

## Effects of capsaicin, tachykinins, calcitonin gene-related peptide and bradykinin in the pig iris sphincter muscle

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**Summary.** Capsaicin-sensitive sensory neurons of the rabbit iris, by releasing tachykinins, exert a major role in the control of pupil motility in response to various noxious stimuli. However, the contribution of sensory innervation to the regulation of iris smooth muscle tone in other mammalian species is not known. We have studied the effects produced by electrical field stimulation, capsaicin, substance P, neurokinin A, calcitonin gene-related peptide (CGRP), and bradykinin in the isolated iris sphincter muscle of the pig.

Capsaicin (10  $\mu$ M): a) contracted the isolated sphincter muscle and; b) released immunoreactivity for substance P (SP-LI) and CGRP (CGRP-LI) from this preparation. These two effects were no longer observed at the second exposure to the drug. Electrical field stimulation (10 Hz, 60 V, 0.5 ms for 5 s) produced a biphasic contractile response. The rapid component was inhibited by atropine (1  $\mu$ M), while the delayed response was blocked by previous exposure to capsaicin (10  $\mu$ M).

Substance P and neurokinin A consistently produced contraction of the pig iris sphincter muscle, substance P being more potent than neurokinin A. CGRP induced a contractile response in more than 50% of the preparations. The tachykinin antagonist [D-Arg<sup>1</sup>, D-Trp<sup>7,9</sup>, Leu<sup>11</sup>]-substance P (3  $\mu$ M) blocked: a) the effect of substance P (1 nM); b) the delayed response to electrical field stimulation and; c) reduced by more than 50% response to capsaicin. Bradykinin (10  $\mu$ M) failed to release either SP-LI or CGRP-LI. The contractile response evoked by bradykinin was unaffected by in vitro pretreatment with capsaicin (10  $\mu$ M).

The existence in the pig iris of capsaicin-sensitive sensory fibres releasing neuropeptides and thus regulating sphincter muscle tone is proposed.

**Key words:** Capsaicin — Tachykinins — Calcitonin gene-related peptide — Bradykinin — Pig iris sphincter muscle

### Introduction

Capsaicin, the pungent principle of hot red pepper of the plant genus *Capsicum* has become a useful pharmacological tool to investigate the contribution of sensory neurons in various biological phenomena (Buck and Burks 1986). This is due to the unique ability of capsaicin to excite selectively small diameter sensory fibres and to produce a subsequent prolonged impairment of their functions (Holzer 1988; Maggi and Meli 1988). The presence of capsaicin-sensitive primary sensory neurons has been demonstrated in several mammalian species, including man (Holzer 1988; Maggi and Meli 1988). However, a major issue, extensively investigated in a large array of tissues of small rodents (e.g. production of local response due to neuropeptide release from peripheral terminals of capsaicin-sensitive sensory neurons) remains to be fully clarified in other species. For example, birds seem to lack capsaicin receptive structures (Szolcsanyi et al. 1986), while broad variations in the sensitivity to capsaicin have been reported among various mammal species (Jancso et al. 1967; Maggi et al. 1987).

Recently attention has been given to the contribution of neuropeptides released from sensory fibres to the production of local responses in various tissues of the pig. The effects of sensory neuron activation or direct application of sensory neuropeptides in the broncho-pulmonary and coronary circulation has been analyzed in this species (Franco-Cereceda et al. 1987; Martling et al. 1989). Of particular interest is the observation that in pig skin capsaicin produced a flare response by activating an axon reflex, a phenomenon not found in small rodents but present in the human skin (Pierau and Szolcsanyi 1988).

The rabbit eye has been the most widely used model for investigating the role of neuropeptide released from sensory neurons in the control of pupillary tone (Cole and Unger 1974; Butler and Hammond 1980; Hakanson et al. 1987). Substance P or other related tachykinins are considered to be the transmitter(s) of the non-cholinergic non-adrenergic miosis in the rabbit iris (Leander et al.

1981; Ueda et al. 1982; Wahlestedt et al. 1985a), while CGRP, which did not exert per se any pupillary motor response, induced marked inflammatory effects (Unger et al. 1985; Wahlestedt et al. 1986). Furthermore, bradykinin exerts in the rabbit eye inflammatory and miotic actions that are ascribed largely to release of sensory neuropeptides from capsaicin-sensitive fibres of trigeminal origin (Cole and Unger 1974; Zhang et al. 1982; Wahlestedt et al. 1985b).

It has been reported that substance P contracted the isolated sphincter muscle of the pig iris (Unger and Tighe 1984). However, detailed information on the effects produced by excitation of sensory nerves of the pig iris is lacking. In the iris sphincter muscle of the pig we have studied the effects of capsaicin (neuropeptide release and functional responses) particularly focusing on its ability to induce rapid tachyphylaxis. The influence of in vitro capsaicin pretreatment on the response produced by bradykinin and electrical field stimulation was also analyzed. Finally, we have investigated the motor effects produced in the isolated pupillary sphincter muscle of the pig by exogenous substance P, neurokinin A or CGRP.

## Materials and methods

**Sample collection.** Pig eyes were obtained from the local slaughterhouse. The eyes were enucleated immediately after death, transported chilled over ice and experiments were commenced within 1–2 h. The eye was opened with a cut posteriorly to the limbus, followed by excision of the iris from the ciliary margin. The iris was freed from the dilator muscle, leaving the approximately 2 mm wide sphincter muscle.

**Release experiments.** Slices (400  $\mu$ m) of the pig iris sphincter muscle were prepared at 4°C using a tissue slicer (Stoelting, IL). Tissues (60–100 mg) were transferred to 2 ml perfusion chambers. The slices were superfused with oxygenated (96% O<sub>2</sub>–4% CO<sub>2</sub>) normal Krebs solution (NaCl 119, KCl 4.7, CaCl<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 11) containing 0.1% bovine serum albumin and 10  $\mu$ M thiorphan at 37°C. A 30 min equilibration period was allowed to elapse before commencing the experiments. Fractions (2.8 ml) before and during drug administration were collected every 4 min in tubes containing acetic acid (2 N final concentration) and freeze-dried. At the end of the experiments tissues were blotted 1–2 times on filter paper and weighed. In some iris the neuropeptide content was measured. Extraction was performed as described previously (Geppetti et al. 1988a). Tissues were kept at 95°C for 20 min in 2 N acetic acid and homogenized. After centrifugation at 20000  $\times$  g for 20 min at 4°C the supernatant was freeze-dried. All samples were reconstituted with assay buffer (0.1 M, pH 7.4 phosphate buffer containing 0.1% bovine serum albumin and 0.01% NaN<sub>3</sub>) and their peptide content was measured by radioimmunoassay.

**SP-LI and CGRP-LI radioimmunoassay.** Substance P-like immunoreactivity (SP-LI) radioimmunoassay was performed as reported previously (Geppetti et al. 1988b) by using substance P as standard, [<sup>125</sup>I]-Bolton and Hunter substance P and 144 anti-substance P antiserum, which cross-reacted to 1% with neurokinin A, 0.5% with neurokinin B and less than 0.1% with physalaemin and eledoisin. Sensitivity of the assay was 1.1 fmol/tube. CGRP-like immunoreactivity (CGRP-LI) was measured as already reported (Geppetti et al. 1988b) with rat  $\alpha$ -CGRP as standard [<sup>125</sup>I]-CGRP and RAS-6012 anti-human CGRP-II antiserum, which cross-reacted to 100% with both rat  $\beta$ -CGRP and human CGRP-I. Sensitivity of the assay was 2.1 fmol/tube.

**Functional experiments.** Iris sphincter muscles were mounted between two shaped metal holders in a 5 ml bath for isolated organ, containing an oxygenated (95% O<sub>2</sub>–5% CO<sub>2</sub>) normal Krebs solution as referred to above, maintained at 37°C. A resting tension of 5 mN was applied and tension was recorded by means of an isometric transducer connected to a Basile 7050 pen recorder. Experiments were commenced after a 60 min equilibration period. Responsiveness of each preparation was assessed by means of 10  $\mu$ M carbachol, e.g. a concentration which preliminary experiments had shown to produce the maximal contractile response to this bioassay. The iris sphincter muscles were electrically field stimulated (0.1 Hz, 60 V, 0.5 ms or train of stimuli at 10 Hz, 60 V, 0.5 ms for 5 s) by means of two wire platinum electrodes placed at the top and the bottom of the organ bath.

For each single peptide maximally effective concentrations (1 nM–3  $\mu$ M) were applied until reproducible responses were obtained. Then concentration-response curves were established in a cumulative manner, successive concentrations being applied when the response to the previous concentration had plateaued. In vitro capsaicin desensitization was achieved by prolonged (30 min) exposure of the strips to 10  $\mu$ M capsaicin, followed by washing and re-equilibration. The non selective tachykinin antagonist spantide ([D-Arg<sup>1</sup>, D-Trp<sup>7–9</sup>, Leu<sup>11</sup>]-substance P) (Folkers et al. 1984; Buck and Burks 1988) was used for antagonizing the response to EFS, capsaicin and substance P.

**Statistical analysis.** All data in the text are mean  $\pm$  standard error of the mean. Statistical analysis of the data was performed by means of the Student's *t*-test for paired and unpaired data or by means of the analysis of variance, when applicable. A *P* value <0.05 was considered statistically significant. EC<sub>50</sub> and 95% confidence limits (c.l.) were calculated as the concentrations producing 50% of the maximal contraction. Total peptide-like immunoreactivity released during exposure to capsaicin was calculated by subtracting the mean basal value from those observed in the presence of the drug.

**Drugs and reagents.** Drugs and reagents used were: capsaicin (Sigma, MO); substance P, rat  $\beta$ - and  $\alpha$ -CGRP, human CGRP-I and II, neurokinin A, bradykinin, [D-Arg<sup>1</sup>, D-Trp<sup>7–9</sup>, Leu<sup>11</sup>]-substance P (spantide) (Peninsula, CA); carbachol HCl (Merck, FRG); [<sup>125</sup>I]-Bolton and Hunter substance P and [<sup>125</sup>I]-CGRP (Amersham, UK); RAS 6012 anti-CGRP-II antiserum (Peninsula, CA); 144 anti-substance P antiserum was a kind gift of Dr. P. Pradelles (SPI-LERI, CEN-Saclay, Gif-sur-Yvette, France).

## Results

### Tissue levels of SP-LI and CGRP-LI

SP-LI tissue content in the iris sphincter muscle of the pig (*n* = 6) was 5.41  $\pm$  0.69 pmol/g of wet weight. Higher levels of CGRP-LI were found (24.8  $\pm$  2.8 pmol/g of wet weight, *n* = 6). The CGRP-LI/SP-LI ratio was 4.6.

### Release experiments

The mean basal release of CGRP-LI and SP-LI from slices of iris sphincter muscle averaged 170  $\pm$  32 fmol/g/fraction and 38  $\pm$  11 fmol/g/fraction, respectively. The mean basal outflows of CGRP-LI and SP-LI before the second administration of capsaicin were 90  $\pm$  29 fmol/g/fraction and 33  $\pm$  9 fmol/g/fraction, respectively. A first application of capsaicin (10  $\mu$ M) produced a marked and sustained increase of the CGRP-LI outflow (total evoked release being 5317  $\pm$  603 fmol/g/20 min). A se-

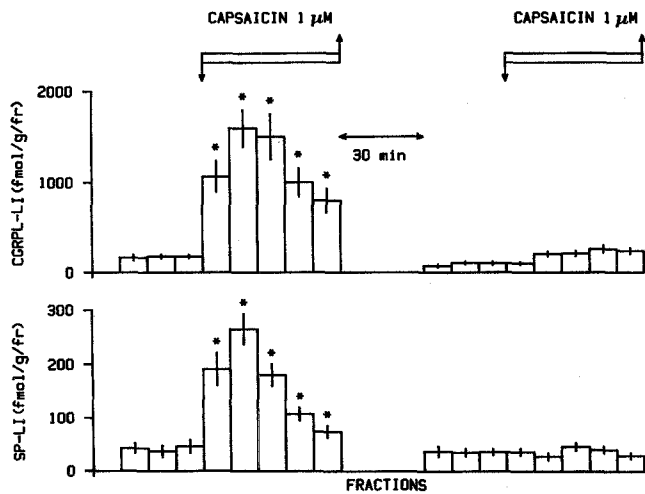


Fig. 1. Effect of exposure to capsaicin ( $1 \mu\text{M}$ ) on the outflow of calcitonin gene-related peptide (CGRP)- and substance P (SP)-like immunoreactivity (LI) in superfusates of the pig iris. A second exposure to capsaicin was performed 30 min after the end of the first administration. \*  $P < 0.01$  vs. the mean of the basal values; fr, fraction

cond challenge with the drug released a small amount of CGRP-LI ( $726 \pm 156 \text{ fmol/g/20 min}$ ,  $n = 5$ ,  $P < 0.001$ ). Likewise, the total evoked release of SP-LI observed at the first capsaicin administration was  $606 \pm 110 \text{ fmol/g/20 min}$ , while at the second exposure to the drug it was reduced to  $35 \pm 16 \text{ fmol/g/20 min}$  ( $n = 5$ ,  $P < 0.001$ ) (Fig. 1). The ratio of CGRP-LI/SP-LI released by capsaicin over a period of 20 min was 7.4. Bradykinin ( $10 \mu\text{M}$ ) failed to increase the outflow of SP-LI in superfusates of pig iris ( $n = 4$ ). The mean basal outflow of CGRP-LI ( $153 \pm 31 \text{ fmol/g/fraction}$ ) was only slightly increased during exposure to  $10 \mu\text{M}$  bradykinin (total evoked release being  $274 \pm 32 \text{ fmol/g/20 min}$ ) without reaching the significance level ( $n = 4$ ).

#### Response to electrical field stimulation and capsaicin

All the preparations ( $n = 10$ ) were mechanically quiescent. Electrical field stimulation with single pulses ( $0.1 \text{ Hz}$ ,  $60 \text{ V}$ ,  $0.5 \text{ ms}$  pulse width) produced twitch contractions ( $2.71 \pm 0.2 \text{ mN}$ ,  $n = 10$ ) which were abolished by atropine ( $1 \mu\text{M}$ ,  $n = 4$ ). The response to a train of stimuli ( $10 \text{ Hz}$ ,  $60 \text{ V}$ ,  $0.5 \text{ ms}$  for  $5 \text{ s}$ ) produced a biphasic response, e.g. a rapid contraction which declined to baseline at the end of the train of stimuli, followed by a delayed slow component (Fig. 2). The rapid response was abolished by atropine ( $1 \mu\text{M}$ ) which left the delayed response unaffected ( $n = 4$ ) (Fig. 2).

Capsaicin ( $10 \mu\text{M}$ ) produced a rapidly developing tonic contraction which slowly ( $2-3 \text{ min}$ ) declined to the baseline. This response to capsaicin ( $n = 7$ ) averaged  $3.23 \pm 0.5 \text{ mN}$ . A second application of capsaicin ( $10 \mu\text{M}$ ,  $20 \text{ min}$  later) had no further contractile effect ( $0.16 \pm 0.05 \text{ mN}$ ,  $n = 7$ ,  $P < 0.001$ ) indicating complete desensitization (Table 1). In preparations pre-exposed to

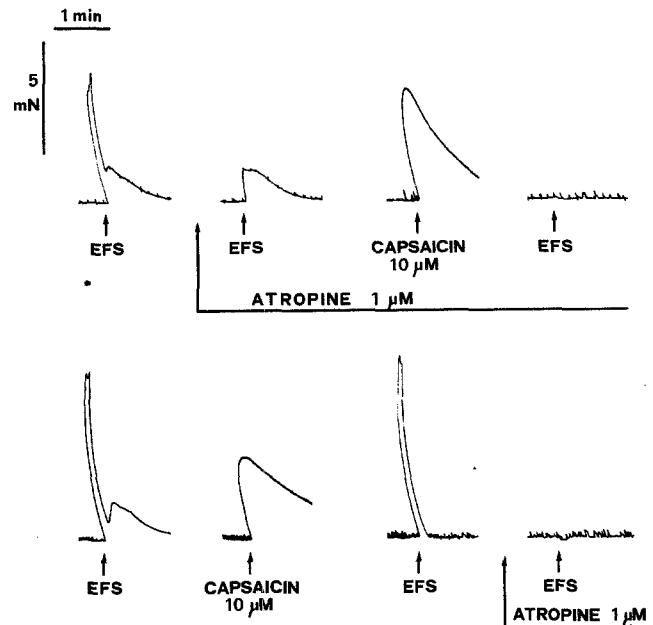


Fig. 2. Typical tracings showing the effect of electrical field stimulation (EFS, trains of stimuli at  $10 \text{ Hz}$ ,  $60 \text{ V}$ ,  $0.5 \text{ ms}$  for  $5 \text{ s}$ ) in the isolated sphincter muscle of the pig iris, in presence of atropine and after exposure to capsaicin (upper tracings), or after exposure to capsaicin followed by addition of atropine (lower tracings)

Table 1. Effect of exposure to bradykinin, [D-Arg<sup>1</sup>, D-Trp<sup>9-11</sup>, Leu<sup>11</sup>]-substance P (spantide) and capsaicin itself on the contractile response to capsaicin ( $10 \mu\text{M}$ ) in the isolated sphincter muscle of the pig iris

Compound added	n	mN (% of the response to EFS <sup>d</sup> )
None	7	$3.23 \pm 0.47$ ( $115 \pm 18\%$ )
Capsaicin <sup>a</sup>	7	$0.16 \pm 0.05^{**}$ ( $6 \pm 2\%$ )
Spantide <sup>b</sup>	4	$1.37 \pm 0.30^*$ ( $49 \pm 11\%$ )
Bradykinin <sup>c</sup>	4	$3.20 \pm 0.58$ ( $110 \pm 21\%$ )

<sup>a</sup> Capsaicin ( $10 \mu\text{M}$ ) was applied in the same preparation 30 min after the first exposure (for  $15 \text{ min}$ ) to the drug

<sup>b</sup> Spantide ( $3 \mu\text{M}$ ) was added 20 min before exposure to capsaicin

<sup>c</sup> Capsaicin was added 20 min after exposure to  $3 \mu\text{M}$  bradykinin

<sup>d</sup> EFS, electrical field stimulation ( $0.1 \text{ Hz}$ ,  $60 \text{ V}$ ,  $0.5 \text{ ms}$  pulse width)

\*  $P < 0.05$ ; \*\*  $P < 0.001$

capsaicin, the rapid phasic response to electrical field stimulation at  $10 \text{ Hz}$  was unaffected, while the delayed response was abolished ( $n = 4$ ) (Fig. 2). After exposure to capsaicin and in the presence of atropine ( $1 \mu\text{M}$ ) no response to electrical field stimulation (at  $10 \text{ Hz}$ ) was detected ( $n = 4$ ) (Fig. 2). Response to capsaicin was unaffected by pre-exposure ( $20 \text{ min}$  before) to  $3 \mu\text{M}$  bradykinin (Table 1).

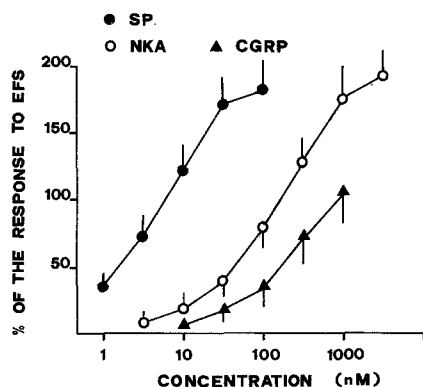


Fig. 3. Concentration-response curves to substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) in the isolated sphincter muscle of the pig iris. Concentration-response curves were obtained in a cumulative manner. All the preparations responded to substance P, neurokinin A. The concentration-related response to CGRP shown in the figure was calculated from data obtained from 5 out of the 9 preparations tested. In the 4 remaining preparations CGRP did not produced any effect or elicited a concentration-unrelated action. Values are expressed as % of the response induced by electrical field stimulation (EFS, 0.1 Hz, 60 V, 0.5 ms pulse width) and are mean  $\pm$  SE of at least 5 experiments

#### Response to substance P, neurokinin A, calcitonin gene-related peptide and bradykinin

Substance P consistently contracted all the preparations tested [ $EC_{50}$  (c.l.) = 3.9 (2.4–6.5) nM;  $n = 6$ ] in a concentration-dependent manner, and its maximal effect averaged  $4.48 \pm 0.4$  mN. Neurokinin A induced a concentration-dependent contractile response in all the preparations tested [ $EC_{50}$  (c.l.) = 120 (100–170) nM;  $n = 5$ ], and its maximal effect averaged  $4.88 \pm 0.79$  mN. A response to CGRP was not consistently observed in all preparations. In 5 cases, CGRP produced a concentration (10 nM–1  $\mu$ M) dependent contraction and the maximal effect averaged  $2.03 \pm 0.5$  mN. In 2 cases a small response to CGRP was observed, but it was not concentration dependent, while in 2 additional preparations CGRP had no effect at all (Fig. 3).

Bradykinin produced a rapid developing and concentration-dependent contractile response in all the preparations tested (Fig. 4).  $EC_{50}$  (c.l.) and maximal response of the concentration-response curve to bradykinin, performed 30 min after exposure to capsaicin (10  $\mu$ M for 30 min,  $n = 5$ ) [289 (246–352) nM and  $3.86 \pm 0.75$  mN, respectively] were not statistically different from those obtained in preparation pre-exposed to capsaicin vehicle (0.01% ethanol for 30 min,  $n = 5$ ) [215 (185–266) nM and  $4.15 \pm 0.68$  mN, respectively] (see also Fig. 4).

#### Effect of spantide on contractile response to electrical field stimulation, substance P and capsaicin

In the isolated sphincter muscle of the pig iris spantide (1–3  $\mu$ M) showed a small contractile effect that waned

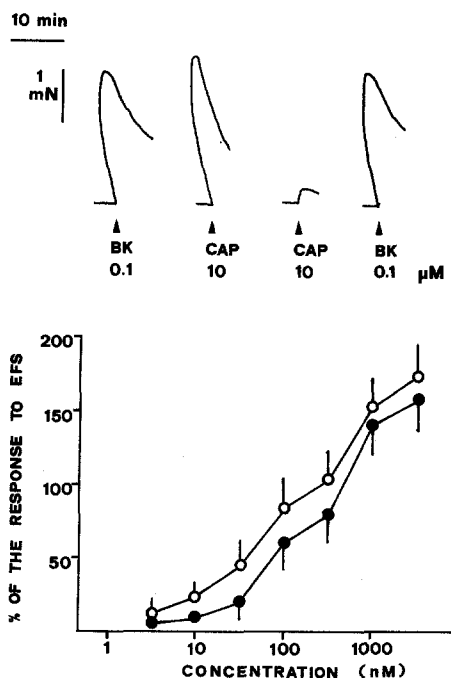


Fig. 4. Upper panel: typical tracings showing the contractile response to bradykinin (0.1  $\mu$ M) in the isolated iris sphincter muscle of the pig before and after in vitro desensitization to capsaicin. Compounds were administered at time intervals of 30 min. BK, bradykinin; CAP, capsaicin. Lower panel: concentration-response curves to bradykinin, obtained after exposure to capsaicin 10  $\mu$ M (filled circles) or its vehicle (open circles). Each point is the mean  $\pm$  SE of 5 experiments

after repeated (2–3) administrations. The delayed response to electrical field stimulation (trains of 10 Hz, 60 V, 0.5 ms, for 5 s) was almost completely blocked in presence of 3  $\mu$ M spantide. In fact in two out of the 4 preparations tested delayed response was abolished, while in the remainders a residual contraction that amounted to 10–15% of that elicited in control preparations was found (Fig. 5). In contrast, the rapid component was totally unaffected. The delayed contractile component elicited by electrical field stimulation was completely restored after prolonged washing of the preparation ( $n = 4$ ). Contraction elicited by a low substance P concentration (1 nM) was abolished in the presence of spantide (3  $\mu$ M) ( $n = 4$ ) (Fig. 5). The contractile response to capsaicin (10  $\mu$ M) was significantly reduced by 58% in the presence of spantide (3  $\mu$ M) ( $n = 4$ ) (Fig. 5 and Table 1).

#### Discussion

In the present experiments we have shown that in the isolated sphincter muscle of the pig iris capsaicin-sensitive sensory fibres regulate pupillary contractility by releasing neuropeptides stored within their peripheral endings. This hypothesis is substantiated by various findings. Consistent levels of immunoreactive substance P and CGRP have been found in this tissue. Exposure to capsaicin

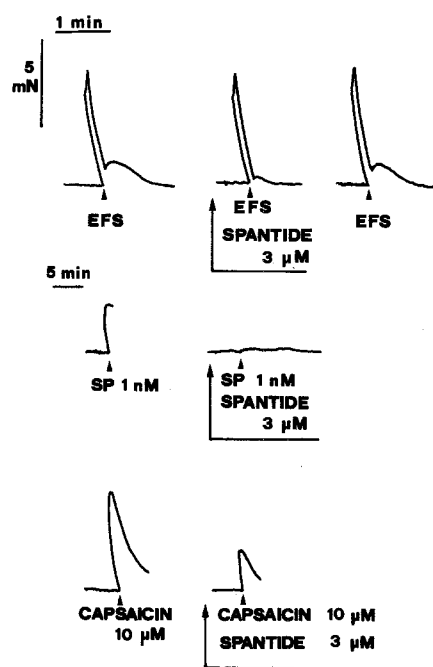


Fig. 5. Typical tracings showing of the inhibitory effect of [D-Arg<sup>1</sup>, D-Trp<sup>7-9</sup>, Leu<sup>11</sup>]-substance P (spantide) in the isolated sphincter muscle of the pig iris, on the response to: electrical field stimulation (EFS, trains of stimuli at 10 Hz, 60 V, 0.5 ms for 5 s) (upper tracings); a low concentration of substance P (SP) (middle tracings); and capsaicin (lower tracings)

produced both neuropeptide release and marked contractile response which underwent rapid and complete tachyphylaxis. Substance P and CGRP both contracted the isolated iris sphincter muscle. Electrical field stimulation (10 Hz) produced a delayed contractile response, unaffected by atropine, but abolished by pre-exposure to capsaicin. Desensitization, e.g. reduction or absence of responses (either functional or neurochemical) following repeated exposures to capsaicin is considered a specific feature of the selective action of this compound on a certain subpopulation of primary sensory neurons (Holzer 1988; Maggi and Meli 1988).

#### *Tachykinins as the putative mediators of both the delayed response to electrical field stimulation and response to capsaicin*

Several lines of evidence indicate substance P or other related tachykinins released from terminals of capsaicin-sensitive trigeminal afferents as the mediator of neurogenic miotic response to injury in this organ (Leander et al. 1981; Ueda et al. 1984; Wahlestedt et al. 1985b). Substance P, which potently contracted the pig iris sphincter muscle, appears as the putative transmitter of the atropine-resistant component of the miotic response to electrical field stimulation, since this response was blocked by spantide that also inhibited a similar response produced by a low substance P concentration. However, it is known that neurokinin A is co-released along with

substance P from capsaicin-sensitive sensory neurons (Saria et al. 1988) and neurokinin A was able to contract the isolated sphincter muscle of the pig iris, although less potently than substance P. Furthermore, since spantide is not a selective substance P antagonist (Buck and Burks 1988), participation of neurokinin A to the delayed contractile response to electrical field stimulation can not be totally excluded.

#### *Species-related variations in the response to capsaicin and calcitonin gene-related peptide*

Some similarity between rabbit and pig eye exists regarding the regulation of pupillary tone by sensory nerves, although relevant differences are also present. Functional response to 10  $\mu$ M capsaicin, although progressively reduced were observed even at the fourth administration of the drug (Ueda et al. 1984; Wahlestedt et al. 1985b) in the rabbit isolated iris sphincter muscle. In the pig desensitization was complete after the second exposure to capsaicin (Fig. 1 and Table 2). Release of substance P and CGRP immunoreactivity from uveal structures of rabbits upon application of electrical or chemical (capsaicin) stimuli has been demonstrated (Bill et al. 1979; Wahlestedt et al. 1986; Hakanson et al. 1987). We show that sensory fibres of the iris sphincter muscle of the pig may release a consistent amount of neuropeptides and that, likewise the miotic response, neuropeptide release by capsaicin underwent rapid and complete tachyphylaxis. CGRP did not affect per se the pupillary tone of the rabbit eye (Unger et al. 1985; Wahlestedt et al. 1986), while in more than half preparations of pig iris it produced a concentration-dependent contractile response. In the pig iris sphincter muscle the contractile response to capsaicin was reduced partially by spantide. The residual effect might be due to an incomplete blockade of tachykinin action by 3  $\mu$ M spantide or to release by capsaicin of compounds other than tachykinins able to contract the isolated sphincter muscle, among which CGRP should be included. In this respect, it is interesting to note that the amount of CGRP-LI released by capsaicin from the iris sphincter muscle was as much as 7-fold higher than that of SP-LI. However, CGRP was much far less potent than substance P in contracting this preparation. All together these observations suggest that CGRP although possessing a contractile activity, does not play a relevant role in the control of pupil motility in the pig. A most likely effect of CGRP released from sensory neurons of the pig iris might be the modulation of vascular tone in this tissue.

#### *Species related variations in the response to bradykinin*

Marked differences are also present in the action of bradykinin between the pig and the rabbit isolated iris sphincter muscle. Bradykinin (0.1–100  $\mu$ M) action in the rabbit iris was almost completely blocked by pre-exposure to capsaicin (Ueda et al. 1984; Hakanson et al. 1987) and strongly inhibited by tachykinin antagonists

(Ueda et al. 1984; Wahlestedt et al. 1985b). Therefore, it has been proposed that the miotic response to bradykinin in this tissue is mainly mediated by tachykinins released from capsaicin-sensitive sensory nerves. Indeed, it has been recently found that bradykinin can release sensory neuropeptides from in vitro guinea-pig tissues (Geppetti et al. 1988b; Saria et al. 1988). However, we failed to induce a significant increase of immunoreactivity for either CGRP or substance P in superfusates of the iris sphincter muscle of the pig after administration of bradykinin. Pre-exposure to a maximally effective concentration of capsaicin (10  $\mu$ M) that produced complete desensitization did not significantly affect response to bradykinin. Furthermore, since the response to capsaicin was not reduced by pre-exposure to a maximally effective concentration of bradykinin, 'cross-tachyphylaxis' between these two agents, as described in the rabbit iris (Wahlestedt et al. 1985b), can be excluded in the pig. Bradykinin action on the isolated sphincter muscle of the pig seems therefore largely independent from neuropeptide released from capsaicin-sensitive sensory neurons.

In conclusion, terminals of capsaicin-sensitive sensory neurons of the sphincter muscle of pig iris may be antidromically invaded by electrical stimuli eliciting contractile response mainly due to tachykinin release. CGRP co-released, along with substance P by capsaicin from these neurons, might have only a minor part in this event. The excitatory action of bradykinin on sensory neurons, which largely contributes to the miotic response induced by this pro-inflammatory peptide in the rabbit eye (Cole and Unger 1974; Butler and Hammond 1980; Ueda et al. 1984; Wahlestedt et al. 1985b) does not seem to play a major role in the genesis of the contractile response produced in the pig. Considering the sensory innervation of the iris as an important structure involved in the neurogenic response to injury, present work shows that marked differences exist between mammalian species. These differences must be taken into account in the design of experimental animal model of ocular inflammation as well as in the development of compounds able to interfere with inflammatory events in pathological conditions involving the eye.

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