscle layer than in the rat. This might be attributed to a less optimal circulation of the inner muscle layer. The probability of capsaicin diffusion from the blood vessels to the nerve axons may also be less, since capsaicin does not produce plasma extravasation in pigeons (Pierau et al. 1987); this certainly causes capsaicin leakage into the vicinity of vessels in mammals.

Capsaicin-sensitive nerves of the mammalian ureter are likely to be involved in the control of contractility and vascular permeability (Saria et al. 1983a; Hua and Lundberg 1986; Maggi et al. 1987). Thus substance P (and neurokinin A) causes contraction of the ureteric smooth muscle and an increase in vascular permeability, whilst CGRP relaxes the ureter and potentiates the action of substance P in enhancing vascular permeability (Saria et al. 1983a; Gamse and Saria 1985; Hua and Lundberg 1986; Maggi et al. 1987).

Action of capsaicin in the pigeon

Capsaicin has two sites of action on primary afferent neurones: the axon and the nerve terminal (Szolcsányi 1982). These actions can be distinguished by their time course (Lembeck and Donnerer 1981), by the route of administration of capsaicin (Jancsó 1981), and by the dose of capsaicin administered. Acute behavioural responses and the release of neuropeptides from the central and peripheral terminals of primary afferent fibres can be attributed to an action of capsaicin at nerve terminals (Gamse et al. 1986). Axonal actions, such as blockade of axonal transport and the depletion of neuropeptides are of a longer time course and, in mammals, occur after the action on nerve terminals (Burks et al. 1985).

In the pigeon, an apparent paradox exists; on the one hand, large doses of capsaicin produce only weak behavioural responses, whereas on the other, peptides localized in the somata of primary afferent neurones are depleted after topical application of capsaicin to the peripheral axons (Pierau et al. 1987). This can be resolved if it is postulated that the pigeon possesses functional capsaicin receptors on the axon, but not on the nerve terminals, of primary afferent neurones. This notion is consistent with the observation that capsaicin releases SP-LI from spinal cord slices of rats in vitro, but not from those of pigeons (Pierau et al. 1987). To date, although a capsaicin receptor has been proposed (Szolcsányi 1982), no studies have been performed to identify its cellular localization.

Another paradox is that, in rats, topical capsaicin application to the peripheral axons depletes SP-LI from the afferent terminals in the dorsal horn, whereas in pigeons, similar application results in an accumulation of SP-LI in the dorsal horn (Pierau et al. 1987). It may be assumed that in rats capsaicin first excites the peptidergic axons, and this in turn leads to a release of peptides from central afferent terminals; the axonal transport is probably impaired by a longer time course. In pigeons, axonal excitation might not precede the impairment of the axonal transport, so that the release of SP from central terminals is not stimulated.

In conclusion, we have demonstrated substance P-LI and CGRP-LI in nerve fibres in the cornea and the ureter of the pigeon. In both tissues, this innervation is sensitive to systemic administration of capsaicin although it was in some respects different to the effects seen in the rat. These results suggest that the behavioural insensitivity of the pigeon to capsaicin is not a result of the absence of neuropeptide-containing capsaicin-sensitive nerves in peripheral tissues of this species.

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References

Alm P, Alumets J, Brodin E, Håkanson R, Nilsson G, Sjöberg NO, Sundler F (1978) Peptidergic (substance P) nerves in the genito-urinary tract. Neuroscience 3:419–425

Amara SG, Jonas V, Rosenfeld MG (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 298:240–244

Avrameas S, Guilbert B (1971) A method for quantitative determination of cellular immunoglobulins by enzyme-labeled antibodies. Eur J Immunol 1:394–396

Bowen B, Steinberg J, Laemmli UK, Weintraub H (1980) The detection of DNA-binding proteins by protein blotting. Nucleic Acids Res 8:1–20

Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I (1985) Calcitonin gene-related peptide is a potent vasodilatator. Nature 313:S4–S6

Buck SH, Burks TF (1986) The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Rev 38:179–226

Burks TF, Buck SH, Miller MS (1985) Mechanism of depletion of substance P by capsaicin. Fed Poc 44:2531–2534

Bynke G, Hakanson R, Sundler F (1984) Is substance P necessary for corneal nociception? Eur J Pharmacol 101:253–258

Camras CB, Bito LZ (1980) The pathophysiological effects of nitrogen mustard on the rabbit eye. II. The inhibition of the initial hypertensive phase by capsaicin and the apparent role of substance P. Invest Ophthamol Vis Sci 19:423–426

Ch'ng JLC, Christofides ND, Anand P, Gibson SJ, Allen YS, Su HC, Tatemoto K, Morrison JFB, Polak JM, Bloom SR (1985) Distribution of galanin immunoreactivity in the central nervous system and the responses of galanin-neuronal pathways to injury. Neuroscience 16:343–354

Colin S, Kruger L (1986) Peptidergic nociceptive axons visualization in whole-mount preparations of the cornea and tympanic membrane in rat. Brain Res 398:199–203

Dalsgaard CJ, Vincent SR, Hökfelt T, Lundberg JM, Dahlström A, Schultzberg M, Dockray GJ, Cuello AC (1982) Coexistence of cholecystokinin- and substance P-like peptides in neurones of the dorsal root ganglia of the rat. Neurosci Lett 33:159–163

Fitzgerald M (1983) Capsaicin and sensory neurones – a review. Pain 15:109–130

Franco-Cereceda A, Henke H, Lundberg JM, Peterman JB, Hökfelt T, Fischer JA (1987) Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: Distribution and release by capsaicin. Peptides 8:399–410

Gamse R, Saria A (1985) Potentiation of tachykinin-induced plasma protein extravasation by calcitonin gene-related peptide. Eur J Pharmacol 114:61–66

Gamse R, Saria A, Lundberg JM, Theodorssen-Norheim E (1986) Behavioral and neurochemical changes after intracisternal capsaicin treatment of the guinea pig. Neurosci Lett 64:287–292

Geisthövel E, Ludwig O, Simon E (1986) Capsaicin fails to produce disturbances of autonomic heat and cold defence in an avian species (*Anas platyrhynchos*). Pflügers Arch 406:343–350

Gibbins IL, Furness JB, Costa M, MacIntyre I, Hillyard CJ, Girgis S (1985) Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett 57:125–130

Gibbins IL, Furness JB, Costa M (1987) Pathway-specific patterns

of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea pig. Cell Tissue Res 248:417–437

Gibson SJ, Polak JM, Bloom SR, Sabate IM, Mulderry PM, Ghatei MA, McGregor GP, Morrison JFB, Kelly JS, Rosenfeld MG (1984) Calcitonin gene-related peptide immunoreactivity in the spinal cord of man and eight other species. J Neurosci 4:3101–3111

Gulbenkian S, Merighi A, Wharton J, Varndell JM, Polak JM (1986) Ultrastructural evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig. J Neurocytol 15:535–542

Håkanson R, Bynke G, Beding B, Wahlestedt C (1985) Tachykinin antagonists suppress responses to ocular injury in the rabbit. In: Hakanson R, Sundler F (eds) Tachykinin antagonists. Elsevier, Amsterdam, pp 91–96

Handwerker HO, Holzer-Petsche U, Heym C, Welk E (1984) C-fibre functions after topical application of capsaicin to a peripheral nerve and after neonatal capsaicin treatment. In: Chahl LA, Szolcsányi J, Lembeck F (eds) Antidromic vasodilatation and neurogenic inflammation. Akadémiai Kiadó, Budapest, pp 57–82

Harti G, Sharkey KA, Rössler W, Pierau Fr-K (1987) Distribution of substance P (SP) and calcitonin gene-related peptide (CGRP) and the effect of capsaicin in sensory neurones of rat and pigeon. Neuroscience 22 [Suppl]:S322

Hökfelt T, Kellerth JO, Nilsson G, Pernow B (1975) Experimental immunohistochemical studies on the localization and distribution of substance P in the cat primary sensory neurons. Brain Res 100:235–252

Hökfelt T, Elde R, Johansson O, Luft R, Nilsson G, Arimura A (1976) Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary sensory neurons in the rat. Neuroscience 1:131–136

Hoyes AD, Barber P (1981) Degeneration of axons in the ureteric and duodenal nerve plexus of the adult rat following in vivo treatment with capsaicin. Neurosci Lett 25:19–24

Hua X-Y, Lundberg JM (1986) Dual capsaicin effects on ureteric 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, Theodorssen-Norheim E, Brodin E, Lundberg JM, Hökfelt T (1985) Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory-neurons in the guinea pig. Regul Pept 13:1–19

Jancsó G (1981) Intracisternal capsaicin selective degeneration of chemosensitive primary afferents in the adult rat. Neurosci Lett 27:41–45

Jancsó G, Kiraly E, Joo F, Such G, Nagy A (1985) Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in adult rat. Neurosci Lett 59:209–214

Jancsó N (1968) Desensitization with capsaicin as a tool for studying the function of pain receptors. In: Proceedings of the 3rd Int Pharmacol Meeting 1966, Pergamon Press, Oxford, pp 33–55

Jancsó N, Szolcsányi J, Jancsó-Gábor A (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol 31:138–151

Jessell TM, Iversen LL, Cuello AC (1978) Capsaicin-induced depletion of substance P from primary afferent neurones. Brain Res 152:183–188

Katz DM, Karten HJ (1980) Substance P in the vagal sensory ganglia: localization in cell bodies and pericellular arborizations. J Comp Neurol 193:549–564

Kawatani M, Low IP, Nadelhaft I, Morgan C, Groat WC de (1983) Vasoactive intestinal polypeptide in visceral afferent pathways to the sacral spinal cord of the cat. Neurosci Lett 42:311–316

Keen P, Tulls AB, Blyth WA, Hill TJ (1982) Substance P in the mouse cornea: effects of chemical and surgical denervation. Neurosci Lett 29:231–235

Kuo D, Kawatani M, Groat WC de (1985) Vasoactive intestinal polypeptide identified in the thoracic dorsal root ganglia of the cat. Brain Res 330:178–182

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lee Y, Kawai Y, Shiosaka S, Takami K, Kiyama H, Hillyard CJ, Girgis S, MacIntyre I, Emson PC, Tohyama M (1985) Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: immunohistochemical analysis. Brain Res 300:194–196

Lembeck F, Donnerer J (1981) Postocclusive cutaneous vasodilatation mediated by substance P. Naunyn-Schmiedeberg's Arch Pharmacol 316:165–171

Lembeck F, Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedeberg's Arch Pharmacol 310:175–183

Lethosalo JI (1984) Substance P-like immunoreactive trigeminal ganglion cells supplying the cornea. Histochemistry 80:273–276

Maggi CA, Giuliani S, Santicioli P, Abelli L, Meli A (1987) Viscero-omotor response to calcitonin gene related peptide (CGRP) in the rat lower urinary tract: evidence for a transmitter role in the capsaicin-sensitive nerves of the ureter. Eur J Pharmacol 142:73–82

Mason RJ, Maruniak JA (1983) Behavioural and physiological effects of capsaicin in red-winged blackbirds. Pharmacol Biochem Behav 19:857–862

Miller A, Costa M, Furness JB, Chubb IW (1981) Substance P immunoreactive sensory nerves supply the rat iris and cornea. Neurosci Lett 23:243–249

Nagy JI (1982) Capsaicin: a chemical probe for sensory neuron mechanisms. In: Iversen LL, Iversen S, Snyder SH (eds) Handbook of psychopharmacology, Vol 15. Plenum Press, New York, pp 185–235

Pierau Fr-K, Gamse R, Harti G, Sann H, Szolcsányi J (1985) Resistance of pigeons to capsaicin is the consequence of differences in their substance P (SP) system. Neurosci Lett Suppl 22:S462

Pierau Fr-K, Sann H, Harti G, Gamse R (1987) Neuropeptides in sensory neurones of pigeons and the insensitivity of avians to capsaicin. In: Schmidt RF, Schaible H-G, Vahle-Hinz C (eds) Fine nerve fibres and pain. VHC, Weinheim, pp 213–223

Potter JD (1974) The content of troponin, tropomyosin, actin, and myosin in rabbit skeletal muscle myofibrils. Arch Biochem Biophys 162:436–441

Rosenfeld MG, Mermod J-J, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304:129–135

Rössler W, Pierau Fr-K, Harti G (1987) Distribution of substance P and calcitonin gene-related peptide in the spinal cord of pigeons and rats. Pflügers Arch Ges Physiol 408 [Suppl 1]:R61

Sann H, Harti G, Pierau Fr-K, Simon E (1987) Effect of capsaicin upon afferent and efferent mechanisms of nociception and temperature regulation in birds. Can J Physiol Pharmacol 65:1347–1354

Saria A, Lundberg JM, Hua X, Lembeck F (1983a) Capsaicin-induced substance P release and sensory control of vascular permeability in the guinea-pig ureter. Neurosci Lett 41:167–172

Saria A, Lundberg JM, Skofitsch G, Lembeck F (1983b) Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. Naunyn-Schmiedeberg's Arch Pharmacol 324:212–218

Sharkey KA, Harti G, Pierau Fr-K (1988) The effects of capsaicin on peripheral peptidergic nerves of rat and pigeon. Pflügers Arch Ges Physiol 411 [Suppl 1]:R133

Sikri KL, Hoyes AD, Barber P, Jagessar H (1981) Substance P-like immunoreactivity in the intramural nerve plexus of the guinea-pig ureter: a light and electron microscopic study. J Anat 133:425–422

Skofitsch G, Jacobowitz DM (1985) Calcitonin gene-related pep-

tide coexists with substance P in capsaicin-sensitive neurons and sensory ganglia of the rat. Peptides 6:747–754
Stefanini M, De Martino C, Zamboni L (1967) Fixation of ejaculated human spermatozoa for electron microscopy. Nature 216:173–174
Stone RA, Kuwayama Y, Terenghi G, Polak JM (1986) Calcitonin gene-related peptide: occurence on corneal sensory nerves. Exp Eye Res 43:279–283
Stjernschantz J (1985) Role of substance P in the acute irritative response of the rabbit eye, and effect of substance P antagonist analogs. In: Håkanson R, Sundler F (eds) Tachykinin antagonists. Elsevier, Amsterdam, pp 119–131
Su HC, Wharton J, Polak JM, Mulderry PK, Ghatei MA, Gibson SJ, Terenghi G, Morrison JFB, Ballesta J, Bloom SR (1986) Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunohistochemistry. Neuroscience 18:727–747
Szolcsányi J (1982) Capsaicin type pungent agents producing pyrexia. In: Milton AS (ed) Pyretics and antipyretics. Handbook of experimental pharmacology, Vol 60. Springer, Berlin Heidelberg New York, pp 437–478
Szolcsányi J, Jancsó-Gábor A, Joo F (1975) Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 287:157–169
Szolcsányi J, Sann H, Pierau Fr-K (1986) Nociception in pigeon is not impaired by capsaicin. Pain 27:247–260
Szolcsányi J, Anton F, Reeh PW, Handwerker HO (1988) Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. Brain Res 446:262–268
Terenghi G, Polak JM, Ghatei MA, Mulderry PK, Butler JM, Unger WG, Bloom SR (1985) Distribution and origin of calcitonin gene-related peptide (CGRP) immunoreactivity in the sensory innervation of the mammalian eye. J Comp Neurol 233:506–516
Tervo K, Tervo T, Eränkö L, Eränkö O (1981) Substance P immunoreactive nerves in the rodent cornea. Neurosci Lett 25:95–97
Tuchscherer MM, Seybold VS (1985) Immunohistochemical studies of substance P, cholecystokinin-octapeptide and somatostatin in dorsal root ganglia of the rat. Neurosciene 14:593–605
Unger WG, Terenghi G, Ghatei MA, Ennis KW, Butler JM, Zhang SQ, Too HP, Polak JM, Bloom SR (1985) Calcitonin gene-related polypeptide as a mediator of neurogenic ocular injury response. J Ocular Pharmacol 1:189–199
Wahlestedt J, Håkanson R, Beding B, Brodin E, Ekman R, Sundler F (1985) Intraocular effects of CGRP, a neuropeptide in sensory nerves. In: Håkanson R, Sundler F (eds) Tachykinin antagonists. Elsevier, Amsterdam, pp 137–146
Wharton J, Polak JM, Probert L, De Mey J, McGregor GP, Bryant MG, Bloom SR (1981) Peptide containing nerves in the ureter of the guinea pig and cat. Neuroscience 6:969–982
Wiesenfeld-Hallin Z, Hökfelt T, Lundberg JM, Forsmann WG, Reinecke M, Tschopp FA, Fischer JA (1984) Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurons to the spinal cord and interact in spinal behavioral responses of the rat. Neurosci Lett 52:199–204
Zaidi M, Breimer LH, MacIntyre I (1987) Biology of peptides from the calcitonin genes. Q J Exp Physiol 72:371–408

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