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Systemic anti-inflammatory effect induced by counter-irritation through a local release of somatostatin from nociceptors

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1 Neurogenic plasma extravasation evoked by topical application of 1% vv^{-1} mustard oil on the skin of the acutely denervated rat hindleg (primary reaction) inhibited the development of a subsequent oil-induced plasma extravasation induced in the skin of the contralateral hindleg by $49.3 \pm 7.06\%$ ($n=9$) and in the conjunctival mucosa due to 0.1% wv^{-1} capsaicin instillation by $33.5 \pm 10.05\%$ ($n=6$). The primary reaction also inhibited the non-neurogenic hindpaw oedema evoked by s.c. injection of 5% wv^{-1} dextran into the chronically denervated hindpaw by $48.0 \pm 4.6\%$ ($n=5$).

2 Capsaicin injection (100 $\mu g\ ml^{-1}$ in 50 μl, s.c.) into the acutely denervated hindleg caused $56.5 \pm 4.0\%$ ($n=5$) inhibition in the intensity of plasma extravasation elicited by 1% vv^{-1} mustard oil smearing on the contralateral side. After chronic denervation, subplantar injection of 5% wv^{-1} dextran elicited a non-neurogenic inflammatory response with intensive tissue oedema without causing any systemic anti-inflammatory effect. Bilateral adrenalectomy did not inhibit the mustard oil-induced anti-inflammatory effect in the contralateral hindleg.

3 Pretreating the rats with polyclonal somatostatin antiserum (0.5 ml rat^{-1}, i.v.) or with the somatostatin depleting agent cysteamine (280 mg kg^{-1}, s.c.) prevented the inhibitory action of mustard oil-induced inflammation on subsequent neurogenic plasma extravasation and strongly diminished the inhibition of non-neurogenic oedema formation evoked by dextran.

4 Exogenous somatostatin (10 $\mu g\ kg^{-1}$, i.p.) caused a $30.3 \pm 8.3\%$ ($n=6$) inhibition of plasma extravasation caused by mustard oil smearing on the acutely denervated hindleg and this inhibitory effect was abolished by somatostatin antiserum (0.5 ml rat^{-1}, i.v.). The plasma level of somatostatin-like immunoreactivity (SST-LI) increased by $40.03 \pm 6.8\%$ ($n=6$) 10 min after topical application of 1% vv^{-1} mustard oil on the acutely denervated hindpaws compared to the paraffin oil treated control group. Chronic denervation of the hindlegs or cysteamine (280 mg kg^{-1}, s.c.) pretreatment prevented the mustard oil-induced elevation of SST-LI in plasma.

5 It is concluded that chemical excitation of the capsaicin-sensitive sensory receptors not only induces local neurogenic plasma extravasation but also inhibits the development of a subsequent inflammatory reaction at remote sites of the body in the rat. A role for somatostatin in this systemic anti-inflammatory effect is suggested.

Keywords: Neurogenic inflammation; anti-inflammatory effect; capsaicin-sensitive primary afferent neurone; mustard oil; dextran-oedema; somatostatin; somatostatin antiserum; cysteamine

Introduction

Antidromic or orthodromic activation of capsaicin-sensitive afferent nerve terminals evokes plasma extravasation and vasodilatation in the innervated skin and mucosal areas (Jancsó *et al*., 1967; Chahl, 1991; Pintér & Szolcsányi, 1995; Szolcsányi, 1996a). These local efferent responses are mediated by the release of substance P (SP) and calcitonin gene-related peptide (CGRP) (Maggi, 1995; Lundberg, 1996; Geppetti & Holzer, 1996). In contrast, neurogenic plasma extravasation in rats induced by antidromic stimulation of dorsal roots (primary reaction) has been shown to inhibit the development of a subsequent inflammatory reaction (secondary reaction) in a distant part of the body (Pintér & Szolcsányi, 1996; Szolcsányi, 1996b). This new type of systemic, neurohumoral response (Szolcsányi, 1996b) is apparently triggered by mediator(s) released from capsaicin-sensitive sensory nerve endings since the anti-inflammatory effect was absent when antidromic stimulation of the dorsal roots was performed after selective sensory degeneration induced by perineural capsaicin treatment (Pintér & Szolcsányi, 1996). It has been assumed that this mechanism might play a significant role in the therapeutic effect of 'counter-irritation' treatments.

Counter-irritation is a traditional, topical remedy for musculoarthritic inflammatory diseases (Gillies, 1895). It still forms a part of medical practice, although the mode of the therapeutic action of counter-irritants, including mustard oil, remains unclear. Early theories for explaining the phenomenon of counter-irritation proposed that local release of hypothetical endogenous substances with anti-inflammatory action might occur. However, the involvement of nociceptive reflex mechanisms with either sympathetic vasoconstriction or glucocorticoid mobilization cannot be excluded (Goldstein *et al*., 1967; Atkinson & Hicks, 1974; Bonta, 1978). Recently, the negative neuroendocrine feed-back control of inflammation through a spinal pathway mediated by activation of the hypothalamic-pituitary-adrenal axis and sympathetic post-ganglionic nerve terminals has been emphasized (Green *et al*., 1997).

The present series of experiments provide evidence that chemical stimulation of sensory nerve endings and neurogenic, but not non-neurogenic inflammation, induce a systemic anti-inflammatory effect in which reflex responses and involvement

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of the adrenal glands can be excluded. It is proposed that the principal mediator of this systemic response is somatostatin which, released from activated sensory nerve endings, reaches remote sites of the body *via* the circulation.

Methods

Animals

Experiments were carried out on female Wistar rats weighing 200–250 g. The animals were housed at the Laboratory Animal Centre of the University Medical School of Pécs under pathogen-free conditions at 24–25°C and were provided with standard rat chow and water *ad libitum*. Sodium pentobarbitone (40 mg kg^{-1}, i.p.) was used for anaesthesia which, in this single dose, was sufficient to maintain narcosis throughout the experiments. All procedures used in this study were in agreement with the rules of the Ethics Committee on Animal Research of the University Medical School of Pécs.

Surgical procedures

Acute denervation Both saphenous and sciatic nerves were exposed and cut 30 min before the experiments in order to avoid the interference of autonomic reflexes.

Chronic denervation The hindlegs were denervated 5 days prior to dextran injection to induce degeneration of the nerve fibres and to exclude the neurogenic component of the inflammation (Jancsó *et al*., 1967). Operating instruments were disinfected and wound infection was not observed in any case.

Adrenalectomy Lateral abdominal incisions were made to expose both adrenal glands. The vessels were ligated, the adrenals were removed 30 min before the application of mustard oil on the skin of the hindpaws and the wounds were sutured. In sham-operated animals the same incisions were performed and the wounds were sutured without ligating the adrenal glands.

Induction of inflammation

Neurogenic inflammation Neurogenic inflammation in the skin of the acutely denervated hindleg was evoked either by topical application of 1% vv^{-1} mustard oil dissolved in paraffin oil or by subplantar injection of capsaicin solution (100 μg ml^{-1}, 50 μl). Plasma extravasation in the conjunctival mucosa was induced by capsaicin instillation (1 mg ml^{-1}, 50 μl).

Non-neurogenic inflammation Non-neurogenic inflammation with tissue oedema was elicited by subplantar injection of 5% wv^{-1} dextran solution (100 μl) into the chronically denervated hindleg.

Drug pretreatments

Somatostatin (10 μg kg^{-1} or 20 μg kg^{-1}) was given i.p. 10 min before mustard oil smearing. In another group of animals, polyclonal somatostatin antiserum (0.5 ml rat^{-1}) was injected i.v., and inflammation was evoked 1 h later. The same volume of serum from untreated sheep served as controls for this group of animals. Cysteamine (280 mg kg^{-1}, s.c.) pretreatment was performed 4 h prior to the experiment.

Determination of plasma extravasation

Extravasation of plasma albumin was measured by the Evans blue leakage method. Evans blue (50 mg kg^{-1}) was injected i.v. and neurogenic inflammation was induced 10 min later. The anaesthetized rats were killed by exsanguination 20 min after the second application of an inflammatory agent. The conjunctival mucosal areas and the skin of the hindpaws were removed and the extravasated dye was extracted with formamide for 72 h at room temperature for photometric determination at 620 nm (Spectromom 195). The amount of the accumulated Evans blue, which quantitatively correlates with the intensity of plasma extravasation, was expressed as μg dye g^{-1} wet tissue.

Measurement of oedema formation

Formation of oedema in the rat hindpaw was determined by plethysmometry (Ugo Basile 7140). The transducer of the instrument records small differences in water level caused by volume displacement. The hindpaw volumes were measured prior to s.c. injection of 0.1 ml 5% wv^{-1} dextran (control value) and at 10, 20 and 30 min after the treatment. The extent of the oedema was expressed as a percentage of control.

Determination of plasma somatostatin-like immunoreactivity (SST-LI)

A specific and sensitive radioimmunoassay (RIA) developed in our laboratory was used to measure plasma SST-LI (Németh *et al*., 1996) in response to 1% vv^{-1} mustard oil smearing on the skin of the hindlegs after acute denervation in untreated or cysteamine (280 mg kg^{-1}, s.c.) pretreated animals and also after chronic denervation. C-terminal sensitive polyclonal somatostatin antiserum was kindly provided by Dr Tamás Görcs (Department of Anatomy, Semmelweis University Medical School, Budapest). This antiserum was raised in sheep using somatostatin-14-bovine thyroglobulin antigen coupled with glutaraldehyde. It was successfully used for the development of a somatostatin radioimmunoassay at 1 : 600,000 dilution and proved able to bind both of the biologically active, 14 and 28 amino acid-containing, molecular forms. Tyr(1)-somatostatin-14 was labelled with ^{125}I isotype by Iodogen and the mono-iodinated peptide was separated from the other fragments by Merck HPLC system (Németh *et al*., 1996). Intra- and inter-assay variation coefficients of the assay were 7.18% and 12.0% respectively. The detection limit was 1 fmol ml^{-1}. In controls paraffin oil (the solvent for mustard oil solution) was applied on both acutely denervated hindpaws. Arterial blood samples (3 ml rat^{-1}) were taken into ice-cold glass tubes containing EDTA and Trasylol. This procedure started 10 min after topical application of mustard oil or paraffin oil and lasted for 1.5 min. Following centrifugation (10,000 × *g* for 10 min at 4°C) the peptide from the plasma was extracted by addition of 3 vol of absolute alcohol. After precipitation and a second centrifugation (10,000 × *g* for 10 min at 4°C) the samples were dried under nitrogen flow. The samples were resuspended in assay buffer before RIA determination.

Drugs

Sodium pentobarbitone was obtained from May and Baker (England, U.K.), mustard oil (allyllisothiocyanat) and dextran from Fluka (Buchs, Switzerland), Evans blue dye, capsaicin

(8-methyl-N-vanillyl-6-nonenamide), somatostatin-14, cysteamine (2-mercaptoethylamine) and Tyr(1)-somatostatin-14 from Sigma. Capsaicin was dissolved in 10% ethanol, 10% Tween 80 and 80% saline (0.9% wv^{-1} NaCl).

Statistical analysis

The data are expressed as means ± s.e.mean and were evaluated by means of ANOVA followed by a modified Student's *t*-test for multiple comparisons according to Bonferroni's method or Mann-Whitney's U-test when appropriate. The level of significance was $P<0.05$ throughout the study.

Results

Systemic anti-inflammatory effect of chemically-induced neurogenic inflammation

Effect of neurogenic inflammation on a subsequent neurogenic plasma extravasation evoked by topical application of mustard oil or capsaicin In the first series of experiments 1% vv^{-1} mustard oil smeared on the skin of the acutely denervated hindleg induced local cutaneous dye accumulation. If mustard oil was simultaneously applied on the skin of both hindlegs, no difference was observed between the Evans blue accumulation on the two sides. When the first mustard oil smearing on the left hindleg (primary reaction) was followed by a similar treatment of the contralateral hindpaw 10 min later (secondary reaction), this latter response was inhibited by 49.3 ± 7.1% ($n=7$). In the control group, where the first application was vehicle (paraffin oil) the intensity of the mustard oil-induced secondary reaction was unaltered and did not differ significantly from the primary reaction of the previous group of rats ($n=9$) (Figure 1).

The effect of similar mustard oil application on neurogenic plasma extravasation evoked by instillation of 0.1% wv^{-1} capsaicin (Szolcsányi, 1988) into the conjunctival sack was also investigated. 2 min after capsaicin application into the left eye, 1% vv^{-1} mustard oil was smeared on one acutely denervated hindleg and a further 5 min later capsaicin instillation was repeated into the right eye. This second application elicited significantly less dye accumulation as compared to the first response (inhibition of 33.5 ± 10.5%, $n=6$). In control rats application of paraffin oil to the hindleg did not alter the response to the first and second capsaicin instillations (Figure 2).

In a third series of experiments, primary neurogenic inflammation was evoked by subplantar injection of capsaicin (100 $\mu g\ ml^{-1}$ in 50 μl) into the acutely denervated left hindleg. The secondary mustard oil-induced inflammatory reaction induced 10 min later on the contralateral side was reduced by 56.5 ± 4.0% ($n=5$) when compared with the control group of rats in which saline was given instead of capsaicin (Figure 3).

Effect of mustard oil-induced neurogenic inflammation on a subsequent non-neurogenic oedema formation evoked by s.c. injection of dextran Oedema of the chronically denervated hindleg elicited by subplantar injection of 5% wv^{-1} dextran was also inhibited by mustard oil smearing on the acutely denervated contralateral hindleg 10 min earlier. This inhibition was 58.0 ± 10.9% ($n=5$), 47.4 ± 9.2% ($n=5$) and 48.0 ± 4.6% ($n=5$) at 10, 20 and 30 min after the injection of dextran, respectively (compared with the control group in which paraffin oil was applied prior to the induction of dextran oedema, Figure 4).

Effect of dextran-induced non-neurogenic inflammation on a subsequent neurogenic inflammation Injection of 5% wv^{-1} dextran (s.c.) into the chronically denervated hindpaw evoked intense plasma extravasation but did not inhibit the mustard

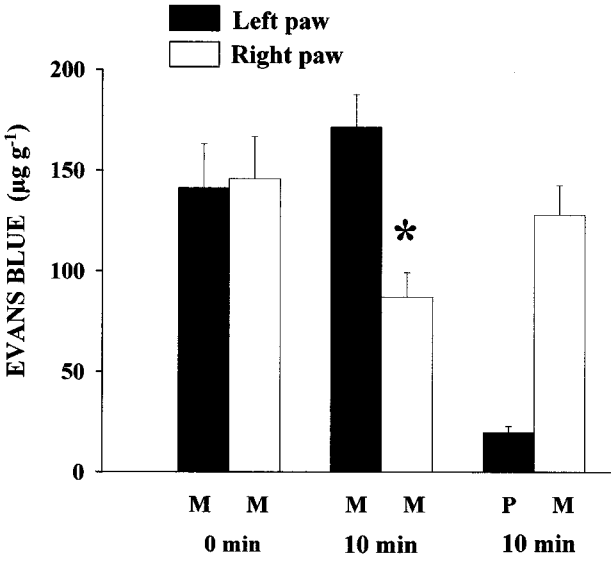

Figure 1 Effect of 1% vv^{-1} mustard oil (M)-induced plasma extravasation in the acutely denervated left hindpaw of the rat on a simultaneously (0 min) or 10 min later induced neurogenic inflammation on the right side. In the control group the solvent paraffin oil (P) was applied to the skin. Both saphenous and sciatic nerves were cut 30 min before the experiment. Each pair of columns shows the mean of 9 experiments with s.e.mean, $*P<0.05$.

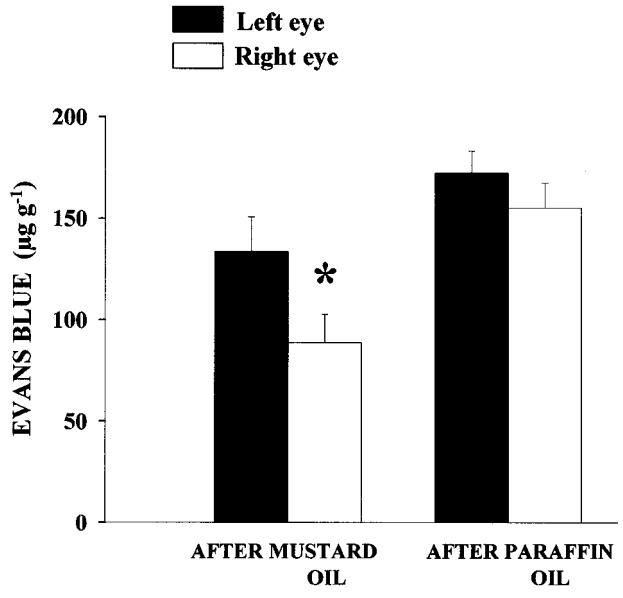

Figure 2 Plasma extravasation elicited by instillation of 0.1% wv^{-1} capsaicin into the conjunctival sack before (left eye) and after (right eye) treatment of one hindleg with 1% vv^{-1} mustard oil or paraffin oil. Mustard oil or paraffin oil was smeared on the skin of the acutely denervated hindleg 2 min after capsaicin administration into the left eye. 5 min later capsaicin was instilled into the right eye. Values are means ± s.e.mean, $n=6$. $*P<0.05$ right eye vs left eye.

oil-evoked dye accumulation in the contralateral, acutely denervated hindleg 10 min later. The secondary reaction was similar to that of the control group, in which saline was injected instead of dextran. The reduced plasma extravasation of the control group (c.f. Figure 1) might be attributed to the fact that these animals underwent surgery 5 days before the experiment causing hindleg paralysis and possible release of corticosteroids. When mustard oil was applied after chronic denervation, it had no significant inflammatory action and did not inhibit plasma extravasation induced by mustard oil in the acutely denervated contralateral hindleg (Figure 5).

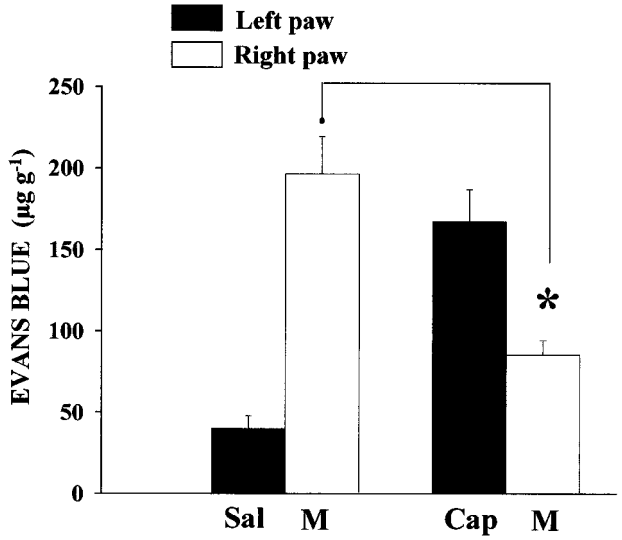

Figure 3 Effect of subplantar injection of capsaicin (Cap) (100 μg ml^{-1} in 50 μl) into the acutely denervated left hindpaw on neurogenic plasma extravasation elicited by 1% mustard oil (M) in the right hindleg 10 min later. In the control group 50 μl saline (Sal) was given s.c. instead of capsaicin. Data are presented as means $\pm$s.e.mean, $n=5$. *$P<0.01$ mustard oil-induced plasma extravasation after capsaicin vs after saline.

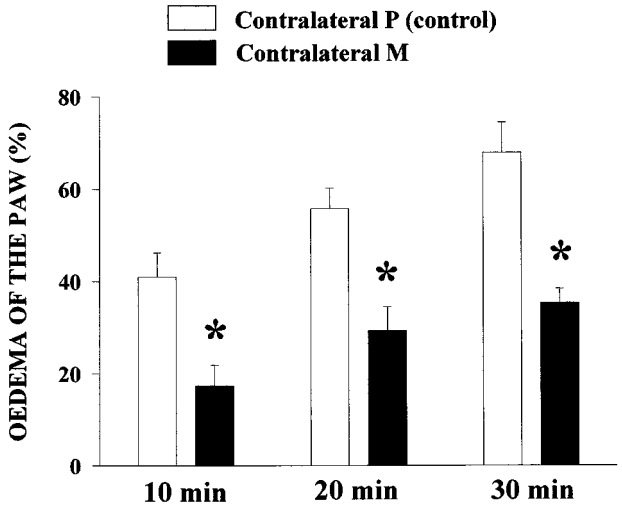

Figure 4 Effect of 1% vv^{-1} mustard oil (M)-induced neurogenic inflammation in the acutely denervated hindleg on non-neurogenic hindpaw oedema due to dextran (5% wv^{-1}, 100 μl) injected s.c. into the contralateral chronically denervated hindpaw. In the control group paraffin oil (P) was applied on the skin instead of mustard oil. Oedema was measured by plethysmometry before administration of dextran, then 10, 20 and 30 min afterwards. The increase in volume of the hindpaw is expressed as percentage of the preinjected values. Results are means$\pm$s.e.mean, $n=5$. (*$P<0.01$) vs control.

Neurotransmitter background of the anti-inflammatory action of neurogenic inflammation

Effect of adrenalectomy on the mustard oil-induced anti-inflammatory activity Topical application of 1% vv^{-1} mustard oil on the skin of the acutely denervated hindleg induced similar inhibition of plasma extravasation evoked by mustard oil 10 min later in the acutely denervated contralateral hindleg both in the adrenalectomized (from 129.5 ± 26.1 to 59.7 ± 6.7 μg g^{-1}) and sham-operated (from 125.1 ± 10.7 to 63.9 ± 7.8 μg g^{-1}) group of animals ($n=5$).

Effect of somatostatin antiserum and cysteamine pretreatment on the anti-inflammatory action of neurogenic inflammation The inhibitory effect of mustard oil-induced neurogenic inflammation on a subsequent neurogenic plasma extravasation evoked by mustard oil 10 min later in the contralateral hindleg was prevented by pretreatment of the animals with somatostatin antiserum (0.5 ml rat^{-1}, i.v.) or by the selective somatostatin depleting agent cysteamine (280 mg kg^{-1}, s.c.) (Figure 6a). Control rats received serum from untreated sheep (0.5 ml rat^{-1}). When the secondary reaction was a non-neurogenic inflammation elicited by dextran after chronic denervation the systemic anti-inflammatory action observed during a 30 min period was also decreased in the presence of the antiserum (Figure 6b).

Effect of somatostatin antiserum pretreatment on the anti-inflammatory action of exogenous somatostatin Injection of somatostatin (10 μg kg^{-1}, i.p.) caused $30.3\pm8.3\%$ ($n=6$) inhibition of mustard oil-induced plasma extravasation in the acutely denervated hindleg and this effect was not significantly different to that observed after injection of 20 μg kg^{-1} of this peptide. The inhibitory action of exogenous somatostatin was

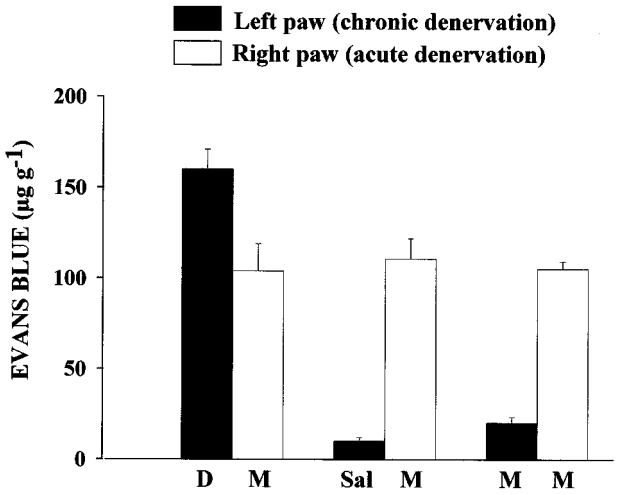

Figure 5 Effect of dextran (D)-induced (5% wv^{-1}, 100 μl, s.c.) non-neurogenic oedema in the chronically denervated left hindpaw on neurogenic plasma extravasation evoked by 1% vv^{-1} mustard-oil (M) smearing on the skin of the right acutely denervated hindleg 10 min later. In control animals 100 μl saline (Sal) was injected s.c. into the chronically denervated hindpaw. Mustard oil application on the skin after chronic denervation elicited neither local plasma extravasation nor inhibitory action on the subsequent neurogenic inflammation. Values are means$\pm$s.e.mean, $n=6$ or $n=12$ (Sal). Note that non-neurogenic plasma extravasation evoked by dextran did not diminish the contralateral mustard oil-induced response (the three open columns do not differ from each other and give P values of 0.81, 0.73, 0.95 respectively according to ANOVA followed by Bonferroni's test).

prevented by polyclonal somatostatin antiserum (0.5 ml rat^{-1}, i.v.) pretreatment (Figure 7).

Plasma concentration of SST-LI in response to topical 1% vv^{-1} mustard oil smearing on the skin

Plasma SST-LI increased by 40.03 ± 6.81% ($n=6$) 10 min after 1% vv^{-1} mustard oil smearing on the skin of the acutely denervated hindlegs compared to the control group in which solvent was topically applied. Chronic denervation of the hindlimbs or cysteamine pretreatment (280 mg kg^{-1}, s.c.) 4 h prior to mustard oil administration prevented the stimulation-evoked increase of SST-LI of the plasma, and in the latter case the concentration of the peptide was significantly lower than the basal value (Figure 8).

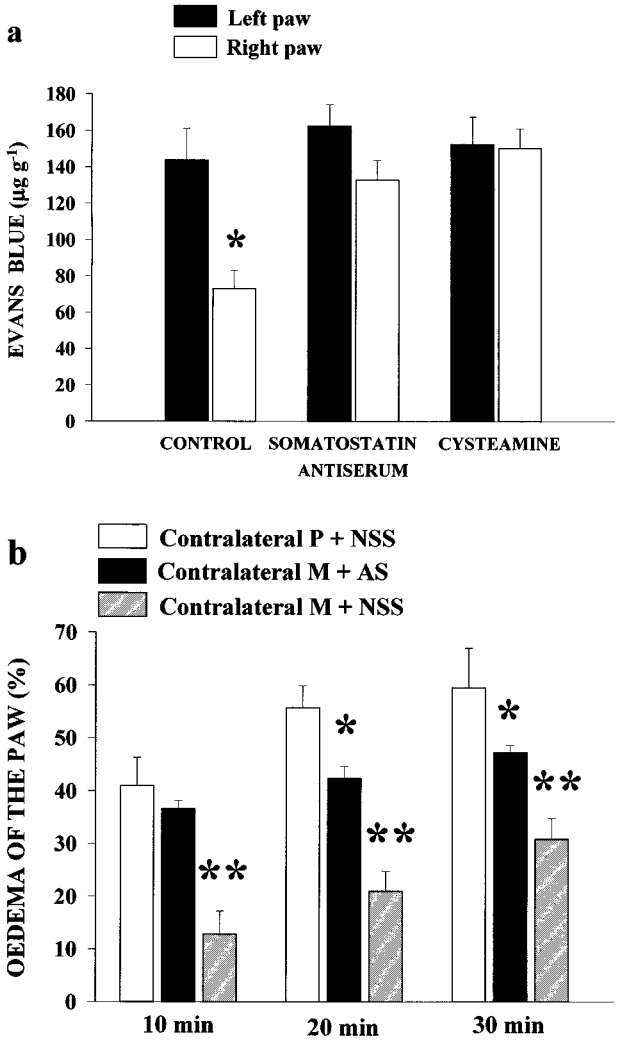

Figure 6 (a) Effect of 1% vv^{-1} mustard oil smearing on the skin of the acutely denervated left hindleg on a subsequent neurogenic plasma extravasation evoked also by mustard oil 10 min later on the right side in control (normal sheep serum; 0.5 ml rat^{-1}, i.v., 1 h before testing), polyclonal somatostatin antiserum (0.5 ml rat^{-1}, i.v., 1 h prior to testing) or cysteamine (280 mg kg^{-1}, s.c., 4 h before testing) pretreated animals. Results are shown as means ± s.e.mean of $n=7$ experiments, $*P<0.01$. (b) Oedema of the chronically denervated right hindpaw in response to subplantar injection of 100 μl 5% wv^{-1} dextran in three groups of rats. The contralateral, acutely denervated hindleg was smeared with paraffin oil (P) or 1% vv^{-1} mustard oil (M). One group of rats was pretreated with polyclonal somatostatin antiserum (AS, 0.5 ml rat^{-1}, i.v.) and two groups with normal sheep serum (NSS; 0.5 ml rat^{-1}, i.v.) 1 h prior to testing. Oedema was measured by plethysmometry before dextran, and 10, 20, 30 min afterwards. The percent increase in tissue swelling is indicated. Values are means ± s.e.mean, $n=5$. $*P<0.05$, $**P<0.01$ mustard oil treated vs paraffin oil treated animals.

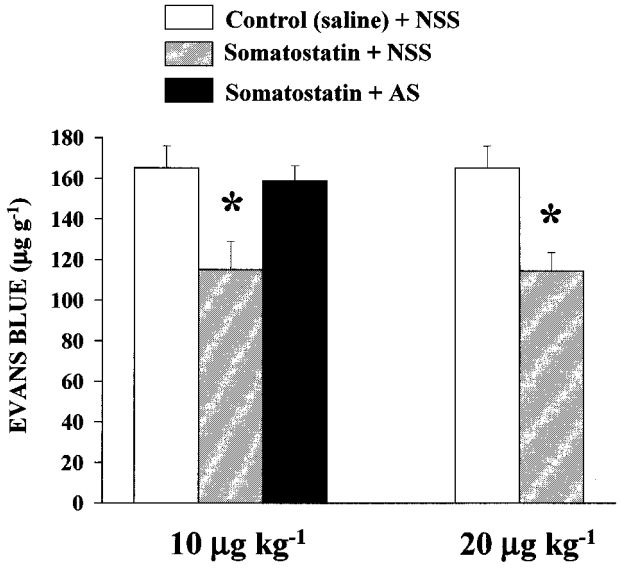

Figure 7 Effect of somatostatin injection (10 μg kg^{-1} or 20 μg kg^{-1}, i.p.) on plasma extravasation induced by 1% mustard oil smearing on the acutely denervated hindlegs 10 min later. Control rats were given isotonic saline i.p. instead of somatostatin. One group of rats was pretreated with polyclonal somatostatin antiserum (AS; 0.5 ml rat^{-1}, i.v.) and two groups with normal sheep serum (NSS; 0.5 ml rat^{-1}, i.v.) 1 h prior to testing. All data are means ± s.e.mean of $n=$ five to seven experiments, $*P<0.01$ indicates significant differences vs control group.

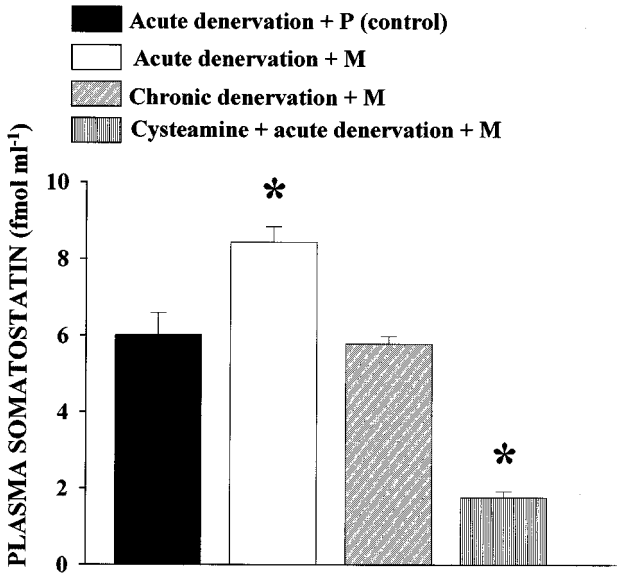

Figure 8 SST-LI plasma levels in rats from which blood samples were taken 10 min after topical application of 1% vv^{-1} mustard oil (M) on the skin of both acutely denervated hindlegs. In control animals the solvent paraffin oil (P) was smeared on the hindpaws. Chronic denervation of the hindlegs or administration of cysteamine (280 mg kg^{-1}, s.c.; 4 h prior to mustard oil smearing) prevented the increase of plasma SST-LI. In the latter case the concentration of the peptide was significantly diminished compared to the control values. Results are means ± s.e.mean, $n=6$. $*P<0.01$ vs controls.

Discussion

This study provides the first evidence that chemical agents used for counter-irritation, like mustard oil or capsaicin, applied to the acutely denervated hindleg of the rat elicit a systemic anti-inflammatory action. This inhibition of plasma extravasation was caused by low concentration of mustard oil (Jancsó *et al*., 1967), occurred within 5–15 min and developed to a similar extent in adrenalectomized animals. Therefore, in the experimental circumstances of this study, reflex activation of sympathetic nerves and glucocorticoid mobilization from the adrenal glands, both of which are known to elicit anti-inflammatory activity (Bonta, 1978; Green *et al*., 1997), can reasonably be excluded.

It is clear that capsaicin and mustard oil, at the applied concentrations, evoke a neurogenic inflammation, since, after chronic denervation, they produce no dye accumulation when compared with untreated or solvent-treated tissues (Jancsó *et al*., 1967; Szolcsányi, 1996a, 1988). These agents are selective sensory stimulants and the major subgroup of cutaneous receptors that they stimulate are the C-polymodal nociceptors. Furthermore, a smaller number of C-afferents known as 'silent' (mechano-heat insensitive) units, warm receptors and heat sensitive nociceptors with C and A δ fibres should also be taken into consideration as possible targets (Handwerker *et al*., 1987; Szolcsányi 1987, 1993, 1996b; Lynn, 1996). Mechanical nociceptors with C and A δ fibres, as well as various types of low threshold mechanoreceptors, are insensitive to capsaicin or mustard oil. Thus, capsaicin-sensitive cutaneous nociceptive afferents which are activated also by mustard oil form a substantial group (around 50%) of primary afferent neurones (Szolcsányi, 1993, 1996b). They express capsaicin receptor-coupled ion channels which are also sensitive to noxious heat (Caterina *et al*., 1997). The systemic anti-inflammatory effect of antidromic stimulation of the dorsal roots is prevented by degeneration of the capsaicin-sensitive afferent fibres by perineural capsaicin pretreatment (Pintér & Szolcsányi, 1996). Therefore, it is suggested that the neurohumoral mediator which elicits the systemic anti-inflammatory effect observed in this study is released from a capsaicin-sensitive subset of sensory nerve endings and reaches remote sites of the body *via* the circulation (Szolcsányi, 1996b).

Mustard oil application inhibited not only neurogenic plasma extravasation elicited by mustard oil or capsaicin but also non-neurogenic oedema formation evoked by dextran in the chronically denervated contralateral hindleg. Dextran exerts its oedematogenic effect through mast cell degranulation (Selye, 1965). This observation indicates that the inhibitory neurohumoral mediator(s) have direct vascular effects irrespective of their ability to diminish the release of SP and CGRP which elicit neurogenic inflammation and antidromic vasodilatation (Lembeck *et al*., 1982; Chahl, 1991; Maggi, 1995; Geppetti & Holzer, 1996). It is interesting to note, however, that plasma extravasation but not increased blood flow evoked by antidromic stimulation of sensory fibres was inhibited by stimulation of the sciatic nerve in the contralateral hindleg (Szolcsányi *et al*., 1998).

Inflammation induced by dextran in the chronically denervated hindleg did not induce inhibition of a distant inflammatory response. Consequently, agents released during the early stage of inflammation initiated by mast cell degranulation and venular plasma extravasation cannot be the mediators of the observed systemic anti-inflammatory effect. Therefore, it is concluded that the anti-inflammatory mediator(s) are released from capsaicin-sensitive sensory nerve terminals.

On the basis of earlier data, anti-inflammatory sensory neuropeptides with a putative neurohumoral role include somatostatin (Lembeck *et al*., 1982; Karalis *et al*., 1994; Fioravanti *et al*., 1995), opioid peptides (Barthó & Szolcsányi, 1981; Lembeck *et al*., 1982) and galanin (Xu *et al*., 1991). The following results favour the role of somatostatin in the anti-inflammatory effect elicited by mustard oil or capsaicin.

Somatostatin stored in capsaicin-sensitive afferents is depleted by capsaicin pretreatment and there is also some evidence that it is released by capsaicin (Gamse *et al*., 1981). In agreement with previous reports (Lembeck *et al*., 1982; Karalis *et al*., 1994; Fioravanti *et al*., 1995), we found that exogenous somatostatin inhibits neurogenic inflammation. The role of somatostatin was also supported by the observation that somatostatin antiserum and cysteamine both reduce the systemic anti-inflammatory effect of mustard oil application. Cysteamine is a sulphydryl agent that induces a loss of both biologically and immunologically active somatostatin by forming mixed disulphide bonds with the neuropeptide (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 (Paklovits *et al*., 1982). Concomitant with the reduction in somatostatin, cysteamine has also been shown to alter growth hormone secretion and increase insulin and glucagon release in rodents (see McLeod *et al*., 1995).

Mustard oil applied to the acutely denervated hindlegs has been shown to elicit a significant increase in plasma SST-LI. In contrast, this response was absent after chronic denervation, thus indicating the neural source of the increased plasma somatostatin concentration. Cysteamine, which depletes somatostatin both from neural and non-neural tissues (Patel & Pierzchala, 1985), not only inhibited mustard oil-induced elevation of the plasma concentration of SST-LI but also markedly decreased the basal plasma level. These data are consistent with observations that stimulation of the peripheral stump of the sciatic nerves of the rat induces a pronounced rise in somatostatin concentration in the blood which was absent after cysteamine pretreatment (Szolcsányi *et al*., 1998).

It is concluded that somatostatin is released from cutaneous noxious heat responsive capsaicin-sensitive nerve endings (Szolcsányi 1993; Caterina *et al*., 1997) in the rat in response to chemical stimulation by counter-irritants and thereby induces a systemic anti-inflammatory effect.

Counter-irritation by chemicals and mild irritation by acupuncture are traditional therapeutic remedies for the treatment of inflammatory diseases including arthritis and bronchial asthma (Nicholas, 1994; Lewith & Watkins, 1996). Neurogenic plasma extravasation plays a significant role in the pathogenesis of inflammation in several organs (Chahl, 1991; Geppetti & Holzer, 1996; Szolcsányi, 1996b). Consequently, release of somatostatin from the activated capsaicin-sensitive sensory nerve endings and its anti-inflammatory effect detected in other parts of the body forms of novel neurohumoral regulatory mechanism and a new target for drug development for the treatment of inflammation.

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