Naunyn-Schmiedeberg's Arch Pharmacol (1981) 317:140–148

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Differential Effects of Capsaicin on the Content of Somatostatin, Substance P, and Neurotensin in the Nervous System of the Rat

Rainer Gamse[1,2], Susan E. Leeman[1,*], Peter Holzer[2], and Fred Lembeck[2]

[1] Department of Physiology, Harvard Medical School, 45 Shattuck Street, Boston, MA 02115, USA
[2] Institut für Experimentelle und Klinische Pharmakologie, Universität Graz, Universitätsplatz 4, A-8010 Graz, Austria

Summary. The distribution of immunoreactive substance P (I-SP), somatostatin (I-SRIF), and neurotensin (I-NT) and the effect of capsaicin treatment on the concentration of these peptides was studied in the peripheral and central nervous system of the rat.

Neonatal capsaicin treatment (50 mg/kg s.c.) caused a depletion of I-SRIF as well as of I-SP in sensory nerves and in the dorsal half of the spinal cord. No recovery of the peptide content was found when examined 4 months later suggesting an irreversible effect. I-NT, not a constituent of primary sensory neurons, was not changed in the spinal cord. None of the peptides studied was depleted in the hypothalamus or preoptic area.

Capsaicin treatment of adult rats also led to a decrease of I-SRIF and I-SP in primary sensory neurons. The highest dose used (950 mg/kg s.c.) induced no greater depletion than the lowest one (50 mg/kg), except for I-SP in dorsal root ganglia. Intraperitoneal injection of capsaicin led to a higher degree of depletion than subcutaneous administration as examined 1 week after treatment. In contrast to neonatal treatment, the I-SRIF content was completely restored within 4 months after treatment of adult rats. The I-SP content, however, did not completely recover in all areas but remained reduced in cornea, vagus nerve, dorsal spinal cord, and medulla oblongata for up to 9 months.

Intraventricular administration of capsaicin (200 µg) caused a depletion of I-SP in the medulla oblongata but had no effect on the content of all 3 peptides in hypothalamus or preoptic area. In contrast to systemic treatment, no depletion of I-SP or I-SRIF was found in the trigeminal ganglion. Chemosensitivity of the eye was abolished after intraventricular or systemic treatment. Repeated topical application of a capsaicin solution (10 mg/ml) to the eye led within 4 h to a nearly complete depletion of I-SP in the cornea.

These experiments show that capsaicin treatment of rats causes a depletion of both I-SRIF and I-SP in primary sensory neurons. While topical or systemic capsaicin administration causes depletion in terminals, the failure of intraventricular injections of capsaicin to deplete the peptides in the trigeminal ganglion suggests that depletion of the entire neuron requires an action of capsaicin on the peripheral branch and/or the cell body.

Key words: Substance P – Somatostatin – Neurotensin – Capsaicin – Sensory neurons – Chemogenic pain

Send offprint requests to R. Gamse, Institut für Experimentelle und Klinische Pharmakologie, Universitätsplatz 4, A-8010 Graz, Austria
* Present address: Dept. of Physiology, University of Massachusetts Medical School, 55 Lake Ave North, Worcester, MA 01605, USA

0028-1298/81/0317/0140/$01.80

Introduction

Treatment of newborn rats with capsaicin results in a substantial degeneration of unmyelinated primary sensory neurons (Jancsó et al. 1977; Jancsó and Király 1980; Jancsó et al. 1980; Scadding 1980). No signs of axonal degeneration were found, however, when adult rats were injected with capsaicin (Jóo et al. 1969). Systemic administration of capsaicin to adult rats leads to a depletion of immunoreactive substance P (I-SP) in the dorsal horn of the spinal cord (Jessell et al. 1978; Hayes and Tyers 1980) but not in the gastrointestinal tract (Holzer et al. 1980). Similarly, neonatal capsaicin treatment causes a decrease of I-SP in regions of the peripheral and central nervous system containing primary sensory neurons but not in other brain areas (Gamse et al. 1980; Nagy et al. 1980; Cuello et al. 1981) nor in the intestine (Holzer et al. 1980). This decrease seemed irreversible in rats treated on the second day of life, but it was reversible in most areas when 20 days old rats were injected (Gamse et al. 1980).

While neonatal capsaicin treatment causes degeneration of about 70% of the unmyelinated fibers in the saphenous nerve (Jancsó et al. 1977; Scadding 1980), substance P-positive cells account for not more than 20% of the cells in spinal ganglia (Hökfelt et al. 1976). This suggests that capsaicin also affects fiber systems containing other putative neurotransmitters, like somatostatin. Somatostatin-positive cell bodies have been found in spinal ganglia (Hökfelt et al. 1975, 1976). The upper dorsal horn of the spinal cord also contains a high density of somatostatin-positive fibers (Hökfelt et al. 1975, 1976; Forssmann 1978) and fibers staining for neurotensin (Uhl et al. 1977a), but no staining for neurotensin was detected in dorsal root ganglia (Chan-Palay and Palay 1977; Seybold and Elde 1980).

It was investigated, therefore, whether capsaicin treatment leads to a selective depletion of substance P or whether it also affects somatostatin or neurotensin in various areas of the central and peripheral nervous system. Capsaicin was also administered by different routes and in various doses in order to study the dose–depletion relationship, and possible differences in the effect of capsaicin on substance P and somatostatin neurons.

Methods

Animals. Male rats (Charles River, Wilmington, USA; or Sprague Dawley, strain OFA, Himberg, Austria) were used for all experiments. Newborn rats obtained from Charles River or the departmental animal house were weaned at the age of 3 weeks and, like all other rats, kept on a 12 h light–dark cycle with food and water ad libitum.

Capsaicin Treatment. Newborn rats were injected subcutaneously with 50 mg/kg capsaicin on the second day of life as described (Gamse et al. 1980).

Adult rats (200 – 300 g) received capsaicin according to the following protocols:

(a) 50 mg/kg s.c. were given as a single injection. (b) 125 mg/kg s.c. were given over 2 days, with 25 + 50 or 10 + 20 + 45 mg/kg on the first and 50 mg/kg on the second day. (c) 950 mg/kg s.c. were administered over 5 days as described by Jessell et al. (1978, 50 + 100 + 200 + 200 + 400 mg/kg). (d) In one set of experiments, rats injected with 125 mg/kg s.c. were given additional injections of 20 and 30 mg/kg i.p. on the following 2 days. Twenty minutes before the i.p. injections of capsaicin, these rats were pretreated i.p. with atropine (10 mg/kg), antazoline (5 mg/kg) and isoprenaline (10 mg/kg). (e) In another group of rats, receiving 125 mg/kg capsaicin i.p. only, capsaicin was administered in 8 injections with increasing doses from 2 – 20 mg/kg on the first day and in 3 injections with up to 50 mg/kg on the second day. On the first day 5 mg/kg atropine was given twice. (f) Intraventricular injections of capsaicin were performed under light ether anaesthesia with 1 mg/kg atropine given 15 min prior to the first 2 injections. A total dose of 200 µg capsaicin (50 + 50 + 100 µg at 1 h intervals; 10 mg/ml) dissolved in 60 % dimethylsulfoxide was injected with a Hamilton syringe into the lateral ventricle (posterior margin of coronal suture, ML 2 mm, DV 4 mm). (g) For local desensitization, a drop of a 1 % capsaicin solution was instilled into the eye every 30 min for 2 h as described (Szolscànyi et al. 1975).

In all experiments a comparable number of control rats was pretreated in the same way as capsaicin rats and they received equal amounts of control solutions (10 % ethanol, 10 % Tween 80 in saline or 60 % dimethylsulfoxide in saline).

Tissue Extraction and Radioimmunoassay. Tissue samples were immediately frozen on dry ice, weighed and extracted with more than 10 volumes of 2 N acetic acid. Samples from the peripheral nervous system were boiled for 5 min in a water bath or heated for 2 × 30 s in a microwave oven: The samples were homogenized in a glass-teflon homogenizer or by sonication, centrifuged and the supernatants were lyophilized. Substance P was determined using antibodies R6P or Rd2 and iodinated Tyr^8-substance P as tracer (Mroz and Leeman 1979). Both antibodies are directed towards the C-terminal part of substance P and display a similar degree of cross-reactivity with related peptides (Mroz and Leeman 1979). The antibody R6P has a 3 times higher sensitivity (limit 1.5 fmol per tube) but tissue concentrations obtained with the two antibodies were the same. Somatostatin was measured using the antibody M-6 (Arnold and Fernstrom 1980) and iodinated N-Tyr- or Tyr^1-somatostatin as tracer. The antibody HC-8 (Carraway and Leeman 1976a) was used in the neurotensin assay; tracer was prepared according to Carraway and Leeman (1976a) or Uhl et al. (1977b). All three peptides were determined in the same samples.

For characterization of the immunoreactivities measured in the 3 assays, an extract of whole spinal cord was chromatographed on Sephadex G-25 using assay buffer as eluent (Gamse et al. 1979).

The concentrations of the peptides varied up to 2-fold between groups of control rats for no obvious reasons. Therefore, we included a group of control rats for every capsaicin-treated group and dissected and extracted samples from control and capsaicin treated rats in alternating order. All results with capsaicin are presented as percent peptide concentration of the corresponding control group.

In one experiment (8 days after treatment of adult rats with 125 mg/kg s.c. capsaicin), protein was measured in the acetic acid extract according to Markwell et al. (1978). The results obtained were identical when peptide concentrations were calculated in fmol/mg protein or fmol/mg wet weight. Therefore, all results reported in this paper are based on wet weight.

Wiping Test. The sensitivity towards chemogenic pain was tested with the wiping test (Jancsó 1968), e.g. by instillation of a drop of a 100 µg/ml capsaicin solution into one eye and counting the number of wipings with the forepaws.

Statistics. The significance of differences between capsaicin-treated and the corresponding control rats was calculated using Student's *t*-test.

Results

Distribution of Peptides

Since the 3 peptides were measured by radioimmunoassay we use the terms "immunoreactive" substance P (I-SP), somatostatin (I-SRIF), and neurotensin (I-NT) throughout the paper. Identity of I-SP and I-NT with the synthetic peptides is suggested by single peaks of immunoreactivity on Sephadex G-25 chromatography and by parallel dilution curves of samples and standard in the assay. While the dilutions of I-SRIF were also parallel to the standard curve, the chromatogram of a spinal cord extract showed 3 immunoreactive peaks: 91.8 % eluted at the position of somatostatin (relative elution volume 2.3), 3 % in the void volume, and 5.2 % with a relative elution volume of 1.35.

I-SP and I-SRIF were found in peripheral nerves (Table 1). While the concentrations of I-SRIF were similar to those of I-SP in most areas, the vagus nerve contained much less I-SRIF, and no I-SRIF was detected in the cornea. I-NT could not be detected in the peripheral nerves investigated, except for dorsal root and trigeminal ganglia.

Table 1
Distribution of I-SP, I-SRIF, and I-NT in nervous tissue of rats treated with control solution (10 % ethanol, 10 % Tween 80 in saline). Values are given in fmol/mg wet weight ($n = 27-85$)

	I-SP	I-SRIF	I-NT
Saphenous nerve	25.7 ± 1.5	24.8 ± 2.0	< 0.2[a]
Dorsal root ganglia	15.9 ± 0.9	16.4 ± 1.5	2.5[a]
Dorsal roots	13.8 ± 0.6	10.6 ± 0.9	< 0.1[a]
Dorsal spinal cord	234 ± 14.7	387 ± 42.7	16.5 ± 1.0
Ventral spinal cord	36.5 ± 2.1	53.3 ± 6.5	2.1 ± 0.1
Ventral roots	1.9 ± 0.3	1.5 ± 0.2	< 0.1
Cornea	5.2 ± 0.3	< 0.1[a]	< 0.1[a]
Trigeminal ganglion	19.5 ± 1.0	9.7 ± 1.0	1.2[a]
Medulla oblongata	137 ± 6.1	178 ± 9.9	22.2 ± 1.1
Vagus nerve	37.2 ± 2.4	4.4 ± 0.3	< 0.3[a]
Preoptic area	224 ± 11.0	715 ± 66.0	91.8 ± 5.3
Hypothalamus	167 ± 11.0	1,164 ± 84.0	84.4 ± 3.8

[a] Tissue from 5 untreated rats pooled and assayed

Table 2
Effect of neonatal capsaicin treatment (50 mg/kg s.c.) on peptide levels measured 6 and 16 weeks after treatment. Data are given in % of the values of corresponding control rats ($n = 6-10$)

	Weeks	I-SP	I-SRIF	I-NT
Saphenous nerve	6	13 ± 2**	28 ± 4**	
	16	19 ± 2**	14 ± 3**	
Dorsal root ganglia	6	26 ± 3**	28 ± 2**	
	16	39 ± 3**	11 ± 3**	
Dorsal roots	6	14 ± 2**	34 ± 6**	
	16	19 ± 2**	23 ± 5**	
Dorsal spinal cord	6	35 ± 3**	85 ± 5	106 ± 5
	16	37 ± 4**	77 ± 6*	92 ± 9
Ventral spinal cord	6	108 ± 8	107 ± 6	97 ± 7
	16	85 ± 7	96 ± 6	107 ± 6
Ventral roots	6	51 ± 11**	73 ± 9	
	16	49 ± 5**	122 ± 11	
Cornea	6	<20[a]		
	16	14 ± 2**		
Trigeminal ganglion	6	24 ± 5*	44 ± 5**	
	16	29 ± 2**	30 ± 6**	
Medulla oblongata	6	49 ± 8**	98 ± 8	108 ± 4
	16	49 ± 3**	91 ± 5	100 ± 7
Vagus nerve	6	8 ± 4**	72 ± 10	
	16	29 ± 3**	85 ± 6	

* $P < 0.01$; ** $P < 0.001$
[a] 20% represents limit of the assay

The immunoreactivity found in these two regions did, however, not dilute parallel to neurotensin in the assay. No chromatography could be carried out because of the low quantity of I-NT in the ganglia. All 3 peptides were found in the spinal cord, the concentration in the dorsal half exceeding that in the ventral half by a factor of about seven. The absolute concentrations of I-NT in the spinal cord and medulla oblongata were, however, much lower than those of I-SP and I-SRIF (Table 1).

General Observations after Capsaicin Treatment

After neonatal capsaicin treatment about 50% of the rats died within the first 3–5 weeks. Extensive atelectatic areas were found in the lungs of most of these rats. In some litters the capsaicin treated rats gained weight much slower than the control rats. While rats treated neonatally with capsaicin in Boston (Charles River rats) showed consistently wounds, particularly around their nostrils and behind the ears, this was almost never found in rats treated in Graz (Sprague Dawley rats), confirming our first observations made on this strain of rats (Gamse et al. 1980). Whether this finding reflects differences in strain, housing, food or anything else was not investigated.

When adult rats were treated, 10–80% died immediately after the capsaicin injections or within the following day. Thereafter no deaths were recorded up to 9 months. A decrease in body weight was found after capsaicin treatment of adult rats: the decrease varied from 5.5% ($P < 0.001$) 1 day after 50 mg/kg s.c. to 19% ($P < 0.001$) 2 days after 950 mg/kg s.c. The original body weight was regained within 2–4 days after treatment with 50 or 125 mg/kg s.c. Self-inflicted wounds were never found in rats treated as adults. Vehicle treated rats did not differ from untreated rats in weight, appearance, or death rate.

Capsaicin Treatment of Newborn Rats

The depletion of I-SP after neonatal capsaicin treatment (Table 2) was somewhat higher but otherwise identical with that reported by Gamse et al. (1980). In addition to the earlier paper, a depletion of I-SP was found also in ventral roots and dorsal root ganglia, as well as in the cornea and the trigeminal ganglion. I-SRIF was decreased to a similar extent as I-SP in peripheral nerves, except for ventral roots and the vagus nerve. In contrast to I-SP, I-SRIF was unchanged in the medulla oblongata and only moderately decreased in the dorsal half of the spinal cord. The concentration of I-NT in these two regions was unaltered (Table 2), as was the content of all 3 peptides in preoptic area and hypothalamus. The extent of the depletion of I-SP and I-SRIF was similar 6 weeks after treatment of Charles River rats and 4 months after treatment of Sprague Dawley rats.

Capsaicin Treatment of Adult Rats: Doses and Routes of Administration

Systemic capsaicin treatment of adult rats resulted in a depletion of I-SP and I-SRIF in those regions where a decrease was found after neonatal treatment (Table 3). In almost all areas the extent of the depletion was, however, smaller in adult treated rats. While I-SP was depleted in dorsal root ganglia 4 days after treatment with 125 and 950 mg/kg s.c., no depletion but rather an increase was found with 50 mg/kg. This result was confirmed in two other sets of experiments. In dorsal and ventral roots, the depletion of I-SP was also smaller 4 days after treatment with 50 mg/kg than with higher doses. Except for dorsal root ganglia the depletion of I-SP caused by 950 mg/kg s.c. capsaicin was not higher than that by 125 mg/kg s.c. An additional intraperitoneal administration of capsaicin did not increase the depletion found after 125 mg/kg s.c. (Table 3).

Table 3. Effect of capsaicin treatment of adult rats on peptide levels measured 4 or 8 days (125 mg/kg i.p.) after the last capsaicin injection (for treatment protocols see Methods). Data are given in % of the values of rats treated in the same way with an equal volume of control solution (10% ethanol, 10% Tween 80 in saline); $n = 6-10$

		50 mg/kg s.c.	125 mg/kg s.c.	950 mg/kg s.c.	125 mg/kg s.c. + 50 mg/kg i.p.	125 mg/kg i.p.
Saphenous nerve	I-SP	66 ± 9*	47 ± 5**	50 ± 9**	56 ± 3*	32 ± 2**
	I-SRIF	53 ± 2**	65 ± 8*	71 ± 8*	66 ± 3**	33 ± 2**
Dorsal root ganglia	I-SP	141 ± 41	67 ± 7*	33 ± 5**	45 ± 1**	33 ± 2**
	I-SRIF	76 ± 3*	70 ± 5*	71 ± 7*	71 ± 2*	58 ± 3*
Dorsal roots	I-SP	62 ± 0**	28 ± 6**	32 ± 3**	49 ± 3**	20 ± 1**
	I-SRIF	71 ± 5**	61 ± 5**	63 ± 8**	67 ± 2	38 ± 1**
Dorsal spinal cord	I-SP	67 ± 5*	51 ± 6**	68 ± 4**	58 ± 3**	65 ± 2**
	I-SRIF	81 ± 6*	82 ± 5*	n.d.	82 ± 3	100 ± 6
Ventral spinal cord	I-SP	n.d.	86 ± 6	89 ± 9	92 ± 4	105 ± 4
	I-SRIF	n.d.	114 ± 15	n.d.	100 ± 5	91 ± 13
Ventral roots	I-SP	65 ± 14	<20[a]	n.d.	21 ± 3**	n.d.
	I-SRIF	n.d.	n.d.	n.d.	91 ± 5	n.d.

* $P < 0.05$; ** $P < 0.01$
n.d. = not determined
[a] 20% represents limit of the assay

Table 4
Effect of intraventricular capsaicin administration (total dose 200 µg) on peptide levels measured 1 and 10 days after treatment. Data are given in % of the values of rats treated with control solution (total volume 20 µl of 60% DMSO in saline); $n = 6$

	Days	I-SP	I-SRIF	I-NT
Preoptic area	1	104 ± 9	97 ± 9	95 ± 7
	10	108 ± 7	97 ± 4	99 ± 6
Hypothalamus	1	85 ± 6	84 ± 4	96 ± 3
	10	98 ± 6	99 ± 10	104 ± 5
Medulla oblongata	1	75 ± 5*	87 ± 6	102 ± 5
	10	69 ± 3**	104 ± 8	92 ± 10
Trigeminal ganglion	1	95 ± 11	91 ± 8	n.d.
	10	102 ± 9	81 ± 8	n.d.
Lumbosacral dorsal spinal cord	1	n.d.	n.d.	n.d.
	10	85 ± 5	119 ± 12	n.d.

* $P < 0.05$; ** $P < 0.01$
n.d. = not determined

Depletion of I-SRIF in dorsal roots and the dorsal half of the spinal cord was smaller than that of I-SP. This was also observed in dorsal root ganglia after high doses of capsaicin. Four days after 50 mg/kg, I-SRIF but not I-SP was found to be decreased in the ganglia. In all areas examined, a dose of 950 mg/kg did not result in a higher depletion of I-SRIF than 50 or 125 mg/kg.

Peptide concentrations were measured in regions in addition to those shown in Table 3 in rats treated with 125 mg/kg s.c. plus 50 mg/kg i.p. I-SP was depleted in cornea (12 ± 1% of controls, $P < 0.001$), trigeminal ganglion (37 ± 2%, $P < 0.01$), medulla oblongata (75 ± 2%, $P < 0.05$) and vagus nerve (22 ± 4%, $P < 0.01$). A significant decrease of I-SRIF was found in the trigeminal ganglion only (68 ± 19%, $P < 0.01$). No change of the I-NT concentration was found in spinal cord and medulla oblongata. The concentrations of all 3 peptides were unchanged in preoptic area and hypothalamus.

A pure intraperitoneal administration of 125 mg/kg capsaicin caused a high depletion of I-SP and I-SRIF in saphenous nerve, dorsal root ganglia and dorsal roots as measured 8 days afterwards (Table 3). While I-SRIF was not changed in the vagus nerve, its I-SP content was decreased to 8 ± 1% ($n = 7$) of control rats. The decrease in the peptide content in these areas exceeded that found 1 week after subcutaneous treatment with 125 mg/kg (Fig. 2) and was the most pronounced ever seen in rats treated as adults.

The first intraventricular injection of capsaicin evoked characteristic behavioral responses: the rats exhibited signs of pain, scratching and wiping movements restricted to the head, the ears turned intensively red, and respiration was impaired. Instillation of capsaicin into the eye 15 min after this injection, e.g. when the rats were already fully awake, did not evoke blepharospasm and wiping movements in capsaicin treated rats, while control rats reacted normally. The second and third intraventricular injections, applied in 1 h intervals, had no effects on behavior. On the day after the injections, capsaicin treated rats produced 2 ± 1 wipings (controls: 30 ± 4, $n = 5$) and 9 days later 0.4 ± 0.4 wipings (controls: 24 ± 1). The peptide concentration measured in rats treated with dimethylsulfoxide vehicle were within the range of those in untreated or ethanol-Tween-saline treated rats. After capsaicin, the only change in peptide content of the regions examined was a decrease of I-SP

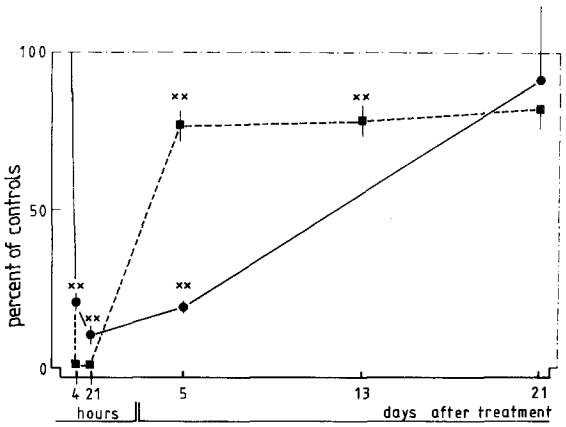

Fig. 1. Effect of instillation of capsaicin into the eye (5 × 1 drop of a 10 mg/ml solution) on I-SP content of the cornea and on responsiveness in the wiping test. Data are given in % of values of rats instilled with control solution (10% ethanol, 10% Tween 80 in saline); $n = 8-10$. $^{\times\times}P < 0.01$. (●——●) I-SP content, (■——■) wiping movements

in the medulla oblongata (Table 4). It should be noted, that I-SP and I-SRIF remained unchanged in the trigeminal ganglion and the lumbosacral spinal cord.

The first and sometimes also the second instillation of a 10 mg/ml capsaicin solution into the eye caused pain, blepharospasm and reddening of the conjunctiva. Already 4 h after the last of 5 instillations of capsaicin, the content of I-SP was decreased to 20% of control values and the rats did not respond in the wiping test (Fig. 1). Five days later, the number of wipings was 75% of those in control rats, the I-SP content was, however, still only 20% of controls. The sensitivity towards chemogenic pain and the I-SP content recovered within 3 weeks.

Capsaicin Treatment of Adult Rats: Long-Term Effects

Adult rats were treated with 50 or 125 mg/kg capsaicin s.c. and the sensitivity towards chemogenic pain and peptide levels were determined up to 9 months. One day after treatment all rats showed no reaction in the wiping test, while control rats produced 25 ± 3 ($n = 38$) wipings. Four and nine months later 1 ± 1 wipings were counted for rats treated with 125 mg/kg capsaicin, but 25 ± 2 ($n = 6$) and 28 ± 2 ($n = 7$) wipings, respectively, for control rats.

Four and 7 days after treatment with 125 mg/kg s.c. capsaicin, I-SP was depleted in all areas investigated containing primary sensory neurons (Figs. 2, 3). One month after treatment the I-SP level in the saphenous nerve was as high as in control rats but still decreased in dorsal root ganglia and dorsal roots. At this time the extent of the depletion found in rats treated with 50 mg/kg (dorsal root ganglia 43 ± 5%, dorsal roots 55 ± 4%, $n = 10$, $P < 0.001$) was similar to the depletion caused by 125 mg/kg (Fig. 2). This was also seen 4 months after treatment, when the I-SP content in dorsal root ganglia had reached control values in both groups, but was still depleted in dorsal roots and the dorsal half of the spinal cord. In this latter region as well as in cornea and vagus nerve, recovery was incomplete even 9 months after treatment with 125 mg/kg capsaicin. No tendency towards restoration of the depleted I-SP content was found in the medulla oblongata (Fig. 3).

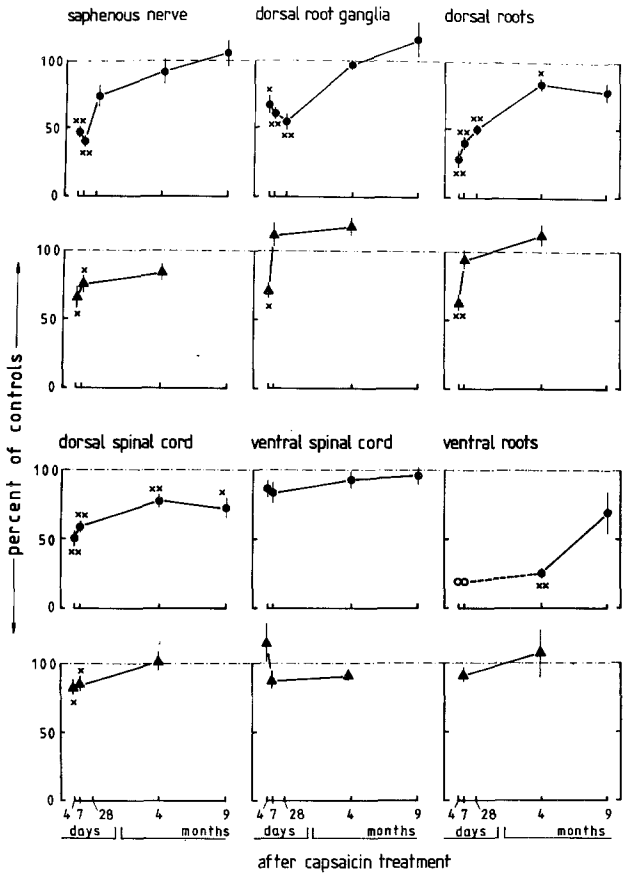

Fig. 2. Effect of capsaicin treatment (125 mg/kg, s.c.) of adult rats on the content of I-SP (●——●) and I-SRIF (▲——▲) measured at various time points after treatment. Data are given in % of values of control rats; $n = 5-9$. $^{\times}P < 0.05$, $^{\times\times}P < 0.01$. ○ For ventral roots represents assay limit

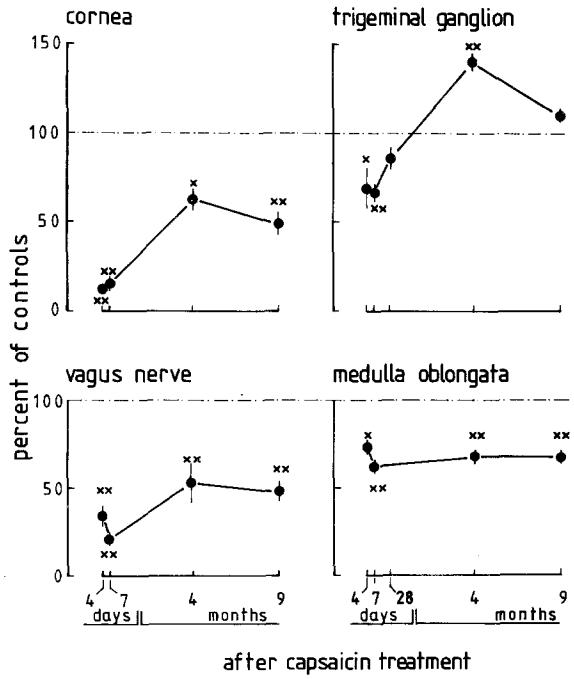

Fig. 3. Effect of capsaicin treatment (125 mg/kg, s.c.) of adult rats on the content of I-SP measured at various time points after treatment. Data are given in % of values of control rats; $n = 5-9$. $^{\times}P < 0.05$, $^{\times\times}P < 0.01$

In contrast to these results for I-SP, the content of I-SRIF recovered completely within 4 months in all areas where a depletion was found 4 days after treatment with 125 mg/kg s.c. (Fig. 2). The difference in the time course between the recovery of I-SP and I-SRIF was most pronounced in dorsal root ganglia and dorsal roots.

Discussion

Distribution of I-SP, I-SRIF, and I-NT

The results on the distribution of I-SRIF confirm immunohistochemical data showing somatostatin-positive cells and fibers in sensory structures (Hökfelt et al. 1975, 1976; Lundberg et al. 1978; Seybold and Elde 1980). While the concentrations of I-SP and I-SRIF were similar in nerves derived from dorsal root ganglia, lower levels of I-SRIF were found in cerebral nerves and I-SRIF was apparently absent from the cornea. This may suggest a differential innervation of peripheral organs by SP- and SRIF-containing neurons and is in line with the finding of Hökfelt et al. (1976) that SP- and SRIF-containing neurons constitute different fiber populations.

A chromatographic analysis of I-SRIF in the spinal cord indicated that the antibody recognizes also high molecular weight forms of somatostatin which may represent precursor molecules (Zingg and Patel 1979). Since these compounds contributed to less than 8% of the total immunoreactivity and since they can e.g. be released like SRIF (Zingg and Patel 1979), it seems unlikely that changes in their concentrations could mask or markedly exaggerate changes of the SRIF content caused by capsaicin treatments.

The concentrations of I-NT were found to be similar to that reported for CNS-regions by Carraway and Leeman (1976b) and Kobayashi et al. (1977). As already revealed by immunocytochemistry (Uhl et al. 1977a; Seybold and Elde 1980), I-NT is like I-SP and I-SRIF highly concentrated in the dorsal half of the spinal cord. Unlike I-SP and I-SRIF, I-NT is not present in primary sensory neurons, since it could not be measured in dorsal roots or saphenous nerve and since no cell bodies were found in dorsal root ganglia (Chan-Palay and Palay 1977; Seybold and Elde 1980). I-NT detected in sensory ganglia cannot be identical with neurotensin because serial dilutions were not parallel to the standard curve. While I-NT was also undetectable in rat lumbosacral ventral roots, neurotensin-positive fibers possibly originating in the spinal cord, have been described in cat ventral roots at the thoracic level (Lundberg et al. 1980).

Differences in Depletion of I-SP and I-SRIF

Capsaicin treatment was found to cause depletion of I-SP and I-SRIF in structures containing primary sensory neurons. Likewise, capsaicin releases not only I-SP but also I-SRIF from spinal cord tissue in vitro (Gamse et al. 1981a). Some differences between the depletion of I-SP and I-SRIF were, however, found. Most conspicuous was the lack of depletion of I-SRIF in medulla oblongata and vagus nerve, and the small decrease in the dorsal half of the spinal cord. The results may suggest, that primary afferent SRIF neurons contribute to the I-SRIF content of spinal cord and medulla oblongata less than afferent SP neurons to the I-SP content. Another explanation could be that secondary changes of intrinsic SRIF neurons (Forssmann 1978; Seybold and Elde 1980) masked a decrease in primary sensory neurons. It has been reported, that depletion of SP-positive fibers in the dorsal horn after rhizotomy was followed by sprouting of remaining SP fibers (Tessler et al. 1980). No evidence for sprouting was, however, obtained in the present experiments, since after neonatal capsaicin treatment the I-SP content did not recover and the increase of I-SP in the spinal cord of adult treated rats was accompanied by an increase in dorsal root ganglia and dorsal roots. Plasticity of intrinsic SRIF neurons can also not explain the lack of depletion of I-SRIF in the medulla found 1, 4, 7, 10 and 112 days after capsaicin treatment. The higher degree of depletion of I-SP in the medulla compared to earlier findings (Gamse et al. 1980) arises from the fact that the region termed medulla in the first paper also contained the pons which appears to contain few, if any capsaicin-sensitive SP-fibers.

The finding of a more than 8-fold higher concentration of I-SP than I-SRIF in the vagus nerve agrees with earlier data (Gilbert et al. 1980; Lundberg et al. 1978). SP-positive and some SRIF-positive cell bodies found in the nodose ganglion (Lundberg et al. 1978; Katz and Karten 1980), suggest that both peptides are present in sensory neurons. Since, however, capsaicin treatment was never found to deplete I-SRIF in the vagus nerve, it would appear that these I-SRIF neurons are capsaicin-insensitive.

Capsaicin-treatment caused a depletion of I-SP in lumbosacral ventral roots. Since I-SP is not depleted by capsaicin in the ventral horn, the depletion of I-SP in ventral roots suggests that I-SP is not contained in efferent but in afferent neurons. Unmyelinated fibers having their cell bodies in spinal ganglia and entering the spinal cord via the ventral root have been described (Coggeshall et al. 1974; Maynard et al. 1977). Furthermore, there is functional evidence for the presence of sensory fibers in ventral roots (Longhurst et al. 1980; Hosobuchi 1980). Assuming that capsaicin sensitive SP-fibers in ventral roots are afferent fibers, they are most likely to terminate in the upper dorsal horn, because capsaicin-evoked release of I-SP in vitro could only be demonstrated from slices of the upper dorsal but not the ventral horn (Gamse et al. 1981a).

Effect of Local Capsaicin Treatment

While capsaicin treatment has been reported to deplete I-SP in sensory neurons and their terminals in the CNS (Jessell et al. 1978; Gamse et al. 1980; Nagy et al. 1980; Hayes and Tyers 1980; Cuello et al. 1981), the I-SP content remained unchanged in other areas of the CNS (Gamse et al. 1980; Nagy et al. 1980; Cuello et al. 1981). The lack of depletion cannot be due to a failure of capsaicin to cross the blood-brain-barrier since it was also seen after intraventricular capsaicin administration. Furthermore, capsaicin does not evoke release of I-SP in vitro from hypothalamus or substantia nigra (Gamse et al. 1979). The entrance of capsaicin into the CNS after subcutaneous injection was recently directly measured by Saria et al. (submitted for publication), revealing a high CNS concentration already after 30 min. This can explain that after systemic administration of capsaicin morphological changes occur in the preoptic area (Szolscányi et al. 1971) which probably account for the effect of capsaicin on thermoregulation (Jancsó 1968). Since we did not find changes in the content of I-SP, I-SRIF or I-NT in the preoptic area in any experiment, neither of these peptides seems involved in the effects of capsaicin on thermoregulation.

The first intraventricular injection of capsaicin caused signs of pain restricted to the trigeminal system, an effect similar to that observed in the caudal portions of the body after intrathecal administration to the lumbar cord (Yaksh et al. 1979). Already 15 min after the intraventricular injection of capsaicin, the rats were insensitive to a noxious chemical stimulus applied to the cornea. This may be interpreted in that the early response after intraventricular injection of capsaicin is caused by a direct action on terminals of primary afferent fibers in the medulla leading to the release of pain transmitter(s) which is followed by desensitization of the terminals or depletion of the releasable transmitter pool. Alternatively, capsaicin could directly activate and then block postsynaptic sites for pain transmission. The first explanation seems more likely since capsaicin has direct effects on primary sensory neurons (Szolcsányi 1977; Ault and Evans 1980; Godfraind et al. 1981) and it can evoke release of, e.g. I-SP from central (Gamse et al. 1979, 1981a; Theriault et al. 1979) and peripheral (Gamse et al. 1981b) endings of sensory neurons.

While I-SP and I-SRIF were found to be depleted in the trigeminal ganglion after systemic capsaicin treatment, no change occurred after intraventricular capsaicin injections, e.g. when capsaicin had access only to the central terminals and possibly to the intracranial part of the trigeminal nerve. This finding may indicate that in order to deplete I-SP or I-SRIF from cell bodies of primary sensory neurons, capsaicin must act on the cell body and/or the peripheral branch of the sensory neuron. A striking similarity thus seems to exist between the effects induced by capsaicin administrations and that of nerve lesions at different parts of sensory neurons, since section of the peripheral but not the central branch of sensory neurons leads to chromatolysis of the perikaryon (Cragg 1970) which is accompanied e.g. by a depletion of I-SP (Jessell et al. 1979).

Depletion of I-SP in the cornea was achieved not only by systemic but also by repeated local capsaicin administration suggesting a localization of I-SP in sensory trigeminal neurons. If so, the effect of topically applied capsaicin would indicate that it can deplete I-SP by a direct action also from peripheral endings of sensory neurons. The rapid time course of the I-SP decrease (80% in 4 h) suggests an effect on storage and release rather than on synthesis or axonal transport.

Long-Term Effects of Capsaicin Treatment

Various doses of capsaicin were used mainly to investigate whether the treatment protocols reported in the literature (50–950 mg/kg s.c.) result in different biochemical changes. In interpreting the I-SP results of Table 3 one must consider that, while the measurement were made 4 days after the last capsaicin injection, the time elapsed between first and last dose varied between 4 and 8 days. The apparent pronounced dose relationship of the extent of the I-SP depletion in dorsal root ganglia may, therefore, be overlapped by differences in the time course of the depletion. The significantly higher depletion seen 4 days after 950 mg/kg than 7 days after 125 mg/kg, e.g. 8 days after the first dose, indicates, however, a dose–depletion relationship for I-SP in the ganglia. In interpreting the increase of I-SP 4 days after 50 mg/kg, also found by Lembeck and Donnerer (1981) one could speculate that the initial neurotoxic effect of 50 mg/kg is lower than that of higher doses, so that the cell body can respond with increased synthesis. The results obtained 1 month after treatment indicated, however, that this low dose causes long term depletions similar in magnitude as the higher doses tested.

Axonal degeneration of primary sensory neurons caused by neonatal capsaicin treatment (Jancsó et al. 1977; Jancsó and Király 1980; Scadding 1980) coincides with long lasting and most likely irreversible depletions of I-SP and I-SRIF. In adult treated rats it would appear that I-SRIF neurons are less affected by capsaicin than are I-SP neurons. Release experiments in vitro also showed an almost 9-fold higher capsaicin-induced release of I-SP than of I-SRIF from upper dorsal horn tissue (Gamse et al. 1981a). The data obtained after 125 mg/kg indicated, however, a faster restoration of the I-SRIF content. Thus at 4 days after treatment, the earliest time point examined, the I-SRIF content may already have been partly restored. The rapid and in all areas complete recovery of I-SRIF contrasts with the slow, and in some areas incomplete restoration of I-SP. Whether this reflects differences in synthesis, axonal transport or turnover of the two peptides remains to be investigated. It shows, however, that, unlike in newborn treated rats, the relation between the I-SP and I-SRIF content differs markedly with time after capsaicin treatment of adult rats. The early restoration of I-SP in the saphenous nerve may be explained by the high amount of I-SP transported towards the periphery (Brimijoin et al. 1980). The reason for the incomplete recovery of I-SP in the cornea and vagus nerve is, however, unknown. Continued presence of capsaicin cannot account for this long-term depletion since capsaicin is eliminated from the body within 1 day (Saria et al. submitted for publication).

Functional Implications

Substance P has been proposed to be involved in transmission of pain in the trigeminal system (Hökfelt et al. 1977; Henry et al. 1980; Del Fiacco and Cuello 1980). It is therefore of interest to compare the sensitivity of the eye to noxious chemical stimuli with the I-SP content in trigeminal fibers. Depletion of I-SP in the medulla oblongata after intraventricular or systemic capsaicin treatment coincided with insensitivity of the eye to pain. This is consistent with a role of substance P in pain transmission. On the other hand, changes in the I-SP content of the cornea did not parallel pain sensitivity of the eye. This does not necessarily argue against a function of peripheral substance P in nociception since capsaicin administration to the eye excites both corneal and conjunctival nociceptors while I-SP was determined in cornea only.

Summarizing the results one can conclude that capsaicin can release (Gamse et al. 1981a) and deplete I-SP as well as I-SRIF from primary sensory neurons but apparently not from neurons intrinsic to the CNS suggesting that peptidergic primary afferent neurons contain a specific recognition site for capsaicin. An acute effect of capsaicin administration appears to be release of neuronal substances, which leads to the initial behavioral responses. Depletion and thus reduced release of the same substances may explain some of the impaired functions after capsaicin treatment. The finding of Lembeck and Donnerer (1981) that functional impairment preceded depletion of I-SP makes it likely, however, that capsaicin has multiple actions on primary sensory neurons. While the present study has substantiated the usefulness of capsaicin in studying primary sensory neurons, it revealed, however, that the action of capsaicin is not con-

fined to I-SP containing fibers, which complicates the interpretation of functional changes.

Acknowledgements. The skilful assistance of Mr. J. Donnerer, Mrs. G. Gamse and Mrs. R. Schuligoi is gratefully acknowledged. We thank Dr. Fernstrom for somatostatin antibody and Dr. Carraway for neurotensin antibody. The study was supported by grant N. 3400 and 3506 of the Scientific Austrian Research Funds.

References

Arnold MA, Fernstrom JD (1980) Administration of anti-somatostatin serum to rats reverses the inhibition of pulsatile growth hormone secretion produced by injection of metergoline but not yohimbine. Neuroendocrinology 31:194–199

Ault B, Evans RH (1980) Depolarizing action of capsaicin on isolated dorsal root fibres of the rat. J Physiol 306:22P–23P

Brimijoin S, Lundberg JM, Brodin E, Hökfelt T, Nilsson G (1980) Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. Brain Res 191:443–457

Carraway R, Leeman SE (1976a) Radioimmunoassay for neurotensin, a hypothalamic peptide. J Biol Chem 251:7035–7044

Carraway R, Leeman SE (1976b) Characterization of radioimmunoassayable neurotensin in the rat. J Biol Chem 251:7045–7052

Chan-Palay V, Palay SL (1977) Immunocytochemical identification of substance P cells and their processes in rat sensory ganglia and their terminals in the spinal cord: Light microscopic studies. Proc Natl Acad Sci USA 74:3597–3601

Coggeshall RE, Coulter JD, Willis WD (1974) Unmyelinated axons in the ventral roots of the cat lumbosacral enlargement. J Comp Neurol 153:39–58

Cragg BG (1970) What is the signal for chromatolysis? Brain Res 23: 1–21

Cuello AC, Gamse R, Holzer P, Lembeck F (1981) Substance P immunoreactive neurons following neonatal administration of capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 315:185–194

Del Fiacco M, Cuello AC (1980) Substance P and enkephalin-containing neurones in the rat trigeminal system. Neuroscience 5:803–815

Forssmann WG (1978) A new somatostatinergic system in the mammalian spinal cord. Neurosci Lett 10:293–297

Gamse R, Molnar A, Lembeck F (1979) Substance P release from spinal cord slices by capsaicin. Life Sci 25:629–636

Gamse R, Holzer P, Lembeck F (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br J Pharmacol 68:207–213

Gamse R, Lackner D, Gamse G, Leeman SE (1981a) Effect of capsaicin pretreatment on capsaicin-evoked release of immunoreactive somatostatin and substance P from primary sensory neurons. Naunyn-Schmiedeberg's Arch Pharmacol 316:38–41

Gamse R, Wax A, Zigmond RE, Leeman SE (1981b) Immunoreactive substance P in sympathetic ganglia: Distribution and sensitivity towards capsaicin. Neuroscience 6:437–441

Gilbert RFT, Emson PC, Fahrenkrug G, Lee CM, Penman E, Wass J (1980) Axonal transport of neuro-peptides in the cervical vagus nerve of the rat. J Neurochem 34:108–113

Godfraind JM, Jessell TM, Kelly JS, McBurney RN, Mudge AW, Yamamoto M (1981) Capsaicin prolongs action potential duration in cultured sensory neurones. J Physiol 312:32P

Hayes AG, Tyers MB (1980) Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. Brain Res 189:561–564

Henry JL, Sessle BJ, Lucier GE, Hu JW (1980) Effects of substance P on nociceptive and non-nociceptive trigeminal brain stem neurons. Pain 8:33–45

Hökfelt T, Elde R, Johansson O, Luft R, Arimura A (1975) Immunohistochemical evidence for the presence of somatostatin, a powerful inhibitory peptide, in some primary sensory neurons. Neurosci Lett 1:231–235

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 afferent neurons in the rat. Neuroscience 1:131–136

Hökfelt T, Ljungdahl A, Terenius L, Elde R, Nilsson G (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: Enkephalin and substance P. Proc Natl Acad Sci USA 74:3081–3085

Holzer P, Gamse R, Lembeck F (1980) Distribution of substance P in the rat gastrointestinal tract – lack of effect of capsaicin pretreatment. Eur J Pharmac 61:303–307

Hosobuchi Y (1980) The majority of unmyelinated afferent axons in human ventral roots probably conduct pain. Pain 8:167–180

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

Jancsó G, Király E (1980) Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. J Comp Neurol 190:781–792

Jancsó G, Király E, Jancsó-Gábor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270:741–743

Jancsó G, Király E, Jancsó-Gábor A (1980) Chemosensitive pain fibres and inflammation. Int J Tiss React II:57–66

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

Jessell T, Tsunoo A, Kanazawa I, Otsuka M (1979) Substance P: Depletion in the dorsal horn of rat spinal cord after section of the peripheral processes of primary sensory neurons. Brain Res 168: 247–260

Joó F, Szolcsányi J, Jancsó-Gábor A (1969) Mitochondrial alterations in the spinal ganglion cells of the rat accompanying induced by capsaicin. Life Sci 8:621–626

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

Kobayashi RM, Brown M, Vale W (1977) Regional distribution of neurotensin and somatostatin in rat brain. Brain Res 126:584–588

Lembeck F, Donnerer J (1981) Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. Naunyn-Schmiedeberg's Arch Pharmacol 316: 240–243

Longhurst JC, Mitchell JH, Moore MB (1980) The spinal cord ventral root: An afferent pathway of the hind-limb pressor reflex in cats. J Physiol 301:467–476

Lundberg JM, Hökfelt T, Nilsson G, Terenius L, Rehfeld J, Elde R, Said S (1978) Peptide neurons in the vagus splanchnic and sciatic nerves. Acta Physiol Scand 104:499–501

Lundberg JM, Hökfelt T, Änggard A, Uvnäs-Wallenstein K, Brimijoin S, Brodin E, Fahrenkrug J (1980) Neural peptides and neural communication. In: Costa E, Trabucchi (eds) Peripheral peptide neurons: distribution, axonal transport and some aspects on possible functions. Raven Press, New York, pp 25–36

Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Analyt Biochem 87:206–210

Maynard CW, Leonard RB, Coulter JD, Coggeshall RE (1977) Central connections of ventral root afferents as demonstrated by the HRP method. J Comp Neurol 172:601–608

Mroz E, Leeman SE (1979) Substance P. In: Jaffe BM, Behrmann MR (eds) Methods of hormone radioimmunoassay. Academic Press, New York, pp 121–137

Nagy JI, Vincent SR, Staines WA, Fibiger HC, Reisine TD, Yamamura HI (1980) Neurotoxic action of capsaicin on spinal substance P neurons. Brain Res 186:435–444

Scadding JW (1980) The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. J Anat 131:473–484

Seybold JW, Elde R (1980) Immunohistochemical studies of peptidergic neurons in the dorsal horn of the spinal cord. J Histochem Cytochem 28:367–370

Szolcsányi J (1977) A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiol (Paris) 73:251–259

Szolcsányi J, Joó F, Jancsó-Gábor A (1971) Mitochondrial changes in preoptic neurones after capsaicin desensitization of the hypothalamic thermodetectors in rats. Nature 229:116–117

Szolcsányi J, Jancsó-Gabor A, Joó F (1975) Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 287:157–169

Tessler A, Glazer E, Artymyshyn R, Murray M, Goldberger ME (1980) Recovery of substance P in the cat spinal cord after unilateral lumbosacral deafferentation. Brain Res 191:459–470

Theriault E, Otsuka M, Jessell T (1979) Capsaicin-evoked release of substance P from primary sensory neurons. Brain Res 170:209–213

Uhl GR, Kuhar MJ, Snyder SH (1977a) Neurotensin: Immunohistochemical localization in rat central nervous system. Proc Natl Acad Sci USA 74:4059–4063

Uhl GR, Bennett JP, Snyder SH (1977b) Neurotensin, a central nervous system peptide: Apparent receptor binding in brain membranes. Brain Res 130:299–313

Yaksh TL, Farb DH, Leeman SE, Jessell TM (1979) Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science 206:481–483

Zingg HH, Patel YC (1979) Somatostatin precursors: Evidence for presence in and release from rat median eminence and neurohypophysis. Biochem Biophys Res Commun 90:466–472

Received May 4/Accepted June 9, 1981