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Anti-inflammatory effect of synthetic somatostatin analogues in the rat

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1 Somatostatin (6.11 nmol kg^{-1} i.p.) inhibited neurogenic plasma extravasation evoked by 1% mustard oil and non-neurogenic oedema induced by 5% dextran in the rat skin.

2 Cyclic synthetic octapeptide (TT-248 and TT-250) and heptapeptide (TT-232) somatostatin analogues proved to be more effective in reducing neurogenic and non-neurogenic inflammatory reactions but octreotide had no influence on either neurogenic or non-neurogenic inflammation.

3 TT-232 administered i.p. or i.v. (1.06–42.40 nmol kg^{-1}) inhibited in a dose-dependent manner the plasma extravasation evoked by mustard oil in the rat's paw. Neither diclofenac (15.78–315.60 μmol kg^{-1}) nor the selective COX-2 inhibitor meloxicam (2.95–569.38 μmol kg^{-1}) attenuated the mustard oil-induced neurogenic plasma extravasation.

4 TT-232, diclofenac and meloxicam dose-dependently diminished non-neurogenic dextran-oedema of the paw the ED_{35} values were 1.73 nmol kg^{-1} for TT-232 and 34.37 μmol kg^{-1} for diclofenac.

5 TT-232 inhibited in the dose range of 1.06–21.21 nmol kg^{-1} the bradykinin-induced plasma extravasation in the skin of the chronically denervated paw.

6 Mustard oil-induced cutaneous plasma extravasation was dose-dependently diminished by s.c. TT-232 1, 2, 4, 6 or 16 h after the treatment. TT-232 (2×106, 2×212 and 2×530 nmol kg^{-1} per day s.c. for 18 days) caused dose-dependent inhibition of chronic Freund adjuvant-induced arthritis during the experimental period.

7 TT-232 (200 and 500 nM) inhibited the release of SP, CGRP and somatostatin from the rat isolated trachea induced by electrical field stimulation (40 V, 0.1 ms, 10 Hz, 120 s) or by capsaicin (10^{-7} M), but did not influence the basal, non-stimulated peptide release.

8 It is concluded that somatostatin analogues without endocrine functions as TT-232 are promising compounds with a novel site of action for inhibition of non-neurogenic and neurogenic inflammatory processes. *British Journal of Pharmacology* (2001) **134,** 1571–1579

Keywords: Neurogenic inflammation; anti-inflammatory effect; neuropeptide release; mustard oil; dextran-oedema; Freund adjuvant; somatostatin analogues; TT-232; diclofenac; meloxicam

Abbreviations: CGRP, calcitonin gene-related peptide; COX, cyclo-oxygenase; GH, growth hormone; NK1, neurokinin1; RIA, radioimmunoassay; SP, substance P; SRIF, somatotropin release inhibiting factor; sstr, somatostatin receptor

Introduction

It has been shown that activation of nociceptors sensitive to capsaicin, noxious heat or inflammatory mediators (Szolcsányi, 1996a, b; Caterina *et al*., 1997) results in not only pain sensation but a release of sensory neuropeptides. Among these neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP) elicit local neurogenic inflammation (vasodilatation and plasma extravasation) (Chahl, 1991; Holzer, 1992; Maggi, 1995; Geppetti & Holzer, 1996; Szolcsányi, 1988; 1996a). Neurogenic inflammation participates in this sense in various extent in all inflammatory responses where nociception or pain sensation occurs. This type of inflammation is resistant to the inhibitory effect of non-steroidal anti-inflammatory agents like indomethacin, phenylbutazone, amidopyridine or flufenamic acid (Jancsó-Gábor & Szolcsányi, 1970). It has been described that somatostatin is also stored in the capsaicin-sensitive subpopulation of nociceptors from where it can be released and depleted (Gamse *et al*, 1981; Holzer, 1988; Szolcsányi *et al*., 1994). It has been known for a long time that somatostatin inhibits neurogenic inflammation (Lembeck *et al*., 1982; Karalis *et al*., 1994; Fioravanti *et al*., 1995) and nociception but our studies were the first to reveal that suitable amount of somatostatin could be released from the activated primary afferent nerve terminals to elicit a systemic anti-inflammatory (Szolcsányi *et al*., 1998a, b) and anti-nociceptive (Helyes *et al*., 2000) action.

Somatostatin is widely expressed in the central nervous system (Gamse *et al*., 1981) and the peripheral tissues in 14 and 28 amino acid-containing forms (Patel *et al*., 1995; ten

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Bokum *et al*., 2000). A great variety of its effects has already been described including modulation of hormone (growth hormone, glucagon, insulin) and neurotransmitter (SP, dopamine, 5-hydroxytriptamine, acetylcholine and somato-statin) release, cognitive and behavioural processes, the gastrointestinal tract, the cardiovascular system and tumour-cell proliferation (ten Bokum *et al*., 2000). These effects are mediated *via* five different somatostatin receptor subtypes which have been cloned and which are known to be members of the G-protein associated receptor family (Hoyer *et al*., 1995; Reisine & Bell, 1995). The therapeutic use of native somatostatin is limited by its broad range of effects at these somatostatin receptors and also by its very short (3 min) plasma half life-time (ten Bokum *et al*., 2000). Functional characterization of these receptor subtypes has revealed two main groups: $SRIF_1$ group comprising the sst_2, sst_3 and sst_5 receptors and $SRIF_2$ group comprising sst_1 and sst_4 receptors (Reisine & Bell, 1995; Hofland *et al*., 1995). Recently a series of new potent, stable, analogues (Table 1) has been synthesized in our laboratories to study the relative importance of specific substitutions in selectivity between these receptor subtypes. An analogue with a cyclopenta-ring structure, called TT-232, was found to be unique because it had no endocrine activity. This analogue failed to inhibit growth hormone (GH) release or gastrin secretion *in vivo* but had a strong antiproliferative and apoptotic effect on tumour cells *in vivo* and *in vitro* (Kéri *et al*., 1993; 1996).

The aim of the present study was to analyse the effect of somatostatin and its cyclic, synthetic analogues, particularly the heptapeptide TT-232 (Kéri *et al*., 1993; 1996) on neurogenic and non-neurogenic inflammatory reactions *in vivo* and on sensory neuropeptide release *in vitro*.

Methods

Animals

Experiments were performed on female Wistar rats weighing 150–250 g. The animals 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 water *ad libitum*. All experimental procedures used in this study were in agreement with the rules of the Ethics Committee on Animal Research of Pécs University.

Induction and determination of acute cutaneous inflammation

Experiments were carried out under sodium pentobarbitone (40 mg kg^{-1} i.p.) anaesthesia.

Neurogenic inflammation Neurogenic inflammation in the skin of the acutely denervated hindleg was evoked by topical application of 1% mustard oil dissolved in paraffin oil. Both saphenous and sciatic nerves were exposed and cut 30 min before the experiments in order to avoid the interference of autonomic reflexes. 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. Rats were killed by exsanguination 20 min after the application of the inflammatory agent. 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 neurogenic inflammation, was expressed as μg dye g^{-1} wet tissue.

Non-neurogenic inflammation Non-neurogenic inflammation was elicited by dextran (100 μl, 5%) or bradykinin (50 μl, 0.25 μg) administered s.c. under the plantar skin of the chronically denervated hindleg to produce tissue oedema and plasma extravasation. The hindlimbs were denervated 5 days prior to dextran or bradykinin injection to elicit degeneration of the leg's nerve supply and therefore exclude the neurogenic part of the inflammation (Jancsó *et al*., 1967). Oedema formation in the rat hindpaw was measured by plethysmometry (Ugo Basile 7140). The transducer of the instrument records small differences in water level caused by volume displacement. The paw volumes were measured prior to s.c. injection of 0.1 ml 5% dextran (control value) and 10, 20, 30 min after the treatment. The extent of the oedema was expressed as a percentage of control. Non-neurogenic plasma extravasation elicited by bradykinin was determined by the Evans blue technique.

Somatostatin in a dose of 10 μg kg^{-1} i.p. (6.11 nmol kg^{-1}), 10 μg kg^{-1} i.p. of its cyclic, synthetic analogues, TT-248, TT-

Table 1 Amino acid sequence and molecular weight of the somatostatin analogues under investigation (Ind: indolinil)

Compound	*Sequence*	*Molecular weight*
Somatostatin-14	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys–NH_2	1638.0
TT-232	D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2	947.0
TT-250	D-Phe-Cys-Tyr-D-Trp-Lys-Leu-Cys-Thr-NH_2	1060.2
TT248	β Asp(Ind)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2	1235.0
RC-160	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2	1232.4
OCTREOTIDE	Phe-Cys-Phe-Trp-Lys-Thr-Cys-CH-CHOH-CH_3 (CH-CH_2OH)	1019.6

250, RC-160 and octreotide (8.12, 9.43 and 8.11 nmol kg^{-1}, respectively); 1, 2.5, 5, 10, 20 and 40 10 $\mu g\ kg^{-1}$ TT-232 i.p. and i.v. (1.06, 2.65, 5.30, 10.60, 21.2, 42.40 nmol kg^{-1}); 5, 10, 20 i.v. and 100 mg kg^{-1} i.m. diclofenac (15.75, 31.56, 63.12 μmol kg^{-1} and 314.4 μmol kg^{-1}) or 1, 10, 20, 200 mg kg^{-1} i.v. selective cyclo-oxygenase-2 (COX-2) inhibitor meloxicam (2.95, 29.5, 56.94 and 569.38 μmol kg^{-1}) were given 10 min before the induction of neurogenic or non-neurogenic inflammation. In a subset of experiments 10, 20, 40, 80, 160, 320 $\mu g\ kg^{-1}$ TT-232 (10.6, 21.2, 42.4, 84.8, 169.6, 339.2 nmol kg^{-1}) was administered s.c. 1, 2, 4 and 6 h before mustard oil smearing. Animals of the control groups were pretreated with the solvent of the respective compound.

Induction and determination of chronic arthritis

Oedema of the ankle joint was measured by plethysmometry before and on the 2nd, 5th, 8th, 12th, 15th and 18th day after 0.1 ml (1 mg ml^{-1}) injection of complete Freund-adjuvant (mixture of killed Mycobacteria and liquid paraffin) into the plantar skin of the left hindpaw. In order to enhance the systemic effects additional intradermal injections (0.1 ml, 1 mg ml^{-1}) were given into the root of the tail on the same and on the following day. Joint swelling was expressed as a percentage of pre-injection control values. TT-232 in doses of 2×100, 2×200 or $2\times500\ \mu g\ kg^{-1}$ (2×106, 2×212 or 2×530 nmol kg^{-1}, respectively) per day was administered s.c. throughout the experimental period of 18 days.

Measurement of sensory neuropeptide release in vitro

After exsanguination the tracheae of 2–2 female Wistar rats were removed and perfused (1 ml min^{-1}) in an organ bath (1.8 ml) at 37°C for 60 min with oxygenated (95% O_2 and 5% CO_2) Krebs solution of the following composition (in mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5, glucose 11. After stopping the flow the solution was changed three times for 8 min (prestimulated–stimulated–poststimulated). Electrical field stimulation (40 V, 0.1 ms, 10 Hz, 120 s) and chemical stimulation (capsaicin 10^{-7} M) were performed to induce release of sensory neuropeptides from the tissue pieces in the presence or absence of TT-232 (200, 500 or 1500 nM). The fractions were collected in ice-cold tubes and the wet weight of the tracheae were measured. Concentrations of SP, CGRP and somatostatin were determined by specific radioimmunoassay (RIA) methods developed in our laboratory (Németh *et al*., 1996; 1998a, b), and were expressed as the released amount of peptide per tissue weight.

Drugs

Sodium pentobarbitone was obtained from May and Baker (England, U.K.), mustard oil (allyllisothiocyanate) and dextran from Fluka (Buchs, Switzerland), complete Freund adjuvant, Evans blue dye, capsaicin (8-methyl-N-vanillyl-6-nonenamide), bradykinin, and somatostatin-14 from Sigma (St. Louis, MO, U.S.A.), rat α-CGRP, [Tyr^1]somatostatin-14 and Tyr-α-CGRP(23–37) from Bachem (Bubendorf, Switzerland), substance P RIA-tracer from Amersham (Amersham, U.K.), octreotide (Sandostatin) from Sandoz (U.K.), diclofenac sodium from RBI (U.S.A.) and meloxicam from Boehringer Ingelheim (Biberach, Germany). Capsaicin was dissolved in 10% ethanol, 10% Tween 80 (Reanal, Hungary) and 80% isotonic saline. TT-248, TT-250 and TT-232 were synthetized in the Central Research Institute for Chemistry of the Hungarian Academy of Sciences. Somatostatin and the analogues were dissolved in isotonic saline with 200 μl 0.1 M acetic acid for i.p. application. For i.v. and s.c. administration TT-232 (1 mg) was dissolved in 1 ml acetate-acetic acid buffer (0.2 mol l^{-1}, pH: 3.4) as a stock solution, further dilutions were made in the same buffer and the injection form of the solution contained 5% mannitol. Diclofenac in low doses and meloxicam were dissolved in saline, but owing to the limited solubility of diclofenac, the 100 mg kg^{-1} dose was dissolved in DMSO and made up to the final volume with 0.9% saline. Substance P antiserum was kindly provided by Prof D.J. Dockray, University of Liverpool and C-terminal sensitive somatostatin antiserum and CGRP antiserum by Dr T. Görcs, Semmelweis University Medical School of Budapest. ^{125}I-labelled Tyr-α-CGRP(23–37) and ^{125}I-labelled [Tyr^1]somatostatin-14 were prepared in our laboratory.

Statistical analysis

Results are presented as means ± s.e.mean. Non-parametric (Mann–Whitney) test was used for statistical evaluation of the *in vivo* data and Student's *t*-test for paired comparison for the *in vitro* experiments. Probability values $P<0.05$ or less were regarded as significant. For evaluation of the anti-inflammatory effects of i.v. or s.c. TT-232, diclofenac and meloxicam dose-response curves of per cent inhibitions with 95% confidence intervals were calculated and presented with the ID_{50} or ID_{35} values.

Results

Effect of i.p. administered somatostatin and its analogues on neurogenic and dextran-induced non-neurogenic inflammation

Somatostatin (6.11 nmol kg^{-1} i.p.) inhibited the 1% mustard oil-induced neurogenic plasma extravasation in the skin of the acutely denervated hindlegs by 24.85% ($n=7$). This inhibition was 46.26% ($n=7$), 48.86% ($n=5$), 53.98% ($n=6$) and 63.62% ($n=5$), after TT-248 (8.12 nmol kg^{-1} i.p), TT-250 (9.43 nmol kg^{-1} i.p), RC-160 (8.11 nmol kg^{-1} i.p) and TT-232 (10.6 nmol kg^{-1} i.p) pretreatments, respectively. Octreotide did not show inhibitory action compared to the solvent treated group ($n=5$) (Figure 1). Oedema formation of the chronically denervated hindlegs elicited by subplantar injection of 100 μl 5% dextran was diminished by 30.96% ($n=7$), 22.2% ($n=7$), 24.93% ($n=5$), 20.74% ($n=5$) and 51.92% ($n=5$) 30 min after the induction of inflammation in rats pretreated with somatostatin, TT-248, TT-250, RC-160 and TT-232 (6.11, 8.12, 9.43, 8.11 and 10.6 nmol kg^{-1} i.p), respectively. Octreotide had no influence on non-neurogenic oedema either ($n=5$) (Figure 2).

Efficacy and time-course of anti-inflammatory action of TT-232 on neurogenic inflammation and ineffectiveness of diclofenac and meloxicam

The heptapeptide TT-232 administered i.p. or i.v. (1.06, 2.65, 5.30, 10.6, 21.21, 42.42 nmol kg^{-1}) inhibited in a dose-

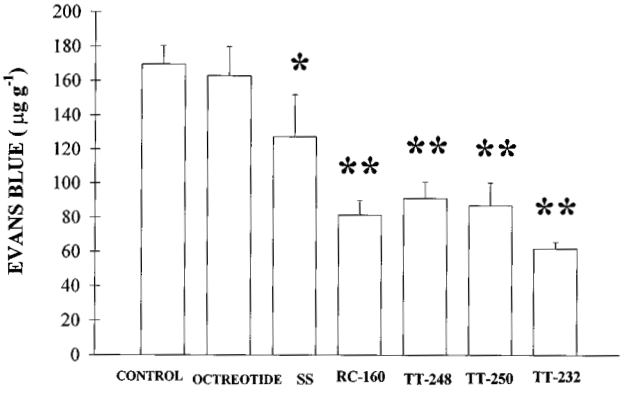

Figure 1 Effect of 10 μg kg^{-1} i.p. somatostatin (SS) and its cyclic, synthetic analogues, octreotide, TT-248, TT-250, RC-160 and TT-232 (6.11, 8.12, 9.43, 8.11 and 10.6 nmol kg^{-1}, respectively) on 1% mustard oil-induced neurogenic Evans blue accumulation in the skin of the acutely denervated hindlegs. In the control group saline (solvent) was applied i.p. in the same volume. Each column shows the mean of $n=5-7$ experiments with s.e.mean. *$P<0.05$; **$P<0.01$.

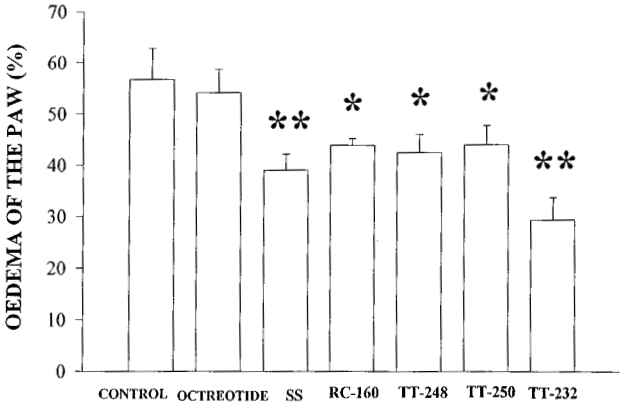

Figure 2 Effect of 10 μg kg^{-1} i.p. somatostatin (SS), octreotide and its new peptidomimetic analogues, TT-248, TT-250, RC-160 and TT-232 (6.11, 8.12, 9.43, 8.11 and 10.6 nmol kg^{-1}, respectively) on non-neurogenic oedema induced by subplantar injection of dextran (100 μl 5%) in the chronically denervated hindleg 30 min after the induction of inflammation. In the control group of rats the same volume of saline was given. Oedema was measured by plethysmometry before and 30 min after the administration of dextran. Columns indicate per cent increase of the volume of the hindpaws as compared to the original values (means$\pm$s.e.mean, $n=5-7$ per group). *$P<0.05$; **$P<0.01$.

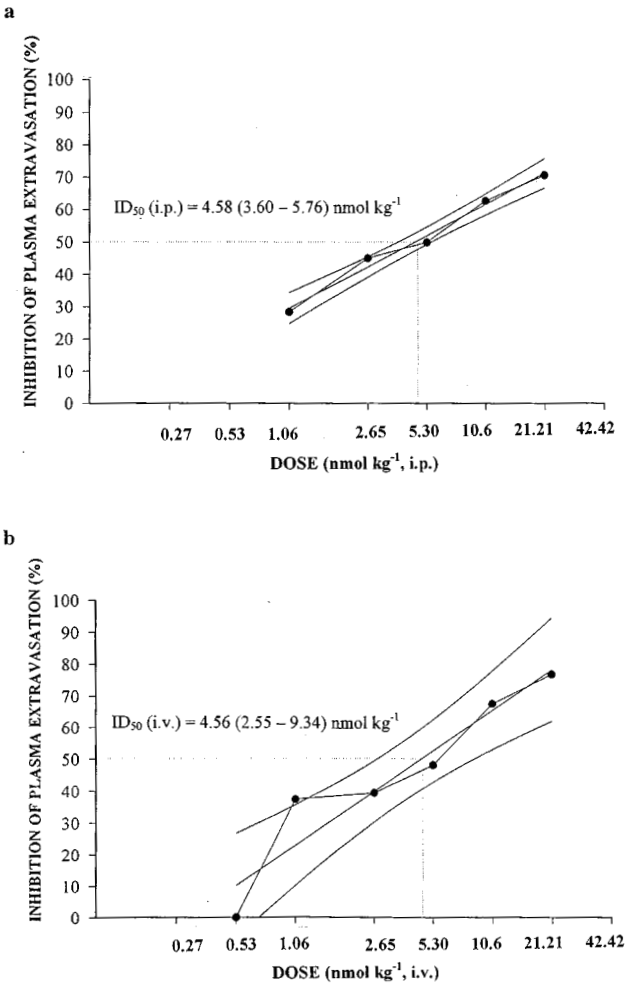

Figure 3 Dose-response curves of TT-232 after (a) i.p. and (b) i.v. administration for inhibiting the 1% mustard oil-induced neurogenic Evans blue accumulation in the skin of the acutely denervated hindlegs. Results are expressed in per cent inhibition as compared to the solvent-treated control group. The per cent inhibitions with 95% confidence intervals are calculated and presented with the ID_{50} values, $n=5-7$ animals in each group.

dependent manner the plasma extravasation evoked by mustard oil in the skin of the rat's paw. Dose-response curves of per cent inhibitions with 95% confidence intervals for i.p. and i.v. administrations gave almost identical values. The ID_{50} values were 4.58 (3.60–5.76) nmol kg^{-1} (i.p.) ($n=29$) and 4.56 (2.55–9.34) nmol kg^{-1} (i.v.) ($n=28$) (Figure 3a,b). The anti-inflammatory effect lasted for 2 h after a dose of 2.65 nmol kg^{-1} i.v. ($n=15$). Neither diclofenac given i.v. in doses of 15.72, 31.56, 63.12 μmol kg^{-1} and given i.m. in a high dose of 314.37 μmol kg^{-1} nor the selective COX-2 inhibiting agent meloxicam (2.95, 29.50, 56.91 and 569.38 μmol kg^{-1} i.v.) inhibited the mustard oil-induced neurogenic plasma extravasation. Mustard oil-induced cutaneous plasma extravasation was dose-dependently diminished by s.c. TT-232 (10.6, 21.21, 42.42, 84.84, 169.86, 339.36 and 2×530 nmol kg^{-1}) 1, 2, 4, 6 and 16 h after the pretreatment. Although the dose-response curves for s.c. administrations were not steep enough to determine the 95% confidence intervals, the 1, 2, 4 and 6 h ID_{35} values were 21.75, 34.31, 42.09 and 82.60 nmol kg^{-1}, respectively. Administration of 2×530 μg kg^{-1} per day TT-232 s.c. caused 52.09% inhibition of neurogenic plasma extravasation evoked by mustard oil 16 h after the second injection (Table 2).

Effect of TT-232, diclofenac and meloxicam on non-neurogenic dextran-oedema and bradykinin-evoked Evans blue accumulation

TT-232, diclofenac and meloxicam dose-dependently diminished non-neurogenic dextran-oedema of the paw. The

Table 2 Effects of different s.c. doses (nmol kg $^{-1}$) of TT-232 on neurogenic plasma extravasation 1, 2, 4, 6 and 16 h after 1% mustard oil smearing on the skin of the hindleg

		Plasma extravasation	*Inhibition (%)*
Control 1 (buffer)		199.73±8.56	
1 h	10.60	122.99±9.25**	38.42±2.65
	21.21	136.41±14.73**	31.70±3.21
	42.42	124.39±14.27**	37.70±3.18
	84.84	147.01±13.52*	26.40±2.03
2 h	21.21	143.69±13.10**	28.05±1.96
	42.42	118.13±15.15**	40.85±2.98
	84.84	114.86±13.40**	42.49±3.09
	169.68	89.68±7.97**	55.09±4.52
4 h	21.21	179.14±11.54	10.2±0.96
	42.42	114.77±16.04**	42.53±6.99
	84.84	94.65±10.54**	52.61±3.41
	169.68	104.48±10.06**	47.68±2.59
	339.36	97.49±8.47**	51.23±3.29
6 h	42.42	149.75±15.55*	25.02±2.41
	84.84	116.86±7.01**	41.48±3.85
	169.68	120.11±10.18**	39.86±3.12
	339.36	103.92±6.99**	47.96±3.25
16 h		152.42±10.63	
Control 2 (buffer)			
	2×530	73.02±8.17**	52.09±6.99

Plasma extravasation values are means±s.e.mean. Evans blue accumulation (μg) per g wet tissue, per cent inhibitions compared to the respective controls are also indicated. Mann–Whitney *U*-test was used to evaluate statistical significances (*$P<0.05$, **$P<0.01$), $n=5-7$ in each group.

respective ID_{35} (i.v.) values for TT-232 and diclofenac were 1.73 (1.61–6.22) nmol kg^{-1} and 34.37 (22.41–59.43) μmol kg^{-1}, respectively. The slope of the dose-response curve of meloxicam was not steep enough to determine this value (Figure 4a,b,c) and 50% inhibition in the applied dose range of the three drugs could neither be reached.

TT-232 inhibited in the dose range of 1.06–21.21 nmol kg^{-1} the bradykinin-induced plasma extravasation in the skin of the chronically denervated paw. Although the inhibition was statistically significant at all doses the dose-response curve was flat and did not reach the ID_{35} level (Table 3).

Effect of TT-232 on Freund-adjuvant induced arthritis

In control animals definite oedema developed in the Freund-adjuvant treated left leg and slight swelling on the contralateral side. Chronic arthritis was dose-dependently diminished by TT-232 (2×106, 2×212 and 2×530 nmol kg^{-1} per day s.c.) throughout the period of 18 days, more dominant inhibition was observed in the left leg (Table 4, Figure 5). Systemic symptoms (decreased mobility and appetite, fever, weariness) and local hyperalgesia were seen during the experiment.

Effect of TT-232 on sensory neuropeptide release from isolated rat trachea

Both capsaicin (10^{-7} M) and electrical field stimulation (40 V, 0.1 ms, 10 Hz, 120 s) evoked a significant increase in SP, CGRP and somatostatin release from isolated rat tracheae in control samples. TT-232 (500 nM) reduced the release of these

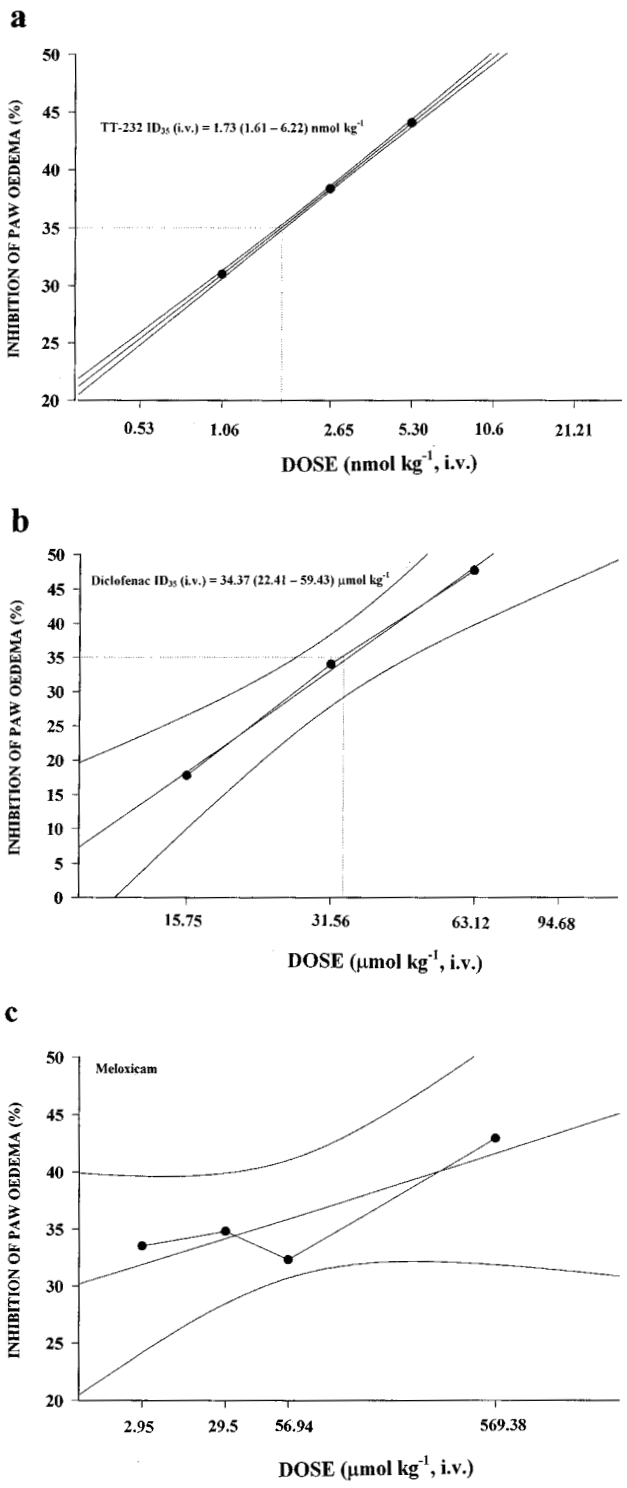

Figure 4 Dose-response curves of i.v. given TT-232 (a) diclofenac (b) and meloxicam (c) for inhibiting the dextran-induced oedema of the chronically denervated hindlegs. Tissue swelling was measured by plethysmometry 30 min after the induction of inflammation and the results were expressed in per cent inhibition as compared to the solvent-treated control group. The per cent inhibitions with 95% confidence intervals were calculated and presented with the ID_{35} values, $n=5-7$ animals in each group.

neuropeptides elicited by capsaicin by 79% (SP), 48% (CGRP), 74% (somatostatin), respectively (Figure 6). The corresponding

values in the presence of 500 nM TT-232 in response to electrical field stimulation were 36, 48 and 42% (Figure 7). TT-232 did not influence the basal, non-stimulated peptide release.

Discussion

Somatostatin and its novel cyclic synthetic analogues (the octapeptides RC-160, TT-248 and TT-250 and the heptapeptide TT-232) inhibited in nmol kg^{-1} dose range both the mustard oil-induced neurogenic plasma extravasation and the non-neurogenic dextran oedema. Mustard oil in concentrations under 5% causes pure neurogenic inflammation without involvement of mast cells (Inoue *et al*., 1997; Szolcsányi *et al*., 1998b) by selectively stimulating the capsaicin-sensitive sensory nerve endings (Jancsó *et al*., 1967). On the other hand dextran is known to exert oedema formation through mast cell degranulation (Selye, 1965). Thus, these data show that somatostatin and its analogues elicit acute anti-inflammatory effect either by acting directly on venular plasma extravasation process or by inhibition the release of inflammatory mediators both from the peptidergic sensory nerve terminals and also from the mast cells. Experiments with TT-232 indicate a slight diminution of bradykinin-induced plasma extravasation in chronically denervated hindleg. However, in contrast to the potent anti-inflammatory action of TT-232 against neurogenic plasma extravasation (ID_{50} = 4.56 nmol kg^{-1} i.v.) the significant inhibition of the bradykinin response did not reach the ID_{50} level up to a dose of 21.2 nmol kg^{-1} i.v. It is concluded that the potent acute anti-inflammatory action of the compound is mainly due to the inhibition of proinflammatory mediator release both from capsaicin-sensitive nociceptors (Szolcsányi, 1996a, b) and also from mast cells. Similar dual inhibition was observed in the case of another neuropeptide, nociceptin which inhibited simultaneously the release of sensory neuropeptides and mast cell degranulation, producing similar anti-inflammatory effect as somatostatin (Helyes *et al*., 1997; Németh *et al*., 1998b).

TT-232 failed to inhibit growth hormone release or gastrin secretion *in vivo*, but it had a strong antiproliferative,

Table 3 Effect of i.v. TT-232 (nmol kg^{-1}) on bradykinin-induced (50 μl, 0.25 μg s.c.) plasma extravasation in the skin of the chronically denervated hindleg

		Plasma extravasation	*% Inhibition*
Control (buffer)		127.35 ± 7.11	
TT-232	1.06	98.21 ± 7.44*	22.88 ± 2.04
	2.65	85.02 ± 7.33**	33.24 ± 2.34
	5.30	81.95 ± 7.64**	35.64 ± 3.19
	10.60	86.31 ± 8.47**	32.23 ± 2.81
	21.21	77.40 ± 4.59***	39.22 ± 3.06

Values are means ± s.e.mean. Evans blue accumulation (μg per g wet tissue), $n = 5$ in each group. Mann–Whitney *U*-test was used to evaluate statistical significances, $^*P < 5.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the control value.

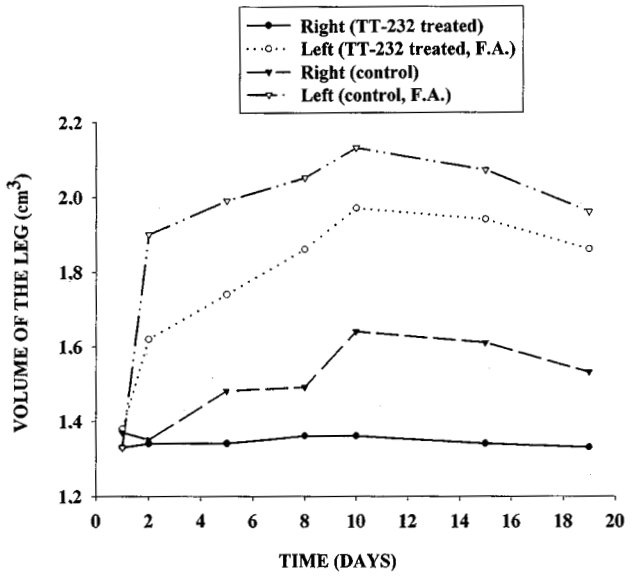

Figure 5 Freund-adjuvant induced oedema of the hindlegs in control and TT-232 (2 × 530 nmol kg^{-1} per day s.c.) pretreated rats ($n = 5–7$ per group) throughout the experimental period of 18 days. In control animals pronounced swelling developed in the treated left leg and slight swelling on the contralateral side. Responses of both legs were significantly inhibited by TT-232.

Table 4 Effect of TT-232 s.c. treatment (2 × 106, 2 × 212 and 2 × 530 nmol kg^{-1} per day) on Freund adjuvant-induced arthritis

	Control (buffer)		*TT-232*(2 × 106)		*TT-232*(2 × 212)		*TT-232*(2 × 530)	
Days	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*
2.	–	45.19 + 5.63	–	33.92 + 2.98	0.79 + 1.31	36.82 + 4.79	1.47 + 3.12	19.66 + 3.06 **
5.	12.14 + 3.33	62.36 + 3.61	14.88 + 2.81	58.99 + 6.37	8.82 + 2.22	48.97 + 4.31	6.50 + 2.74	33.08 + 4.75 ***
8.	15.69 + 3.11	69.79 + 3.90	17.01 + 3.74	62.22 + 4.01	11.54 + 3.23	53.08 + 4.21 *	8.9 + 3.29	36.88 + 3.97 ***
12.	21.35 + 2.83	75.13 + 4.30	18.43 + 3.25	58.62 + 3.08 **	16.11 + 2.24	54.83 + 4.08 **	8.63 + 2.67 *	46.01 + 4.33 ***
15.	25.34 + 3.28	70.87 + 4.19	26.38 + 4.22	61.06 + 3.72	15.72 + 2.20	55.51 + 3.01 *	10.47 + 3.49 **	44.15 + 3.27 ***
19.	24.16 + 3.41	71.62 + 6.30	25.88 + 3.13	60.64 + 3.31	20.34 + 2.14	59.96 + 1.23	10.76 + 4.39 *	41.38 + 4.77 **

Swelling of the hindleg compared to the first day volumes is indicated as increase in per cent (means ± s.e.mean, $n = 8$. Mann–Whitney *U*-test was used for statistical analysis of differences between values of the control and treated groups $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Left: Freund adjuvant-treated leg, right: untreated leg.

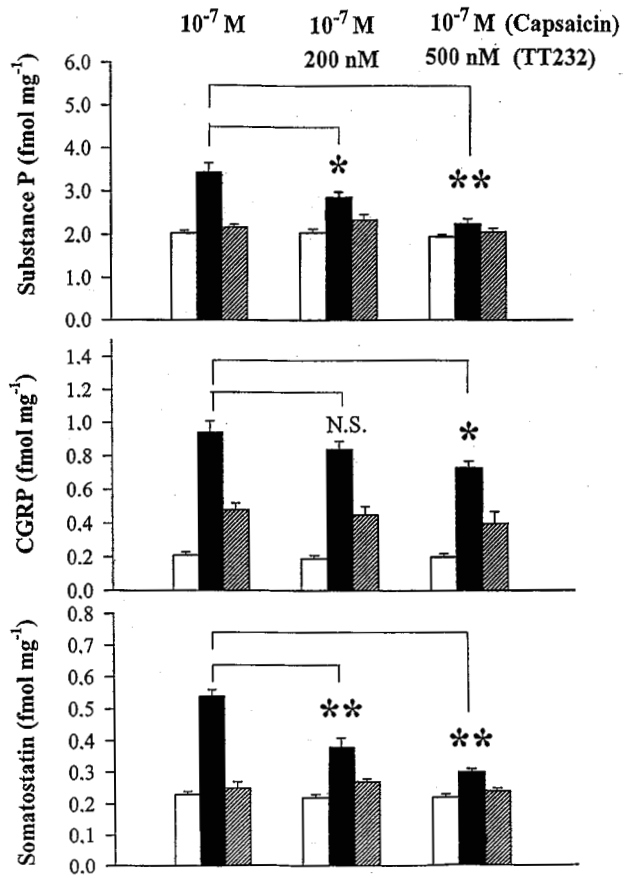

Figure 6 Effect of TT-232 (200 and 500 nM) on substance P, CGRP and somatostatin release from isolated rat tracheae in response to 10 M^{-7} capsaicin. Columns indicate prestimulated (open), stimulated (solid) and poststimulated (hatched) values. Results are shown as means ± s.e.mean obtained from six experiments (*$P<0.05$; **$P<0.01$).

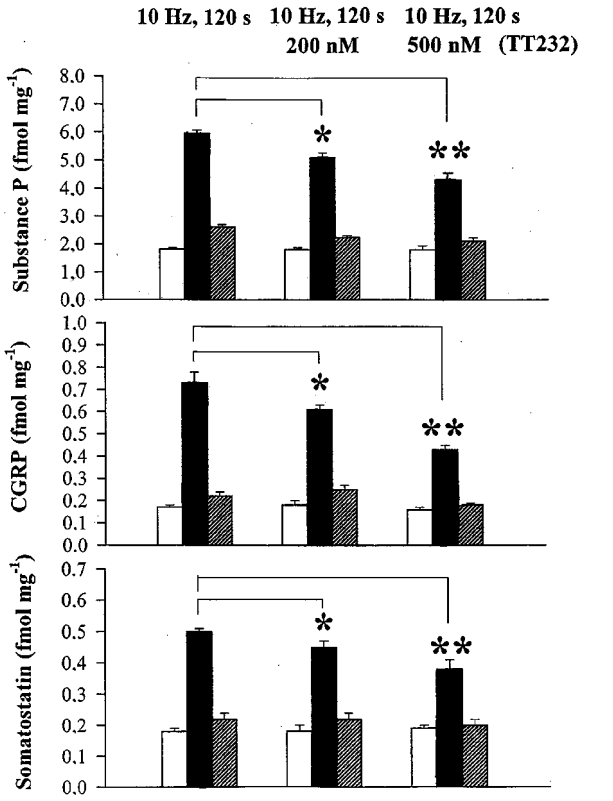

Figure 7 Effect of TT-232 (200 and 500 nM) on substance P, CGRP and somatostatin release from isolated rat tracheae in response to electrical field stimulation (40 V, 0.1 ms, 10 Hz, 120 s). Columns indicate prestimulated (open), stimulated (solid) and poststimulated (hatched) values. Results are shown as means ± s.e.mean obtained from six experiments (*$P<0.05$; **$P<0.01$).

apoptotic (Kéri *et al*., 1996) and anti-nociceptive effect (Helyes *et al*., 2000). The octapeptide analogue, octreotide, which is effectively used in the treatment of hormone-secreting tumours (Pincus *et al*., 1989), in the applied dose had no influence on neurogenic inflammation and dextran oedema, although there is some evidence, that octreotide inhibits carrageenin-induced oedema and leukocyte accumulation (Karalis *et al*., 1994) and formalin-induced nociception (Carlton *et al*., 2001). Consequently, it is concluded that the receptor subtypes being responsible for the anti-inflammatory and endocrine effects of somatostatin are different. Octreotide which has been reported to mediate its hormone secretion inhibitory action through the sst_2, sst_3 and sst_5 receptors (Hofland *et al*., 1995; Siehler & Hoyer, 1999) proved to be less effective than TT-248, TT-250 and RC-160 compounds, which also inhibit GH secretion (Jaspers *et al*., 1994). Structure-activity relationship study of these compounds on somatostatin receptor subtypes has not been done, but the heptapeptide TT-232 without endocrine effect showed the greatest anti-inflammatory potency, indicating pivotal role for sst_1 and/or sst_4 respors in this response. TT-232 in contrast to the other compounds contains a five-residue ring, which makes the molecular structure more rigid (Jaspers *et al*., 1994). This seems to be favourable to enhance its receptor selectivity. The present data has revealed that TT-232 inhibited the release of sensory neuropeptides from the rat trachea *in vitro* providing biochemical evidence for involvement of somatostatin receptors on capsaicin-sensitive sensory nerve endings in inhibition of neurogenic inflammation. Nevertheless, an additional neurokinin 1 (NK 1) receptor blocking activity described in the case of vapreotide (RC-160), a long-lasting octapeptide somatostatin analogue with analgesic effect (Betoin *et al*., 1995; Helyes *et al*., 2000) cannot be excluded. In rat lung tissues mRNA of two somatostatin receptor subtypes were indentified. Predominantly sst_4 receptor, and a lesser extent sst_1 subtype were expressed (Schloos *et al*., 1997). Therefore it is tempting to assume that sst_4 and probably sst_1 receptors are responsible for the anti-inflammatory effect of somatostatin and TT-232 on the neurogenic inflammation. Although recently a non-peptide somatostatin agonist with sst_4 selectivity was described the action of this compound on neurogenic inflammation has not been tested (Ankersen *et al*., 1998).

The classical non-steroidal anti-inflammatory agents (Jancsó-Gábor & Szolcsányi, 1970), diclofenac and the selective COX-2 inhibitor meloxicam (Engelhardt *et al*., 1995) diminished non-neurogenic oedema in a dose-dependent manner but did not influence neurogenic plasma extravasation. High dose steroids are able to attenuate neurogenic

inflammation (Piedimonte *et al*., 1990), but their many serious side-effects limit their usage in clinical practice. Neurogenic and non-neurogenic components simultaneously take part in most of the inflammatory processes, and neurogenic inflammation plays significant role in the pathogenesis of rheumatoid arthritis, allergic rhinitis and conjunctivitis, asthma bronchiale, urticaria, psoriasis, migraine (Geppetti & Holzer, 1996) and also in adjuvant arthritis in the rat (Donaldson *et al*., 1995), and murine delayed-type hypersensitivity reactions (Girolomoni & Tigelaar, 1990).

Daily s.c. pretreatment of rats with doses of TT-232 which inhibited neurogenic inflammation for 6–16 h diminished also the development of Freund adjuvant-induced bilateral arthritis. It has been described in several experimental models that somatostatin suppresses a number of immune functions among others the release of proinflammatory cytokines, lymphocyte proliferation, and immunoglobulin production (ten Bokum *et al*., 2000). In the Freund adjuvant arthritis model activation of T-cell subtypes by Mycobacterium cross-reacts with articular tissues causing in this way joint destruction and enhancement of local immune responses (Wooley, 1991). The fact that this type of systemic chronic inflammatory response is also inhibited by somatostatin analogue which devoid of endocrine effects opens new horizons for development of broad spectrum anti-inflammatory agents which are effective also against neurogenic inflammation. TT-232 is a promising, effective, stable and selective somatostatin analogue for parenteral application. It is proposed as a lead molecule for a new class of anti-inflammatory, analgesic agents.

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