Characterization of novel somatostatin 4 receptor agonists in different pain models

PhD thesis

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I. GENERAL INTRODUCTION, LITERATURE REVIEW

I.1. Neuropathic pain

Neuropathic pain is a subjective sensory symptom resulting from damage or dysfunction of the central or peripheral nervous system, which has no useful protective function for the body and the exact pathomechanism of its development is not clear. Pain sensations caused by a not essentially painful stimulus (e.g., tactile) are named allodynia, whereas increased pain sensations caused by moderately painful stimulus are named hyperalgesia [1]. Neuropathy can be caused by a variety of aetiological factors and thus affects a large and heterogeneous group of patients.

In the treatment of neuropathic pain, conventional analgesics are not effective (NSAIDs) or have limited efficacy (opioids). In pharmacological therapy, so-called adjuvant analgesics are used, which include antidepressant (e.g. amitriptyline, nortriptyline, desipramine, duloxetine, venlafaxine) and antiepileptic (e.g. gabapentin, pregabalin) agents. Lidocaine and capsaicin in topical form can also relieve the neuropathic pain, but in some patients they cause severe skin irritation. A therapeutic challenge is that the analgesic effect of adjuvant analgesics is often unsatisfactory for patients, therapy-resistant cases are not uncommon, and their use is limited by several unpleasant side effects. Patient compliance is significantly impaired by the latency period of several weeks required for adjuvant analgesics to develop their analgesic effect, while unpleasant side effects appear almost immediately after the start of therapy. These therapeutic difficulties require the development of a new analgesics with completely novel mechanism of action [2].

I.2. Neurogenic inflammation

In the peripheral nervous system, a group of pain-sensing nerves involved in inflammation are selectively excited by capsaicin and, at higher doses, desensitized by it, the so-called capsaicin-sensitive afferents [3]. Capsaicin activates the Transient Receptor Potential Vanilloid 1 (TRPV1) non-selective cation channel, which is activated by heat stimulation above 43°C in addition to capsaicin [4], pH below 6, other exogenous irritants (e.g. resiniferatoxin (RTX), piperine) and endogenous mediators (e.g. anandamide, lipoxygenase products) can activate the receptor. Upon activation of the receptor, a conformational change occurs and the channel opens, Na+ and Ca2+ ions flow in, leading to depolarization of the nerve terminal and the formation of an action potential. The influx of Ca2+ ions from the nerve terminal leads to the exocytosis of neuropeptides stored

there, and Ca2+ ions also play an important role in the eventual subsequent desensitisation of the neuron [5].

During the activation of capsaicin-sensitive sensory nerve endings, several neuropeptides are released, resulting in vasodilatation and plasma extravasation [6]. This phenomenon is called neurogenic inflammation.[7] During activation of the same nerve endings, in addition to pro-inflammatory neuropeptides (substance P, neurokinin A and B), neuropeptides with anti-inflammatory effects (somatostatin, galanin, PACAP-38) are released.

Neurogenic inflammation plays an important role in the pathogenesis of many diseases, the neurogenic inflammatory component of which is not controlled by conventional NSAIDs [8], and thus, as with neuropathic pain, the treatment of neurogenic inflammation is a major challenge in medical practice.

1.3. Somatostatin and its receptors

Somatostatin is a cyclic neuropeptide expressed in many areas of the body in a biologically active form of 14 to 28 amino acids [9]. It inhibits the secretion of several hypophyseal (somatotropin, prolactin, thyrotropin) and peripheral (e.g. insulin, glucagon, gastrin, secretin, motilin, cholecystokinin) hormones. Somatostatin can be detected in a significant population of GABAergic interneurons, from where it is released together with GABA and acts as an inhibitory neuromodulator on neurons of the central nervous system [10]. Decreased inhibitory neurotransmission, and thus lack of somatostatin, plays an important role in the development of mood disorders (e.g. major depression, bipolar disorder) and anxiety [11]. Somatostatin exerts its diversified effects through its own heptahelical membrane-bound Gi-protein coupled receptors (SST_1 - SST_5), of which SST_1 and SST_4 receptors are responsible for anti-inflammatory and analgesic effects [12].

Due to the broad spectrum of action of native somatostatin and its short plasma elimination half-life, it is not therapeutically applicable [13], so its receptor-selective, stable agonists may represent a new therapeutic perspective.

SST_4 is present in both the peripheral and central nervous system, and its peripheral activation can be associated with analgesic and anti-inflammatory effects without endocrine side effects [14], making it a promising target for drug development. In terms of its brain localization, it can be detected by immunohistochemistry in the cerebral cortex, striatum, hippocampus and amygdala, among others [15]. It plays a role in the regulation of locomotor activity [16] and learning processes [17], memory formation[18], and mood regulation [19].

Over the past decades, our team has focused on SST_4 receptor-activating compounds. TT-232 is a cyclic heptapeptide that binds to SST_4/SST_1 receptors with higher affinity compared to other somatostatin receptors. Experiments with this compound have demonstrated a significant antiproliferative effect [20], as well as inhibition of acute nociception and anti-inflammatory effects in rat models. Toxic side effects were not detected at doses much higher than sufficient to produce anti-inflammatory effects, and no effects on the central nervous system were observed [21]. Subsequent studies have shown that it increases thermal tolerance and improves diabetes-induced neuropathic hyperalgesia [12]. Unlike non-selective somatostatin agonists, it does not affect gastrin and growth hormone secretion.

I.5. Background of our current investigations

The per os active compounds with pyrrolo-pyrimidine structure (C1-C6) were patented by the University of Pécs and synthesized by Avicor Ltd. The starting 4-chloro-pyrrolo-pyrimidines were obtained from commercial sources, which were coupled with various phenylethylamines after N-benzylation.

The SST_1/SST_4 receptor agonist peptide, TT-232 was ordered from Tocris Bioscience (Cat. No. 4639) and stored at -20 °C until use.

Rita Börzsei and Csaba Hetényi modeled the structure of the SST_4 receptor using the Maestro program, and then docked different ligand structures to the extracellular region of the SST_4 receptor using AutoDock 4.2.6. The structural calculations showed that C1-6 molecules bind with similar interaction energy (-8.24 ± 0.41 kcal/mol) to the same high-affinity binding site of the SST_4

receptor. TT-232 fits into the deep binding pocket of the SST_4 receptor, with an interaction energy (-11.03 kcal/mol) that is similar or better than the binding strength of the superagonist J-2156 [22].

Based on in silico studies, Éva Szőke performed G-protein activation assays with promising C1-6 compounds, in which all six molecules induced G-protein activation in stably SST_4 receptor expressing cell cultures. The compounds proved to be potent and effective agonists of the SST_4 receptor.

The β-arrestin accumulation assay was carried out by Junaid Asghar and Lina Hudhud. β-arrestin accumulation, which referes to desensitization of G-protein coupled receptors, was not detectable.

Éva Szőke investigated the binding of TT-232 to the SST_4 receptor in the presence of [125I-Tyr11]somatostatin-14 in a cell culture stably expressing the receptor. As a result of this study, TT-232 is able to displace somatostatin from the SST_4 receptor in a concentration-dependent manner.

I. AIMS

The aim of my PhD thesis is to investigate the antihyperalgesic effects of novel potential SST_4 receptor agonists, the per os active pyrrolo-pyrimidine compounds and the intraperitoneally active, SST_1/SST_4 receptor agonist TT-232 in pain models with different mechanisms:

I. Examination of C1-C6 compounds and TT-232 in a mouse model of neuropathic pain

II. Examination of C5, C6 compounds in a mouse model of neurogenic inflammation

III. Testing of compound C2 in behavioural studies

III. EXPERIMENTAL MODELS AND METHODS

III.1. Animals

To model neuropathic pain and neurogenic inflammation, 8-12 week old male NMRI mice were used, which have the highest pain threshold compared to other mouse strains. We also worked with 8-12-week-old male *Sstr4* gene-deficient (*Sstr4*$^{-/-}$) and wild-type (*Sstr4*$^{+/+}$) mice generated from C57Bl/6J mice. Behavioural tests were performed on 12-16-week-old male C57Bl/6 mice, this strain is most commonly used for this purpose.

III.2. Ethical statement

All experimental procedures complied respects with the recommendations of the No. 1998/XXVIII act and the of European Parliament directive (63/2010) and were approved by the Ethics Committee on Animal Research of Pecs University according to the Ethical Codex of Animal Experiments; license was given (license No. BA1/35/55-50/2017). We made all efforts to minimize the number and suffering of the animals used in this study.

III.3. Protocols for treatment

For the experiments, 1 mg of the C1-C6 compounds was rubbed dry in a braying mortar, suspended thoroughly in 1 ml 1.25% methylcellulose solution. Microsuspensions of 1, 5, 25, 50 and 100 μg/ml of the stock solutions in 1.25% methylcellulose were prepared and used to treat the animals. The animals treated a 20 ml/kg suspension by feeding tube, i.e. they were treated with doses of 20, 100, 500, 1000 and 2000 μg/kg. The control group treated 20 ml/kg of 1.25% methylcellulose via a feeding tube. The per os treatments were administered 60 min before the experimental measurements.

A solution of TT-232 was prepared at a concentration of 1 mg/ml using acetate buffer (pH 3.5), and further dilutions were made with phosphate buffer (PBS - pH 7.3). The animals were treated intraperitoneally with 100 μg/kg and 200 μg/kg doses 30 min before the experimental measurements. The control group was treated intraperitoneally a mixture of acetate and phosphate buffer at the same time.

III.4. *In vivo* models and methods

III.4.1. Neuropathic pain model

Traumatic sensory mononeuropathy was induced by the partially ligation of the right sciatic nerve, also known as Seltzer-operation [23]. During the operation animals were anaesthetised with a combination of ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.). After isolation of the right sciatic nerve, the dorsal 1/3-1/2 part of the nerve was tightly ligated with 8-0 nonabsorbable yarn and the wound was closed with 6-0 suture.

During the control measurements and on the 7th postoperative day, the mechanonociceptive thresholds of the animals were measured by dynamic plantar aesthesiometer (DPA, Ugo Basile, Comerio, Italy). Compared to the mean of the control measurements, mice with less than 20% reduction in mechanonociceptive threshold after the operation were excluded. Animals that underwent successful surgery were treated with different doses of our compounds and their vehicle. Mechanonociceptive thresholds were measured again 60 min after the treatment with compounds C1-C6 and 30 min after treatment with TT-232.

III.4.2. RTX-induced acute neurogenic inflammation model

In this experiment, we investigated the ability of C5 and C6 compounds to prevent RTX-induced acute neurogenic inflammation, the p.o. treatment was administered 60 min before RTX-injection.

RTX is an ultrapotent capsaicin analogue, thus activates the non-selective cation channel of Transient Receptor Potential Vanilloid 1 (TRPV1). According to the Scovill scale, RTX is 1000 times more potent than capsaicin, which is responsible for the pungency of peppers. Intraplantar injection of RTX (Sigma, St. Louis, MO, USA) (20 µl, 0.1 µg/ml) was used to induce an acute neurogenic inflammatory response in the right hind paw of animals, which within minutes produced thermal allodynia due to peripheral sensitization mechanisms, followed by mechanical hyperalgesia via central sensitization [24].

During one habituation and two control measurements, the mechanonociceptive threshold of the hind paws were determined by DPA and the heat threshold by Hot plate (IITC Life Science, Woodland Hills, CA, USA), which heats up from 25°C to a maximum of 50°C at a rate of 12°C/minute. During the measurement, the animals indicate when the Hot plate has reached the painful temperature by showing pain responses (leg jerking, shaking, foot licking), at this point the measurement ends immediately and the Hot plate shows the exact temperature in °C. On the day of the experiment, the heat thresholds were determined at 10, 20 and 30 min after RTX-injection and their mechanical pain thresholds at 30, 60 and 90 min, and compared with the results of the control measurements.

III.4.3. Open field test (OFT)

To determine spontaneous locomotor activity and anxiety levels, we used OFT 60 min after treatment. Animals were placed individually in a 39 cm × 39 cm × 39 cm box with a white floor and grey walls, open at the top, for 5 min of observation [25]. The box was cleaned with 70% ethanol after each animal [26]. The movements of the animals were recorded and evalueted by EthoVision XT 8.0 (Noldus Information Technology, Wageningen, Netherlands) motion analysis software.

III.4.4. Elevated plus maze (EPM)

The EPM is a widely used test to determine the anxiety level of rodents. The arms of a cross-shaped plank, placed 1 m off the ground, are 5 cm wide and 30 cm long, with two opposite arms closed on three sides and the other two open. 60 minutes after treatment, mice were placed one by one in the middle of the cross with their noses towards one of the open arms. During the 5 min observation period the movements of the animals were video-recorded and analysed by measuring the time the animals spent on the open arms of the board, a time inversely proportional with the anxiety level of the animals. The apparatus was cleaned with 70% ethanol after each animal [27].

IV. STATISTICAL ANALYSIS

The antihyperalgesic effects of the compounds were calculated from mechanonociceptive thresholds and compared with the control group by unpaired t-test. Unpaired t-tests was also used to evaluate the behavioural tests, except for the number of branching, where Mann-Whitney U test was used, which is the non-parametric equivalent of the unpaired t-test. In case of TT-232, in addition to antihyperalgesic effect, mechanonociceptive thresholds were plotted, these values were compared with paired t-test.

The changes of the mechanonociceptive threshold and heat threshold induced by RTX-injection were evaluated by two-way analysis of variance (ANOVA) and Bonferroni correction.

When comparing the results of each group, values of *$p<0.05$; **$p<0.01$; ***$p<0.001$ and ****$p<0.0001$ were considered significant. Statistical analysis was performed by GraphPad Prism 8 statistical software.

V. RESULTS

V.1. Effect of pyrrolo-pyrimindine compounds in neuropathic pain model

By the 7th postoperative day, the mechanonociceptive threshold of the experimental animals was reduced by 37.3±13.4% compared to baseline. Significant antihyperalgesic effects were observed in case of treatment with 500 µg/kg dose of C1 (C1: 52.1±5.4% vs. Vehicle: 14.7±6.1%), 100 and 500 µg/kg doses of C2 (C2: 64.4±14.3%; 54.6±13.7% vs. Vehicle: 7.8±8.1%), 500 µg/kg dose of C3 (C3: 57.0±16.1% vs. Vehicle: 12.0±7.2%), C4 500 µg/kg dose (C4: 57.2±14.6% vs. Vehicle: 10.0±7.6%), C5 100, 500 and 2000 µg/kg doses (C5: 46.7±9.5%; 57.6±10.8%; 48.1±7.2% vs. Vehicle: 8.1±6.0%), and C6 500 and 2000 µg/kg doses (C6: 44.7±8.3%; 45.0±13.2 vs. Vehicle: 16.6±7.8%).

V.2. Effect of C5 and C6 molecules in the RTX-induced acute neurogenic inflammation model

In a mouse model of neurogenic inflammation, the effects of C5 and C6 molecules were investigated at a dose of 500 µg/kg. After intraplantar injection of RTX (20 µl, 0.1 µg/ml), the heat

threshold decreased from 46.1 ± 0.4 °C to 34.7 ± 1.5; 41.2 ± 2.3 and 41.1 ± 1.7 °C at 10, 20 and 30 min (-24.6 ± 3.5%; -10.4 ± 5.2% and -10.7% ± 4.1%) and their mechanonociceptive threshold decreased from 9.7 ± 0.1 g to 5,1 ± 0,4; 6,6 ± 0,3 and 6,7 ± 0,3 g at the 30., 60. and 90. min, thus 47,3% ± 4,4%; 31,5% ± 2,9% and 31,4% ± 3,6% mechanical hyperalgesia developed. Following the oral pretreatment with the compounds, C5 significantly inhibited the development of the heat threshold at the 10. and 30 min (C5: 43.0±1.0°C; 48.5±0.9°C vs. Vehicle: 41.0±1.7 °C), and the development of mechanical hyperalgesia at 30. min (C5: 24.6±4.5% vs. Vehicle: 47.2±4.4%), while C6 had no detectable effect at the tested dose.

V.3. Effect of the C2 molecule in behavioural studies

There was no significant difference between C2-treated and control mice in the time spent on the open arms of the EPM (C2: 52.8±7.4 s vs. Vehicle: 51.0±8.5 s) and the time spent on the outer 1/3 of the open arms (C2: 9.1±3.1 s vs. Vehicle: 6.2±2.8 s).

Measured parameters during OFT, i.e., distance moved (C2: 1798 ± 180.8 cm vs. Vehicle: 1824 ± 130.2 cm), velocity (C2: 6.0±0.6 m/s vs. Vehicle: 6.1±0.4 m/s), time spent moving (C2: 56.0±5.2 s vs. Vehicle: 56.3±3.7 s), time spent in the middle zone (C2: 59.8±8.7 s vs. Vehicle: 59.5±4.0 s), and number of rearings (C2: 31.1±4.1 vs. Vehicle: 30.6±3.2) were not affected by C2 at the tested dose.

V.4. Effect of TT-232 in neuropathic pain model

After the Seltzer-operation, the average reduction in mechanonociceptive threshold of the mice was 37.3±1.0%. In wild-type mice, TT-232 significantly attenuated the reduction in mechanonociceptive threshold at doses of 100 and 200 μg/kg (TT-232: 24.9±3.4%; 19.6±3.2% vs. Vehicle: 38.2±2.3%), whereas the mechanonociceptive threshold of gene-deficient mice was not affected by either dose (TT-232: 34.2±1.9%; 35.8±1.4% vs. Vehicle: 35.8±1.6%).

Antihyperalgesic effect was calculated from the changes of the mechanonociceptive threshold, TT-232 showed 35.7±8.3% antihyperalgesic effect at 100 μg/kg dose and 50.4±8.4% antihyperalgesic effect at 200 μg/kg dose in wild-type mice.

VI. DISCUSSION

Neuropathic pain is particularly resistant to conventional analgesic treatment: opioids and NSAIDs are almost completely ineffective in neuropathic conditions [28], and the effect of adjuvant analgesics (e.g. antiepileptics, antidepressants) is often limited, in addition several side effects limiting their use [29].

The examined pyrrolo-pyrimidine compounds (C1-6) were selected based on preliminary in silico and in vitro studies as potential SST_4 receptor agonists. As a result of in silico modeling, all 6 molecules showed binding similar with interaction energy to the high affinity binding pocket of the SST_4 receptor, and induced G-protein activation in vitro studies, nevertheless β-arrestin accumulation, which suggesting receptor desensitization, was not detected [30].

The SST_1/SST_4 agonist TT-232 showed in silico binding to the deep binding pocket of the SST_4 receptor with similar interaction energy to the superagonist J-2156 [22](S. Liu et al., 1999; Szőke et al., 2020) and concentration-dependently displaces somatostatin binding from SST_4 receptor in vitro (unpublished results pending).

As a result of Seltzer-operation, significant damage develops in thin myelinated and non-myelinated nerve fibres, leading to the development of abnormal sensory function, hyperalgesia and allodynia without impairment of motor function [31]. Our group has previously demonstrated the role of capsaicin-sensitive sensory nerves in chronic neuropathic pain models. In these studies, wild-type mice with the TRPV1 receptor developed significantly lower mechanonociceptive threshold reductions than TRPV1 gene-deficient animals, and their plasma somatostatin concentrations were significantly higher compared to plasma concentrations in gene-deficient animals [32]. These results showed the importance the role of TRPV1-dependent somatostatin release in the antinociceptive mechanisms in chronic neuropathic conditions and suggest that compounds acting on specific receptors of somatostatin such as our selected compounds, may also have antihyperalgesic effects.

Following pretreatment with a single oral dose of 500 μg/kg of pyrrolo-pyrimidine compounds, all of them significantly increased the mechanonociceptive threshold and each produced similar

antihyperalgesic effects, the maximum antihyperalgesic effect was 50-60%. Interestingly, these compounds did not show conventional dose-response correlation, each compound has bell-shaped dose-response curves, where the two lower and two higher doses were ineffective. Compound C2, unlike the others, was effective at a lower dose, 100 μg/kg. These novel compounds have significant antihyperalgesic effect in relatively low doses, which refers to high potency. At present, the explanation of bell-shaped dose-response curves or the extent of its central or peripheral components are not known. Using an ultrasensitive RNAscope technique, our group has provided data about the localisation of the SST_4 receptor in the central nervous system. Based on these data the receptor is present in several important pain and mood regulating brain regions, such as prelimbic cortex, hippocampus, habenula, amygdala, primary somatosensory cortex [33]. The widespread brain localisation of the receptor may be one possible explanation for the bell-shaped dose-response curves, whereby somatostatin agonist compounds administered at higher doses may also inhibit their own inhibitory effect on pain pathways directly or even indirectly, but it is also possible that other receptors are activated at higher doses. The potential inhibitory effect of SST_4 agonists on the release of endogenous inhibitory mediators such as somatostatin and opioid peptides cannot be excluded, which may explain the lack of dose-response or the bell-shaped dose-response curves.

Similar to the compounds mentioned above, the SST_4 receptor agonist heptapetide TT-232 was also tested in a mouse model of traumatic sensory mononeuropathy, showing 36 and 50% antihyperalgesic effects in wild-type mice at 100 and 200 μg/kg doses, but it did not influence the mechanonociceptive threshold of SST_4 KO mice. This results demonstrate the antihyperalgesic effect of TT-232 and that the antihyperalgesic effect is mediated through activation of the SST_4 receptor [34]. Previous results from our group have also demonstrated the inhibitory effect of TT-232 on the mechanical allodynia in rat model of streptozotocin-induced diabetes [12].

RTX is a selective, ultrapotent agonist of the TRPV1 capsaicin receptor, which intraplantar injection induces an acute neurogenic inflammatory response with rapid onset and short duration of thermal allodynia, mediated predominantly by peripheral sensitization mechanisms. RTX releases proinflammatory neuropeptides, such as P-matter and calcitonin gene-related peptide, in the innervated area, which trigger a local inflammatory cascade and lead to peripheral sensitization

of nociceptive nerve endings [35]. Thermal allodynia is followed by mechanical hyperalgesia, mediated by peripheral mechanisms as well as central sensitization processes in the spinal cord and various pain processing brain regions [36].

C5 inhibited the development of thermal allodynia and mechanical hyperalgesia in RTX-induced acute neurogenic inflammation in mice, while C6 was ineffective.

Based on its physicochemical properties and the results of Lipinsky's RO5, which is suitable for estimating the kinetic parameters of drugs, C5 is likely to cross the blood-brain barrier. It can be assumed that the inhibitory effect of C5 on mechanical hyperalgesia is not only due to peripheral mechanisms, it also reduces central pain sensitivity. This is supported by the localization of SST_4 receptors [33], which is detailed above. However, in the background of the different effect of this two compounds in this model can be pharmacodynamics and mechanism-of-action differences (e.g., SST1 or opioid receptor agonist effect and/or kinase inhibition in case of C5).

Several conclusions can be drawn from the results of the OFT based on how the spontaneous locomotor activity of the animals changed. The parameters tested include distance moved, speed, time spent moving, time spent in the middle zone and number of rearings, which provide information not only on spontaneous locomotor activity, they provide information about exploratory behaviour, anxiety level and the test can be used to detect possible sedative side effects [17], while EPM assesses the anxiety level of animals [25], which may be potentially influenced by our compounds.

Based on the results, we can conclude that C2 at the dose with antihyperalgesic effect has no effect on the behaviour of the animals and does not have sedative side effect. It is possible that C2 acts selectively on pain pathways, so that common brain structures involved in pain and mood regulation are not affected, but it is also possible that a much higher dose would be required to influence animal behaviour than to achieve the antihyperalgesic effect. Completely intact were used in the experiments, they that had not been stressed in any way prior to testing, but the fact that C2 did not improve the mice's baseline mood does not rule out the possibility that it may have a mood-enhancing or anti-anxiety effect following stress.

Stable, orally active, non-peptide SST_4 agonists clearly have significant broad-spectrum antihyperalgesic effect in both inflammatory and neuropathic pain models. Their mechanism of action is similar to opioid analgesics, which also activate Gi-protein-coupled receptors, typically located presynaptically/preadaptively, which leads the release of several proinflammatory and/or pronociceptive mediators from, among others, the peripheral and central terminals of primary sensory neurons. This mechanism, which potentiates inhibitory effects, is thought to be more effective than antagonism of stimulatory mediators such as glutamate receptors. Since the SST_4 receptor is not involved in the endocrine actions of somatostatin (mediated by SST_2, SST_3 and SST_5 receptors), it is expected that our compounds do not affect hormonal regulation.

Our present discovery studies provide a good basis for the initial steps of preclinical drug development, where our task is the selection and optimisation of the lead molecule in a co-development with our industrial partner.

VII. SUMMARY OF NEW RESULTS

During my PhD work, we were the first to demonstrate in vivo:

I. Our patented C1-C6 pyrrolo-pyrimidine compounds, following a low dose (100, 500 or 2000 μg/kg) single oral administration, and the heptapeptide TT-232 somatostatin analogue, also following a low dose (200 μg/kg) single intraperitoneal administration, exert significant antihyperalgesic effects in a mouse model of neuropathic pain.

II. Compound C5 following a single oral administration of a low dose (500 μg/kg) inhibits the development of thermal allodynia and mechanical hyperalgesia during neurogenic inflammation in mice.

III. An effective dose of compound C2 in neuropathic pain (500 μg/kg) does not influence spontaneous locomotor activity and anxiety levels in animals.

VIII. CONCLUSIONS

Our novel compounds have a clear analgesic effect in neuropathic pain after a single oral administration and also inhibit mechanical hyperalgesia and thermal allodynia caused by neurogenic inflammation. Therefore, these compounds are promising candidates for the development of completely new type of analgesic drug that is essentially needed for the effective treatment of neuropathic pain conditions.

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X. PUBLICATIONS

X.1. Publication list

Kántás Boglárka, Börzsei Rita, Szőke Éva, Bánhegyi Péter, Horváth Ádám, Hunyady Ágnes, Borbély Éva, Hetényi Csaba, Pintér Erika, Helyes Zsuzsanna. Novel drug-like somatostatin receptor 4 agonists are potential analgesics for neuropathic pain. Int. J. Mol. Sci. 2019, 20(24), 6245. doi:10.3390/ijms20246245

IF: 4,56

Kántás Boglárka, Szőke Éva, Börzsei Rita, Bánhegyi Péter, Asghar Junaid, Hudhud Lina, Steib Anita, Hunyady Ágnes, Horváth Ádám, Kecskés Angéla, Borbély Éva, Hetényi Csaba, Pethő Gábor, Pintér Erika, Helyes Zsuzsanna. In Silico, In Vitro and In Vivo Pharmacodynamic Characterization of Novel Analgesic Drug Candidate Somatostatin SST_4 Receptor Agonists. Frontiers in Physiology. 11:601887 (2021). doi: 10.3389/fphar.2020.601887

IF: 5,33

X.2. Other publications, not related to the topic of the PhD thesis

Horváth Ádám, Biró-Sütő Tünde, **Kántás Boglárka**, Payrits Maja, Skodáné-Földes Rita, Szánti-Pintér Eszter, Helyes Zsuzsanna, Szőke Éva. Antinociceptive effects of lipid raft disruptors, a novel carboxamido-steroid and methyl β-cyclodextrin, in mice by inhibiting Transient Receptor Potential Vanilloid 1 and Ankyrin 1 ion channel activation. Frontiers in Physiology. 11, 559109 (2020) DOI: 10.3389/fphys.2020.559109

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Horváth Ádám, Payrits Maja, Steib Anita, **Kántás Boglárka**, Biró-Sütő Tünde, Erostyák János, Makkai Géza, Sághy Éva, Helyes Zsuzsanna, Szőke Éva. Analgesic effects of lipid raft disruption by sphingomyelainse and myriocin via Transient Receptor Potential Vanilloid 1 and Transient Receptor Potential Ankyrin 1 ion channel modulation. Frontiers in Pharmacology. 11, 593319 (2021) DOI: 10.3389/fphar.2020.593319

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