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The heptapeptide somatostatin analogue TT-232 exerts analgesic and anti-inflammatory actions via SST$_4$ receptor activation: In silico, in vitro and in vivo evidence in mice

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ABSTRACT

Since the conventional and adjuvant analgesics have limited effectiveness frequently accompanied by serious side effects, development of novel, potent pain killers for chronic neuropathic and inflammatory pain conditions is a big challenge. Somatostatin (SS) regulates endocrine, vascular, immune and neuronal functions, cell proliferation through 5 G$_i$ protein-coupled receptors (SST$_1$-SST$_5$). SS released from the capsaicin-sensitive peptidergic sensory nerves mediates anti-inflammatory and antinociceptive effects without endocrine actions via SST$_4$. The therapeutic use of the native SS is limited by its diverse biological actions and short plasma elimination half-life. Therefore, SST$_4$ selective SS analogues could be promising analgesic and anti-inflammatory drug candidates with new mode of action. TT-232 is a cyclic heptapeptide showing great affinity to SST$_4$ and SST$_1$. Here, we report the in silico SST$_4$ receptor binding mechanism, in vitro binding (competition assay) and cAMP-decreasing effect of TT-232 in SST$_4$-expressing CHO cells, as well as its analgesic and anti-inflammatory actions in chronic neuropathic pain and arthritis models using wildtype and SST$_4$-deficient mice. TT-232 binds to SST$_4$ with similar interaction energy (-11.03 kcal/mol) to the superagonist J-2156, displaces somatostatin from SST$_4$ binding (10 nM to 30 μM) and inhibits forskolin-stimulated cAMP accumulation (EC$_{50}$: 371.6 ± 58.03 nmol; E$_{max}$: 78.63 ± 2.636 %). Its i.p. injection (100, 200 μg/kg) results in significant, 35.7 % and 50.4 %, analgesic effects upon single administration in chronic neuropathic pain and repeated injection in arthritis models in wildtype, but not in SST$_4$-deficient mice. These results provide evidence that the analgesic effect of TT-232 is mediated by SST$_4$ activation, which might open novel drug developmental potentials.

Chemical compounds

Chemical compounds studied in this article TT-232 (PubChem CID: 74053735).

1. Introduction

Somatostatin (SS) also called as somatotropin release-inhibiting factor (SRIF) was firstly described as a growth hormone (GH) inhibiting factor. It has two active forms containing 14 or 28 amino acids, due to the alternative splicing of a single preproprotein [1]. SS-14 is

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ordinarily found in the brain, while SS-28 in intestinal enteroendocrine cells. SS regulates endocrine functions such as inhibiting GH, prolactin, thyreotropin, insulin, glucagon, gastrin, secretin, motilin, cholecystokinin secretion, modulates vascular, immune and neuronal functions, as well as cell proliferation via 5 G_i protein-coupled receptors [2,3]. These SST_{1-5} receptors are grouped according to their agonist binding abilities: the SRIF1 group receptors bind octapeptide analogues with high affinity (SST_2, SST_3 and SST_5), while the SRIF2 ones (SST_1 and SST_4) with low affinity [4]. The SRIF2 group receptors were proposed to be crucial for the anti-inflammatory and antinociceptive effects of SS without inducing endocrine actions [5–7]. Administration of SS inhibits several pain conditions in patients, such as postoperative, cancer-related [8], and osteoarthritic pain [9]. Since 1998 our group has provided numerous data that SS originated from the activated capsaicin-sensitive peptidergic sensory nerve endings mediates anti-inflammatory and antinociceptive effects at remote parts of the body [6,10–13]. Among others, in rat models, exogen SS significantly inhibited the mustard oil-induced vasodilatation and plasma protein extravasation [13] and mechanical hyperalgesia in carragenin-induced inflammation [14]. Octreotide is a stabile SS analogue which binds with high affinity to the SST_2, SST_3, SST_5 receptors. In a double-blind study, severe headache suffered acromegalic patients were treated by single dose of octreotide that exerted rapid and permanent analgesic effect was not revoked by intravenous naloxone. Then the patients received octreotide for months without any sign of tolerance, dependence or unwanted sedative side effect [15]. A study involving patients with rheumatoid arthritis, showed that the intraarticular injection of SS-14 for 15 days decreases inflammation acute phase parameters, improves the result of telethermography and relieves the pain at rest and on the movement of the knee joint [16]. The epidural injection of SS resulted in complete postoperative pain relief of the patients who had undergone abdominal surgery and in other study octreotide i.v. administration also reduced the postoperative pain after major abdominal surgery [8,17].

However, due to their endocrine effects, neither SS nor octreotide can be applicable as analgesics. Among 5 receptors of SS, SST_2, SST_3 and SST_5 are primarily responsible for the endocrine effects, while SST_1 and SST_4 mediate the analgesic and anti-inflammatory actions [5–7,18]. SST_4 is expressed in both peripheral and the central nervous system, where it plays an important role in the pain transmission, such as in primer afferent neurons, dorsal root ganglions, dorsal horn of the spinal cord, somatosensory cortex, hippocampus, amygdala and periaqueductal grey matter [19,20]. TT-232 is a cyclic heptapeptide which was suggested to be a SST_4 / SST_1 agonist with greater affinity compared to other somatostatin receptors [21,22]. Furthermore, it is also a tyrosine-kinase inhibitor, thus it was originally developed as antitumor drug candidate with significant antiproliferative effects. It reduced the proliferation of 20 different human tumors and exerted apoptotic actions in vitro [21]. In our earlier studies TT-232 also inhibited nociceptive processes such as mechanical allodynia and exerted a wide range of anti-inflammatory effects in rat models. TT-232 in 5–20 µg/kg range, dose-dependently inhibited the partial sciatic nerve injury-induced mechano-nociceptive hyperalgesia and the carragenin-induced paw oedema in rats, but did not showed a dose–response correlation during the inhibition of bradykinin-induced plasma extravasation. In other study with rats, somatostatin and its synthetic analogues, such as TT-232 inhibited the non-neurogenic dextran oedema and the mustard oil-induced neurogenic plasma extravasation in µg/kg dose range. The subcutaneous pre-treatment of TT-232 (2 × 530 nmol/kg/day) for 18 days, significantly inhibited the Complete Freund's Adjuvant (CFA)-induced bilateral arthritis [5]. Toxic side effects were not observed at doses which reduced inflammation [5]. Subsequent studies provided data that it increases the thermal nociceptive threshold and improves diabetic neuropathic hyperalgesia at doses of 10–100 µg/kg, inhibits the nocifensive behaviour in the first and the second phase (55 % and 66 % inhibition) of formalin test at 80 µg/kg [7]. In mouse models, capsaicin-induced ear oedema was inhibited by 10 and 20 µg/kg dose of TT-232

[18] and it significantly diminishes the number of phenylquinone-induced writhes at doses of 20 and 200 µg/kg (70 % and 75 % inhibition) [7]. In contrast to native somatostatin and other analogues, TT-232 did not affect gastrin and GH secretion [23] which opens new horizons for development of SST_4 selective new analgesic agents [22,24–26].

Here, we report on the in silico modelling of the SST_4 receptor binding of TT-232 using a SST_4 receptor model generated by homology modelling, competitive binding and inhibition of cAMP in SST_4-expressing Chinese hamster ovary (CHO) cells. SST_4-mediated antinociceptive actions of chronic neuropathic and arthritic pain and the effects of TT-232 on oedema formation in CFA-induced arthritis were also investigated in mouse models.

2. Materials and methods

2.1. In silico modelling

2.1.1. Target and ligand preparation

The SST_4 receptor model was created with homology modelling approach as detailed in our previous study [27]. In the present study, the µ opioid receptor structure (PDB code: 5c1m) was used as a template for homology model construction. 10 models were built with the Modeller program package [28] and ranked by the objective function calculated by the program. The first ranked model was subjected to energy-minimization as described in the next paragraph, and used as a target structure. SST_4 receptor amino acids are numbered according to UniProt entry P31391.

The TT-232 ligand (D-Phe-c[*Cys-Tyr-D-Trp-Lys-Cys*]-Thr-NH$_2$) was built in Maestro [29] taking attention to the correct configuration of D-amino acids. The raw structures of the ligand and the target were equilibrated separately using a two-step energy-minimization procedure performed in AMBER99SB-ILDN force field by Gromacs program package [30]. The structures were centred in a cubic box, in which the distance was 10 Å between the box and the solute atoms. Explicit TIP3P water molecules [31] and neutralizing counter-ions (sodium or chloride) were added to the systems. Convergence thresholds were set to 10^3 kJ/mol/nm and 10 kJ/mol/nm for the steepest descent and the conjugate gradient minimization steps, respectively. The energy-minimized structures were used for docking calculations.

2.1.2. Docking calculations

The minimized and equilibrated ligand and target structures were submitted to focused docking calculations by AutoDock 4.8 [32] program package. Addition of Gasteiger-Marsilli partial charges to both the ligand and target atoms were performed by AutoDock Tools [32] and united atom representation was applied for hydrogen atoms in apolar bonds. All active torsions of the ligand were flexible, while the target was set to be rigid. The grid box was centred to target residue Asp126 that plays fundamental role in ligand bonding and receptor activation according to the literature [33–36]. The box size was set to 80x80x80 grid points with 0.375 Å spacing using AutoGrid 4. Global search was accomplished by Lamarckian genetic algorithm. After 10 docking runs, ligand conformation ranking and subsequent clustering were based on the corresponding calculated interaction energy values and a tolerance of 3.5 Å root mean square deviation (RMSD) between cluster members [37], respectively. Representative ligand structure of Rank 1 (the rank with the best interaction energy) was further analysed.

2.2. In vitro examinations

2.2.1. Competition binding analysis

CHO cells expressing SST_4 receptor were analysed in competition binding assays using TT-232 in increasing concentrations (100 nM to 300 µM). Confluent cells were used on 24-well plates. Cells were washed two times with assay buffer (5 mM KH$_2$PO$_4$ (7778–77-0), 5 mM MgCl$_2$ (7786–30-3), 10 mM HEPES (7365–45-9), 1 % (wt/vol) bovin serum

albumin (9048–46-8) and 150 mM NaCl (7647–14-5) (pH 7.4)) and incubated in the same buffer at room temperature for 30 min. Bacitracin (1 mg/ml, 1405–87-4) and [^{125}I-Tyr11]somatostatin-14 were added in 1 ml buffer for 30 min at room temperature (SS-14 (38916–34-6) was obtained from Sigma-Aldrich Ltd, Hungary, [^{125}I-Tyr11]somatostatin-14 was labelled by us using ^{125}I isotope purchased from Isotope Institute, Hungary). We washed the cells twice with ice-cold assay buffer and collected in 8 M urea/3 M acetic acid (1 ml) and measured radioactivity by a γ-counter [38]. Materials used for cell cultures and binding analysis were purchased from Sigma-Aldrich Ltd, Hungary.

2.2.2. cAMP accumulation assay

CHO-K1 cell line was maintained in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, USA) supplemented with 2 mM L-glutamine (Thermo Fischer Scientific, USA, 56–85-9), 10 % fetal bovine serum (FBS), 1x penicillin/streptomycin (Thermo Fisher Scientific, USA, 61–33-6/57–92-1). Cells were cultured and kept at 37 °C, 5 % CO_2 incubator until 70–80 % confluence.

CHO-K1 cells expressing the human SST$_4$ receptor-expressing (Eurofins DiscoverX, Fremont, CA, USA) were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12, Thermo Fisher Scientific, USA) and kept at 37 °C, 5 % CO_2 incubator until 70–80 % confluence. This media was supplemented with 2 mM L-glutamine (Thermo Fischer Scientific, USA), 10 % fetal bovine serum (FBS), 1x penicillin/streptomycin (Thermo Fisher Scientific, USA), and 800 µg/ml selection antibiotic G418 (Eurofins DiscoverX, Fremont, CA, USA).

Level of cAMP was measured using the DiscoverX HitHunter™ cAMP assay kit (Eurofins DiscoverX, Fremont, CA, USA). 10 mM stock solutions were prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich Ltd, Hungary, 67–68-5) and kept at −20 °C until future use. Cells were seeded into a white 96-well assay plate in 100 µl cell plating reagent (Eurofins DiscoverX, Fremont, CA, USA) at a density of 20,000 cells/well and incubated overnight at 37 °C, 5 % CO_2. In the next day, cell plating reagent (Eurofins DiscoverX, Fremont, CA, USA) was aspirated and replaced with PBS. A series of serial dilutions (10 pmol – 10 µmol) of compounds with PBS containing the phosphodiesterase inhibitor rolipram (Sigma-Aldrich Ltd, Hungary, 61413–54-5) and the adenylate cyclase stimulator forskolin (Sigma-Aldrich Ltd, Hungary, 66575–29-9) (100 µmol) was performed. Then, cells were treated with different concentrations of the SST$_4$ ligands for 30 min at 37 °C, each in duplicates. Once ligand treatment was completed, each following step involved incubations with the assay reagents (Eurofins DiscoverX, Fremont, CA, USA) at room temperature. The chemiluminescent signal corresponding to the cAMP concentration was detected using a PerkinElmer EnSpire Alpha plate reader. The data were expressed as cAMP accumulation in proportion to the percentage of the forskolin response.

2.3. In vivo investigations

2.3.1. Partial sciatic nerve ligation: Traumatic mononeuropathy pain model

One conditioning and two initial measurements of mechano-nociceptive threshold were performed on three consecutive days. On the fourth day mice were anesthetized with ketamine (100 mg/kg, i.p., 6740–88-1) and xylazine (10 mg/kg, i.p., 7361–61-7). The proximal 1/3–1/2 part of the right sciatic nerve was tightly ligated with one 8–0 silk suture under a dissection microscope to evoke traumatic sensory mononeuropathy [39]. During the operation animals were placed on a heating blanket and monitored until complete awakening. The mechano-nociceptive threshold of the hindpaws was determined again on the 7th postoperative day to demonstrate the mechanical hyperalgesia (decrease of the mechano-nociceptive threshold in response to the nerve ligation expressed as percentage compared to the mean pre-surgery values). Animals not showing at least 20 % mechanical hyperalgesia were excluded, since they did not show significant neuropathic pain behaviour.

Mechano-nociceptive thresholds of the hindpaws were investigated by Dynamic Plantar Aesthesiometry (DPA, Ugo Basile Dynamic Plantar Aesthesiometer 37400; Comerio, Italy). This is an electronic device with a blunt-end needle, which evolves pressure to the plantar surface of the hindpaw through the metal mesh floor of the cages. The maximal of 10 g force is reached in 4 s. The equipment automatically turns off the stimulus when mice withdrawal response occurs and registers the mechano-nociceptive threshold.

In the in vivo experiments 1 mg/ml stock solution was made from the TT-232 (Tocris Bioscience, Cat. No. 4639) with acetate buffer (pH 3.5) freshly every experimental day. Further dilutions were made with phosphate buffer (PBS - pH 7.3) to get 10 and 20 µg/ml solution (10 ml/kg body weight for the 100, and 200 µg/kg dose). The vehicle was always the mixture of acetate and phosphate buffer.

The TT-232 (in 100 or 200 µg/kg doses) or the vehicle were given intraperitoneally and threshold measurements were repeated 30 min later to compare pre- and post-treatment mechanical hyperalgesia. The analgesic effect of the TT-232 was expressed in percentage as described earlier: ((hyperalgesia before drug treatment—hyperalgesia after drug treatment)/hyperalgesia before drug treatment) · 100 [40].

2.3.2. Chronic arthritis model

Arthritis was evoked by intraplantar injection of 50 µl of Complete Freund's Adjuvant (CFA, killed Mycobacteria in paraffin oil, 1 mg/ml; Sigma, St. Louis, MO) into the right hindpaw and s.c. into the root of the tail. The s.c. injection was repeated on the next day into the tail to achive systemic effects and to show more similarities with the human disease [41–43]. The mechano-nociceptive threshold of the hindpaw was determined by DPA, similarly to neuropathy model, before and 4, 6, 8, 11, 13, 15, 18, 20, 21 days after CFA administration. The TT-232 (in 100 µg/kg dose) or the vehicle were applied i.p. (in a volume of 10 ml/kg body weight) every measuring day 30 min before the measurements. Mechanical hyperalgesia was expressed as % of control mechano-nociceptive threshold compared to the initial values [41,43–45]. The paw volume was determined by plethysmometry (Ugo Basile Plethys-mometer 7140, Comerio, Italy) [43,45]. Volumes were measured before and 4, 6, 8, 11, 13, 15, 18, 20 and 21 days after CFA-injection. Oedema was expressed in percentage compared to the initial values [41,42].

2.3.3. Animals and ethics

Male C57Bl/6J based SST$_4$ receptor gene deficient (KO) [46] and wild type C57Bl/6J (WT) mice (8–12 weeks old) were bred and kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pecs in standard polycarbonate cages under a 12–12 h light–dark cycle, at 24–25 °C and provided with standard rodent chow and water ad libitum. SST$_4$ knockout mice were generated by pairing heterozygote animals donated by the research group of Dr. Pierce C. Empson (Laboratory of Molecular Neuroscience, The Babraham Institute, Babraham Research Campus, Babraham, Cambridge CB22 3AT, United Kingdom). The genotype of their offsprings was identified by PCR analysis [46]. Experimental procedures complied with the recommendations of the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (63/2010) and were approved by the Ethics Committee on Animal Research of Pecs University according to the Ethical Codex of Animal Experiments (license No. BA1/35/55–50/2017).

2.4. Statistical analysis

Graphs and calculations were made using GraphPad Prism version 8.0.1 statistical software. Results are expressed as means ± S.E.M. The number of the animals were n = 5–6/group and n = 9–16/group in CFA-induced arthritis and sciatic nerve ligation-evoked neuropathy model, respectively.

The cAMP levels were normalized considering forskolin response as

100 %. Curves were fit by nonlinear regression using the sigmoidal dose–response equation.

Data were tested by Shapiro-Wilk normality test and showed normal distribution. Baseline values of the WT and KO groups were compared with unpaired *t*-test, the mechano-nociceptive thresholds before and after the treatment with TT-232 of vehicle were compared with one paired *t*-test. The analgesic effect of the TT-232 was calculated from the mechano-nociceptive thresholds compared to the results of the control group using two-sample t-tests.

Mechanical hyperalgesia- and paw oedema induced by CFA were evaluated by two-way analysis of variance (ANOVA) followed by Bonferroni posttest.

When comparing the results of the respective groups, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ and $****p < 0.0001$ were considered to be significant.

3. Results

3.1. Binding of TT-232 to the SST$_4$ receptor determined by in silico modelling

Docking results showed that TT-232 fits to a deep binding pocket of the SST$_4$ receptor accessible from the extracellular region. The pocket is located in a crevice between TM3-7 helices and ECL2-3 loops. The low negative interaction energy between the representative structure of TT-232 and SST$_4$ receptor was −11.03 kcal/mol. The SST$_4$ receptor residues interacting with TT-232 (within 3.5 Å heavy atom distance threshold) are listed in Table 1. TT-232 is stabilized mainly by H-bonds in the binding pocket (Table 1, Fig. 1.), other non-covalent interactions have lower importance. The Lys5 of TT-232 resembles to residue of Lys9 of SS, the endogen ligand that plays a critical role in receptor binding and activation from the aspect of the ligand.

3.2. TT-232 displaces somatostatin from the SST$_4$ receptor binding

The binding ability of TT-232 (100 nM to 300 μM) on the SST$_4$ receptor expressed in CHO cells was determined by competition binding assay using [^{125}I-Tyr11]SS-14. TT-232 displaced the labelled SS from the SST$_4$-expressing CHO cells by concentration-dependent manner. The maximal value of the displacement was 94 ± 5.2 % after the administration 100 μM TT-232 (13 964 count per minute value decreased to 838). The IC$_{50}$ value was 21.5 μM (Fig. 2.).

Table 1
The list of interacting residues and H-bridges in the complex of SST$_4$ and TT-232.

Ligand residues	Target residues within 3.5 Å distance from the corresponding ligand residue	H-bond between the target and the ligand residues
Phe1	Asn282	
Tyr3	Val278	
	Gln279	
	Asn282	X
Trp4	Asn199	
	Leu200	
Lys5	Leu123	
	Asp126	X
	Tyr301	X
Cys6	Phe211	
Thr7	Asp126	X
	Gly127	
	Met130	
	Phe131	
	Phe211	
	Thr215	X

3.3. TT-232 decreases intracellular cAMP concentration via SST$_4$ receptors

TT-232 showed a robust concentration-dependent inhibitory effect on the forskolin-stimulated cAMP production in SST$_4$-expressing CHO cells with similar efficacy, but lower potency as the selective SST$_4$ agonist reference compound J-2156. The E$_{max}$ values for TT-232 and J-2156 were 78.63 ± 2.636 %, and 90.53 ± 1.776 %, the EC$_{50}$ values were 371.6 ± 58.03 nmol, and 681.4 ± 63.91 pmol, respectively (Fig. 3A). Meanwhile, neither compound had any effect in CHO cells not expressing the SST$_4$ receptor (Fig. 3B.).

3.4. TT-232 inhibits neuropathic hyperalgesia via SST$_4$ receptor activation in the mouse

In the partial sciatic nerve ligation model, the mechano-nociceptive threshold dropped with approximately 37 % on the seventh postoperative day, while the thresholds of the contralateral paws remained unchanged compared to the baseline values. In case of WT mice the treatment with the 100 and 200 μg/kg dose of TT-232 significantly reduced the drop of the mechano-nociceptive threshold of the treated paw 30 min later with the following values: TT-232: 6.77 ± 0.30 g; 7.23 ± 0.29 g (24.9 ± 3.4 %; 19.6 ± 3.2 %), while the vehicle had no effect (5.57 ± 0.23 g 38.2 ± 2.3 %) (Fig. 4A). In contrast with the WT mice, TT-232 did not influence the mechano-nociceptive threshold in the SSTR$_4$-gene deficient mice. The corresponding values were the following in the 100 and 200 μg/kg dose of TT-232 or vehicle-treated groups: TT-232: 5.31 ± 0.16 g; 5.14 ± 0.15 g (34.2 ± 1.9 %; 35.77 ± 1.4 %) vs vehicle: 5.32 ± 0.11 g (35.3 ± 1.75 %) (Fig. 4B).

Analgesic effect was calculated from the changes of the mechano-nociceptive threshold. TT-232 showed 35.7 ± 8.3 % and 50.4 ± 8.4 % analgesic effect in 100 and 200 μg/kg doses in WT mice (Fig. 4C, D).

### 3.5. TT-232 inhibits chronic arthritic hyperalgesia via SST$_4$ receptor activation

Initial mechano-nociceptive thresholds of WT (7.78 ± 0.10 g) and SST$_4$ KO (8.09 ± 0.08 g) animals did not differ ($p = 0.1102$; $F = 1.51$) at the beginning of the experiment. CFA injection induced an approximately 30 % drop of the threshold (mechanical hyperalgesia) on the treated paw in both groups by the fourth day (saline-treated WT: 5.66 ± 0.26 g, TT-232-treated WT: 5.72 ± 0.32 g; saline-treated SST$_4$ KO: 5.20 ± 0.27 g, TT-232-treated SST$_4$ KO: 5.59 ± 0.14 g), which was stably maintained during the 21-day experimental period. TT-232 treatment (100 μg/kg, i.p. 30 min before the measurements) significantly reduced this chronic inflammatory mechanical hyperalgesia from day 6 (saline-treated WT: 5.35 ± 0.16 g, TT-232-treated WT: 6.86 ± 0.39 g), and almost abolished from day 11 (saline-treated WT: 5.91 ± 0.25 g, TT-232-treated WT: 7.4 ± 0.17 g) in WTs (Fig. 5A), but not in the SST$_4$ KOs (saline-treated SST4 KO: 5.62 ± 0.30 g, TT-232-treated SST4 KO: 5.72 ± 0.14 g) (Fig. 5B). Control paw volumes of WT (0.15 ± 0.003 cm^3) and SST$_4$ KO (0.15 ± 0.002 cm^3) animals were also similar ($p = 0.68$; $F = 1.85$). CFA injection evoked remarkable, about 100 % paw oedema by day 8 (saline-treated WT: 0.31 ± 0.02 cm^3, saline-treated SST4 KO: 0.29 ± 0.01 cm^3), which persisted throughout the experiment in all groups, it was not affected by TT-232 treatment in either WT ($0.29 \pm 0,007$ cm^3) (Fig. 5C) or SST$_4$ KO mice (0.30 ± 0.01 cm^3) (Fig. 5D).

4. Discussion

In the present study we demonstrated the SST$_4$ receptor binding and activation of the heptapeptide somatostatin analogue TT-232 by in silico and in vitro methods, as well as its SST$_4$-mediated anti-nociceptive effects in chronic inflammatory and neuropathic pain mouse models.

In silico modelling revealed that TT-232 interacts with the SST$_4$ receptor with similar or stronger binding strength than the selective SST$_4$
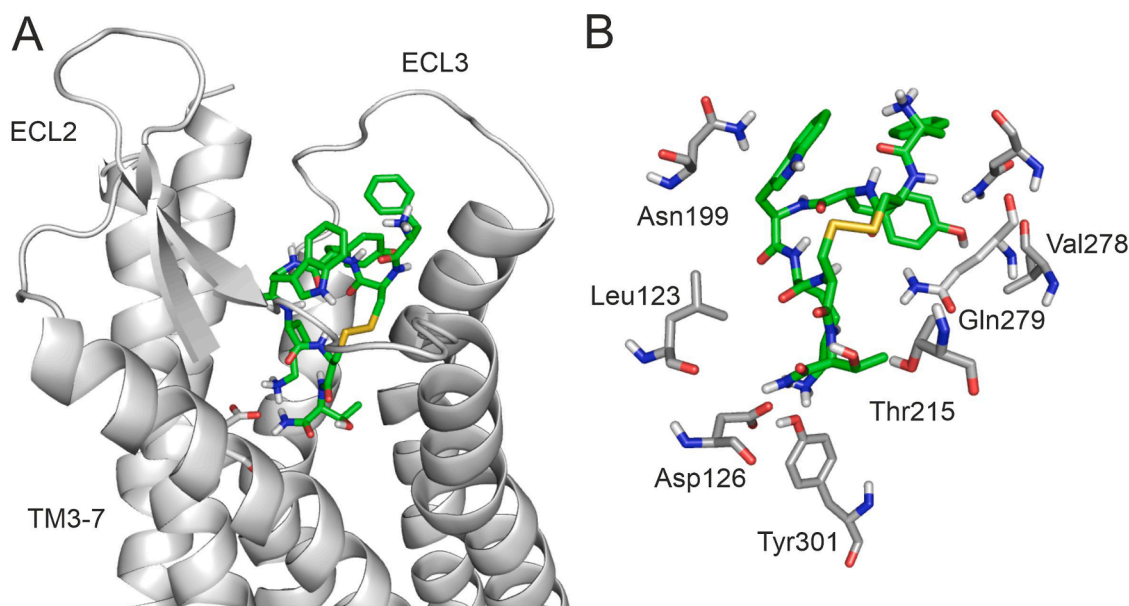
Fig. 1. A) A global view of SST$_4$ receptor (grey, cartoon representation) in complex with TT-232 (Rank 1 representative, green sticks) B) The close-up view of TT-232 (Rank 1 representative) in the binding pocket of SST$_4$ receptor. Target residues interacting with the ligand within 3.5 Å and TT-232 are colored by grey and green, respectively.
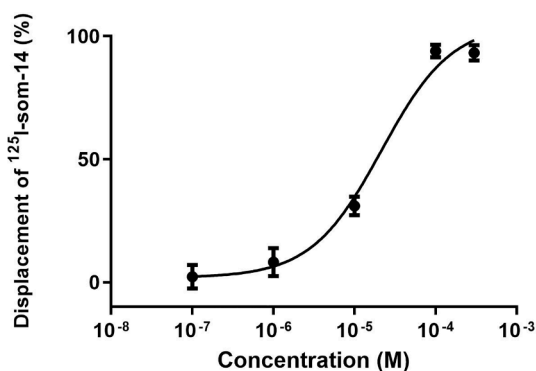
Fig. 2. Concentration-dependent displacement of [^{125}I-Tyr11]SS-14 binding from SST$_4$ receptors by TT-232 (100 nM-300 µM) on SST$_4$-expressing CHO cells. Each data point represents the mean ± SEM of n = 4 experiments.

agonist reference compounds J-2156 and NNC-269100 [40] and some novel pyrrolo-pyrimidine molecules [27]. The Lys5 of the TT-232 creates H-bonds with the conserved Asp126 of SST$_4$ receptor that is essential for the receptor activation proved by several experimental studies [33–36], furthermore, Lys5 is stabilized by a further H-bond with Tyr301 as well. The Lys5 of TT-232 resembles the residue of Lys9 of somatostatin, the endogen ligand that plays a critical role in receptor binding and activation from the aspect of the ligand. The intramolecular cyclization of TT-232 results in a relatively constrained structure with low conformational freedom for the peptide backbone [47] allowing rotations only at the side-chains. When previously studying the SST$_1$ binding ability of TT-232 it was shown that all conformations of this molecule have almost the same backbone structure in aqueous solution resulting in high inner stability [47]. Thus, the pre-formed cyclic conformation of TT-232 allows a favourably low entropy loss during binding to the SST$_4$ receptor. At the same time, the new interactions with Tyr301 provide a considerably large enthalpic contribution to the overall binding strength.

The in vitro binding ability of TT-232 to the SST$_4$ receptor confirmed the in silico results. The displacement of the radiolabelled SS by TT-232
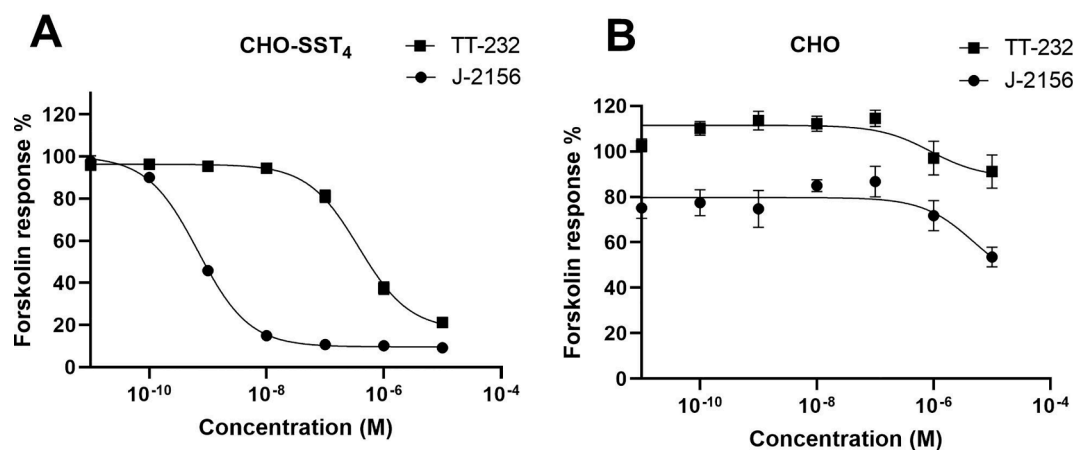
Fig. 3. Concentration-response curves of TT-232 showing cAMP accumulation levels in (A) SST$_4$ expressing CHO and (B) CHO cells in comparison to J-2156. All values are means ± SEM of n = (A) 10, (B) n = 8 for TT-232 and 4 for J-2156.
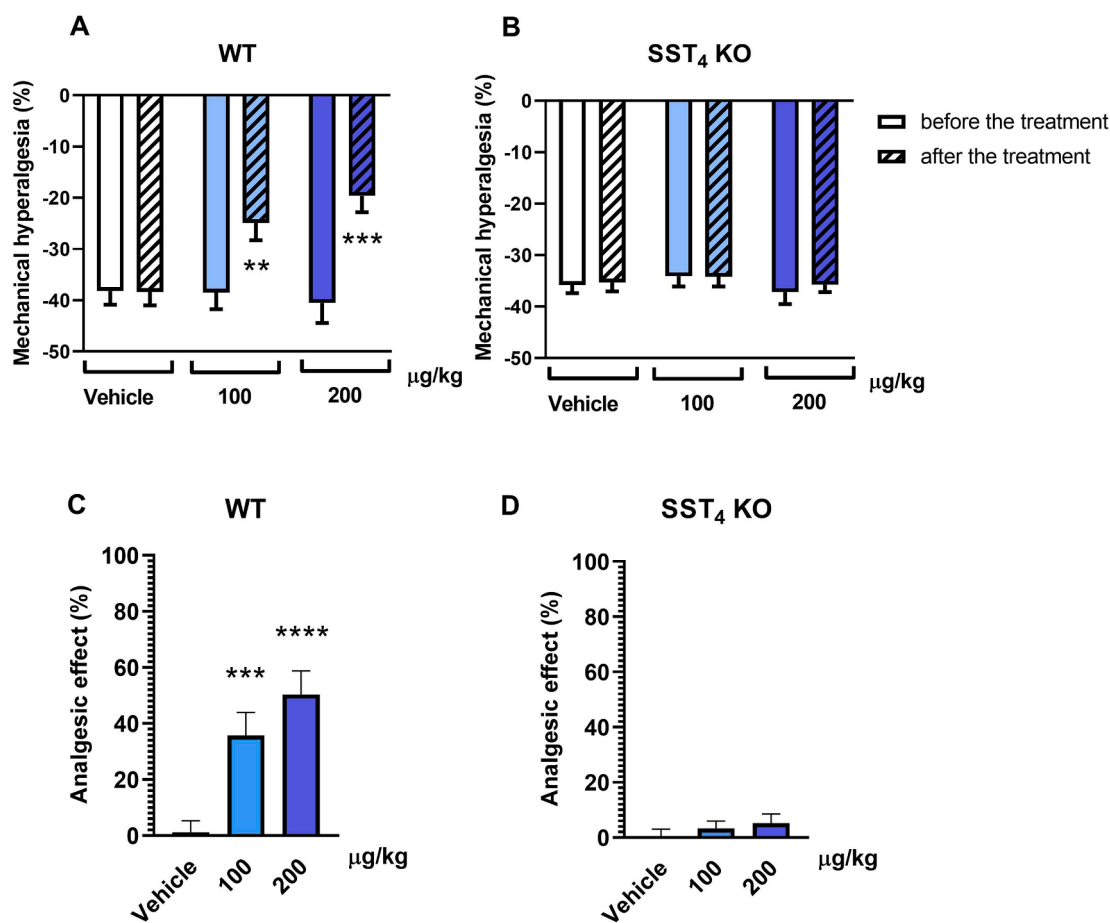
Fig. 4. The changes of the mechano-nociceptive thresholds (A, B) and analgesic effect (C, D) of TT-232 in WT (A, C) and SST$_4$ KO (B, D) mice. The data were compared with paired *t*-test and represented in mean ± SEM format (*p < 0,05; **p < 0,01 and ***p < 0,001 vs before the treatment, n = 9–16/group).

on SST$_4$ receptor-expressing CHO cells was concentration dependent and it was almost 95 % at 100 μM concentration in the competition binding assay. After proving the receptor-binding to the SST$_4$ receptor, we measured the intracellular cAMP level. Since SST$_4$ is a G$_i$ protein-coupled receptor, it inhibits adenylate cyclase and consequent cAMP formation. Forskolin is a cell-permeable diterpene that directly activates adenylate cyclase, therefore raises the intracellular cAMP level [48]. TT-232 exerted robust concentration-dependent inhibitory effect on the forskolin-stimulated cAMP production in SST$_4$-expressing CHO cells similarly to the reference compound J-2156 [49], but there was no effect in control CHO cells demonstrating specific SST$_4$-dependent action. TT-232 has similar efficacy, but lower potency than the reference compound J-2156 in the cAMP assay.

Our previous studies have provided substantial evidence that TT-232 reduces neurogenic and non-neurogenic inflammatory processes in rats and mice [5,18], furthermore the acute and chronic airway inflammation in rats [50]. Therefore, in the present experiments its effects on neuropathic and chronic inflammatory pain conditions were investigated. Partial sciatic nerve ligation [39] is a widely used well established method to model traumatic neuropathic pain in rodents. Following the operation, significant nerve damage develops leading to abnormal sensory functions without disabling motor functions [39,51]. Both examined single administered doses (100, 200 μg/kg, i.p.) of TT-232 were able to increase the mechano-nociceptive threshold evoking dose-dependent analgesic effects on the 7th postoperative day when the neuropathic pain was fully developed. This is consistent with previous data showing that both TT-232 and the non-peptide superagonist J-2156 exerted analgesic effects (10–100 μg/kg i.p.) in rat and mouse models, TT-232 also reversed mechanical hyperalgesia [18,52].

In the adjuvant-induced chronic arthritis model, daily injections of TT-232 also inhibited mechanical hyperalgesia, but did not affect oedema formation. However, in our earlier studies, in different acute inflammation models, TT-232 (5, 10, 20 μg/kg i.v. or s.c.) significantly and dose-dependently reduced the bradykinin-, carragenin- and capsaicin-induced oedema in rats [18]. The moderate variability of the effective doses of TT-232 in the different animal models could be explained by the strain differences (rat and mouse) and the distinct pathomechanisms of the examined processes.

Since the inhibitory actions of TT-232 on hyperalgesia were not observed in SST$_4$-deficient mice in either the neuropathy or the arthritis model, it is suggested that its analgesic actions are mediated by SST$_4$ activation. This hypothesis can also be supported by our previous results related to J-2156, the selective SST$_4$ superagonist, which did not relieve the carragenin-evoked mechanical hyperalgesia [46]. Furthermore, increased inflammatory reactions were developed both in the oxazolone-induced allergic contact dermatitis and the endotoxin-evoked airway inflammation model in SST$_4$ gene deficient mice compared to the WT counterparts [46].

Original compounds with new mechanisms of action are needed for the adequate treatment of neuropathic pain conditions, since the conventional analgesics, such as NSAIDs and opioids show only limited effectiveness. The widely used adjuvant analgesics, such as amitriptyline and gabapentin only partly diminish the symptoms and often exert serious side effects. Amitriptyline, a tricyclic antidepressant, used to treat neuropathic pain caused by shingles, often causes anticholinergic side effects (obstipation, dry mouth, vomiting), palpitation, blurring of vision, drowsiness, dizziness and weight gain. Gabapentin, has been developed as an antiepileptic drug, is also used for peripheral
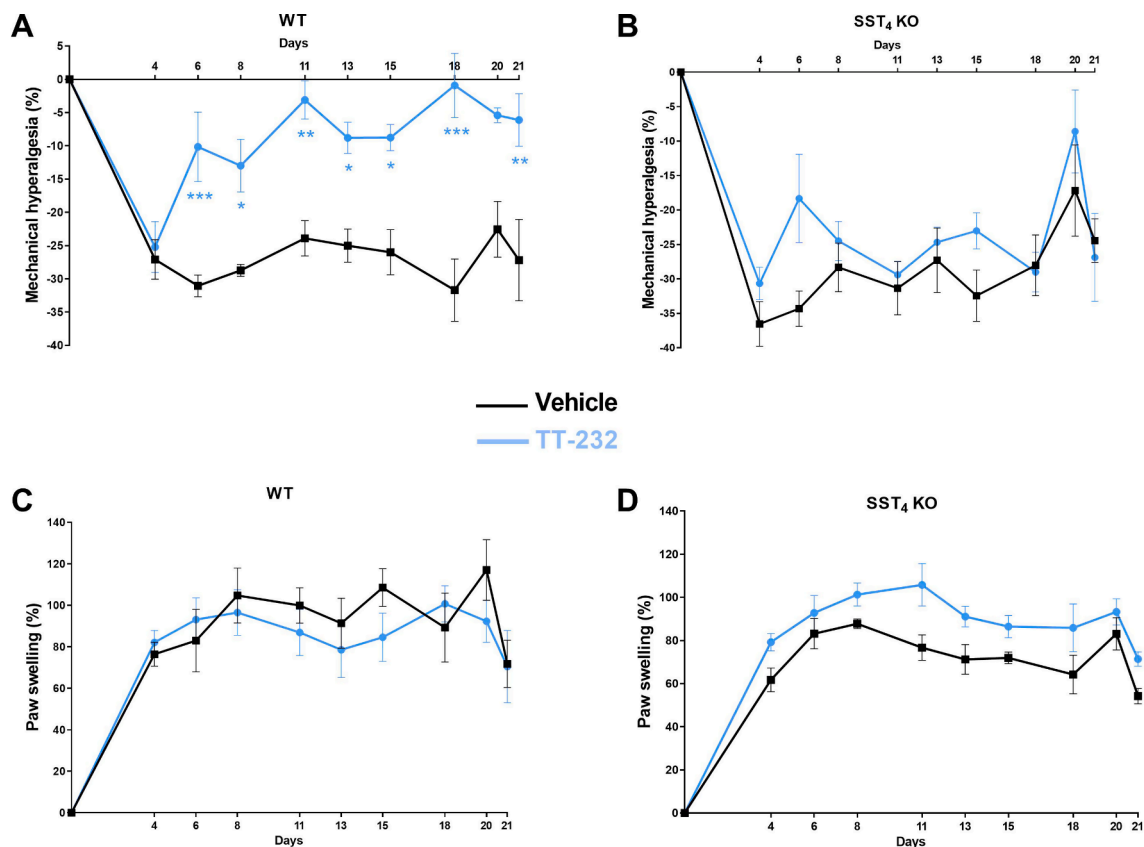
Fig. 5. Changes of the (A, B) mechano-nociceptive thresholds and (C, D) paw volumes over the 21-day investigation period after intraplantar and tail root injection of complete Freund's adjuvant in TT-232-treated (A, C) WT and (B, D) SST$_4$ KO mice compared to the saline vehicle-treated group. Each data point represents the mean ± SEM of n = 5–6/group (two-way ANOVA; *p < 0,05; **p < 0,01 and ***p < 0,001 vs control group).

neuropathy. It frequently causes somnolence, dizziness, ataxia, vomiting, as well as, diarrhea [53–55]. Since the SST$_4$ receptor selective agonists have completely different molecular mode of action than NSAIDs, opioids and adjuvant analgesics they might have reduced, more tolerable side effect spectrum. Furthermore, SST4 selective agonists compared to the non-selective somatostatin analogues do not exert endocrine actions. Octreotide and lanreotide commonly cause GI-related adverse effects such as diarrhea, nausea and abdominal pain [56,57], increased gallstone formation [58]. Exocrine pancreatic insufficiency is a common but under-recognized adverse effect induced by octreotide via inhibiting the production of cholecystokinin and secretin [59,60]. Elevated blood glucose levels occurred in case of all marketed somatostatin analogues [61,62].

In summary the present results strongly support that SST$_4$ could be a promising therapeutic target in the therapy of neuropathic and inflammatory chronic pain conditions. These data clearly show the effectiveness of TT-232 in chronic pain models via SST$_4$ receptor. Although its peptide characteristics prevent oral administration, alternative formulations, such as subcutaneous or intravenous injections, as well as nasal spray might be applicable.

CRediT authorship contribution statement

Rita Börzsei: Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Éva Borbély:** Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization, Funding acquisition. **Boglárka Kántás:** Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Lina Hudhud:** Methodology, Investigation, Writing – original draft, Visualization. **Ádám Horváth:** Methodology, Formal analysis, Investigation. **Éva Szőke:** Methodology, Investigation, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition. **Csaba Hetényi:** Conceptualization, Writing – original draft, Supervision, Project administration, Funding acquisition. **Zsuzsanna Helyes:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Erika Pintér:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] E. Ampofo, L. Nalbach, M.D. Menger, M.W. Laschke, Regulatory mechanisms of somatostatin expression, Int. J. Mol. Sci. 21 (2020) 4170, https://doi.org/10.3390/ijms21114170.

[2] Handbook of Hormones - 2nd Edition, (n.d.). https://www.elsevier.com/books/handbook-of-hormones/ando/978-0-12-820649-2 (accessed October 6, 2022).

[3] Y.C. Patel, Somatostatin and its receptor family, Front. Neuroendocrinol. 20 (1999) 157–198, https://doi.org/10.1006/frne.1999.0183.

[4] D. Hoyer, G.I. Bell, M. Berelowitz, J. Epelbaum, W. Feniuk, P.P.A. Humphrey, A.-M. O'Carroll, Y.C. Patel, A. Schonbrunn, J.E. Taylor, T. Reisine, Classification and nomenclature of somatostatin receptors, Trends Pharmacol. Sci. 16 (1995) 86–88, https://doi.org/10.1016/S0165-6147(00)88988-9.

[5] Z. Helyes, E. Pintér, J. Németh, G. Kéri, M. Thán, G. Oroszi, A. Horváth, J. Szolcsányi, Anti-inflammatory effect of synthetic somatostatin analogues in the rat, Br. J. Pharmacol. 134 (2001) 1571–1579, https://doi.org/10.1038/sj.bjp.0704396.

[6] E. Pintér, Z. Helyes, J. Szolcsányi, Inhibitory effect of somatostatin on inflammation and nociception, Pharmacol. Therapeutics. 112 (2006) 440–456, https://doi.org/10.1016/j.pharmthera.2006.04.010.

[7] J. Szolcsányi, K. Bölcskei, A. Szabó, E. Pintér, G. Petho, K. Elekes, R. Börzsei, R. Almási, T. Szuts, G. Kéri, Z. Helyes, Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia, Eur. J. Pharmacol. 498 (2004) 103–109, https://doi.org/10.1016/j.ejphar.2004.07.085.

[8] J. Chrubasik, J. Meynadier, S. Blond, P. Scherpereel, E. Ackerman, M. Weinstock, K. Bonath, H. Cramer, E. Wunsch, Somatostatin, a potent analgesic, Lancet 324 (1984) 1208–1209, https://doi.org/10.1016/S0140-6736(84)92761-2.

[9] F. Silveri, P. Morosini, D. Brecciaroli, C. Cervini, Intra-articular injection of somatostatin in knee osteoarthritis: clinical results and IGF-1 serum levels, Int. J. Clin. Pharmacol. Res. 14 (1994) 79–85.

[10] C. Hernández, A.I. Arroba, P. Bogdanov, H. Ramos, O. Simó-Servat, R. Simó, A.M. Valverde, Effect of topical administration of somatostatin on retinal inflammation and neurodegeneration in an experimental model of diabetes, J. Clin. Med. 9 (2020) E2579, https://doi.org/10.3390/jcm9082579.

[11] N. Schuelert, S. Just, R. Kuelzer, L. Corradini, L.C.J. Gorham, H. Doods, The somatostatin receptor 4 agonist J-2156 reduces mechanosensitivity of peripheral nerve afferents and spinal neurons in an inflammatory pain model, Eur. J. Pharmacol. 746 (2015) 274–281, https://doi.org/10.1016/j.ejphar.2014.11.003.

[12] J. Szolcsányi, Z. Helyes, G. Oroszi, J. Németh, E. Pintér, Release of somatostatin and its role in the mediation of the anti-inflammatory effect induced by antidromic stimulation of sensory fibres of rat sciatic nerve, Br. J. Pharmacol. 123 (1998) 936–942, https://doi.org/10.1038/sj.bjp.0701685.

[13] J. Szolcsányi, E. Pintér, Z. Helyes, G. Oroszi, J. Németh, Systemic anti-inflammatory effect induced by counter-irritation through a local release of somatostatin from nociceptors, Br. J. Pharmacol. 125 (1998) 916–922, https://doi.org/10.1038/sj.bjp.0702144.

[14] M.M. Corsi, C. Ticozzi, C. Netti, A. Fulgenzi, M. Tiengo, G. Gaja, F. Guidobono, M.E. Ferrero, The effect of somatostatin on experimental inflammation in rats, Anesth. Analg. 85 (1997) 1112–1115, https://doi.org/10.1097/00000539-199711000-00028.

[15] K. Schmidt, P.H. Althoff, A.G. Harris, H. Prestele, P.M. Schumm-Draeger, K.H. Usadel, Analgesic effect of the somatostatin analogue octreotide in two acromegalic patients: a double-blind study with long-term follow-up, Pain 53 (1993) 223–227, https://doi.org/10.1016/0304-3959(93)90084-3.

[16] A. Fioravanti, M. Govoni, G. La Montagna, G. Perpignano, G. Tirri, F. Trotta, A. Bogliolo, A. Ciocci, M.T. Mauceri, R. Marcolongo, Somatostatin 14 and joint inflammation: evidence for intraarticular efficacy of prolonged administration in rheumatoid arthritis, Drugs Exp. Clin. Res. 21 (1995) 97–103.

[17] A.A. Dahaba, G. Mueller, G. Mattiassich, G. Rumpold-Seitlinger, H. Bornemann, P.H. Rehak, G. Linck, H.-J. Mischinger, H. Metzler, Effect of somatostatin analogue octreotide on pain relief after major abdominal surgery, Eur. J. Pain. 13 (2009) 861–864, https://doi.org/10.1016/j.ejpain.2008.10.006.

[18] E. Pintér, Z. Helyes, J. Németh, R. Pórszász, G. Pethő, M. Thán, G. Kéri, A. Horváth, B. Jakab, J. Szolcsányi, Pharmacological characterisation of the somatostatin analogue TT-232: effects on neurogenic and non-neurogenic inflammation and neuropathic hyperalgesia, Naunyn. Schmiedebergs Arch. Pharmacol. 366 (2002) 142–150, https://doi.org/10.1007/s00210-002-0563-9.

[19] A. Kecskés, K. Pohóczky, M. Kecskés, Z.V. Varga, V. Kormos, É. Szőke, N. Henn-Mike, M. Fehér, J. Kun, A. Gyenesei, É. Renner, M. Palkovits, P. Ferdinandy, I.M. Ábrahám, B. Gaszner, Z. Helyes, Characterization of neurons expressing the novel analgesic drug target somatostatin receptor 4 in mouse and human brains, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21207788.

[20] I.S. Selmer, M. Schindler, P.P. Humphrey, H.J. Waldvogel, R.L. Faull, P.C. Emson, First localisation of somatostatin sst(4) receptor protein in selected human brain areas: an immunohistochemical study, Brain Res. Mol. Brain Res. 82 (2000) 114–125, https://doi.org/10.1016/s0169-328x(00)00186-8.

[21] G. Kéri, J. Erchegyi, A. Horváth, I. Mezö, M. Idei, T. Vántus, A. Balogh, Z. Vadász, G. Bökönyi, J. Seprödi, I. Teplán, O. Csuka, M. Tejeda, D. Gaál, Z. Szegedi, B. Szende, C. Roze, H. Kalthoff, A. Ullrich, A tumor-selective somatostatin analog (TT-232) with strong in vitro and in vivo antitumor activity, Proc. Natl. Acad. Sci. USA 93 (1996) 12513–12518, https://doi.org/10.1073/pnas.93.22.12513.

[22] G. Kéri, I. Mezö, Z. Vadász, A. Horváth, M. Idei, T. Vántus, A. Balogh, G. Bökönyi, T. Bajor, I. Teplán, Structure-activity relationship studies of novel somatostatin analogs with antitumor activity, Pept. Res. 6 (1993) 281–288.

[23] A. Ben-Shlomo, N.-A. Liu, S. Melmed, Somatostatin and dopamine receptor regulation of pituitary somatotroph adenomas, Pituitary 20 (2017) 93–99, https://doi.org/10.1007/s11102-016-0778-2.

[24] J.-U. Lee, R. Hosotani, M. Wada, R. Doi, T. Koshiba, K. Fujimoto, Y. Miyamoto, S. Tsuji, S. Nakajima, M. Hirohashi, T. Uehara, Y. Arano, N. Fujii, M. Imamura, Antiproliferative activity induced by the somatostatin analogue, TT-232, in human pancreatic cancer cells, Eur. J. Cancer 38 (2002) 1526–1534, https://doi.org/10.1016/S0959-8049(02)00101-6.

[25] A. Steták, P. Csermely, A. Ullrich, Gy. Kéri,, Physical and Functional Interactions between Protein Tyrosine Phosphatase α, PI 3-Kinase, and PKCδ, Biochem. Biophys. Res. Commun. 288 (2001) 564–572, https://doi.org/10.1006/bbrc.2001.5811.

[26] M. Tejeda, D. Gaál, L. Hullán, R. Schwab, O. Szokoloczi, G. Kéri, Antitumor activity of the somatostatin structural derivative (TT-232), against mouse and human melanoma tumor models, Anticancer Res. 27 (2007) 4015–4019.

[27] B. Kántás, R. Börzsei, É. Szőke, P. Bánhegyi, Á. Horváth, Á. Hunyady, É. Borbély, C. Hetényi, E. Pintér, Z. Helyes, Novel drug-like somatostatin receptor 4 agonists are potential analgesics for neuropathic pain, IJMS. 20 (2019) 6245, https://doi.org/10.3390/ijms20246245.

[28] A. Šali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815, https://doi.org/10.1006/jmbi.1993.1626.

[29] Schrödinger Relase 2017–4: Maestro., Schrödinger LLC, New York, NY, USA, 2017.

[30] M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, E. Lindahl, GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers, SoftwareX. 1–2 (2015) 19–25, https://doi.org/10.1016/j.softx.2015.06.001.

[31] P. Mark, L. Nilsson, Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K, J. Phys. Chem. A. 105 (2001) 9954–9960, https://doi.org/10.1021/jp003020w.

[32] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791, https://doi.org/10.1002/jcc.21256.

[33] L. Chen, C. Hoeger, J. Rivier, V.D. Fitzpatrick, R.L. Vandlen, A.H. Tashjian, Structural basis for the binding specificity of a SSTR1-selective analog of somatostatin, Biochem. Biophys. Res. Commun. 258 (1999) 689–694, https://doi.org/10.1006/bbrc.1999.0699.

[34] K. Kaupmann, C. Bruns, F. Raulf, H.P. Weber, H. Mattes, H. Lübbert, Two amino acids, located in transmembrane domains VI and VII, determine the selectivity of the peptide agonist SMS 201–995 for the SSTR2 somatostatin receptor, EMBO J. 14 (1995) 727–735.

[35] R.B. Nehring, W. Meyerhof, D. Richter, Aspartic acid residue 124 in the third transmembrane domain of the somatostatin receptor subtype 3 is essential for somatostatin-14 binding, DNA Cell Biol. 14 (1995) 939–944, https://doi.org/10.1089/dna.1995.14.939.

[36] J. Strnad, J.R. Hadcock, Identification of a critical aspartate residue in transmembrane domain three necessary for the binding of somatostatin to the somatostatin receptor SSTR2, Biochem. Biophys. Res. Commun. 216 (1995) 913–921, https://doi.org/10.1006/bbrc.1995.2708.

[37] C. Hetényi, D. van der Spoel, Blind docking of drug-sized compounds to proteins with up to a thousand residues, FEBS Lett. 580 (2006) 1447–1450, https://doi.org/10.1016/j.febslet.2006.01.074.

[38] É. Szőke, M. Bálint, C. Hetényi, A. Markovics, K. Elekes, G. Pozsgai, T. Szűts, G. Kéri, L. Őrfi, Z. Sándor, J. Szolcsányi, E. Pintér, Z. Helyes, Small molecule somatostatin receptor subtype 4 (sst4) agonists are novel anti-inflammatory and analgesic drug candidates, Neuropharmacology 178 (2020) 108198, https://doi.org/10.1016/j.neuropharm.2020.108198.

[39] Z. Seltzer, R. Dubner, Y. Shir, A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury, Pain 43 (1990) 205–218, https://doi.org/10.1016/0304-3959(90)91074-S.

[40] B. Kántás, É. Szőke, R. Börzsei, P. Bánhegyi, J. Asghar, L. Hudhud, A. Steib, Á. Hunyady, Á. Horváth, A. Kecskés, É. Borbély, C. Hetényi, G. Pethő, E. Pintér, Z. Helyes, In silico, in vitro and in vivo pharmacodynamic characterization of novel analgesic drug candidate somatostatin SST4 receptor agonists, Front. Pharmacol. 11 (2021) 601887, https://doi.org/10.3389/fphar.2020.601887.

[41] É. Borbély, Z. Hajna, K. Sándor, L. Kereskai, I. Tóth, E. Pintér, P. Nagy, J. Szolcsányi, J. Quinn, A. Zimmer, J. Stewart, C. Paige, A. Berger, Z. Helyes, Role of Tachykinin 1 and 4 gene-derived neuropeptides and the Neurokinin 1 receptor in adjuvant-induced chronic arthritis of the mouse, PLoS One 8 (2013) e61684.

[42] Z. Helyes, E. Pintér, J. Németh, K. Sándor, K. Elekes, G. Szabó, D. Pozsgai, L. Keszthelyi, M. Kereskai, S. Engström, J.S. Wurster, Effects of the somatostatin receptor subtype 4 selective agonist J-2156 on sensory neuropeptide release and inflammatory reactions in rodents, Br. J. Pharmacol. 149 (2006) 405–415, https://doi.org/10.1038/sj.bjp.0706876.

[43] Á. Szabó, Z. Helyes, K. Sándor, A. Bite, E. Pintér, J. Németh, Á. Bánvölgyi, K. Bölcskei, K. Elekes, J. Szolcsányi, Role of Transient Receptor Potential Vanilloid 1 receptors in adjuvant-induced chronic arthritis: in Vivo study using gene-

deficient mice, J. Pharmacol. Exp. Ther. 314 (2005) 111–119, https://doi.org/10.1124/jpet.104.082487.

[44] K. Bölcskei, Z. Helyes, Á. Szabó, K. Sándor, K. Elekes, J. Németh, R. Almási, E. Pintér, G. Pethő, J. Szolcsányi, Investigation of the role of TRPV1 receptors in acute and chronic nociceptive processes using gene-deficient mice, Pain 117 (2005) 368–376, https://doi.org/10.1016/j.pain.2005.06.024.

[45] Z. Helyes, Á. Szabó, J. Németh, B. Jakab, E. Pintér, Á. Bánvölgyi, L. Kereskai, G. Kéri, J. Szolcsányi, Antiinflammatory and analgesic effects of somatostatin released from capsaicin-sensitive sensory nerve terminals in a Freund's adjuvant-induced chronic arthritis model in the rat: Function of Somatostatin in Chronic Inflammation, Arthritis Rheum. 50 (2004) 1677–1685, https://doi.org/10.1002/art.20184.

[46] Z. Helyes, E. Pinter, K. Sandor, K. Elekes, A. Banvolgyi, D. Keszthelyi, E. Szoke, D. M. Toth, Z. Sandor, L. Kereskai, G. Pozsgai, J.P. Allen, P.C. Emson, A. Markovics, J. Szolcsanyi, Impaired defense mechanism against inflammation, hyperalgesia, and airway hyperreactivity in somatostatin 4 receptor gene-deleted mice, Proc. Natl. Acad. Sci. 106 (2009) 13088–13093, https://doi.org/10.1073/pnas.0900681106.

[47] Á. Simon, A. Czajlik, A. Perczel, G. Kéri, L. Nyikos, Z. Emri, J. Kardos, Binding crevice for TT-232 in a homology model of type 1 somatostatin receptor, Biochem. Biophys. Res. Commun. 316 (2004) 1059–1064, https://doi.org/10.1016/j.bbrc.2004.02.161.

[48] R.H. Alasbahi, M.F. Melzig, Forskolin and derivatives as tools for studying the role of cAMP, Pharmazie 67 (2012) 5–13.

[49] M. Engström, J. Tomperi, K. El-Darwish, M. Åhman, J.-M. Savola, S. Wurster, Superagonism at the Human Somatostatin Receptor Subtype 4, J. Pharmacol. Exp. Ther. 312 (2005) 332–338, https://doi.org/10.1124/jpet.104.075531.

[50] K. Elekes, Z. Helyes, L. Kereskai, K. Sándor, E. Pintér, G. Pozsgai, V. Tékus, A. Bánvölgyi, J. Németh, T. Szuts, G. Kéri, J. Szolcsányi, Inhibitory effects of synthetic somatostatin receptor subtype 4 agonists on acute and chronic airway inflammation and hyperreactivity in the mouse, Eur. J. Pharmacol. 578 (2008) 313–322, https://doi.org/10.1016/j.ejphar.2007.09.033.

[51] B. Botz, A. Imreh, K. Sándor, K. Elekes, J. Szolcsányi, D. Reglődi, J.P. Quinn, J. Stewart, A. Zimmer, H. Hashimoto, Z. Helyes, Role of Pituitary Adenylate-Cyclase Activating Polypeptide and Tac1 gene derived tachykinins in sensory, motor and vascular functions under normal and neuropathic conditions, Peptides 43 (2013) 105–112, https://doi.org/10.1016/j.peptides.2013.03.003.

[52] K. Sándor, K. Elekes, Á. Szabó, E. Pintér, M. Engström, S. Wurster, J. Szolcsányi, Z. Helyes, Analgesic effects of the somatostatin sst4 receptor selective agonist J-2156 in acute and chronic pain models, Eur. J. Pharmacol. 539 (2006) 71–75, https://doi.org/10.1016/j.ejphar.2006.03.082.

[53] B. Botz, K. Bölcskei, Z. Helyes, Challenges to develop novel anti-inflammatory and analgesic drugs, WIREs Nanomed. Nanobiotechnol. 9 (2017) e1427.

[54] E. Cavalli, S. Mammana, F. Nicoletti, P. Bramanti, E. Mazzon, The neuropathic pain: An overview of the current treatment and future therapeutic approaches, Int J Immunopathol Pharmacol. 33 (2019) 2058738419838383. https://doi.org/10.1177/2058738419838383.

[55] K. Jefferies, Treatment of neuropathic pain, Semin. Neurol. 30 (2010) 425–432, https://doi.org/10.1055/s-0030-1267286.

[56] R. Cozzi, M. Montini, R. Attanasio, M. Albizzi, G. Lasio, S. Lodrini, P. Doneda, L. Cortesi, G. Pagani, Primary treatment of acromegaly with octreotide LAR: a long-term (up to nine years) prospective study of its efficacy in the control of disease activity and tumor shrinkage, J. Clin. Endocrinol. Metab. 91 (2006) 1397–1403, https://doi.org/10.1210/jc.2005-2347.

[57] M. Mercado, F. Borges, H. Bouterfa, T.-C. Chang, A. Chervin, A.J. Farrall, A. Patocs, S. Petersenn, J. Podoba, M. Safari, J. Wardlaw, SMS995B2401 Study Group, A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly, Clin. Endocrinol. (Oxf) 66 (2007) 859–868, https://doi.org/10.1111/j.1365-2265.2007.02825.x.

[58] J.S. Redfern, W.J. Fortuner, Octreotide-associated biliary tract dysfunction and gallstone formation: pathophysiology and management, Am. J. Gastroenterol. 90 (1995) 1042–1052.

[59] P. Ros-Pérez, T. Golmayo, M.L. Cilleruelo, C. Gutiérrez, P. Celaya, N. Lacámara, I. Martínez-Badás, M. Güemes, J. Argente, Octreotide-related exocrine pancreatic insufficiency (EPI) in congenital hyperinsulinism, J Pediatr. Endocrinol. Metab. 33 (2020) 947–950, https://doi.org/10.1515/jpem-2019-0565.

[60] M.W. Saif, H. Larson, K. Kaley, W. Shaib, Chronic octreotide therapy can induce pancreatic insufficiency: a common but under-recognized adverse effect, Expert Opin. Drug Saf. 9 (2010) 867–873, https://doi.org/10.1517/14740338.2010.510130.

[61] R. Baldelli, C. Battista, F. Leonetti, M.-R. Ghiggi, M.-C. Ribaudo, A. Paoloni, E. D'Amico, E. Ferretti, R. Baratta, A. Liuzzi, V. Trischitta, G. Tamburrano, Glucose homeostasis in acromegaly: effects of long-acting somatostatin analogues treatment, Clin. Endocrinol. (Oxf). 59 (2003) 492–499, https://doi.org/10.1046/j.1365-2265.2003.01876.x.

[62] M. Boscaro, W.H. Ludlam, B. Atkinson, J.E. Glusman, S. Petersenn, M. Reincke, P. Snyder, A. Tabarin, B.M.K. Biller, J. Findling, S. Melmed, C.H. Darby, K. Hu, Y. Wang, P.U. Freda, A.B. Grossman, L.A. Frohman, J. Bertherat, Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial, J. Clin. Endocrinol. Metab. 94 (2009) 115–122, https://doi.org/10.1210/jc.2008-1008.