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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

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1 The effect of antidromic stimulation of the sensory fibres of the sciatic nerve on inflammatory plasma extravasation in various tissues and on cutaneous vasodilatation elicited in distant parts of the body was investigated in rats pretreated with guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium (200 μg kg^{-1}, i.v.).

2 Antidromic sciatic nerve stimulation with C-fibre strength (20 V, 0.5 ms) at 5 Hz for 5 min elicited neurogenic inflammation in the innervated area and inhibited by $50.3 \pm 4.67\%$ the development of a subsequent plasma extravasation in response to similar stimulation of the contralateral sciatic nerve. Stimulation at 0.5 Hz for 1 h also evoked local plasma extravasation and inhibited the carrageenin-induced (1%, 100 μl s.c.) cutaneous inflammation by $38.5 \pm 10.0\%$ in the contralateral paw. Excitation at 0.1 Hz for 4 h elicited no local plasma extravasation in the stimulated hindleg but still reduced the carrageenin-induced oedema by $52.1 \pm 9.7\%$ in the paw on the contralateral side.

3 Plasma extravasation in the knee joint in response to carrageenin (2%, 200 μl intra-articular injection) was diminished by $46.1 \pm 12.69\%$ and $40.9 \pm 4.93\%$ when the sciatic nerve was stimulated in the contralateral leg at 0.5 Hz for 1 h or 0.1 Hz for 4 h, respectively.

4 Stimulation of the peripheral stump of the left vagal nerve (20 V, 1 ms, 8 Hz, 10 min) elicited plasma extravasation in the trachea, oesophagus and mediastinal connective tissue in rats pretreated with atropine (2 mg kg^{-1}, i.v.), guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium (200 μg kg^{-1}, i.v.). These responses were inhibited by $37.8 \pm 5.1\%$, $49.7 \pm 9.9\%$ and $37.6 \pm 4.2\%$, respectively by antidromic sciatic nerve excitation (5 Hz, 5 min) applied 5 min earlier.

5 Pretreatment with polyclonal somatostatin antiserum (0.5 ml/rat, i.v.) or the selective somatostatin depleting agent cysteamine (280 mg kg^{-1}, s.c.) prevented the anti-inflammatory effect of sciatic nerve stimulation (5 Hz, 5 min) on a subsequent neurogenic plasma extravasation of the contralateral paw skin. The inhibitory effect of antidromic sciatic nerve excitation on plasma extravasation in response to vagal nerve stimulation was also prevented by somatostatin antiserum pretreatment.

6 Cutaneous blood flow assessment by laser Doppler flowmetry indicated that antidromic vasodilatation induced by sciatic nerve stimulation was not inhibited by excitation of the sciatic nerve of the contralateral leg (1 Hz, 30 min) or by somatostatin (10 μg/rat, i.v.) injection.

7 Plasma levels of somatostatin increased more than 4 fold after stimulation of both sciatic nerves (5 Hz, 5 min) but the stimulus-evoked increase was not observed in cysteamine (280 mg kg^{-1}, s.c.) pretreated rats.

8 These results suggest that somatostatin released from the activated sensory nerve terminals mediates the systemic anti-inflammatory effect evoked by stimulating the peripheral stump of the sciatic nerve.

Keywords: Capsaicin-sensitive primary afferent neurones; sciatic nerve; neurogenic inflammation; anti-inflammatory; carrageenin; somatostatin; cysteamine; antidromic vasodilatation

Introduction

Direct evidence for neurogenic inflammation evoked by antidromic stimulation of sensory fibres was presented thirty years ago in this journal (Jancsó *et al*., 1967). These findings suggest that beyond the old phenomenon of arteriolar 'antidromic vasodilatation' and 'flare reaction' (Bayliss, 1923; Lewis, 1927) venular plasma extravasation forms a cardinal sign of neurogenic inflammation. Subsequently it was realised that sensory neuropeptides such as substance P (SP) and calcitonin-gene related peptide (CGRP) released from these capsaicin-sensitive sensory nerves act on specific receptors in many organs to elicit a variety of smooth muscle and other tissue responses (Holzer, 1992; Maggi, 1995; Szolcsányi, 1996a,b; Lundberg, 1996; Geppetti & Holzer, 1996). Thus, the proposed concept (Szolcsányi, 1984) suggesting that capsaicin-sensitive sensory receptors, particularly the cutaneous C-polymodal nociceptors, subserve various local sensory-efferent functions, has been confirmed and extended with the identification of their regulopeptide mediators.

An unexpected observation made by our group (Pintér & Szolcsányi, 1988) raised the possibility that mediators released from capsaicin-sensitive nerve terminals possess not only local, but systemic neurohumoral effects as well (Szolcsányi, 1996b). In rats antidromic stimulation of dorsal roots inhibited the inflammatory response due to antidromic stimulation of the contralateral dorsal roots or that evoked by carrageenin or capsaicin (Pintér & Szolcsányi, 1996). The intensity of the secondary plasma extravasation was reduced by 50% and 40% when the time difference between the beginning of the two stimulations was 10 min and 20 min, respectively. This

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inhibitory action was not observed after 60 min (Pintér & Szolcsányi, 1988; Pintér & Szolcsányi, 1996).

The aim of the present series of experiments was to analyse further the systemic anti-inflammatory effect of antidromic stimulation of sensory fibres. Particular emphasis was put on clarifying the chemical nature of the released mediator, as well as revealing the effectiveness of low frequency stimulation and its anti-oedema action in the airways and the joint. The results support the functional significance of somatostatin, which is released into the blood circulation from the activated sensory nerve endings by antidromic stimulation, when acting at remote sites of the body as an anti-inflammatory agent.

Methods

Animals

The study was carried out on female Wistars rats weighing 200–250 g which were kept in the Laboratory Animal Centre of the University Medical School of Pécs under pathogen free conditions at 24–25°C and provided with standard rat chow and tap water *ad libitum*. The animals were anaesthetized with sodium thiopentone (100 mg kg^{-1}, i.p.) which in this single dose was sufficient to induce anaesthesia in pipecuronium-untreated rats for 5–6 h, as indicated by the absence of voluntary movements or blood pressure fluctuations (Pintér & Szolcsányi, 1996; Pintér *et al*., 1997b). All experimental procedures used in this study were in agreement with the rules of the Ethics Committe on Animal Research of the University Medical School of Pécs.

Experimental procedures

The sciatic nerve was exposed and cut in the thigh and the surrounding skin flaps were fixed to a metal ring to make a pool which was filled with liquid paraffin. The peripheral stump of the nerve was placed on a pair of platinum hook electrodes and stimulated with C-fibre strength of 20 V, 0.5 ms (Pintér & Szolcsányi, 1996). Guanethidine (8 mg kg^{-1}, i.p.) was injected 1 h before nerve stimulation for abolishing the vascular effects of the admixed sympathetic fibres (Pintér *et al*., 1997b) in the sciatic nerve. Pipecuronium bromide (200 μg kg^{-1}, i.v.) was given to block the neuromuscular transmission. One of the external jugular veins was cannulated to inject drugs and a T-tracheal tube was inserted for artificial respiration.

When plasma extravasation was induced by carrageenin injection (subplantar, 100 μl 1 % or inra-articular, 200 μl 2 %) both saphenous and sciatic nerves were cut in the thigh 30 min before the experiments to avoid nociceptive reflexes.

Plasma extravasation in the trachea, oesophagus and mediastinal connective tissue was evoked by stimulation of the peripheral stump of the left vagal nerve with 20 V, 1 ms, 8 Hz, 10 min. Atropine sulphate (2 mg kg^{-1}, i.v.) was given 10 min before the electrical stimulation to prevent parasympathetic responses.

Determination of plasma extravasation

Evans blue accumulation method was used to measure the extravasation of plasma albumin. Evans blue dye (50 mg kg^{-1}, i.v.) was given 30 min after the nerve had been cut. Rats were exsanguinated 20 min after the termination of the last inflammatory stimulus. For determination of the extent of inflammation in the sciatic nerve supply, the plantar and the lateral dorsal skin of the hindpaw was cut (Pintér & Szolcsányi, 1996). When the peripheral stump of the vagal nerve was stimulated the distal two-thirds of the oesophagus, the trachea and the mediastinal connective tissue around the great vessels with some parts of the thymus were removed. In the case of knee joint inflammation, the articular capsule with the ligaments and the swollen synovial tissue which showed intensive blueing were excised. The extravasated dye content of the tissues was extracted with formamide for 72 h at room temperature for photometric determination at 620 nm (Spectromom 195). The amount of accumulated Evans blue, which quantitatively correlates with the intensity of plasma extravasation, was expressed as μg dye g^{-1} wet tissue.

Measurement of cutaneous microcirculation

Microcirculatory changes in the plantar skin in response to antidromic stimulation of the sensory fibres in the sciatic nerve were measured by laser Doppler flowmeter (Moor Instruments MBF 3D, U.K.). Arterial blood pressure and heart rate were simultaneously recorded through an arterial cannula by a polygraph (Grass, model 7). Both devices were connected to an IBM compatible personal computer. The experimental data were evaluated by using a specific computer programme (Geopolita Ltd., Hungary). As laser Doppler flowmetry gives only relative flow values they were recorded in arbitrary units (AU). For quantitative evaluations the integrated blood flux responses (areas under the curve) were compared to the prestimulated control values and expressed as % changes of cutaneous blood flux as described in other studies (Escott & Brain, 1993; Holzer & Jocic, 1994).

Determination of plasma somatostatin concentration

Specific and sensitive radioimmunoassay (RIA) developed in our laboratory was used to measure plasma somatostatin levels (Németh *et al*., 1996) in response to bilateral antidromic sciatic nerve stimulation. In control cases both sciatic nerves were proximally cut but excitation was not performed. Blood samples (3 ml/rat) were taken into ice-cold glass tubes containing EDTA and Trasylol. This procedure was started 2 or 10 min after stimulation (5 Hz, 5 min) and lasted for 1.5 min. Following centrifugation at 4°C the peptide from the plasma was extracted by addition of 3 volumes of absolute alcohol. After precipitation and second centrifugation the samples were dried under nitrogen flow. The samples were resolved in assay buffer before RIA determinations.

Drugs

Sodium thiopentone was obtained from Byk (Germany), guanethidine, cysteamine (2-mercaptoethylamine), somatostatin-14, carrageenin, Evans blue and Tyr(1)-somatostatin-14 from Sigma, pipecuronium bromide from Richter (Hungary) and atropine sulphate from Egis Gyógyszergyár Rt. (Hungary). C-terminal sensitive somatostatin-14 antiserum was kindly provided by Dr Tamás Görcs (Dept. Anatomy, Semmelweis University Medical School, Budapest). This antiserum was raised against somatostatin-14-bovine thyroglobulin antigen coupled by glutaraldehyde in sheep. It was successfully used for the development of somatostatin radioimmunoasay 1 : 600000 dilution and proved to be able to bind both of the biologically active, 14 and 28 amino acid-containing, molecular forms (Németh *et al*., 1996). Serum from untreated sheep was used in control animals.

Statistical analysis

Data are presented as means ± s.e.mean. Non-parametric (Mann-Whitney) test was used for statistical evaluation. Probability values $P<0.05$ or less were considered to be significant.

Results

Inhibitory effect of sciatic nerve stimulation on cutaneous plasma extravasation of the contralateral leg

In the first group of animals the distal stump of the sciatic nerves of the guanethidine and pipecuronium pretreated rats was stimulated with 5 Hz for 5 min (1500 pulses). Stimulation of the right sciatic nerve was followed by similar stimulation of the left nerve 5 min later and Evans blue accumulation in the skin of the hindpaws was determined. The secondary response was inhibited by 50.3% compared to the primary reaction ($n=7$). Plasma extravasation evoked by subplantar injection of carrageenin (1%, 100 μl) was also inhibited by simultaneous contralateral stimulation of the sciatic nerve. In this series of experiments the peripheral stump of the nerve was stimulated with either 0.5 Hz for 1 h (1800 pulses) or 0.1 Hz for 4 h (1440 pulses). Even though in the former case the electrical stimulation elicited pronounced plasma extravasation, no Evans blue accumulation was observed after 0.1 Hz stimulation and inflammation evoked by carrageenin was inhibited in both cases. Carrageenin-induced plasma extravasation without contralateral sciatic nerve stimulation served as controls. The inhibition induced by stimulation at 0.5 Hz for 1 h and 0.1 Hz for 4 h was 38.9% and 52.1% respectively ($n=6$) (Figure 1).

Inhibition of carrageenin-induced inflammation in the knee joint by contralateral sciatic nerve stimulation

Carrageenin (2%, 200 μl) injected into the knee joint elicited Evans blue accumulation in the capsule, ligaments, and synovial tissue which were removed 1 h or 4 h after the injection ($n=5$). In rats where concommitant stimulation of the contralateral sciatic nerve was performed with 0.5 Hz, 1 h or 0.1 Hz, 4 h, plasma extravasation in the joint was inhibited by 46.1% and 40.9%, respectively ($n=7$) (Figure 2).

Inhibition of neurogenic inflammation in response to vagal nerve stimulation in the trachea, oesophagus and mediastinal connective tissue by sciatic nerve stimulation

In 5 control rats the cut peripheral stump of the left vagal nerve was stimulated (8 Hz, 10 min). The animals were pretreated with atropine sulphate (2 mg kg^{-1}, i.v.) besides guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium bromide (200 μg kg^{-1}, i.v.). The trachea, the distal two-thirds of the oesophagus and the mediastinal connective tissue showed intensive Evans blue accumulation in the control group ($n=5$) of rats. If similar vagal stimulation was performed in rats ($n=5$) of which one sciatic nerve was stimulated 5 min earlier with 5 Hz for 5 min, the effect of vagal stimulation was inhibited in the trachea by 32.8% in the oesophagus by 49.7% and in the mediastinal connective tissues by 37.6% (Figure 3).

Effect of sciatic nerve stimulation on contralateral antidromic vasodilatation

Values for the vasodilatator responses were calculated from areas under the curve and expressed as percentages of basal blood flow (mean ± s.e. mean, $n=5$) recorded by laser Doppler flowmetry on the plantar skin. Stimulation of one sciatic nerve with 4, 8 and 16 impulses at 2 Hz elicited increases in blood flow by 30.78 ± 2.39%, 45.44 ± 5.88% and 59.4 ± 6.32%, respectively. These responses as well as the basal flux values were not altered by excitation of the peripheral stump of the contralateral sciatic nerve with 1 Hz for 30 min. The corresponding values were 27.47 ± 3.64%, 41.66 ± 2.67 and 56.89 ± 3.87% during the stimulation and 24.94 ± 3.19%, 40.41 ± 3.65 and 59.92 ± 4.54% in the poststimulation period (Figure 4).

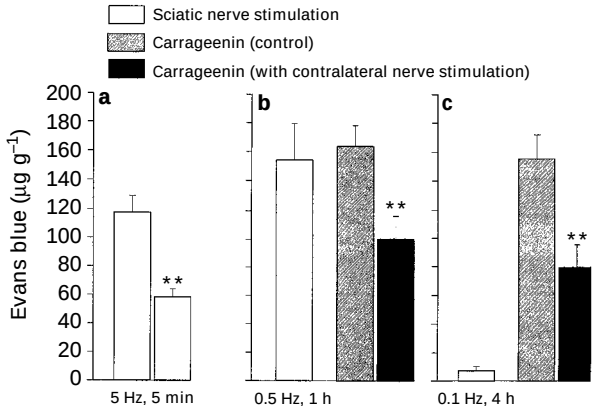

Figure 1 Plasma extravasation in the skin of the hindpaw evoked by stimulation (20 V, 0.5 ms) of the peripheral stump of the cut sciatic nerve of the rat at different frequencies and their effect on (a) the Evans blue accumulation evoked by stimulation of the contralateral sciatic nerve with the same parameters or (b; c) that evoked by subplantar carrageenin injection (1%, 100 μl). The intensity of plasma extravasation in control rats and in animals where the sciatic nerve of the contralateral leg was simultaneously stimulated is presented. Rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium bromide (200 μg kg^{-1}, i.v.). Values are means ± s.e.mean; $n=6-7$. $^{**}P<0.01$. Note that nerve stimulation elicited an inhibitory effect on plasma extravasation in the contralateral leg.

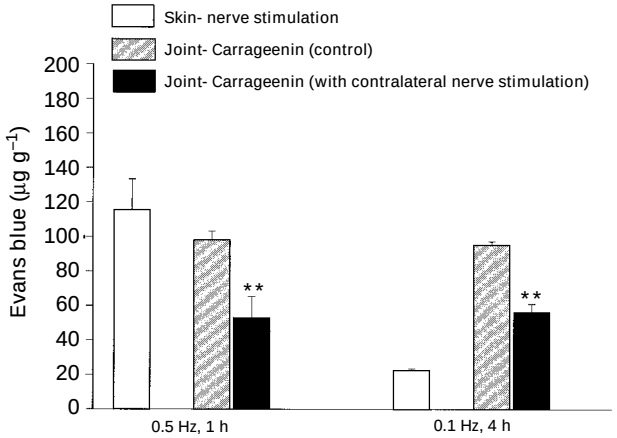

Figure 2 Plasma extravasation in the knee joint of the rat evoked by intra-articular injection of 200 μl 2% carrageenin in control cases and in rats where the peripheral stump of the cut sciatic nerve was simultaneously stimulated on the contralateral leg. Evans blue accumulation in the paw skin in response to nerve stimulation at two different frequencies is also indicated. Rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium bromide (200 μg kg^{-1}, i.v.). Each column represents the means ± s.e.mean; $n=5-7$. $^{**}P<0.01$ vs control.

Effect of somatostatin antiserum or cysteamine pretreatment on the anti-inflammatory response to sciatic nerve stimulation

The inhibitory action of sciatic nerve stimulation (5 Hz, 5 min) on contralateral antidromic neurogenic inflammation in the plantar skin induced 5 min later was abolished in rats pretreated with polyclonal somatostatin antiserum (0.5 ml/rat, i.v.) ($n=5$) or the selective somatostatin depleting agent cysteamine (280 mg kg^{-1}, s.c.) ($n=5$) (Figure 5). Inhibitory action of sciatic nerve excitation on subsequent plasma extravasation in response to vagal nerve stimulation (8 Hz, 10 min) was also prevented by somatostatin antiserum (0.5 ml/rat, i.v.) pretreatment ($n=6-7$) (Figure 6).

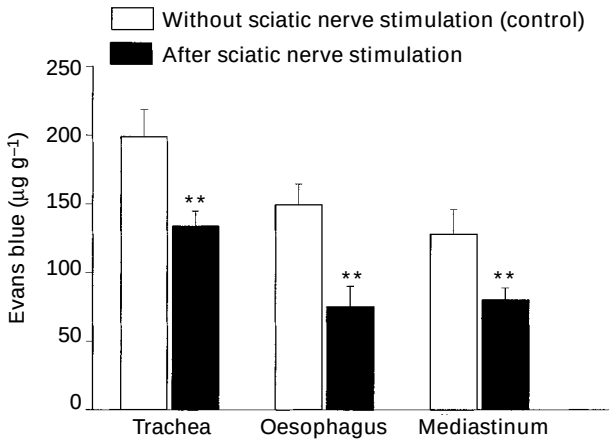

Figure 3 Plasma extravasation in the trachea, oesophagus and mediastinal connective tissue elicited by stimulation of the cut peripheral end of the left vagal nerve (8 Hz, 10 min). Evans blue accumulation in control rats and in animals where one of the sciatic nerves was stimulated (5 Hz, 5 min) 5 min before the vagal stimulation started. Rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.), piperuronium bromide (200 μg kg^{-1}, i.v.) and atropine sulphate (2 mg kg^{-1}, i.v.). Data are presented as means $\pm$s.e.mean from 5–6 rats, $^{**}P<0.01$.

Effect of somatostatin on cutaneous antidromic vasodilatation

Antidromic vasodilatation was recorded on the plantar skin of the rat in response to sciatic nerve stimulation (8 and 16 impulses at 2 Hz) before and 2 min after somatostatin injection (10 μg kg^{-1}, i.v.). The respective values (areas under the curve) were 40.06 ± 2.92 and 53.6 ± 7.04 before somatostatin; 42.82 ± 7.17 and 52.27 ± 6.21 after somatostatin administration. These results indicate that this neuropeptide has no effect on cutaneous blood flux responses ($n=4$).

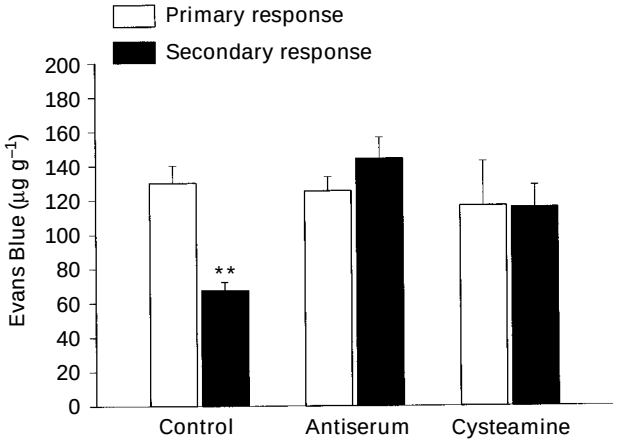

Figure 5 Antidromic plasma extravasation evoked by sciatic nerve stimulation (20 V, 0.5 ms, 5 Hz, 5 min) was inhibited by excitation of the contralateral sciatic nerve applied 5 min earlier (control group of rats). Plasma extravasation values in response to the primary and secondary stimulations are indicated. The second and third pairs of columns represent the results of the same experimental assessments in rats pretreated with polyclonal somatostatin antiserum (0.5 ml/rat, i.v. 1 h before excitation) or cysteamine (280 mg kg^{-1}, s.c. 4 h before stimulation). Rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium bromide (200 μg kg^{-1}, i.v.). Values are means $\pm$s.e. mean, $n=5$; $^{**}P<0.01$.

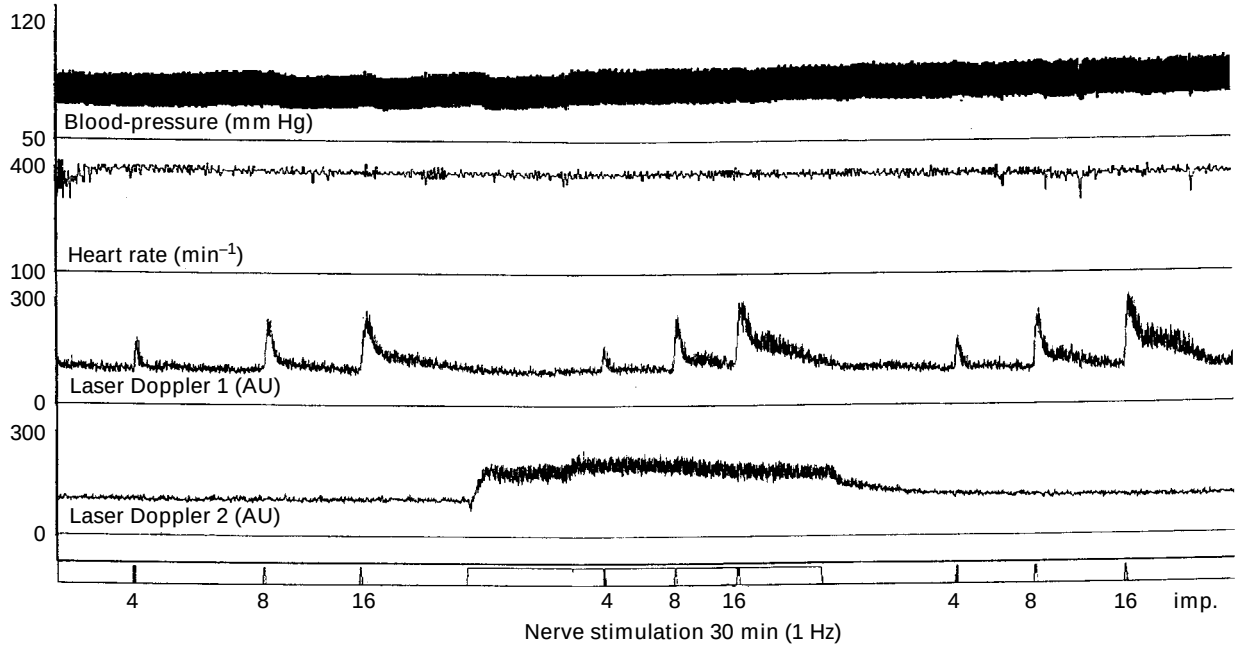

Figure 4 Laser Doppler 1 recording represents antidromic vasodilatation in the plantar skin of the rat in response to sciatic nerve stimulation (20 V, 0.5 ms, 2 Hz, number of impulses as indicated). Laser Doppler 2 shows the response in the contralateral hindpaw skin due to prolonged stimulation of the other sciatic nerve (AU: arbitrary unit).

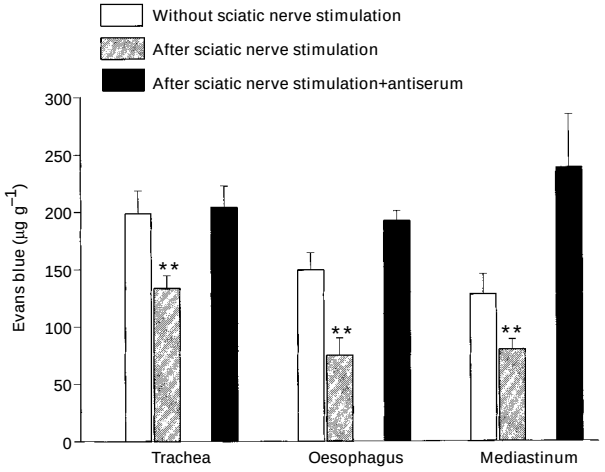

Figure 6 Plasma extravasation elicited by electrical stimulation of the peripheral stump of the vagal nerve (8 Hz, 10 min) in the trachea, oesophagus and mediastinal connective tissue was inhibited by previous antidromic stimulation of the sciatic nerve (5 Hz, 5 min). Control values without sciatic nerve stimulation, responses after sciatic nerve stimulation in rats pretreated with normal sheep serum or with polyclonal somatostatin antiserum (0.5 ml/rat 1 h before stimulation started) are indicated. Rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.), piperuronium bromide (200 μg kg^{-1}, i.v.) and atropine sulphate (2 mg kg^{-1}, i.v.). Results are means $\pm$s.e.mean, $n=6-7$; $^{**}P<0.01$ vs control.

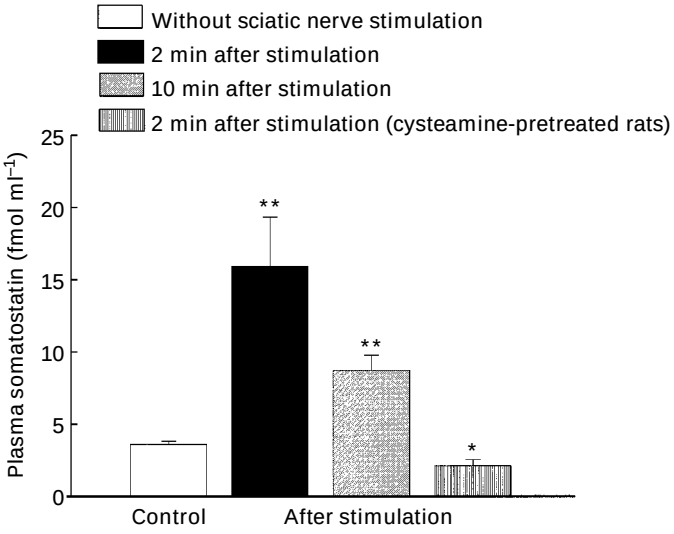

Figure 7 Plasma somatostatin level in control rats, in animals from which blood samples were taken 2 min or 10 min after stimulation of the peripheral stump of the cut sciatic nerves (20 V, 0.5 ms, 5 Hz, 5 min). Administration of cysteamine (280 mg kg^{-1}, s.c.) 4 h before excitation of the sciatic nerves prevented the increase of somatostatin concentration in the plasma (2 min after stimulation). All rats were pretreated with guanethidine (8 mg kg^{-1}, i.p.) and pipecuronium bromide (200 μg kg^{-1}, i.v.). Values are means$\pm$s.e.mean of 5–6 experiments. $^{*}P<0.05$; $^{**}P<0.01$ vs control.

Effect of antidromic sciatic nerve stimulation on the level of plasma somatostatin

Plasma somatostatin level increased more than 4 fold, 2 min after bilateral sciatic nerve stimulation (5 Hz, 5 min), compared to the control group where the nerves were cut but not stimulated. If blood samples were taken 10 min following the stimulation, the level of plasma somatostatin was still elevated by 142%. Administration of cysteamine (280 mg kg^{-1}, s.c.) 4 h before excitation of the sciatic nerves prevented the stimulation-evoked increase of somatostatin concentration in the plasma ($n=5-6$) (Figure 7).

Discussion

Three different models have been developed in the rat to show that activation of capsaicin-sensitive sensory nerve endings results in local release of mediator(s) which produce a systemic anti-oedema effect in several experimental conditions. The phenomenon was observed in response to (1) antidromic stimulation of the dorsal roots (Pintér & Szolcsányi, 1996); (2) chemical excitation of nociceptors by irritants in the acutely denervated hindleg (Szolcsányi, 1997); (3) antidromic stimulation of the sciatic nerve after sympathetic neurone blockade combined with application of a neuromuscular blocking agent (present results). The advantage of the first method is the selective excitation of sensory nerve endings without any chemical intervention. The second approach was necessary to prove that orthodromic natural stimulation of the sensory nerve endings by irritants can also release sufficient amount of mediator for a systemic effect. The advantage of the present series of experiments over the antidromic stimulation of dorsal roots is that it avoids severe surgical intervention and long-lasting experiments can be performed without variation in the sympathetic tone. The guanethidine treatment applied completely abolished the microvascular effect of sympathetic nerve stimulation, as detected by laser Doppler flowmetry in the skin of the rat hindpaw (Pintér *et al*., 1997b). Although these approaches are mutually complementary, the cardinal experiment of inhibiting the neurogenic plasma extravasation in one hindleg by activation of the sensory nerve endings in the other leg was tested by all three techniques. Good correlation is indicated by the fact that stimulation with the same parameters (20 V, 0.5 ms, 5 Hz for 5 min) inhibited the inflammation elicited on the contralateral hindleg 5 min later by 50% both in the case of dorsal root stimulation (Pintér & Szolcsányi, 1988) and when the sciatic nerve was stimulated. Similar inhibition of plasma extravasation was achieved on the contralateral hindleg by topical application of 1% mustard oil or s.c. injection of 0.1% capsaicin (Szolcsányi, 1997).

Plasma extravasation in the skin and knee joint evoked by carrageenin was inhibited by low frequency stimulation of the sciatic nerve of the contralateral hindleg. It is striking that stimulation at 0.1 Hz for 4 h (1440 pulses) did not induce plasma extravasation in the innervated area, but still produced slightly larger inhibition than the pronounced inflammation elicited by 0.5 Hz stimulation for 1 h (1800 pulses). Hence, antidromic stimulation of sensory fibres is efficient to release the anti-inflammatory neurohumoral agent(s) without producing plasma extravasation. Furthermore, oedema with plasma extravasation evoked by dextran after chronic denervation did not induce a systemic anti-inflammatory action (Pintér *et al*., 1997a). On the basis of these two observations, it is concluded that the neurohumoral mediator(s) of the anti-inflammatory effect is released from the sensory nerve endings and not from the inflammatory exudate.

It is interesting to note that the frequency optimum of cutaneous antidromic vasodilatation in response to dorsal root stimulation is also extremely low reaching its maximum already at or below 0.1 Hz, as revealed by laser Doppler flowmetry in the rat (Szolcsányi, 1988; Szolcsányi *et al*., 1992). This local vascular response and the systemic inhibition of plasma extravasation evoked by dorsal root stimulation

(Pintér & Szolcsányi, 1996) are mediated by capsaicin-sensitive fibres and their most numerous group is connected to the C-polymodal nociceptors (Szolcsányi, 1996b). These cutaneous receptors respond to threshold stimuli evoked by chemical agents released from the tissue e.g. due to to ultra-violet irradiation with similar low frequency of discharges starting within minutes and lasting for hours (Szolcsányi, 1987). On the other hand microneurostimulation of the cutaneous C-polymodal nociceptive fibres below 0.5–1 Hz is ineffective in producing pain or other sensations in man (Ochoa & Torebjörk, 1989; Tillman *et al*., 1995). Therefore it has been suggested that this substantial group of sensory nerve terminals serve primarily as effectors at threshold stimuli for local vasodilator tissue responses. Their pain-producing nociceptive function takes place with local plasma extravasation at suprathreshold stimulation (Szolcsányi, 1988; 1996a,b). The present results indicate that a systemic neurohumoral anti-inflammatory mediator is also released by activation of these sensory nerve endings, within this non-painful range of stimulation, without the induction of plasma extravasation in the innervated skin area.

The systemic neurogenic anti-inflammatory response to antidromic stimulation has been demonstrated in the skin, conjunctival mucosa (Pintér & Szolcsányi, 1996) and according to the present findings in the knee joint, trachea, oesophagus and mediastinal connective tissues as well. During electrical stimulation basal cutaneous microcirculation and enhancements to antidromic nerve stimulations are not altered in the contralateral hindleg. Therefore inhibition of plasma extravasation seems to be due to an action on the postcapillary venules and not to a reduction in microvascular circulation.

Evidence for the mediator role of somatostatin in the systemic neurogenic anti-inflammatory effect evoked by orthodromic stimulation of the capsaicin-sensitive sensory receptors by mustard oil or capsaicin has been presented (Pintér *et al*., 1997a; Szolcsányi, 1997). In accordance with these results pretreatment of rats with somatostatin antiserum or cysteamine (Palkovits *et al*., 1982; Helke & Selsky, 1983) prevented the anti-inflammatory effect of sciatic nerve stimulation not only in the skin but also in the trachea, oesophagus and the mediastinal connective tissue. Cysteamine is a sulphhydryl agent which induces a loss of both biologically and immunologically active somatostatin by forming mixed disulphide bonds with the neuropeptide (Lorenson & Jacobs, 1984; Patel & Pierzchala, 1985). In the applied dose range it induced a selective depletion of somatostatin from brain nuclei without affecting the levels of enkephalin, LH-RH, vasopressin, VIP, or cholecystokinin (Palkovits *et al*., 1982). Further evidence for the mediator role of somatostatin was obtained by measuring the plasma level of the peptide. Similar sciatic nerve stimulation induced a pronounced rise in somatostatin level of the blood, which was prevented by cysteamine pretreatment. In fact, it was even lower in these stimulated rats than the basal level of the controls.

Somatostatin is stored in a subgroup of the capsaicin-sensitive primary afferent neurones and their endings from where it can be released by capsaicin (Gamse *et al*., 1981; Holzer, 1992). In the rat 20% of the 'neurofilament poor' cutaneous afferent neurones with C-fibres showed somatostatin-like immunoreactivity, while none of the afferent neurones with A-fibres (Lawson, 1996) or motoneurones of the spinal cord (Senba *et al*., 1982) expressed this neuropeptide. Guanethidine pretreatment completely abolished the adrenergic and neuropeptide Y-mediated vasoconstrictor effect to stimulation of the sympathetic fibres admixed to the saphenous nerve of the rat (Pintér *et al*., 1997b). Furthermore, antidromic stimulation of the capsaicin-sensitive fibres of the dorsal roots or sciatic nerve elicited identical inhibition of plasma extravasation and this was also reproduced by sensory irritants like capsaicin or mustard oil. Taking all these findings together, it is suggested that somatostatin released by sciatic nerve stimulation with C-fibre strength in the guanethidine and pipecuronium pretreated rats was due to antidromic stimulation of a subgroup of the capsaicin-sensitive C-afferent fibres. Hence this substantial group of afferents apparently subserve not only local sensory efferent functions (Szolcsányi, 1984; 1996b; Maggi, 1995) but a systemic humoral effect as well, and therefore they have been defined as 'reguloceptors' (Szolcsányi *et al*., 1997).

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