ELSEVIER

European Journal of Pharmacology 399 (2000) 251–258

ejp

www.elsevier.nl/locate/ejphar

Systemic anti-inflammatory effect of somatostatin released from capsaicin-sensitive vagal and sciatic sensory fibres of the rat and guinea-pig

Márta Thán [a], József Németh [b], Zoltán Szilvássy [c], Erika Pintér [a], Zsuzsanna Helyes [b], János Szolcsányi [a,b,*]

[a] *Department of Pharmacology and Pharmacotherapy, University Medical School of Pécs, H-7601 P.O.Box 99, Pécs, Hungary*
[b] *Neuropharmacology Research Group of the Hungarian Academy of Sciences, H-7601 P.O.Box 99, Pécs, Hungary*
[c] *Department of Pharmacology, University Medical School of Debrecen, H-4032 P.O.Box 12, Debrecen, Hungary*

Received 23 December 1999; received in revised form 26 April 2000; accepted 27 April 2000

Abstract

The systemic anti-inflammatory effect induced by antidromic sensory nerve stimulation was investigated in rats and guinea-pigs. In atropine-pretreated rats, bilateral antidromic stimulation of vagal afferent fibres (8 Hz, 20 min, at C-fibre strength) inhibited plasma extravasation induced by 1% mustard oil on the acutely denervated hindlegs by $36.45 \pm 3.95\%$. Both the prevention of this inhibitory effect by cysteamine pretreatment and the stimulation-evoked rise of plasma somatostatin-like immunoreactivity in the two species suggest a mediator role of neural somatostatin. Since this response was blocked by systemic capsaicin pretreatment and slightly reduced after subdiaphragmal vagotomy, participation of thoracic capsaicin-sensitive afferents is indicated. In guinea-pigs pretreated with guanethidine and pipecuronium, antidromic sciatic nerve stimulation induced $45.46 \pm 5.08\%$ inhibition on the contralateral leg and increased plasma somatostatin-like immunoreactivity. It is concluded that somatostatin released from the activated vagal capsaicin-sensitive sensory nerve terminals of the rat and somatic nerves of the guinea-pigs exerts a systemic humoral function.

Keywords: Vagal afferent; Somatostatin; Capsaicin; Mustard oil; Antidromic stimulation; Anti-inflammatory effect

1. Introduction

It has been shown that activation of capsaicin-sensitive primary afferent nerve terminals results in vasodilatation and plasma extravasation in the relevant innervated areas (Holzer, 1992; Jancsó et al., 1967; Lundberg et al., 1983; McDonald, 1988; McDonald et al., 1988; Pintér and Szolcsányi, 1995; Szolcsányi, 1984a,b; Szolcsányi, 1996a). The local response, defined as antidromic vasodilatation and neurogenic inflammation (Jancsó et al., 1967; Szolcsányi, 1996a), is underlain by the release of sensory neurotransmitters such as tachykinins (neurokinin A, substance P) and calcitonin gene-related peptide (CGRP) (Holzer, 1992; Szolcsányi, 1996a,b). Evidence has been provided by our previous findings that, beyond evoking a local inflammatory reaction, antidromic electrical or orthodromic chemical stimulation of capsaicin-sensitive sensory nerve fibers (dorsal roots, sciatic nerve) elicits a systemic anti-inflammatory effect as well (Pintér and Szolcsányi, 1996; Szolcsányi et al., 1998a,c). The development of this unorthodox systemic humoral response was found to be due to somatostatin release from sensory nerve terminals activated by sciatic nerve stimulation or the cutaneous application of chemical irritants (Szolcsányi, 1996b; Szolcsányi et al., 1998a,c). Since these results provided clear evidence for this novel neuroregulatory mechanism in exteroceptive areas only, the aim of the present work is to study whether the systemic anti-inflammatory effect and the release of somatostatin could also be evoked through the activation of interoceptors, namely by stimulation of the sensory vagal nerve fibres.

* Corresponding author. Department of Pharmacology and Pharmacotherapy, Neuropharmacology Research Group of Hungarian Academy of Sciences, University Medical School of Pécs, H-7601 P.O. Box 99, 7643 Pécs, Hungary. Tel.: +36-72-324-122/1601; fax: +36-72-211-761.
E-mail address: szolcs@apacs.pote.hu (J. Szolcsányi).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00341-1

Substance P- and CGRP-immunoreactive afferent fibres of the trachea originate from the vagal nerve, while the stem bronchi and intrapulmonary airways have a crossed afferent innervation of both vagal and thoracic spinal origin (Lundberg et al., 1983; McDonald et al., 1988; Springall et al., 1987). There is clear evidence that, beyond their classical afferent function, sensory vagal nerve fibres also have a local effector function, which plays an important role in some disease processes (asthma bronchiale) (Lundberg et al., 1983; Szolcsányi, 1996a,b).

All these data made it interesting to examine whether bilateral antidromic electrical stimulation of the cut vagal nerves could evoke a systemic anti-inflammatory action and to analyse its neuromediator background. Furthermore, since all of our previous results were obtained with rats, we investigated somatostatin-mediated systemic anti-inflammatory adaptation in guinea-pigs, as another species.

2. Methods

2.1. Animals

The experiments were carried out on female Wistar rats and guinea-pigs weighing 200–250 and 400–500 g, respectively. The animals were kept in the Laboratory Animal Center of the Medical University of Pécs under pathogen-free conditions (12-h light/dark periods, temperature of 22–25°C, humidity of 50–70%) and had standard rat and guinea-pig chow and tap water ad libitum.

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied was approved by the local ethical committee of Medical School of Pécs, Hungary.

2.2. Surgical procedures and experimental arrangements in rats

2.2.1. Vagal nerve stimulation

The rats were anaesthetized with sodium thiopentone (100 mg/kg, i.p.). Following anaesthesia, both vagal nerves were exposed in the neck, a pool was made and filled with liquid paraffin. The peripheral stumps of the cut vagal nerves were placed on two pairs of platinum hook-electrodes and stimulated (20 V, 1 ms, 8 Hz, 20 min or 20 V, 0.5 ms, 5 Hz, 5 min). In control cases, the nerves were only cut without stimulation. Atropine sulphate (2 mg/kg, i.v.) was given 10 min before vagal nerve excitation to prevent parasympathetic responses. In one set of experiments, hexamethonium bromide (5 mg/kg, i.v.) was also injected 10 min before stimulation to exclude neuropeptide release mediated by activation of autonomic ganglia. For selective immunological and functional inactivation of somatostatin, cysteamine (280 mg/kg, s.c.) was given 4 h prior to nerve stimulation (Palkovits et al., 1982; Patel and Pierzchala, 1985; Szolcsányi et al., 1998a). The effect of this stimulation under these conditions was analysed on chemically induced neurogenic inflammation in the skin of the hindpaws, and on plasma somatostatin-like immunoreactivity (see experimental groups below).

2.2.2. Induction of cutaneous neurogenic inflammation

The left jugular vein was cannulated for drug administration and one of the carotid arteries was cannulated for blood sampling. At the time when electrical stimulation of the vagal nerves started, both acutely denervated hindlegs were smeared with 1% mustard oil dissolved in paraffin oil and the animals were exsanguinated 20 min later (Szolcsányi et al., 1998c). Plasma extravasation was determined by the Evans blue accumulation method.

2.3. Surgical procedures and experimental arrangements in guinea-pigs

2.3.1. Vagal nerve stimulation

The guinea-pigs were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.). In experiments with bilateral antidromic vagal nerve excitation (20 V, 0.5 ms, 5 Hz, 5 min), surgical procedures were performed as described for experiments in rats. The guinea-pigs were also pretreated with atropine sulphate (2 mg/kg, i.v.) 10 min before vagal nerve stimulation to prevent parasympathetic responses, and the alterations of the plasma somatostatin-like immunoreactivity in response to the stimulation were analysed (see experimental groups below).

2.3.2. Stimulation of the sciatic nerve

Stimulation of the peripheral stump of the cut sciatic nerves of the anesthetized guinea-pigs was performed (30 V, 0.5 ms, 20 Hz, 20 min) as described for rats (Szolcsányi et al., 1998a). Guanethidine (8 mg/kg, i.p.) was given 1 h before nerve excitation to counteract the vascular effects of concomitant sympathetic nerve activation. Pipecuronium bromide (200 μg/kg, i.v.) was injected to block neuromuscular transmission and positive pressure ventilation was carried out through a T-tracheal tube connected to a small animal respirator (KUTESZ, Budapest, Hungary) that was supplied with room air. The tidal volume was set for 10 ml/kg and the respiratory rate was adjusted to 50–70 cycles/min.

Stimulation of the right sciatic nerve was followed by stimulation of the left one with an inter-stimulation interval of 5 min and the animals were killed by exsanguination 15 min following the second excitation. Plasma extravasation was determined with Evans blue.

In one set of experiments, we analysed the changes in plasma somatostatin-like immunoreactivity in response to electrical stimulation (30 V, 0.5 ms, 5 Hz, 5 min) of one

sciatic nerve. For this purpose, one of the carotid arteries was cannulated for blood sampling.

2.4. Determination of plasma extravasation

Plasma extravasation was determined by the Evans blue accumulation method. Evans blue dye was given (50 mg/kg, i.v.) 10 min before the inflammatory stimulus and the dye accumulated in the skin samples was determined by spectrophotometry at 620 nm (Spectromom 195, MOM, Budapest, Hungary). The results were expressed as μg dye/g wet tissue weight (Szolcsányi et al., 1998a,c).

2.5. Determination of plasma somatostatin-like immunoreactivity in rats and guinea-pigs

The plasma somatostatin-like immunoreactivity in response to bilateral antidromic stimulation of the vagal nerves (20 V, 0.5 ms, 5 Hz, 5 min) was measured in both species after 12-h starvation. In guinea-pigs, changes of plasma somatostatin-like immunoreactivity provoked by antidromic stimulation (30 V, 0.5 ms, 5 Hz, 5 min) of one sciatic nerve were analysed. Two minutes after the stimulation period, arterial blood samples (3 ml/animal) were taken into ice-cold tubes containing EDTA (6 mg) and Trasylol (1000 U). Plasma somatostatin-like immunoreactivity was measured with a specific and sensitive radioimmunoassay (RIA) (Németh et al., 1996; Szolcsányi et al., 1998a,c).

2.6. Systemic and perineural capsaicin pretreatment

One group of rats received systemic capsaicin pretreatment. Capsaicin was administered (in doses of 30, 60 and 90 mg/kg, s.c.) on 3 consecutive days under anaesthesia (sodium pentobarbitone, 40 mg/kg, i.p.). Bilateral antidromic vagal nerve stimulation and blood sampling for somatostatin-like immunoreactivity measurement was performed 3 days after the last dose was given.

Anaesthetized guinea-pigs received perineural capsaicin pretreatment. A small piece of fibrin sponge (Gelaspon), soaked with 2% capsaicin solution was applied around the sciatic nerve for 30 min. After removal of the cuff, the muscle and the skin were sutured and the animals recovered within the next 2 h. The pretreated sciatic nerve was stimulated 5–6 days later for measurement of plasma somatostatin-like immunoreactivity.

2.7. Drugs and solutions

Sodium thiopentone was purchased from Byk Gulden (Konstanz, Germany), sodium pentobarbitone, somatostatin-14, cysteamine (2-mercaptoethylamine), Evans blue dye, [Tyr^1]somatostatin-(1-14), and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) were from Sigma (St. Louis, USA), atropine sulphate, lidocaine (2%) from Egis (Budapest, Hungary), pipecuronium bromide from Richter (Budapest, Hungary), hexamethonium bromide and mustard oil (allylisothiocyanat) from Fluka, (Buchs, Switzerland), ethanol, formamide and Tween 80 from Reanal (Budapest, Hungary). Capsaicin was dissolved in 10% ethanol, 10% Tween 80 and 80% saline (0.9% NaCl).

2.8. Statistical analysis

The data are expressed as means ± standard error of means (S.E.M.). Mann–Whitney's (non-parametric) *U*-test was used for statistical evaluation in the cases showing plasma extravasation. Plasma somatostatin-like immunoreactivity levels were analysed with an unpaired Student's *t*-test. Changes were considered significant at $P < 0.05$.

3. Results

3.1. Inhibitory effect of bilateral antidromic vagal nerve stimulation on chemically induced cutaneous neurogenic inflammation of the hindpaws in rats

In atropine-pretreated rats, the peripheral stump of both cut vagal nerves was electrically stimulated with 8 Hz for 20 min to induce neurogenic inflammation in the trachea, distal part of the oesophagus, and the mediastinal connective tissue (Szolcsányi, 1984a). The anti-inflammatory effect of this vagal stimulation was investigated on plasma extravasation evoked at the same time by local administration of 1% mustard oil on the hindpaws after acute denervation.

Plasma extravasation in the hindpaws was inhibited by 36.45 ± 3.95% by the simultaneous bilateral vagal excitation, as compared to the controls. In the second group of animals, the same experimental procedures were performed after intravenous hexamethonium (5 mg/kg) treatment. In this case, the inhibition induced by bilateral vagal nerve stimulation was 30.39 ± 2.86% ($n = 5–6$; Fig. 1a). In a subset of experiments, bilateral vagal nerve stimulation started 5 min prior to mustard oil application to the skin of the hindpaws. Under these conditions, plasma extravasation was inhibited by 54.05 ± 5.26% ($n = 5$).

In rats pretreated with cysteamine (280 mg/kg, s.c., 4 h before stimulation), the inhibitory action of bilateral vagal nerve stimulation (8 Hz, 20 min) on neurogenic inflammation in the hindpaws was reduced significantly by 82.37 ± 9.53% ($n = 5$; Fig. 1b).

3.2. Inhibitory effect of sciatic nerve stimulation on cutaneous plasma extravasation of the contralateral hindleg in guinea-pigs

The distal stump of the cut sciatic nerves of the guanethidine and pipecuronium pretreated animals was

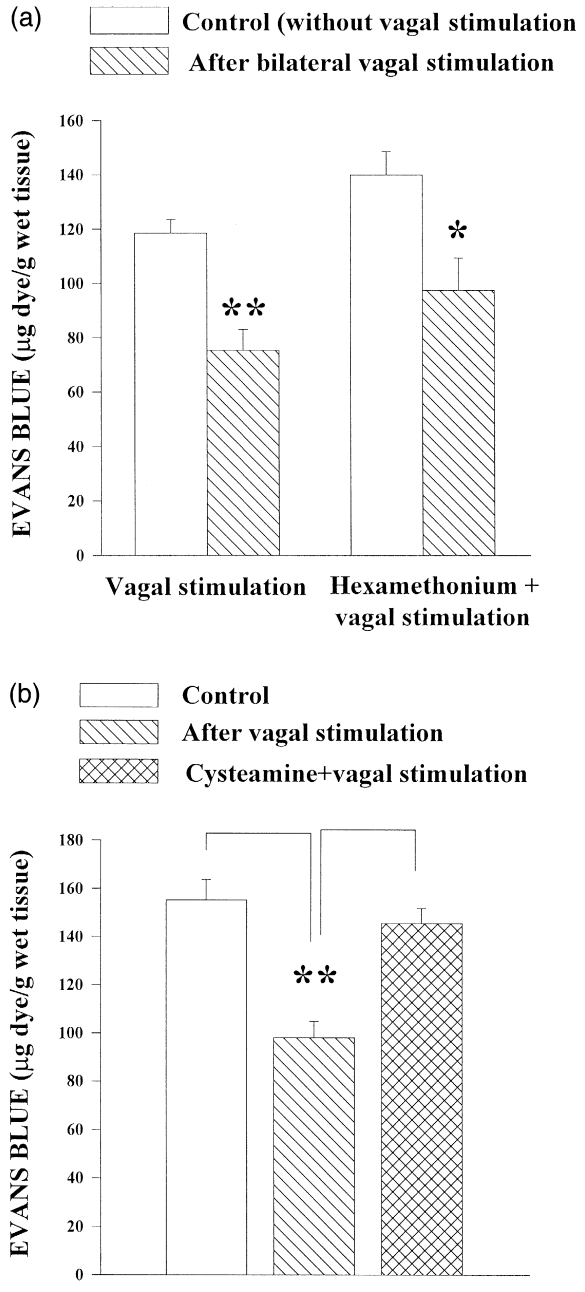

Fig. 1. (a) Cutaneous neurogenic inflammation evoked by 1% mustard oil smeared on the skin of rat hindpaws simultaneously with bilateral antidromic stimulation (20 V, 1 ms, 8 Hz, 20 min) of the peripheral stump of the cut vagal nerve, and in control rats. Rats were treated with atropine sulphate (2 mg/kg, i.v.) or with atropine plus hexamethonium bromide (5 mg/kg, i.v.) 10 min before the stimulation. (b) The inhibitory action of the bilateral antidromic vagal nerve stimulation was reduced by cysteamine (280 mg/kg, s.c.) pretreatment, 4 h before the experiment. Results are shown as means ± S.E.M.; $n = 5–6$; $^{*}P < 0.05$, $^{**}P < 0.01$.

stimulated with 20 Hz for 20 min. Stimulation of the right sciatic nerves was followed by similar stimulation of the left nerve 5 min later. The secondary response was inhibited by 45.46 ± 5.08% compared to the primary reaction ($n = 8$; Fig. 2), a value which was similar to that found in rats on sciatic nerve stimulation (Szolcsányi et al., 1998a).

3.3. Effect of bilateral antidromic vagal nerve stimulation on the level of plasma somatostatin-like immunoreactivity in rats — prevention of somatostatin release by systemic capsaicin pretreatment

In these experiments, the rats were divided into six groups. In the control group, the vagal nerves were only cut in the neck but not stimulated (group 1). In the stimulated group, bilateral antidromic vagal nerve stimulation was performed (20 V, 0.5 ms, 5 Hz, 5 min; group 2). In the third group of animals, the abdominal branches of both vagal nerves were cut above the cardia combined with perioesophageal administration of lidocaine (2%) and ethanol (96%) 30 min before the electrical stimulation of the peripheral stumps. The purpose of the abdominal vagotomy was to exclude the gastrointestinal release of somatostatin in response to nerve stimulation (group 3). In a separate group of animals, the abdominal surgery was performed but the abdominal vagal nerves were left intact. Cervical vagal stimulation started 30 min after abdominal surgery (group 4). In the sham-operated controls, only the abdominal surgical procedure was performed without cutting and stimulating the nerves (group 5). For rats, there was a sixth capsaicin-pretreated group (group 6).

Bilateral vagal nerve stimulation (5 Hz, 5 min) resulted in a 9.9-fold increase of plasma somatostatin-like immunoreactivity level in group 2 (30.5 ± 1.65 fmol/ml), compared to the control rats (group 1; 7.88 ± 1.56 fmol/ml). In the abdominal vagotomised rats (group 3), plasma so-

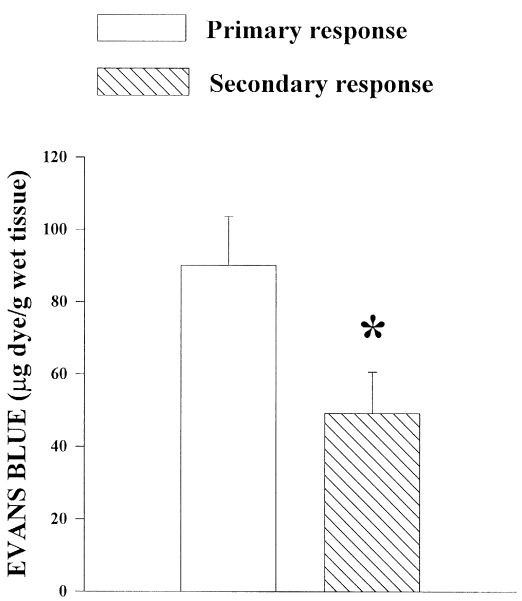

Fig. 2. Plasma extravasation in the skin of the guanethidine (8 mg/kg, i.p.) and pipecuronium bromide (200 μg/kg, i.v.)-pretreated guinea-pig hindpaw evoked by stimulation (30 V, 0.5 ms, 20 Hz, 20 min) of the peripheral stump of the cut sciatic nerve, and its inhibitory effect on the subsequent neurogenic inflammation evoked by stimulation of the contralateral sciatic nerve 5 min later with the same parameters. Results are shown as means ± S.E.M.; $n = 8$; $^{*}P < 0.01$.

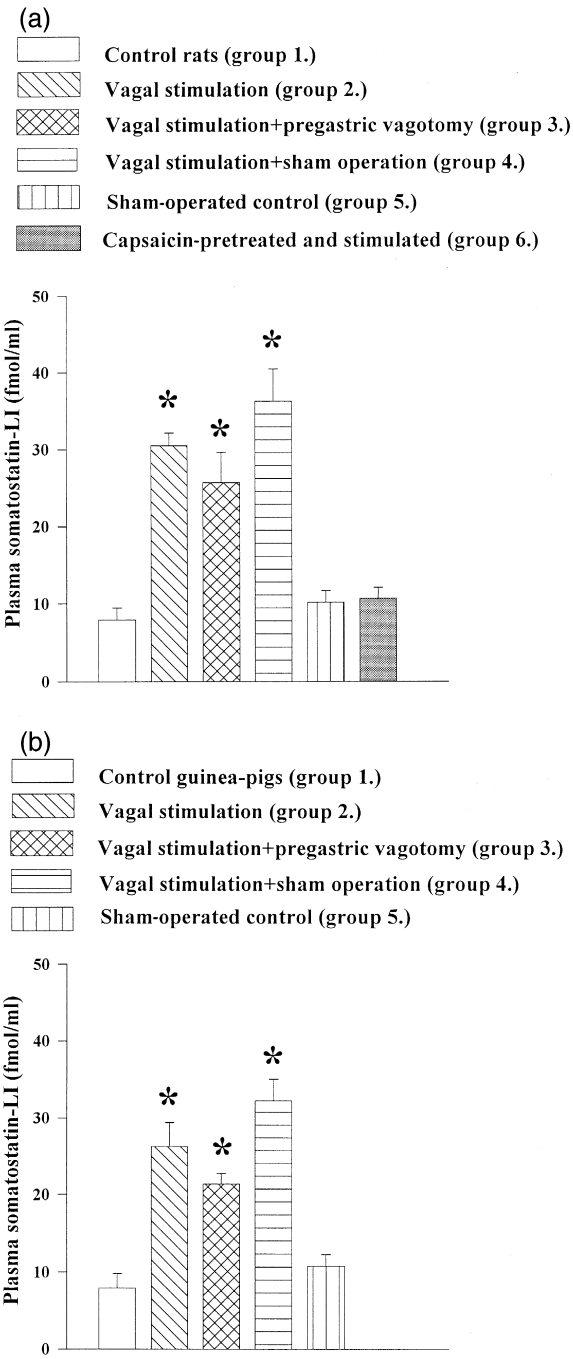

Fig. 3. Effect of bilateral antidromic vagal nerve stimulation (20 V, 0.5 ms, 5 Hz, 5 min) on plasma somatostatin-like immunoreactivity (-LI) in rats (a) and in guinea-pigs (b). Plasma samples were taken 2 min after the stimulation. The concentration of plasma somatostatin-like immunoreactivity was significantly elevated in the vagus-stimulated (group 2), abdominal vagotomised and stimulated (group 3), sham-operated and stimulated animals (group 4), compared to the controls (group 1). No significant elevation was observed after sham operation (group 5) or after vagal stimulation in capsaicin-pretreated animals (group 6). Results are shown as means ± S.E.M.; $n = 6–7$; $^{*}P < 0.01$.

matostatin-like immunoreactivity increased 3.3-fold (25.73 ± 3.92 fmol/ml). In the sham-operated stimulated group (group 4), the increase was 4.6-fold (36.3 ± 4.18 fmol/ml), and in the sham-operated controls (group 5), the basal plasma somatostatin-like immunoreactivity level was slightly but not significantly elevated (10.16 ± 1.51 fmol/ml). Systemic capsaicin pretreatment (group 6) prevented the significant increase of plasma somatostatin-like immunoreactivity level (10.66 ± 1.45 fmol/ml) ($n = 6$; Fig. 3a).

3.4. Effect of bilateral antidromic vagal nerve stimulation on the level of plasma somatostatin-like immunoreactivity in guinea-pigs

The five experimental groups used (Fig. 3b) were similar to those with rats. Bilateral antidromic vagal nerve stimulation (5 Hz, 5 min) elevated the plasma somatostatin-like immunoreactivity level 3.3-fold (26.26 ± 3.15 fmol/ml; group 2), compared to the control animals (group 1; 7.93 ± 1.87 fmol/ml). In the subdiaphragmal vagotomised animals (group 3), the plasma somatostatin-like immunoreactivity level increased 2.7-fold (21.35 ± 1.38 fmol/ml) and in sham-operated stimulated (group 4) animals without vagotomy, the increase was 4.1-fold (32.27 ± 2.81 fmol/ml). In sham-operated controls (group 5), the basal plasma somatostatin-like immunoreactivity level in-

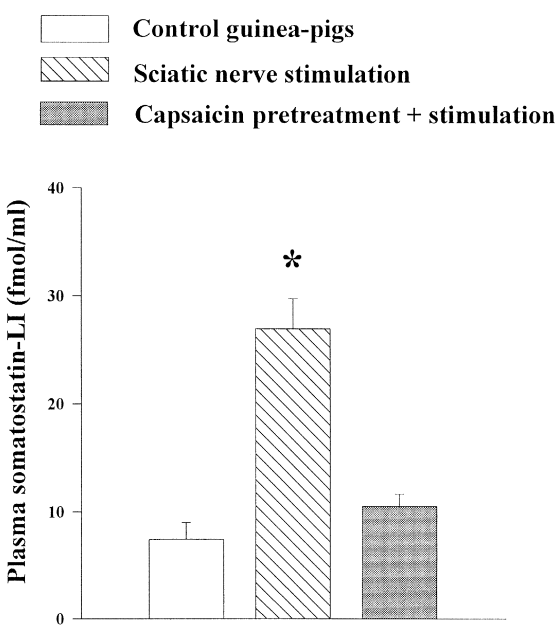

Fig. 4. Plasma somatostatin-like immunoreactivity (-LI) level in guinea-pigs 2 min after stimulation of the peripheral stump of the cut sciatic nerve (30 V, 0.5 ms, 5 Hz, 5 min), and in control and capsaicin-pretreated animals. Perineural administration of capsaicin around the sciatic nerve (2% solution, 30 min) was performed 5–6 days before the experiment. All guinea-pigs were pretreated with guanethidine (8 mg/kg, i.p.) 1 h before nerve excitation and pipecuronium bromide (200 μg/kg, i.v.). Antidromic stimulation of the cut sciatic nerve caused a significant increase in plasma somatostatin-like immunoreactivity compared to the controls, which was prevented by perineural capsaicin pretreatment. Results are shown as means ± S.E.M.; $n = 5–6$, $^{*}P < 0.01$.

creased slightly (10.75 ± 1.49 fmol/ml), but this elevation was not significant compared to the controls ($n = 6–7$; Fig. 3b).

3.5. Changes in plasma somatostatin-like immunoreactivity level in response to sciatic nerve stimulation in guinea-pigs — effect of perineural capsaicin pretreatment

Antidromic electrical stimulation of one sciatic nerve (30 V, 0.5 ms, 5 Hz, 5 min) caused a significant (3.5-fold) increase in plasma somatostatin-like immunoreactivity level in guinea-pigs (26.98 ± 2.79 fmol/ml), compared to the controls (7.45 ± 1.58 fmol/ml). Perineural 2% capsaicin pretreatment almost completely prevented the stimulation-induced release of somatostatin (10.54 ± 1.12 fmol/ml; $n = 5–6$; Fig. 4).

3.6. Circulatory parameters

During anaesthesia and pretreatments, the average heart rate and mean arterial blood pressure did not show significant alterations compared to the baseline in either rats (337 ± 42 bpm and 92 ± 5.8 mm Hg) or guinea-pigs (312 ± 29 bpm and 75 ± 4.4 mm Hg).

4. Discussion

It has been reported that, besides their classical afferent function, sensory vagal nerve fibres contain neuropeptides in their terminals, and that release of these neuromediators elicits local effector responses consisting of vasodilatation, increased vascular permeability of venules with consequent protein extravasation and bronchoconstriction (Jancsó et al., 1967; Lundberg et al., 1983; McDonald, 1988; McDonald et al., 1988; Pintér and Szolcsányi, 1995; Szolcsányi, 1984a,b; Szolcsányi, 1996a).

A sensory nerve-mediated anti-inflammatory effect with a possible adaptive function has been shown by our group, using several experimental conditions with rats (Pintér and Szolcsányi, 1996; Szolcsányi et al., 1998a,c). In these studies, somatostatin release with concomittant anti-inflammatory effect was evoked by (1) antidromic stimulation of the dorsal roots, (2) antidromic stimulation of the sciatic nerve, and (3) excitation of nociceptors by chemical irritants. Furthermore, evidence was obtained that the mediators of the systemic anti-oedema effect were derived from the activated nerve endings, and not from the inflammatory exudate. All of these experiments were carried out on rats and involved activation of nociceptors only in exteroceptive skin areas, but did not test for a similar function of vagal interoceptors.

The present study provided evidence for the first time that excitation of capsaicin-sensitive sensory nerve fibres of the rat vagal nerves elicits not only a local effector response, but a systemic anti-inflammatory effect as well, via somatostatin release from the activated nerve endings. We also reproduced the response in guinea-pigs. A possible contribution of autonomic efferent fibres in this inhibitory action was eliminated by the full development of the response under the effect of hexamethonium, atropine and guanethidine, as well as the blocking effect of capsaicin pretreatment on the release of the proposed mediator, somatostatin. Further, as in the case of antidromic stimulation of the sciatic nerve (Szolcsányi et al., 1998a) or orthodromic excitation of nociceptors by mustard oil (Szolcsányi et al., 1998c), the anti-inflammatory effect evoked by vagal stimulation was also prevented in rats pretreated with cysteamine. Cysteamine is a sulphydryl agent which induces a selective loss, at the dose range applied, of both biologically and immunologically active somatostatin by forming disulphide bonds with the peptide without affecting the levels of encephalin, luteinizing hormone–releasing hormone (LH–RH), vasopressin, vasoactive intestinal polypeptide or cholecystokinin (Palkovits et al., 1982; Patel and Pierzchala, 1985). Prevention by this agent of the appearance of somatostatin-like immunoreactivity in plasma, in response to sensory nerve stimulation, was demonstrated in our previous studies (Szolcsányi et al., 1998a,c).

A pronounced enhancement of somatostatin-like immunoreactivity in the plasma was now shown in response to vagal nerve stimulation in atropine-pretreated rats and guinea-pigs. The respective 3.9-fold and 3.3-fold increases in somatostatin-like immunoreactivity level were only slightly diminished after subdiaphragmal vagotomy (3.3-fold and 2.7-fold, respectively). Consequently, the vagal nerve terminals of thoracic organs are the main sources of the enhancement of this neuropeptide level in the circulating plasma. Systemic pretreatment of adult rats with capsaicin completely prevented this increase of somatostatin-like immunoreactivity in the plasma, providing evidence for the mediating role of capsaicin-sensitive vagal sensory fibres. Capsaicin excites thoracic chemoceptive vagal C-afferents in the heart, epicardium, great vessels, bronchi and lung including the pulmonary J-receptors (Coleridge and Coleridge, 1984; Szolcsányi, 1993). On the other hand, cardiovascular and pulmonary, slowly or rapidly adapting vagal mechanoreceptors, aortic body chemoreceptors, are not stimulated by capsaicin (Coleridge and Coleridge, 1984; Szolcsányi, 1993). Since the long-lasting sensory blocking effect of capsaicin in rats pretreated as adults is restricted to those afferents, which are excited by low doses, the subset of sensory receptors as sites of the released somatostatin could be delineated on the basis of these single-unit studies (Coleridge and Coleridge, 1984; Szolcsányi, 1993). It is worth mentioning that field stimulation of the rat isolated trachea elicits the release of somatostatin into the organ bath and capsaicin-induced release of somatostatin, CGRP and substance P from this preparation is not inhibited by lidocaine or tetrodotoxin (Szolcsányi et al., 1998b), providing clear evidence for the

existence of a sensory neuropeptide releasing process without axon reflexes (Szolcsányi, 1996a,b; Szolcsányi et al., 1998b).

These data, and our previous results, proving the systemic release and humoral effects of somatostatin, establish a novel ''endocrine-like'' or ''systemic efferent'' function of some capsaicin-sensitive sensory nerve endings (Pintér and Szolcsányi, 1996; Szolcsányi, 1996b; Szolcsányi et al., 1998a,b,c; Thán et al., 1999).

Somatostatin released from the hypothalamus serves as a neuroendocrine hormone, with a growth hormone-release inhibitor effect, while released from the gastric D-cells, it has a paracrine function through inhibition of acid secretion (Shubert et al., 1987). To differentiate the sensory nerve-mediated release process of somatostatin from the already described endocrine and paracrine mechanisms, the term ''sensocrine'' function would seem suitable. Sensory neuropeptides, other than somatostatin, are probably released and also serve as sensocrine hormones when the vagal nerves are stimulated, since although cysteamine completely prevented the rise in plasma somatostatin-like immunoreactivity level evoked by sciatic nerve stimulation, about 17% of the anti-inflammatory response to vagal nerve stimulation persisted after this treatment. In the case of sciatic nerve stimulation or cutaneous mustard oil application, both responses were completely abolished (Szolcsányi et al., 1998a,c). These observations are consistent with the immunohistochemical data, which indicate that, of the dorsal root ganglion cells innervating the rat skin, about 20% are somatostatin-like immunoreactive (Lawson, 1996), while in the nodosal, jugular and petrosal ganglia somatostatin-like immunopositive cells seems to be less common (Helke and Hill, 1988) and sparse among those which innervate the rat stomach (Green and Dockray, 1988).

The inhibitory effect of sensory nerve-mediated somatostatin certainly interacts at the release site with the neurogenic inflammation elicited by co-release of substance P and CGRP. This aspect seems to be relevant for consideration, the recently introduced concept (Holzer and Maggi, 1998), suggesting the existence of neurones in the dorsal root ganglia without sensory function in contrast to the gastric vagal chemoreceptive sensory fibres proposed to be without an efferent function. The pronounced release of somatostatin mainly from thoracic, but also from subdiaphragmal vagal fibres, clearly indicates the release of an inhibitory neuropeptide from vagal capsaicin-sensitive nerve terminals.

Acknowledgements

The authors thank Mrs. Csilla Zádor and Mrs. Mária Zsoldos for technical assistance. This study was supported by the Hungarian Academy of Sciences and Hungarian Research Grants OTKA: T-029428, T-030766, T-032002 and ETT: T-04032/99 and T-03031/99.

References

Coleridge, J.C.G., Coleridge, H.M., 1984. Afferent vagal C fibre innervation of the lung and airways and its functional significance. Rev. Physiol. Biochem. Pharmacol. 99, 1–110.

Green, T., Dockray, G.J., 1988. Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. Neuroscience 25, 181–193.

Helke, C.J., Hill, K.M., 1988. Immunohistochemical study of neuropeptides in vagal and glossopharyngeal afferent neurons in the rat. Neuroscience 26, 539–551.

Holzer, P., 1992. Peptidergic sensory neurones in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. Rev. Physiol. Biochem. Pharmacol. 121, 49–146.

Holzer, P., Maggi, C.A., 1998. Dissociation of dorsal root ganglion neurons into afferent and efferent-like neurons. Neuroscience 86, 389–398.

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

Lawson, S.N., 1996. Peptides and cutaneous polymodal nociceptor neurones. In: Kumazawa, T., Kruger, L., Mizumura, K. (Eds.), Progress in Brain Research vol. 113 Elsevier, Amsterdam, pp. 369–385.

Lundberg, J.M., Brodin, E., Saria, A., 1983. Effects and distribution of vagal capsaicin-sensitive substance-P neurons with reference to the trachea and lungs. Acta Physiol. Scand. 119, 243–252.

McDonald, D.M., 1988. Neurogenic inflammation in the rat trachea: Part I. Changes in venules, leukocytes and epithelial cells. J. Neurocytol. 17, 583–603.

McDonald, D.M., Mitchell, R.A., Gabella, G., Haskell, A., 1988. Neurogenic inflammation in the rat trachea: Part II. Identity and distribution of nerves mediating the increase in vascular permeability. J. Neurocytol. 17, 605–628.

Németh, J., Helyes, Zs., Pintér, E., Szolcsányi, J., 1996. Development of somatostatin radioimmunoassay for the measurement of plasma and tissue contents of hormone. Acta Physiol. Hung. 84, 221–223.

Palkovits, M., Brownstein, M.J., Eiden, L.E., Beinfeld, M.C., Russel, J., Arimura, A., Szabó, S., 1982. Selective depletion of somatostatin in rat brain by cysteamine. Brain Res. 240, 178–180.

Patel, Y.C., Pierzchala, I., 1985. Cysteamine induces a loss of tissue somatostatin-28 when measured as somatostatin-$28_{(11-28)}$-like immunoreactivity but not when assessed as somatostatin-$28_{(1-14)}$-like immunoreactivity: evidence for the importance of the disulfide bond for cysteamine action. Endocrinology 116, 1699–1702.

Pintér, E., Szolcsányi, J., 1995. Plasma extravasation in the skin and pelvic organs evoked by antidromic stimulation of the lumbosacral dorsal roots of the rat. Neuroscience 68, 603–614.

Pintér, E., Szolcsányi, J., 1996. Systemic anti-inflammatory effect induced by antidromic stimulation of the dorsal roots in the rat. Neurosci. Lett. 212, 33–36.

Shubert, M.L., Edwards, N.F., Arimura, A., Makhlouf, G.M., 1987. Paracrine regulation of gastric acid secretion by fundic somatostatin. Am. J. Physiol. 252, G485–490.

Springall, D.R., Cadieux, A., Oliveira, H., Su, H., Royston, D., Polak, J.M., 1987. Retrograde tracing shows that CGRP-immunoreactive nerves of rat trachea and lung originate from vagal and dorsal root ganglia. J. Auton. Nerv. Syst. 20, 155–166.

Szolcsányi, J., 1984a. Capsaicin and neurogenic inflammation: history and early findings. In: Chahl, L.A., Szolcsányi, J., Lembeck, F. (Eds.), Antidromic Vasodilatation and Neurogenic Inflammation. Akademiai Kiado, Budapest, pp. 7–25.

Szolcsányi, J., 1984b. Capsaicin-sensitive chemoceptive neural system

with dual sensory-efferent function. In: Chahl, L.A., Szolcsányi, J., Lembeck, F. (Eds.), Antidromic Vasodilatation and Neurogenic Inflammation. Akademiai Kiado, Budapest, pp. 27–56.

Szolcsányi, J., 1993. Actions of capsaicin on sensory receptors. In: Wood, J. (Ed.), Capsaicin in the Study of Pain. Academic Press, London, pp. 1–26.

Szolcsányi, J., 1996a. Neurogenic inflammation: reevaluation of axon reflex theory. In: Gepetti, G., Holzer, P. (Eds.), Neurogenic Inflammation. CRC Press, Boca Raton, USA, pp. 33–42.

Szolcsányi, J., 1996b. Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroregulatory mechanism. In: Kumazawa, T., Kruger, L., Mizumura, K. (Eds.), Progress in Brain Research vol. 113 Elsevier, Amsterdam, pp. 343–359.

Szolcsányi, J., Helyes, Zs., Oroszi, G., Németh, J., Pintér, E., 1998a. Release of somatostatin and its role in the mediation of the anti-inflammatory effect induced by antidromic stimulation of sensory fibres of rat sciatic nerve. Br. J. Pharmacol. 123, 936–942.

Szolcsányi, J., Németh, J., Oroszi, G., Helyes, Zs., Pintér, E., 1998b. Effect of capsaicin and resiniferatoxin on the release of sensory neuropeptides in the rat isolated trachea. Br. J. Pharmacol. Proc. Suppl. 124, 8 pp.

Szolcsányi, J., Pintér, E., Helyes, Zs., Oroszi, G., Németh, J., 1998c. Systemic anti-inflammatory effect induced by counter-irritation through a local release of somatostatin from nociceptors. Br. J. Pharmacol. 125, 916–922.

Thán, M., Németh, J., Helyes, Zs., Szilvássy, Z., Pintér, E., Szolcsányi, J., 1999. Somatostatin mediated systemic anti-inflammatory effect induced by antidromic vagal and sciatic nerve stimulation. Regul. Pept. 80, 137.