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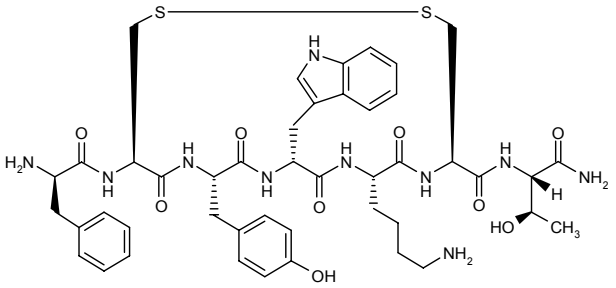
TT-232

Somatostatin sst_1/sst_4 Receptor Agonist
Treatment of Neuropathic Pain
Treatment of Inflammation

D-Phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-cysteinyl-L-threoninamide cyclic disulfide



$C_{45}H_{58}N_{10}O_9S_2$
Mol wt: 947.136
CAS: 147159-51-1
EN: 205408

Abstract

The treatment of the neurogenic component of inflammatory diseases and neuropathic pain remains an unresolved issue in pharmacotherapy. A novel endogenous antiinflammatory/analgesic mechanism mediated by somatostatin released from capsaicin-sensitive sensory nerve terminals has been identified and reported by our research group. These findings raised the possibility of targeting somatostatin receptors to decrease inflammation and nociception. Native somatostatin cannot be used in clinical practice due to its short plasma half-life and broad range of actions, but stable and selective synthetic analogues might open new perspectives for drug development. A cyclic heptapeptide analogue, TT-232, showing the greatest affinity for the somatostatin sst_4 receptor subtype was synthesized at the Peptide Chemistry Research Group of the Hungarian Academy of Sciences and proved to have no endocrine activity. On the other hand, it exerted significant inhibitory effects in several acute and chronic inflammatory and nociceptive animal models, including chronic arthritis, mono- and polyneuropathy conditions. TT-232 is therefore considered a promising lead molecule for the development of a completely new type of antiinflammatory/analgesic agents.

Synthesis

TT-232 is prepared by common methods of solid-phase peptide synthesis in a benzhydrylamine polystyrene resin using Boc- or Fmoc-protected amino acids. The cyclization to form the disulfide bridge is performed by oxidation with I_2 or thallium trifluoroacetate and the removal of protective groups and cleavage of the peptide from the resin is carried out by using HF or trifluoroacetic acid (1, 2).

Introduction

Although the pharmacological sciences have witnessed enormous progress in the 20th century, the area of antiinflammatory and analgesic agents is still restricted to nonsteroidal drugs (cyclooxygenase inhibitors), and in severe cases corticosteroids or opioids. These drugs are double-edged swords because, despite their severe side effects (gastrointestinal erosion/bleeding, renal damage, bone marrow suppression, etc.), they provide the only possibility for the symptomatic treatment of several frequently occurring inflammatory and immune-mediated diseases, such as rheumatoid arthritis, psoriasis and asthma. On the other hand, they are unable to inhibit either the neurogenic component of inflammatory processes mediated by proinflammatory sensory neuropeptides, *i.e.*, tachykinins and calcitonin gene-related peptide (CGRP), or neuropathic pain. Therefore, there is still a great need for new types of antiinflammatory/analgesic agents acting on other targets. Recently, a novel neurohumoral regulatory mechanism was described by our research group. We have provided several lines of evidence in animal experiments that somatostatin released from activated sensory nerve terminals exerts systemic antiinflammatory and antinociceptive actions (3-6). Therefore, it has been suggested that certain

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somatostatin receptors might be promising novel targets for the development of antiinflammatory/analgesic drugs.

Somatostatin is widely expressed in both the central and peripheral nervous systems in 14- or 28-amino-acid forms (7, 8). It is synthesized and stored in the capsaicin-sensitive transient receptor potential vanilloid 1 (TRPV1) receptor-expressing subpopulation of nociceptors, from where it can be released and depleted (9-11). Approximately 20-30% of cutaneous C-fibers show positive somatostatin immunostaining (12).

Numerous effects of somatostatin have already been described, including inhibition of the release of several neurotransmitters (substance P, CGRP, dopamine, acetylcholine and somatostatin itself) and hormones (glucagon, insulin, growth hormone), and modulation of cognitive and behavioral processes, the gastrointestinal tract, the cardiovascular system and tumor cell proliferation (8, 13). These effects are mediated via 5 different somatostatin receptor subtypes (sst_{1-5}), which have been cloned. Structurally they are glycoproteins containing 7-transmembrane α-helical domains connected by short loops, an *N*-terminal extracellular domain and a *C*-terminal intracellular domain (14). They belong to the G-protein-coupled receptor family, which mediates the inhibition of adenylate cyclase activity (7), reduces the conductance of voltage-gated Ca^{2+} channels and activates K^+ channels (15). Furthermore, somatostatin also stimulates tyrosine kinase activity and inhibits cell proliferation (16). According to functional characterization and binding studies using synthetic somatostatin analogues, these receptor subtypes can be divided into 2 main classes: sst_2, sst_3 and sst_5 belong to the SRIF1 group, while sst_1 and sst_4 belong to the SRIF2 group (17, 18).

It has been known for a long time that somatostatin inhibits neurogenic inflammation (19-21) and nociception, both inflammatory pain and the activity of sensitized nociceptors (22-24). We provided several lines of evidence in rat experiments that a sufficient amount of somatostatin could be released from the activated peripheral terminals of primary afferent nerves in response to orthodromic chemical or antidromic electrical stimulation to elicit systemic inhibitory effects on inflammatory and nociceptive responses in distant parts of the body (3-6). Somatostatin release and its systemic inhibitory activity were observed in response to very-low-frequency (0.1 Hz) stimulation, in which case the release of proinflammatory sensory neuropeptides and the consequent neurogenic inflammation could not be evoked (4, 25). In humans, single-unit discharges of C-polymodal nociceptive fibers clearly showed that, under physiological conditions, these afferents signal no pain or other sensation when the frequency action potential is below 1 Hz (25). Pretreatment with polyclonal somatostatin antiserum or cysteamine, which selectively inactivates somatostatin both immunologically and functionally, prevents the systemic antiinflammatory effect of sciatic/vagal nerve stimulation (3, 26) or 1% mustard oil application (4). Plasma somatostatin-like immunoreactivity increases more than 4-fold after stimulation of both sciatic nerves and by 70% after mustard oil smearing on the hindpaw skin. These stimulation-evoked elevations in plasma somatostatin concentrations were not observed in cysteamine-pretreated rats (3, 4).

Furthermore, functional evidence was obtained for the activity of somatostatin released from capsaicin-sensitive nociceptors in the rat adjuvant-induced chronic arthritis model (6). Pretreatment with resiniferatoxin (RTX), which destroys or impairs the function of capsaicin-sensitive nerve terminals (27, 28), increased paw edema on both the adjuvant-treated and the contralateral side. Similar significant enhancement of paw edema was observed in those rats which received daily treatment with cyclosomatostatin. RTX and cyclosomatostatin treatments increased inflammatory mechanical hyperalgesia as well. Plasma somatostatin-like immunoreactivity increased up to more than 3-fold during the 3-week experimental period in control adjuvant-treated rats, but not in those arthritic animals which received RTX pretreatment (6). These findings are supported by data demonstrating the presence and even the upregulation of somatostatin binding sites in affected tissues of infectious and immune-mediated diseases, such as rheumatoid arthritis and inflammatory bowel disease (IBD) (8, 13, 29).

The term ”sensocrine” function based on the described endogenous counterregulatory mechanism was coined to denote the endocrine-like systemic neurohumoral response induced by somatostatin released from activated capsaicin-sensitive nociceptors (25, 26, 28). It is particularly unique, because endocrine-like systemic neurohumoral function evoked by the release of a mediator from sensory nerve endings has not been described previously.

Exogenously applied somatostatin has been shown to inhibit vasodilatation and plasma protein extravasation induced by saphenous nerve stimulation (19). In accordance with these data, we found that somatostatin (10 μg/kg i.p.) reduced plasma leakage induced by mustard oil and sciatic nerve stimulation in rat hindpaw skin by 30% and 42%, respectively (3). Somatostatin inhibits the release of substance P (30) and CGRP from sensory nerve endings, in addition to a negative feedback mechanism whereby it inhibits its own release (31). It also decreases immunoglobulin production in B-lymphocytes (32) and monocyte-macrophage functions (33). Furthermore, it inhibits lymphoid cell proliferation (34), T-lymphocyte functions, such as cytokine production (35), and IL-8/IL-1β secretion from intestinal epithelial cells (36). Somatostatin inhibits the cellular phases of inflammation by decreasing chemotaxis, adhesion, accumulation and the O_2^- production of neutrophilic granulocytes, lymphocyte proliferation, IgG production and inflammatory cytokine release from macrophages. In joints, it decreases the proliferation of synovial cells (Fig. 1) (13, 25).

Although the continuous release of somatostatin resulting in a ”sensocrine function” of capsaicin-sensitive sensory nerve terminals (3, 25, 26, 37) might be a powerful neuroregulatory mechanism, the clinical value of the natural neuropeptide in the treatment of inflammatory or painful diseases is limited due to its broad range of effects

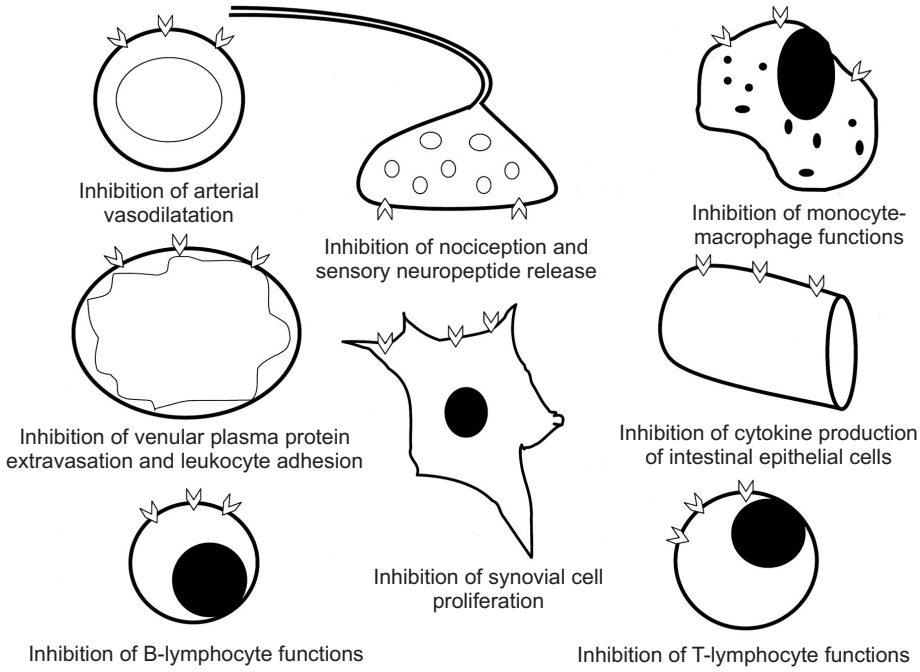

Fig. 1. Potential targets for the inhibitory actions of somatostatin in inflammatory and nociceptive conditions.

and very short half-life in the circulation (< 3 min). These shortcomings, however, can be avoided by using more selective and longer acting synthetic structural analogues. Octapeptide analogues, such as octreotide (SMS-201995, Sandostatin®), bind to receptor subtypes of the SRIF1 family, while they have very low affinity for the other subtypes (14). Octreotide is characterized by greater potency, a longer duration of action, greater metabolic stability and higher selectivity over inhibition of hormone secretion than somatostatin. It is effectively used in the treatment of hormone-secreting tumors, but had no influence on neurogenic inflammation and dextran-induced non-neurogenic edema in our experiments (38). None of the peptide analogues available at present binds selectively to one somatostatin receptor subtype, although there are many new approaches in this field (8, 39). In the group with D-amino acid substitutions, replacing Trp^8 with D-Trp^8 increases the potency by 6-8 times, which might reflect the stabilization of a more active conformation or may result from greater resistance of this peptide to degradation. D-Trp^8 substitution seems to be critical for the potency of somatostatin analogues, as it has been used in all molecules. The aromatic side-chain of D-Phe added to the *N*-terminal occupies the conformational space and protects the disulfide bridge from enzymatic attack (39).

Recently, a series of new potent and stable analogues was synthesized by the Peptide Chemistry Research Group of the Hungarian Academy of Sciences to study the relative importance of specific substitutions in selectivity for the 5 receptor subtypes. TT-232, an analogue with a cyclopenta ring structure and unique conformational characteristics, was found to be particularly promising.

Pharmacological Actions

TT-232 does not inhibit growth hormone release or gastrin secretion, but it has strong antiproliferative and apoptotic effects on tumor cells *in vivo* and *in vitro* (40-44). These effects of TT-232 strongly correlated with its tyrosine kinase-inhibitory action (45). When tumor cells were incubated with this peptide for short periods of time, no inhibition of tyrosine kinase was observed, but significant stimulation of phosphotyrosine phosphatases could be detected, with a biphasic dose-dependency (46).

TT-232 is an effective antiinflammatory and antinociceptive compound, exerting significant inhibitory activity in several rat and mouse models at μg/kg doses. These effects are detailed below and summarized in Tables I and II.

Both capsaicin (0.1 μM) and electrical field stimulation (40 V, 0.1 ms, 10 Hz, 120 s; 1200 impulses) induced a significant increase in the release of the sensory neuropeptides substance P, CGRP and somatostatin from isolated rat trachea. The stimulation-evoked release was significantly inhibited by 500 nM TT-232 in all cases (31, 38), whereas it did not influence basal peptide release. Neither the tyrosine kinase inhibitor genistein (50 μM) nor the G-protein blocker pertussis toxin (PTX; 100 ng/ml) influenced neuropeptide release themselves, but PTX prevented the inhibitory effect of TT-232 on electrical field stimulation-induced peptide release (31). In rat airways the mRNA expression of two somatostatin receptor subtypes was identified, predominantly the sst_4 receptor and to a lesser extent the sst_1 subtype (47). Therefore, it is tempting to assume that an sst_4 or sst_1 receptor-medi-

Table I: Effects of TT-232 in models of inflammation.

Experimental model	Species	Effect	Reference compound	Ref.
Acute models				
Neurogenic plasma protein extravasation induced by mustard oil in paw skin	Wistar rat	ED_{50}: 20-min pretreatment: 4.3 µg/kg i.v. or i.p.; 4-h pretreatment: 65 µg/kg i.p., 78 µg/kg s.c.	Diclofenac/meloxicam 5-100 mg/kg i.p.: no significant effect	38
Neurogenic edema formation induced by capsaicin in the ear	BALB/c mouse	ED_{50}: 6.5 µg/kg i.v.	Diclofenac 5-20 mg/kg i.p.: no significant effect	31
Non-neurogenic edema formation induced by dextran in chronically denervated hindpaw	Wistar rat	ED_{35}: 2.5 µg/kg i.v.	Diclofenac ED_{35}: 35 mg/kg i.v. Meloxicam: significant (40.5%) inhibition at 500 mg/kg i.v.; ED_{35} could not be calculated due to flat dose-response curve	38
Non-neurogenic plasma protein extravasation in the skin induced by bradykinin in chronically denervated hindpaw	Wistar rat	Significant inhibition at 1-20 µg/kg i.v., maximal (39.2%) inhibition at the highest dose; dose-response correlation not found		38
Carrageenan-induced paw edema	Wistar rat	ED_{50}: 7.1 µg/kg i.v. at 1 h, 7.0 µg/kg i.v. at 2 h, 6.9 µg/kg i.v. at 3 h	Diclofenac 20 mg/kg i.v.: significant (32.3-39.4%) inhibition; ED_{50} could not be calculated	31
Plasma extravasation in the knee joint induced by intraarticular bradykinin	Wistar rat	ED_{35}: 15.6 µg/kg i.v.	Diclofenac ED_{35}: 9.8 mg/kg i.v.	31
Carrageenan-induced cutaneous neutrophil accumulation in the hindpaw skin over a 3-h period	Wistar rat	Significant (46.5%) inhibition at 3 x 80 µg/kg i.v.; ED_{35} could not be calculated due to flat dose-response curve	Diclofenac 3 x 10 mg/kg i.v.: no effect	31
IL-1β-induced cutaneous neutrophil accumulation in the hindpaw skin over a 3-h period	Wistar rat	Significant (56.4%) inhibition at 3 x 80 µg/kg i.v.; ED_{35} could not be calculated due to flat dose-response curve	Diclofenac 3 x 10 mg/kg i.v.: similar (54.2%) inhibitory effect	31
Chronic model				
Adjuvant-induced arthritis	Lewis rat	Significant inhibition of edema at 2 x 500 µg/kg/day s.c.; dose-dependent inhibition of edema and histological arthritis score at 2 x 100-400 µg/kg/day i.p.		6, 38

ated action is responsible for the inhibitory effects of TT-232.

Neurogenic inflammation elicited by 1% mustard oil and capsaicin in rats and mice, respectively, and 5% dextran-evoked non-neurogenic edema in the denervated paw of the rat were dose-dependently reduced by TT-232 (38). Acute inflammatory edema and plasma extravasation evoked by carrageenan or bradykinin were also dose-dependently inhibited (31). Furthermore, low doses (5-10 µg/kg) of TT-232 decreased neurogenic plasma extravasation induced by saphenous nerve stimulation in hindpaw skin and bradykinin-induced inflammation in the ankle joint (38). TT-232 proved to be more effective than native somatostatin. Octreotide was without effect in both the mustard oil-induced neurogenic and dextran-induced non-neurogenic inflammatory models, whereas TT-232 was highly effective (38). It was a potent antiinflammatory agent against capsaicin-induced pure neurogenic ear edema formation in the mouse and cutaneous neutrophil accumulation in response to carrageenan or IL-1β in the rat. Thus, TT-232 proved to be effective against both acute vascular and longer lasting cellular inflammatory reactions.

Complete Freund's adjuvant injected s.c. into one paw and the root of the tail of rats induced polyarthritis, with more severe joint inflammation on the injected side and systemic symptoms (fever, loss of appetite, restriction of movement). Inflammatory edema measured with plethysmometry throughout the 21-day experimental period and articular damage evaluated on the basis of histological scoring were dose-dependently reduced by TT-232 (2 x 100-500 µg/kg/day i.p.) (6, 31). In the rat adjuvant arthritis model, sst_3 and sst_4 receptor subtypes were found to be overexpressed in the immune system, and octreotide, which has high affinity for the SRIF2 group of receptors, did not affect the severity of inflammation. It therefore appears likely that the antiinflammatory effect of TT-232 in this model may be mediated by

Table II: Effects of TT-232 in nociceptive models.

Animal model	Species	Effect	Reference compound	Ref.
Acute models				
Nociceptive cardiovascular (blood pressure, heart rate) and respiratory (frequency) reflex responses evoked by topical 1% mustard oil	Wistar rat (anesthetized)	Significant (81-93%) inhibition at 10 µg/kg i.p.		5
Carrageenan-induced mechanical hyperalgesia	Wistar rat	5.1 µg/kg i.v.		31
Nociceptive heat threshold elevation	Wistar rat	1-1.5 °C heat threshold increase at 20-200 µg/kg i.p.	Diclofenac 10-60 mg/kg i.p.: 0.5-1.5 °C increase in heat threshold	25, 49
Resiniferatoxin-induced heat allodynia	Wistar rat	Significant inhibition at 10-100 µg/kg i.p., 50% inhibition at the highest dose; bell-shaped dose-response curve; ED_{50} could not be calculated	Diclofenac ED_{50}: 700 µg/kg i.p.; Paracetamol ED_{50}: 100 mg/kg i.p.	25, 49
Formalin-induced acute somatic chemonociception	Wistar rat	Phase I (chemonociception): significant inhibition at 80 µg/kg i.p.; phase II (acute inflammation): significant inhibition; ED_{50}: 62.8 µg/kg i.p.	Diclofenac: no effect in phase I; 27.5% inhibition of phase II at 50 mg/kg i.p.	25
Phenylquinone-induced acute visceral nociception (writhing test)	BALB/c mouse	Significant inhibition at 10-200 µg/kg s.c., maximal (70-75%) inhibition at 20 and 200 µg/kg; no dose-response correlation		25
Chronic models				
Adjuvant-induced arthritis; inflammatory mechanical hyperalgesia during 21 days	Lewis rat	Dose-dependent inhibition of hyperalgesia at 2 x 100-400 µg/kg/day i.p.		6
Traumatic mononeuropathy model (Seltzer); mechanical hyperalgesia 7 days after operation	Spraque-Dawley rat	Significant inhibition of hyperalgesia at 5 µg/kg i.p., complete reversal to hypoalgesia at 10-20 µg/kg i.p.	Diclofenac 10 mg/kg i.p.: no effect; baclofen 3 mg/kg i.p.: hyperalgesia reversed	31
Streptozotocin-induced diabetic polyneuropathy model; mechanical hyperalgesia 5 weeks after streptozotocin administration	Wistar rat	Significant inhibition at 10-100 µg/kg i.p., minimum effective dose of 10 µg/kg, maximal (56.4%) inhibition at 50 µg/kg; no dose-response correlation		25

sst_1 and/or sst_4 receptor subtypes.

Nocifensive cardiorespiratory reflexes (increase in systemic blood pressure, heart rate and respiration rate) evoked by topical application of 1% mustard oil on the hindpaw skin of the rat were significantly inhibited by pre-treatment with 10 µg/kg TT-232. The effect of TT-232 was greater than that of native somatostatin or the octapeptide analogue vapreotide. Octreotide did not influence any of these reflex responses, suggesting no participation of sst_2, sst_3 or sst_5 receptor subtypes (5).

TT-232 elevated noxious heat threshold and inhibited heat allodynia induced by the potent TRPV1 receptor agonist RTX (25). The minimum effective dose of TT-232 (10 µg/kg) on RTX-evoked heat allodynia was several orders of magnitude lower than that of diclofenac (1 mg/kg) or paracetamol (100 mg/kg) (48). TT-232 produced a maximum inhibition of about 60% in the RTX allodynia test, meaning that it was less effective than morphine or paracetamol, which could abolish the response, but more effective than diclofenac, which produced only 40% inhibition (49). The heat threshold-elevating action of TT-232 is in full accordance with the studies of Carlton *et al.* (23, 24), in which somatostatin receptor agonism inhibited discharge activity of cutaneous polymodal nociceptors evoked by noxious heat stimulation. Diclofenac 10 mg/kg or morphine 3 mg/kg produced similar effects, indicating that the potency of TT-232 is much higher than the reference compounds (25, 50).

The antinociceptive effect of TT-232 (20-160 µg/kg i.p.) and diclofenac (10 and 50 mg/kg i.p.) were assessed on the two phases (51) of the formalin test. In the first phase, the nocifensive behavior was slightly and non-significantly inhibited by TT-232, except at a dose of 80 µg/kg which was associated with a significant 55% decrease. Diclofenac failed to inhibit the first phase of formalin-induced nocifensive behavior over the dose range

tested. In the second phase, TT-232 induced inhibition with a bell-shaped dose-response relationship. The greatest antinociceptive effect (66% inhibition) was achieved at a dose of 80 μg/kg. Antinociception induced by TT-232 at a dose of 40 μg/kg was similar to that produced by 50 mg/kg diclofenac, indicating that the compound is about 1,000 times more potent than the reference drug (25).

TT-232 (10-200 μg/kg) administered s.c. 30 min before i.p. phenylquinone injection significantly reduced the number of abdominal writhing movements compared to the solvent-treated control group, but a clear dose-response relationship could not be detected in this model. The greatest antinociceptive effect was achieved at doses of 20 and 200 μg/kg (70% and 75% inhibition, respectively) (25).

In chronic inflammatory and neuropathic models in which significant mechanical hyperalgesia/allodynia developed, TT-232 produced a pronounced antihyperalgesic effect, although it failed to alter the mechanonociceptive thresholds of the hindpaw of intact rats. The touch sensitivity threshold decreased by 25-35% in the adjuvant-treated paw and only by 5-7% in the contralateral paw. Significant and dose-dependent inhibition of mechanical allodynia was observed in the TT-232-treated (2 x 100-400 μg/kg/day) groups (6).

Traumatic mononeuropathy induced by partial ligation of the sciatic nerve evoked a 25-30% decrease in the mechanonociceptive threshold 7-9 days after the operation. This hyperalgesia was dose-dependently inhibited by TT-232 (2.5-20 μg/kg i.p.) and reversed to hypoalgesia at higher doses (35).

Rats with experimental diabetes induced by a single injection of streptozotocin (50 mg/kg i.v.) displayed polyuria, a reduced growth rate and a marked increase in water and food intake, but otherwise appeared normal. Five weeks after streptozotocin administration, the mechanonociceptive threshold decreased by 28.6 ± 3.1%. TT-232 at doses of 10-100 μg/kg was associated with a pronounced inhibition of mechanical allodynia, although lower doses were ineffective. The minimum effective dose was 10 μg/kg and the maximum effect was achieved at 20 μg/kg (25). Diclofenac was ineffective at a dose of 10 mg/kg. Neuropathy is a relatively early symptom affecting a high proportion of diabetic patients. The mechanism and pharmacological susceptibility of neuropathic pain are different from pain induced by physiological or inflammatory stimuli. Since the analgesic action of opiates is almost absent in neuropathic conditions (52-54), the efficacy of TT-232 is particularly promising.

At the cellular level, somatostatin can open various K^+ channels and inhibit voltage-gated Ca^{2+} channels, which results in inhibition of both spike generation and the release of neurotransmitters (55). In addition, sst_4 agonists enhance signaling through mitogen-activated protein kinase (MAPK), phospholipase C (PLC) and phospholipase A_2 (PLA_2) and activate/inhibit phosphotyrosine phosphatases (PTPases) (55). The effect of TT-232 on intracellular signal transduction was analyzed in several tumor cell lines and the slowly developing tyrosine kinase inhibition seemed to play a pivotal role in its antitumor effect (40, 41). Early responses of these cells resembled those to sst_4 agonism, since the compound activated both PTPases and certain protein kinases, such as extracellular signal-regulated kinase (ERK2/MAPK) (46, 56). Moreover, we obtained experimental evidence suggesting that the antiinflammatory/analgesic effects of the compound are due to an agonist effect on the G-protein-coupled somatostatin sst_4 receptor. Activation of this receptor inhibits the activity of sensory nerve terminals and also inflammatory, vascular endothelial or synovial cells. The involvement of G-proteins in the inhibitory effect of TT-232 on the release of sensory neuropeptides from isolated rat trachea was proposed, since PTX was shown to block this effect (31). TT-232 failed to bind to somatostatin receptors in the brain or pituitary membrane preparations to inhibit growth hormone release and gastric acid secretion, indicating that its actions are not likely to be mediated by sst_2, sst_3 or sst_5 receptors (41). Furthermore, previous findings demonstrated the ineffectiveness of octreotide in mustard oil-induced neurogenic and dextran-induced non-neurogenic inflammatory models in the rat, where TT-232 significantly inhibited the neurogenic plasma extravasation and paw swelling (38). Attenuation of neurogenic inflammation by TT-232 is unlikely to involve tyrosine kinase inhibition, because the release of substance P and CGRP was not inhibited by the tyrosine kinase inhibitor genistein (31).

Based on these data, it was tempting to assume that sst_4, and probably sst_1, receptors are responsible for the antiinflammatory effects of TT-232. This proposed mechanism is supported by specific binding and agonist assays performed by Wurster and Engström at Juvantia Pharma (Turku, Finland). The affinity of TT-232 for the 5 human somatostatin receptor subtypes (sst_1, sst_2, sst_3, sst_4 and sst_5) was determined in competition binding assays using [^{125}I]-Tyr-[Leu^8,D-Trp^{22}]-somatostatin-28 ([^{125}I]-LTT-SRIF-28) and membranes from Chinese hamster ovary (CHO) cells stably transfected with 1 of the 5 human somatostatin receptor subtypes. These binding studies indicated that the compound displayed the greatest affinity for the human sst_4 receptor (Table III). In the functional agonist-mediated [^{35}S]-GTPγS binding assay, TT-232 gave an agonist potency (EC_{50}) at the sst_4 receptor of 1.4 ± 0.2 μM. The estimated efficacy of the compound was about 40% higher than that of somatostatin.

Although tyrosine kinase inhibition or dephosphorylation of the TRPV1 receptor is not likely to participate in the

Table III: Binding affinity of TT-232 for the 5 somatostatin receptor subtypes (sst_1-sst_5 receptors) expressed in CHO cells.

Somatostatin receptor subtype	Affinity (K_i; nM)
sst_1	1300 ± 400
sst_2	3600 ± 900
sst_3	3100 ± 800
sst_4	200 ± 10
sst_5	1500 ± 300

antiinflammatory effects of TT-232, this mechanism may play a role in its antinociceptive actions. Tyrosine kinase A (TrkA) signals the nerve growth factor (NGF)-induced sensitization of nociceptors to heat within minutes (57), and dephosphorylation of the TRPV1 receptor induces antinociception (58). The antiinflammatory effect of TT-232 increased with increasing dose (31, 38), but in the case of its antinociceptive action, a bell-shaped dose-response relationship was observed in the formalin test and RTX-induced heat allodynia (25). This unusual dose-response relationship was less pronounced for heat threshold, writhing behavior and diabetic hyperalgesia. Further experiments are in progress to shed light on the possible contribution of protein dephosphorylation in sensory nerve endings to the analgesic effect of TT-232.

Toxicity

TT-232 had no toxicity over a wide dose range. The lethal dose could not be determined because beyond a small (10%) and transient loss in body weight, no deaths were observed after administration of the highest (120 mg/kg) dose (41). Hematological parameters and blood chemistry were not affected and histological studies of different organs from TT-232-treated mice did not show significant modifications in the tissues (41). Doses of TT-232 up to 1,000 times greater than those inhibiting neurogenic inflammation and bradykinin-induced arthritis, or producing an analgesic effect in a neuropathic pain model, did not cause gastric or duodenal mucosal damage in the rat (31, 50). Since the presently available steroidal and non-steroidal antiinflammatory drugs all damage the gastric and duodenal mucosa, TT-232 may offer a means of avoiding the most frequent serious side effects of antiinflammatory therapy. TT-232 up to a dose of 300 μg/kg caused no significant change in indomethacin-induced gastric mucosal damage. Somatostatin has been shown to have an inhibitory effect on indomethacin-induced gastric injury (48), and the lack of effect of TT-232 may be attributable to its different somatostatin receptor subtype selectivity.

Whole-body autoradiography in rats showed low concentrations in the brain (about 0.1 μg/g) 30 min after a single i.v. dose of 2 mg/kg [^{14}C]-TT-232, indicating low penetration of the blood-brain barrier. Furthermore, central nervous system effects have not been observed even after 5 mg/kg i.v. (unpublished data).

Conclusions

A broad spectrum of antiinflammatory and analgesic activity has been established for TT-232. This compound may thus be effective in vascular and cellular inflammatory responses, in addition to neurogenic inflammation. Rheumatoid arthritis and psoriasis are severe chronic diseases in which the involvement of sensory neuropeptides and neurogenic inflammation play important roles (59) and where treatment with parenteral corticosteroids or cytotoxic anticancer drugs may be required during exacerbations. Protein tyrosine kinases (60), proinflammatory sensory neuropeptides (61) and several other inflammatory mediators, including IL-1β and platelet-activating factor (PAF), appear to play important roles in the pathogenicity of these diseases. Our research (for review, see 13, 25) and other data published in the literature (21, 62) have suggested the therapeutic significance of targeting somatostatin receptors, especially the sst_4 subtype, in these inflammatory and painful conditions. TT-232 has been proposed as a candidate for the treatment of inflammatory skin or joint diseases. Another potential field of application for this compound is neuropathic pain, particularly in diabetes (63), for which no adequate treatment is available and where the analgesic effect of opiates is often compromised (53). The broad antinociceptive spectrum and high potency of the compound, combined with its antiinflammatory action in a wide range of acute and chronic diseases, make this lead molecule very promising for development and it is currently undergoing clinical pharmacological studies. The development of nonpeptide molecules appropriate for oral administration opens further perspectives in the field of antiinflammatory and analgesic drug research.

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Source

Biostatin Research & Development, Ltd (HU).

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