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Effects of capsaicin pretreatment on neuropeptides and salivary secretion of rat parotid glands

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1 'Atropine-resistant' secretion of saliva in response to parasympathetic stimulation may reflect antidromic activation of sensory nerve fibres. In this investigation, the effect of pretreatment in the rat with capsaicin (total dose of 125 mg kg^{-1}, s.c.), was determined.

2 In the parotid glands substance P (SP)/calcitonin gene-related peptide (CGRP)-containing nerve fibres around ducts and blood vessels disappeared after capsaicin, while periacinar SP-containing fibres (devoid of CGRP) and CGRP-containing fibres (devoid of SP) remained. Vasoactive intestinal peptide (VIP)-containing nerve fibres seemed to be unaffected. The parotid content of SP and CGRP was reduced by 11 and 36% respectively, while that of VIP remained unchanged.

3 The weights of the parotid glands and their sensitivity to the secretagogues methacholine and SP, injected intravenously, were unchanged as was the response to stimulation of the auriculo-temporal nerve in the presence and absence of atropine.

4 In contrast to capsaicin pretreatment, parasympathetic denervation of the parotid gland reduced the weight of the gland and produced an increase in the response to methacholine and SP.

5 For comparison, the effectiveness of the capsaicin treatment on neuropeptide content was determined in the urinary bladder. The bladder of capsaicin-pretreated rats increased in weight (21%) and in VIP content (31%), while the content of SP and CGRP was reduced by 86 and 94%, respectively. SP- and CGRP-containing nerve fibres were virtually eliminated, while VIP-containing nerve fibres seemed unaffected.

6 In conclusion, antidromic activation of primary afferent (capsaicin-sensitive) C-fibres does not contribute significantly to the 'atropine-resistant' secretory response of the parotid gland to stimulation of the parasympathetic nerve.

Introduction

In rats, electrical stimulation of the parasympathetic auriculo-temporal nerve evokes salivary secretion from the parotid gland despite pretreatment with atropine and adrenoceptor blockers. This non-cholinergic, non-adrenergic secretion is about one-third of that evoked in the absence of the blocking agents (Ekström *et al.*, 1983a; Ekström, 1987). Parasympathetic nerve stimulation releases neuropeptides such as substance P (SP), vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) (Ekström *et al.*, 1985; 1988) so conceivably, these peptides may mediate the 'atropine-resistant' response. SP injected into the blood stream causes a profuse secretion of saliva (Lembeck & Starke, 1968; Ekström & Wahlestedt, 1982), VIP evokes a small secretion, rich in protein and amylase (Ekström *et al.*, 1983b; Ekström & Olgart, 1986), while CGRP does not evoke any flow of saliva (Ekström *et al.*, 1988). However, CGRP and VIP both enhance the secretory response to SP (Ekström & Olgart, 1986; Ekström *et al.*, 1988).

Stimulation of the auriculo-temporal nerve presumably activates both efferent and afferent nerve fibres and the 'atropine-resistant' nerve-evoked secretion of saliva may therefore reflect antidromic stimulation of sensory nerve fibres. In order to test this

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possibility adult rats were pretreated with capsaicin which is toxic to primary afferent C-fibres (see Buck & Burks, 1986). One to two weeks later we examined the secretory response of the parotid gland to auriculo-temporal nerve stimulation and to injections of various secretagogues. We also examined the effect of capsaicin pretreatment on the content of CGRP, SP and VIP in the parotid gland. A preliminary report of some of these findings has been presented (Ekman *et al.*, 1988).

Methods

Altogether 59 female Sprague-Dawley rats were used. They were killed by an overdose of diethyl ether or, at the completion of the secretion experiments, by air embolus under chloralose anaesthesia. The rats to be treated with capsaicin or to be denervated were paired with age- and weight-matched controls (litter mates). The seven capsaicin-treated rats used for immunocytochemistry were matched against five controls in the same weight range (about 150 g).

Capsaicin pretreatment

Capsaicin (25 mg ml^{-1} in 20% (v/v) ethanol, 10% (v/v) Tween 80 in isotonic saline, stock solution) was injected s.c. in a total dose of 125 mg kg^{-1}: 25 and 50 mg kg^{-1} on the 1st day and 50 mg kg^{-1} on the 2nd day (Holzer-Petsche & Lembeck, 1984). Fifteen min before each capsaicin injection the rats received aminophylline 5 mg kg^{-1} i.p., terbutaline 0.1 mg kg^{-1} i.p. and ketamine 50 mg kg^{-1} i.m. These drugs and the vehicle were also injected in the controls. All the rats survived the capsaicin pretreatment and were killed 1–2 weeks after the last capsaicin injection. The effectiveness of the capsaicin pretreatment was tested (1–2 weeks after the last injection) by instilling a drop of capsaicin solution (0.01% (v/v), dilution in saline from the stock solution) into the eye of the chloralose-anaesthetized animals and counting the number of eye blinks. In pretreated rats this procedure caused only a few blinks of the eye (0.3 ± 0.2, $n = 16$), whereas in control rats blinks were numerous (72 ± 9, $n = 16$, $P < 0.001$). The body weights of the capsaicin-pretreated rats (278 ± 7 g, $n = 16$), used in functional studies, did not differ from those of the controls (283 ± 5 g, $n = 16$).

Parasympathetic denervation

The rats were anaesthetized with diethyl ether and the auriculo-temporal nerve was cut on the right side, where it emerges from the base of the skull (Ekström, 1974; Alm & Ekström, 1976). One week after sectioning the nerve the body weights of the operated rats (319 ± 20 g, $n = 5$) were not different from those of unoperated controls (325 ± 6 g, $n = 5$).

Immunochemistry

Specimens from the parotid gland and urinary bladder were fixed by immersion in an ice-cold mixture of 2% (v/v) formaldehyde and 15% (v/v) of a saturated aqueous picric acid solution in 0.1 M phosphate buffer (pH 7.2) overnight and thoroughly rinsed in Tyrode solution containing 10% sucrose. They were then frozen on dry ice and sectioned at 10 μm thickness in a cryostat. The sections were processed for the immunocytochemical demonstration of SP, CGRP and VIP. The SP antiserum (code SP 8, kind gift from P.C. Emson, Cambridge, UK) was used in a dilution 1:320. The CGRP antiserum (code 8427, Milab, Malmö, Sweden) was used at a dilution of 1:1280. The VIP antiserum (code 7852, Milab, Malmö, Sweden) was used at a dilution of 1:640. The sections were exposed to either of the antisera for 24 h in a moist chamber. The site of the antigen-antibody reaction was revealed by application of fluorescein isothiocyanate (FITC)-labelled antibodies against immunoglobulin G (Milab) at a dilution 1:320 for 1 h at room temperature. Control sections were exposed to peptide antiserum that had been preabsorbed with an excess amounts of the antigen (10–100 μg of synthetic peptide, Peninsula Labs, Belmont, U.S.A.) per ml diluted antiserum. The antibodies used do not cross-react with other known neuropeptides (Sundler *et al.*, 1985; Grunditz *et al.*, 1986). Nevertheless, cross-reaction with still unknown peptides or proteins containing amino acid sequences recognized by the different antisera cannot be excluded and it is appropriate, therefore, to refer to the immunoreactive material as SP-like and CGRP-like. For simplicity, however, the shorter terms 'SP' and 'CGRP' are used henceforth. In order to reveal coexistence of SP and CGRP a simultaneous immunostaining method was used. A CGRP antiserum raised in guinea-pig (code 8513, Milab; dilution 1:1280) was used in combination with the rabbit antiserum against SP and the labels of the second antibodies were FITC and tetramethyl rhodamine isothiocyanate (TRITC), respectively. Sections were first incubated with one of the pair of peptide antibodies and then with the FITC-labelled anti-guinea-pig IgG antibodies. The sections were then incubated with the other peptide antibody. The latter antibodies were visualized using TRITC-labelled anti-rabbit IgG antibodies. The sections were examined in a fluorescence microscope fitted with the appropriate filter settings for viewing FITC and TRITC fluorescence alternately.

Radioimmunoassay

Preparation of samples for radioimmunoassay (RIA) The glands were minced while frozen and extracted in 10 volumes of 0.5 M acetic acid at 100°C for 15 min. After homogenization (Polytron) and centrifugation (3000 *g* for 20 min), the supernatants were collected and lyophilized. The samples were reconstituted and diluted in 0.05 M phosphate buffer (pH 7.4), containing 0.25% human serum albumin (Behringwerke, Marburg, FRG) and assayed for CGRP, SP and VIP in at least two different dilutions.

Radioimmunoassay of calcitonin gene-related peptide A rabbit antiserum (K-8429, Milab, Malmö, Sweden), raised against synthetic rat CGRP conjugated to bovine serum albumin, was used. The final dilution of the antiserum was 1:37,500 and ^{125}I-Tyr^{0}-CGRP served as tracer. The RIA has a detection limit of 20 pmol l^{-1} of standard rat CGRP and the antiserum does not recognize calcitonin, VIP, somatostatin, gastrin-releasing peptide, enkephalins or tachykinins (Grunditz *et al.*, 1986).

Radioimmunoassay of substance P Antiserum SP-2 (kind gift from E. Brodin, Karolinska Institute, Stockholm, Sweden), which is directed against the C-terminal part of SP was used in a final dilution of 1:350,000 with ^{125}I-Tyr^{0}-SP as tracer. The assay detects 10 pmol l^{-1} of synthetic SP and the antiserum does not cross-react with other known tachykinins (Brodin *et al.*, 1986).

Radioimmunoassay of vasoactive intestinal peptide Antiserum 7582 (Milab, Malmö, Sweden) recognizes the N-terminal 15-amino acid sequence of VIP and does not cross-react with peptide histidine isoleucine amide or any known regulatory peptide. The assay detects 5 pmol l^{-1} (Ekström *et al.*, 1984).

Secretory responses

Rats were fasted for 24 h and anaesthetized with chloralose (100 mg kg^{-1} i.v.) after induction with diethyl ether. The trachea was cannulated. The body temperature was maintained at 38°C. The duct of the parotid gland was exposed and cannulated with a fine glass cannula. In experiments on the effect of denervation the ducts were cannulated bilaterally. Saliva was collected in (pre-weighed) plastic tubes and weighed; the specific density of the saliva was taken to be 1.0 g ml^{-1}. Saliva was analysed for amylase by an enzymatic colorimetric test (Boehringer Mannheim, Mannheim, F.R.G.) using α-4-nitrophenyl maltoheptaoside (4NP-G_7) as substrate (Hägele *et al.*, 1982). One unit (u) of catalytic activity of α-amylase is defined as the hydrolysis of 1 μmol of 4NP-G_7 min^{-1} ml^{-1}, which is equivalent to one International Unit.

Drugs were injected through the cannulated femoral vein (polyethylene catheter) in volumes of 0.25–0.3 ml. Each injection of drug was immediately followed by an injection of 0.2 ml saline, a volume considerably exceeding that of the cannula dead-space. The interval between each injection of secretagogue was usually 10 min. The tachykinin antagonist [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}]SP was injected 10–15 min before the start of the test procedure.

The auriculo-temporal nerve was exposed medial to the mandible and divided at the base of the skull. The peripheral end of the nerve was stimulated at supramaximal voltage with a duration of 0.2 ms using a bipolar electrode and a Grass S48 stimulator: (a) one group of rats was stimulated at various frequencies (0.1–60 Hz) in periods of 2 min in the absence of atropine, (b) another group of rats was stimulated in the presence of atropine at 20 Hz for 1 min before and after administration of the tachykinin antagonist and (c) yet another group was stimulated continuously at 40 Hz, producing a maximal secretory response, in the presence of atropine for 60 min; in the latter series of experiments saliva was collected in 10 min periods. In order to exclude adrenergic effects on the secretory cells the rats were injected with the α-adrenoceptor blocker dihydroergotamine, 1 mg kg^{-1} i.p., and the β-adrenoceptor blocker propranolol 1 mg kg^{-1} i.p. before induction of ether anaesthesia. The dose of atropine used was 2 mg kg^{-1} i.v. The parotid glands and urinary bladder were removed and weighed; the urinary bladder and the parotid gland on the non-stimulated side were taken as controls and analysed for neuropeptides.

Capsaicin in acute experiments

(a) Cotton wool was soaked in a solution of capsaicin (2.5%) and applied locally, onto the peripheral stump of the acutely cut auriculo-temporal nerve as distally as possible for a period of 30 min during which any flow of saliva was monitored. This was followed by electrical nerve stimulation proximal to the site of capsaicin application. (b) The effect of a single subcutaneous injection of capsaicin (25–50 mg kg^{-1}) on the secretion was tested; the observation period was about 1 h. (c) The cervical sympathetic chain and the auriculo-temporal nerve were cut together with the facial nerve (just outside the stylomastoid foramen) in order to minimize reflex effects. Immediately afterwards, the parotid duct was cannulated and atropine (2 mg kg^{-1} i.v.),

Table 1 Parotid gland weights (mg) and secretory responses (μl saliva) to methacholine and substance P (both i.v.) of control rats ($n = 5$) and of operated rats ($n = 5$) subjected to parasympathetic denervation of the right gland one week beforehand

	Weight	*Methacholine* ($\mu g\,kg^{-1}$) 1	2	*Substance P* ($\mu g/kg^{-1}$) 0.1	0.2
Control					
Right	206.3 ± 13.6	2.9 ± 0.6	6.5 ± 0.7	5.1 ± 1.2	15.6 ± 2.2
Left	201.2 ± 8.6	3.1 ± 0.5	6.4 ± 0.5	5.0 ± 1.2	15.8 ± 2.7
Operated					
Right	141.2 ± 14.7$^{\dagger b}$	13.0 ± 2.0$^{\dagger a}$	26.3 ± 1.1$^{\ddagger b}$	22.9 ± 1.6$^{\ddagger b}$	39.3 ± 2.2$^{\ddagger b}$
Left	201.0 ± 14.8	3.6 ± 0.3	7.4 ± 0.6	6.7 ± 1.0	16.6 ± 1.8

Values are means ± s.e.mean.
† $P < 0.01$, ‡ < 0.001 when compared with contralateral gland; ^{a} $P < 0.01$, ^{b} < 0.001 when compared with corresponding gland of control litter mate.

dihydroergotamine ($1\,mg\,kg^{-1}$ i.v.) and propranolol ($1\,mg\,kg^{-1}$ i.v.) were injected. The secretory response to a standard dose of substance P was tested, about 15 min before an intraductal injection of capsaicin. Thereafter, the parotid cannula was replaced by another cannula of a similar type, producing drops of the same size and supplied with a three-way tap to which a syringe could be attached. The dead-space of the cannula was filled with capsaicin. Capsaicin (0.01%) in 0.04 ml saline was injected retrogradely into the duct over a 5 s period; 5–10 s later, the tap was turned so as to allow saliva to flow out (see Alm & Ekström, 1976). Immediately a small volume of fluid (0.3–1.9 μl) appeared at the tip of the cannula; this was discarded as it was attributed to overfilling of the system. The standard dose of substance P was injected repeatedly, over a period of 40 min, after cessation of capsaicin-evoked salivary flow. In this type of experiment the concentration of Tween 80 in the capsaicin solution was as low as 0.01%, and the solution was devoid of ethanol. The effect of saline alone (0.04 ml) injected into the duct was also tested.

Drugs

Atropine sulphate (Sigma Chemical Co, MO, St Louis, U.S.A.), capsaicin (Fluka Chemie AG, Buchs, Switzerland), dihydroergotamine methansulphonate (Sandoz, Basel, Switzerland), ketamine (Parke-Davis, Morris Plains, NJ, U.S.A.), methacholine chloride (Sigma), propranolol hydrochloride (ICI Pharmaceuticals, Macclesfield, U.K.), SP (Peninsula Labs, Belmont, CA, U.S.A.), [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}]SP (Peninsula), aminophylline (ACO Läkemedel AB, Solna, Sweden) and terbutaline (AB Draco, Lund, Sweden) were used.

Statistics

Values are means ± s.e.mean. Student's t test for paired data was used.

Results

Parasympathetic denervation

The denervated parotid glands weighed about 30% less ($P < 0.01$) than the contralateral glands and glands of unoperated rats (Table 1). The volume of saliva secreted from the denervated glands in response to i.v. injections of methacholine and SP was 3–4 ($P < 0.01$ or 0.001) and 2–3 ($P < 0.001$) times larger, respectively, than that of the contralateral glands and of glands in unoperated rats (Table 1). There were no differences in either weight or secretory responses between right and left glands of unoperated rats or between these glands and the contralateral glands of the operated rats.

Capsaicin pretreatment

Peptide-containing nerve fibres in the parotid gland In control glands SP-containing nerve fibres were very numerous around acini, and quite prominent around ducts and blood vessels (see also Ekström *et al.*, 1988). CGRP-containing nerve fibres were few by comparison around acini and fairly numerous around ducts and blood vessels. Periacinar SP- and CGRP-containing fibres were fine and varicose, whereas those occurring around ducts and blood vessels were more coarse and less beaded. VIP-containing nerve fibres were numerous around acini, ducts and blood vessels. Double immunostaining showed that the bulk of SP-containing fibres around acini did not contain CGRP, whereas most SP-containing fibres around ducts and blood vessels did. Capsaicin pretreatment resulted in a marked reduction in the number of SP/CGRP-containing nerve fibres around ducts and blood vessels leaving only a few mostly smooth fibres, together with the network of delicate SP-containing fibres around acini. The delicate periacinar CGRP

Table 2 Parotid gland weights (mg, left gland) and secretory responses (μl saliva, right gland) to methacholine and substance P (both i.v.) in control rats ($n = 16$) and in capsaicin-pretreated rats ($n = 16$)

		Methacholine ($\mu g\,kg^{-1}$)		*Substance P* ($\mu g\,kg^{-1}$)	
	Weight	1	2	0.1	0.2
Control	186.3 ± 7.0	2.8 ± 0.4	5.2 ± 0.5	10.6 ± 2.2[a]	17.6 ± 1.7
Capsaicin	191.3 ± 5.4	2.1 ± 0.3	4.8 ± 0.5	9.2 ± 2.3[a]	17.4 ± 1.6

Values are mean ± s.e.mean.
There were no significant differences when the gland of the capsaicin-pretreated rat was compared with the corresponding gland of control litter mate. [a] $n = 7$.

fibres were also persistent. The latter fibres were devoid of SP and the many SP-containing fibres around acini were devoid of CGRP. The VIP-containing nerve fibres were apparently unaffected by treatment with capsaicin.

Neuropeptide content of the parotid gland In the control glands ($n = 9$), the total amounts (pmol) of CGRP, SP and VIP were 0.14 ± 0.02, 1.0 ± 0.2 and 2.0 ± 0.2, respectively. In the glands of the capsaicin-pretreated rats ($n = 9$) the total amounts of CGRP and SP were 63.7 ± 6.6% ($P < 0.02$) and 88.6 ± 6.6% ($P < 0.05$), respectively, of controls, whereas the total amount of VIP was 102.7 ± 11.8% of controls.

Secretory responses to secretagogues and parasympathetic nerve stimulation

The parotid gland weights and the secretory responses of capsaicin-pretreated rats to methacholine and SP did not differ from control rats (Table 2). The volume of saliva secreted in response to parasympathetic nerve stimulation for periods of 2 min (0.1–60 Hz) was the same in five capsaicin-pretreated and five control rats (Figure 1); at 40 Hz the glands of pretreated rats secreted 136 ± 8 μl saliva ($n = 5$) and those of controls 131 ± 4 μl saliva ($n = 5$). The volume of saliva secreted and the output of amylase in response to continuous maximal (40 Hz) parasympathetic nerve stimulation did not differ between capsaicin-pretreated rats and controls (Figure 2). The fluid secretion and the amylase output during the initial period of stimulation (0–10 min) was 174 ± 26 μl and 201 ± 36 u, respectively, in seven pretreated rats and 158 ± 18 μl and 185 ± 25 u in seven controls. The total volume of saliva secreted over a period of 60 min and the total output of amylase over 40 min were the same in the capsaicin-pretreated (381 ± 82 μl and 291 ± 61 u) and control rats (383 ± 65 μl and 279 ± 60 u).

Effects of a tachykinin antagonist

The tachykinin antagonist [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP (0.75–1 $mg\,kg^{-1}$, i.v.) abolished the secretory effect of SP, 0.2 $\mu g\,kg^{-1}$ i.v., in four capsaicin-pretreated rats and in four control rats. The atropine-resistant parasympathetic nerve-evoked secretion was also reduced by the tachykinin

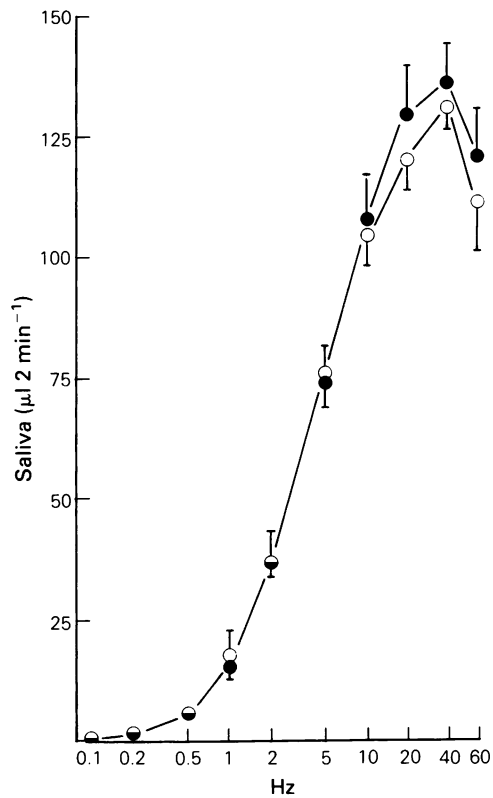

Figure 1 Amount of parotid saliva secreted in capsaicin-pretreated ($n = 5$, ●) and control rats ($n = 5$, ○) in response to stimulation of the parasympathetic (auriculo-temporal) nerve at various frequencies in the presence of adrenoceptor blockers. Ordinate scale: amount of saliva, in μl, per 2 min. Abscissa scale: frequency of stimulation in Hz. Values are means, and vertical bars indicate s.e.mean; in some cases s.e.mean was smaller than the size of the symbols.

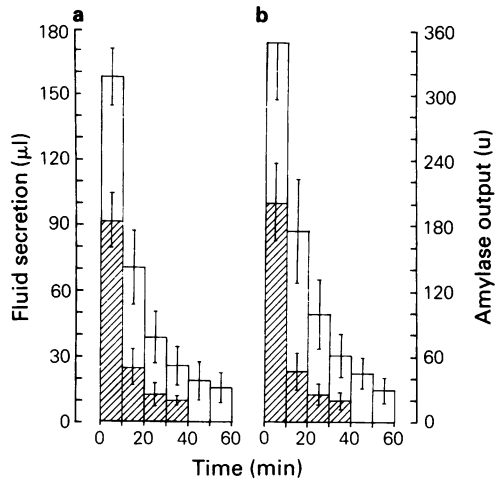

Figure 2 Amount of parotid saliva secreted and output of amylase in capsaicin-pretreated ($n = 7$, b) and control rats ($n = 7$, a) in response to continuous stimulation of the parasympathetic nerve (40 Hz) for 60 min in the presence of atropine and adrenoceptor blockers. Saliva was collected in 10 min periods; the two last samples were not analysed for amylase. Ordinate scales: left, the amount of saliva, in μl, per 10 min; right, the output of amylase, in units, per 10 min. Abscissa scale: time in min. Open columns: mean amount of saliva secreted. Hatched columns: mean output of amylase. Vertical bars indicate s.e.mean.

antagonist: in the capsaicin-pretreated rats the tachykinin antagonist reduced the secretion in response to stimulation for 1 min at 20 Hz to 70.3 ± 5.4% ($n = 4$, $P < 0.05$) of that obtained before administration of the antagonist, while the corresponding figure in control rats was 65.2 ± 6.2% ($n = 4$, $P < 0.05$).

Acute effects of capsaicin

Capsaicin, in a 2.5% solution, applied locally to the auriculo-temporal nerve ($n = 4$) evoked no salivation. Subsequent stimulation of the nerve (40 Hz, 2 min) proximal to the site of the capsaicin application caused a secretory response of the same magnitude as that obtained before the toxin was applied. Capsaicin, in a dose of 25–50 mg kg^{-1}, injected s.c. evoked no secretion from otherwise unstimulated glands ($n = 5$). In three out of four rats where capsaicin was injected retrogradely into the duct a small amount of saliva (0.8–5.8 μl) was secreted; the secretion started 1–2 min after the injection and ceased 2–6 min later. SP, 1 μg kg^{-1} i.v., was injected 15 min before the intraductal capsaicin injection and again after the capsaicin-induced secretion had stopped. In

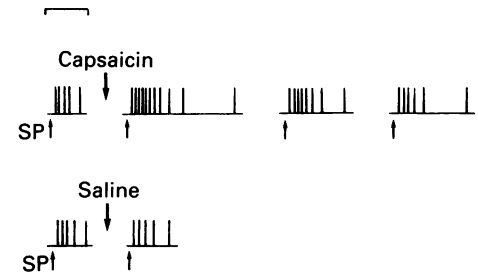

Figure 3 Effects of capsaicin (0.01%), in a volume of 0.04 ml, (upper tracing) or saline, 0.04 ml, (lower tracing) injected retrogradely in the parotid duct on the secretory response to substance P, 1 μg kg^{-1} i.v., in one rat. Substance P was given (arrow) 15 min before the intraductal injection, and then 8, 15, and 25 min after the injection (at the two last time of observations only after capsaicin). Each mark on the tracing represents one drop of saliva (corresponding to 10 μl). Vertical bar indicates one min. The tracings show that after capsaicin, but not after saline, the response to substance P was increased. The auriculo-temporal nerve, the facial nerve and the sympathetic cervical chain had been cut, and atropine and adrenoceptor blockers had been given just before the start of the experiment.

all four of these rats the SP-evoked response was enhanced after capsaicin, by between 100 and 30% over the next 25 min (Figure 3). About 45 min after the capsaicin injection the SP response was the same as before the capsaicin injection. Injection of saline into the duct evoked no secretion, and the SP-evoked response was not enhanced (Figure 3).

Urinary bladder weight, peptide-containing nerve fibres and neuropeptide content

The bladder weights of the capsaicin-pretreated rats (113.7 ± 5.9 mg, $n = 9$) were 21% higher ($P < 0.05$) than those of the untreated controls (89.6 ± 3.2 mg, $n = 9$). In controls SP- and CGRP-containing nerve fibres were numerous in the bladder wall, particularly in the subepithelial layer, where they formed a dense plexus with single fibres penetrating into epithelium. Double immunostaining showed that most CGRP-containing fibres contained SP and *vice versa*. VIP-containing fibres were moderate in number and occurred predominantly in the smooth muscle layer and around blood vessels. Capsaicin treatment virtually abolished the SP- and CGRP-containing fibres, whereas those storing VIP remained unaffected. In the control bladders ($n = 9$) the total amounts (pmol) of CGRP, SP and VIP were 2.6 ± 0.2, 0.31 ± 0.03 and 0.17 ± 0.02, respectively. In the bladders of the capsaicin-pretreated

rats ($n = 9$) the total amounts of CGRP and SP were $5.5 \pm 1.5\%$ ($P < 0.001$) and $13.8 \pm 2.0\%$ ($P < 0.001$), respectively, of controls. The total amount of VIP in the bladders of capsaicin-pretreated rats was $130.7 \pm 10.6\%$ ($P < 0.05$) of that in controls.

Discussion

This study did not provide evidence for any involvement of primary afferent C fibres in the response of the parotid gland to stimulation of the auriculo-temporal nerve as judged by fluid secretion and amylase output. The 'atropine-resistant' response to nerve stimulation persisted undiminished after pretreatment with capsaicin. Further, in the absence of atropine the responses over a whole range of frequencies in capsaicin-pretreated rats were the same as those in the controls. Local application of capsaicin to the auriculo-temporal nerve had no apparent effect on parotid secretion *per se* and did not modify the secretory response to subsequent nerve stimulation. A tachykinin antagonist reduced the 'atropine-resistant' nerve-evoked response just as effectively in capsaicin-pretreated rats as in controls.

There were no signs of 'denervation supersensitivity' following capsaicin treatment. In untreated rats, there is probably a positive interaction not only between certain peptides but also between acetylcholine and VIP as well as CGRP released in response to parasympathetic nerve stimulation (Ekström, 1987). However, the secretory cells of the parotid glands in the capsaicin-pretreated rats were just as sensitive to i.v. injections of the muscarinic agonist methacholine and to SP as those in the control rats. This makes it unlikely that acetylcholine, SP or other substances released during nerve stimulation acted on sensitized cells thereby masking a sensory contribution to the response of the gland.

It is difficult to avoid the conclusion that capsaicin-sensitive sensory nerve fibres in the parotid gland have little or no effect on the secretory activity of the gland. Neither do they seem to play a trophic role, since capsaicin failed to affect the weight of the gland. The time which passed between capsaicin pretreatment and the subsequent acute experiment (1–2 weeks) was thought to be long enough to allow any changes in weight or sensitivity of the secretory cells to secretagogues to reveal themselves because there were marked changes in these parameters within one week following parasympathetic denervation (see also Ekström, 1980; Ekström & Wahlestedt, 1982).

Several peptides have been demonstrated in capsaicin-sensitive primary afferent nerve fibres (Lembeck, 1985; Ju *et al.*, 1987). Assuming that capsaicin eliminates all SP and all CGRP in primary afferent C-fibres, these results suggest that only a minor proportion of SP (11%) and a rather larger proportion of CGRP (36%) are located in primary afferent C-fibres in the parotid gland of the rat. VIP content was unaffected by capsaicin which suggests that VIP-containing fibres either do not have a sensory origin or are insensitive to capsaicin. SP-containing nerve fibres in the parotid gland of control rats were found around acini, ducts and blood vessels (in order of predominance), while CGRP-containing nerve fibres seemed to be mainly localized around ducts and blood vessels; periacinar CGRP-containing fibres were comparatively few (see also Ekström *et al.*, 1988). Double immunostaining showed that most CGRP-containing fibres around ducts and blood vessels contained SP. However, most periacinar SP-storing nerve fibres were devoid of CGRP and most periacinar CGRP-containing fibres were devoid of SP. Following capsaicin treatment, the bulk of SP/CGRP-containing nerve fibres around large ducts and blood vessels had disappeared. Nerve fibres that contain both SP and CGRP are believed to be sensory (Saria *et al.*, 1985; Sundler *et al.*, 1985).

Systemic administration of capsaicin failed to give rise to salivation. However, when capsaicin was injected retrogradely into the duct secretion, albeit very sparse, did occur. When this secretion had ceased, the secretory response to SP was found to be enhanced for about 30 min. The secretory response to SP is known to be enhanced by CGRP (Ekström *et al.*, 1988). It is therefore tempting to speculate that the small secretion that occurred in response to ductal injection of capsaicin involved the combined action of CGRP and SP released from the sensory nerve endings, and that the CGRP that was released enhanced the effect of exogenous SP.

Although the capsaicin-evoked reduction in neuropeptide content was relatively small (11% for SP and 36% for CGRP) and the loss of immunoreactive nerve fibres was modest in the parotid gland we conclude that the capsaicin treatment was effective in eliminating capsaicin-sensitive nerve fibres because it almost abolished the sensitivity of the eye to chemical irritation and more particularly, it greatly reduced the content of SP and CGRP (but not VIP) in the urinary bladder and virtually eliminated nerve fibres storing these two peptides.

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