Transient Receptor Potential Vanilloid 1 és Ankyrin 1 receptors activation and inhibition mechanisms in *in vitro* and *in vivo* experiments

PhD thesis

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I. RESEARCH CONCEPT

Since the treatment of neuropathic pain and the neurogenic components of the inflammatory processes is an unresolved problem due to a grate range of severe side effect and the presently available drug groups cannot be administered for longer time periods, nowadays, the effects of these drug groups are widely investigated. The examination of sensory receptors on nociceptive nerve endings is in the focus of the analgesic drug development strategies. Drug candidates which could act directly at the level of the sensory nerve terminals and selectively inhibit nociceptor activation may have a better side effect profile and might be more effective in the treatment of neuropathic pain, than the currently available anti-inflammatory drugs.

Transient Receptor Potential (TRP) ion channels have a crucial role in the mechanism of pain and neurogenic inflammation via the activation of sensory nerves. Our research group examines the activation of TRP ion channels on trigeminal and dorsal root sensory neurons and on nerve endings of peripheral sensory neurons. These receptors might serve as targets for the development of new anti-inflammatory and analgesic drugs with different mechanisms of action. Therefore, there is a particularly great need to investigate the role of these receptors, especially the Transient Receptor Potential Vanilloid 1 (TRPV1) and Transient Receptor Potential Ankyrin 1 (TRPA1) ion channels, in pain and inflammatory processes and understand their mechanisms of action.

II. INTRODUCTION

1. The investigation of pain and pain relief

Pain is a subjective sensory quality, which is developed by tissue damage, sensed by nociceptors, and the sensory stimulus is transmitted from the whole body to the central nervous system. Pain perception has a useful role in the detection of a dangerous exogenous effect or the pathological process in the body. Even in the ancient times the opium-containing moisture of poppy-head was used for pain killing and anaesthesia and opioid compounds are still widely used analgesics.

Nowadays opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and adjuvant analgesics have been administered in pain therapy. Neuropathic pain developes due to the damage of the peripheral or central nervous system. NSAID type anti-inflammatory drugs and pain killers reduce the synthesis of prostaglandin by inhibiting the cyclooxygenase (COX) enzyme and inhibition of inflammatory neuropeptide's production resulting pain relief. Although they have been used in the clinical practice, they are unable to affect neuropathic pain and the neurogenic components of the inflammatory processes. Opioid type anti-inflammatory drugs and their derivatives reduce neuropathic pain, but due to a great range of severe side effects (e.g. gastrointestinal bleeding) and the development of drug tolerance they cannot be administered for longer time periods. The treatment of neuropathic pain is still an unresolved problem, there is a great need for the development of drugs with novel mechanisms of action. The characterization of capsaicin sensitive sensory nerve endings and the identification of capsaicin receptors was a new direction in the study of analgesia.

2. The TRPV1 and TRPA1 receptors

The TRP cation channels, including Ankyrin repeat domain 1 (TRPA1) and Vanilloid type 1 (TRPV1) are important regulators of nociceptive and inflammatory processing (Akopian et al. 2007, Salas et al. 2009). Besides a variety of exogenous TRPA1 agonists, such as cynnamaldehide, allyl-isothiocyanate (AITC in mustard oil), 4-hydroxynonenal and allicin, there are a lot of endogenous activators produced by tissue injury and inflammation, e.g. formaldehyde and the reactive cytotoxic metabolite methylglyoxal which can activate the TRPA1 channel (Bandell et al. 2004, Jordt et al. 2004, Bautista et al. 2005, Macpherson et al. 2005, 2007, Trevisiani et al. 2007, Wang et al. 2008). In addition, mediators of oxidative stress, cold and mechanical stimuli also gate the TRPA1 (Story et al 2003, Corey et al. 2004, Andersson et al. 2008). Besides the two classical vanilloid type agonists, capsaicin (CAPS) and resiniferatoxin (RTX), TRPV1 is activated by several highly lipophylic compounds as endogenous arachidonic acid or other fatty acid metabolites like anandamide and N-oleoyldopamine (Raisinghani et al. 2005, Smart et al. 2000, Bianchi et al. 2006). Protons (pH<6.0) and noxious heat (>43°C) are also able to directly open this channel (Welch et al. 2000; Myers et al. 2008). Importantly, the TRPV1 receptor is sensitized by several inflammatory mediators and tropomyosin-related kinase A receptor (TrkA) ligand, nerve growth factor (NGF) (Zhang et al. 2005).

3. The role of semicarbazide-sensitive amine-oxidase in inflammation, and SZV-1287

Semicarbazide-sensitive amine-oxidase (SSAO) also known as vascular adhesion protein-1 is expressed predominantly in vascular smooth muscle cells, and can be found as membrane-bound and circulating forms (Lyles, 1996). It catalyzes oxidative deamination of primary amines, resulting in the production of aldehydes, as well as the release of ammonia and hydrogen peroxide (Buffoni and Ignesti 2000, O'Sullivan et al. 2004). It produces tissue irritants, such as formaldehyde and methylglyoxal, which are TRPA1 channel activators (Macpherson et al. 2007), but a link between SSAO activity and TRPA1 stimulation has not yet been thought of. The roles of SSAO and the effects of its inhibitors were investigated in inflammation, angiogenesis, cancer and diabetes (Salmi et al. 1993, O'Sullivan et al. 2004, Noda et al. 2009, Énzsöly et al. 2011), but there are no data regarding its function in pain and nociception.

SZV-1287 is our new 3-(4,5-Diphenyl-l,3-oxazol-2-yl)propanal oxime SSAO inhibitor developed from the oxime analogue of the cyclooxigenase (COX) inhibitor oxaprozin. It exerts both SSAO and COX inhibitory actions, and inhibits both acute and chronic inflammation in rats more effectively than the reference SSAO inhibitor, LJP 1207 (N′-(2-Phenylallyl)hydrazine hydrochloride; (Wang et al. 2006, Tábi et al. 2013). Since the chemical structure of SZV-1287 is similar to other oxime derivatives shown to be selective TRPA1 antagonists (Baraldi et al. 2010, DeFalco et al. 2010), we investigated its effects on TRPA1 receptor activation-induced responses of primary sensory neuronal cell bodies and peripheral terminals. HC030031, the frequently used reference TRPA1 antagonist (Eid et al. 2008), and a conventional SSAO inhibitor LJP 1207 (Wang et al. 2006, Tábi et al. 2013) of different structure (Salter-Cid et al. 2005, Tabi et al. 2013) served as reference compounds for comarison. TRPA1 is present on the capsaicin-sensitive, TRPV1-expressing, subpopulation of sensory neurones (Story et al. 2003,

Caterina and Park 2006, Holzer, 2008; Szolcsányi, 2008) and there is an interaction between these ion channels (Akopian et al. 2007, Salas et al. 2009).

4. The role of estradiol in the pain

Pain is modulated by gonadal steroids (Holdcroft and Berkley 2006) and well-established sex differences exist in chronic pain conditions (Ruau et al. 2012). Great body of clinical evidence demonstrated the higher sensitivity of women to chronic pain (Riley et al. 1998, Hurley and Adams 2008). Furthermore, experimental data revealed that pain sensitivity is higher in females and it is affected by the stage of estrous cycle in rodents (Lacroix-Fralish et al. 2006). Estrogens exert both pro- and anti-nociceptive effects through different neuronal and non-neuronal mechanisms via slow genomic actions (Kumar et al. 2015). Estrogen receptors (ERα and ERβ) are expressed in primary sensory neurons especially in small and medium diameter nociceptors (Papka and Storey-Workley 2002). 17β-estradiol (E2) acts on ERα in sensory neurons to modulate pain transmission (Bereiter et al. 2005). The membrane G protein-coupled estrogen receptor (GPER1) in primary sensory neurons (Dun et al. 2009, Lu et al. 2013) and in spinal somatosensory neurons mediates rapid, non-genomic estrogen pain-modulating intracellular signaling (Takanami et al. 2010).

Interactions between ERs and TRPV1 receptors have been shown in primary sensory neurones indicating a potential mechanism for the explanation of sex differences in pain. E2 directly increases nociceptor excitability, reduces action potential thresholds, and facilitates TRPV1 activation in primary sensory neurons (Flake et al. 2005, Diogenes et al. 2006). TRPV1 sensitization has been shown by other factors, such as NGF via TrkA signaling (Zhang et al. 2005). Clinical studies demonstrated that women feel capsaicin-evoked pain more intensely than men (Gazerani et al. 2005). Furthermore, the number of TRPV1 receptors significantly decreased in ERα and ERβ knock out mice (Cho and Chaban 2012) suggesting that E2 may regulate the expression of TRPV1 via ERs. Although these data suggest that E2 might be an important regulator of TRPV1-mediated nociception, little attention has been given to the sex differences and role of E2 in TRPV1 receptor related mechano- and thermonociceptive action.

III. AIMS

The treatment of neurogenic inflammation and neuropathic pain is still unresolved, because there the presently available drug groups do not provide satisfactory relief in most cases and cause sedation or euphoria. Therefore, there is a great need to investigate promising novel drug candidates and potential new drug targets. Accordingly, we aimed to investigate new molecules that are acting via TRP channels and might have more favorable side effects than that of currently used therapeutic agents. Furthermore, we intended to investigate gender differences in pain sensation to better understand TRPV1 receptor function.

Therefore the objectives of my research work were the following:

1. Investigating the effect of SZV-1287 on the activation of TRPA1 and TRPV1 receptors on cell bodies and peripheral nerve endings of primary sensory neurons and on TRPA1 and TRPV1 receptor-overexpressing CHO cells with *in vitro* methods.

2. Examined the sex differences and action of endogenous E2 in mechano- and thermonociceptive responses in mice, as well as the effects of E2 administration on the TRPV1 channel and related signaling *in vivo* and *in vitro*.

IV. EXPERIMENTAL MODELS

1. Animals and treatments

Eight-week-old adult female and male C57BL/6J wild-type (WT) and TRPV1 knock out ($TRPV1^{-/-}$) mice (Jackson Laboratories (USA, Maine)) were used for the experiment. The animals were kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy, University of Pécs under a 12-hour light/dark cycle at 24-25°C and had access to food and water ad libitum.

In one group, female mice were bilaterally ovariectomized (OVX, n=8) under Avertin anesthesia (1.9% tribromoethanol and 1.2% amyl-hydrate in saline; 0.1 ml per 10 g body weight). Mice were treated with E2 (33 ng/g s.c.; in 0.1 ml of ethyl-oleate vehicle; Sigma, Budapest, Hungary) 14 days after OVX. Age-matched sham-operated mice served as controls. All experimental procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988) and complied with the recommendations of the International Association for the Study of Pain. All experiments were approved by the Ethics Committee on Animal Research of Pécs University according to the Ethical Codex of Animal Experiments (licence: BA 02/2000-2/2012).

2. Primary cultures of primary sensory neurons of the trigeminal ganglion (TRG) and dorsal root ganglia (DRG)

Cultures of TRG were made from Wistar rat pups of 1-4 days old and the DRG from 2-4-day-old C57BL/6J WT mouse pups. The ganglia were cut in ice cold phosphate-buffered solution (PBS), incubated at 37 °C for 35 minutes in PBS containing collagenase (Type XI, 1 mg/ml) and then for 8 min in PBS with deoxyribonuclease I (1000 units/ml). After washing with Ca^{2+} and Mg^{2+} free PBS, the ganglia were dissociated by trituration. Neurones were plated on poly-D-lysin-coated glass coverslips and grown in nutrient-supplemented medium containing 180 ml Dulbecco′s-Modified Eagle Medium (D-MEM), 20 ml horse serum, 20 ml bovine serum albumin, 2 ml insulin-transferrin-selenium-S, 3,2 ml putrescin dihydrocloride (100 μg/ml), 20 μl triiodo-thyronine (0.2 mg/ml), 1,24 ml progesterone (0.5 mg/ml), 100 μl penicillin, 100 μl streptomycin. Cell cultures were maintained at 37 °C in a humid atmosphere with 5 % CO_2, nerve growth factor (NGF, 200 ng/ml) was added, as described earlier (Szőke et al. 2000).

3. TRPV1 and TRPA1 receptor-expressing cell lines

During our investigation CHO cells stably expressing TRPV1 and TRPA1 receptors were used (Sándor et al. 2005).

V. METHODS

1. Determination of intracellular free calcium with microfluorimetry in response to TRPA1 or TRPV1 activation

One-two-day-old cell cultures were stained for 30 min at 37°C with 1 μM of fluorescent Ca^{2+} indicator dye, fura-2-AM (Molecular Probes). Dye loading was followed by at least 5 min washing in extracellular solution (ECS) containing (in mM): NaCl, 160; KCl, 2.5; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; glucose, 10; (pH 7.3). ECS was gravity fed to the cells using a triple outlet tube, test solutions arrived to the outlet via separate tubes using a fast step perfusion system (VC-77SP, Warner Instrument Corporation, Harvard Apparatus GmbH, Germany). Calcium transients of TRG or DRG neurons to capsaicin were examined with microfluorometry as described elsewhere in detail. Briefly, fluorescence images were taken with an Olympus LUMPLAN FI/x20 0.5 W water immersion objective and a digital camera (CCD, SensiCam PCO, Germany), connected to a computer. Up to 12 dye-loaded cells were selected in each plate to monitor their fluorescence individually. Cells were illuminated alternately with 340 and 380 nm light generated by a monochromator (Polychrome II., Till Photonics, Germany) under the control of Axon Imaging Workbench 2.1 (AIW, Axon Instruments, CA). The emitted light was measured > 510 nm, the R = F340/F380 was monitored (rate 1Hz) continuously, while a few sample images were also recorded. The R values were generated by AIW 2.1 software then processed by the Origin software version 7.0 (Originlab Corp. Northampton, USA).

During the investigation of SZV-1287 two administration methods were used, co-administration with SZV-1287 (100, 500 and 1000 nM) or LJP 1207 (500 nM) and capsaicin (330 nM) or AITC (200 μM), and pre-incubation the plates with different concentrations of SZV-1287 (100, 500 and 1000 nM) compound for 60 min, at 37 °C in a humid atmosphere with 5 % CO_2.

In the investigation of estradiol effect with Ca^{2+} imaging the neurons were incubated with 100 pM or 1 nM E2 overnight (14hrs) or were untreated controls. Five-repeated stimulations of 330 nM capsaicin for 10 second were performed during a 50 min period, then capsaicin stimulus was repeated after a 10 min washout period. A short (10 min) E2 treatment or E2 and AG879 co-administration was performed after the third capsaicin.

2. Measurement of CGRP release from the peripheral terminals of primary sensory neurones in response to TRPA1 or TRPV1 activation

The method has been described in detail elsewhere (Helyes et al. 1997, Németh et al. 1998, Helyes et al. 2001). In brief, rats were exsanguinated in deep anaesthesia (sodium thiobarbital 50 mg kg-1 i.p.), then tracheae were removed, cleaned of fat and adhering connective tissues. Trachea were placed into an organ bath to achieve sufficient amount of peptide release and perfused (1 ml min-1) with pH 7.2 controlled oxygenated Krebs solution for 60 min (equilibration period) at 37°C, then incubated in the presence of SZV-1287 or LJP 1207 (100 nM, 500 and 1000 nM, respectively) or the solvent of SZV-1287 or LJP 1207 alone. After discontinuation of the flow, the solution was changed three times for 8 min to produce prestimulated, stimulated, poststimulated fractions. Chemical activation was performed in the second 8-minute period with the TRPA1 agonist mustard oil (100 μM) or the selective TRPV1 agonist capsaicin (100 nM) to elicit CGRP release. CGRP concentrations were determined from

200 µl samples of organ fluid of the preparations by means of radioimmunoassay methods developed in our laboratories as described (Németh et al. 1996, Helyes et al. 1997, Németh et al. 1998). The trachea samples were weighed CGRP release was calculated as fmol/mg wet tissue. The absolute peptide release were calculated by adding CGRP release measured in the stimulated and post-stimulated fractions after taking off the basal release measured in the pre-stimulated 8-min fraction. In each group 3 independent experiments were performed, in every experiment 12 tracheae were investigated (2 tracheae in each organ bath chamber) to provide n=3x6 data per group.

3. Radioactive $^{45}Ca^{2+}$ uptake experiments

Cells were plated in 15 µl cell culture medium onto 72-well miniplate. The following day, cells were washed with calcium-free Hank's solution, then were incubated in 10 µl of the same buffer containing the test solution and $^{45}Ca^{2+}$ isotope. After washing with ECS the residual buffer was evaporated in 75 °C the retained isotope was collected in 15 µl 0.1% SDS and the radioactivity was measured in scintillation liquid in a Packard Tri-Carb 2800 TR scintillation counter.

4. Quantitative Real-time Polymerase Chain Reaction (qPCR)

With qPCR technique the expression levels of the following genes were measured: Gapdh, Trpv1, GPER1, ERα, ERβ. The total RNA was extracted from treated and untreated mouse DRG cells with TRI Reagent (Molecular Research Centre. Inc., Cincinatti, OH, USA) and Direct-Zol RNA isolation kit, following the manufacturer's instructions. RNA samples were treated with Dnase1 (Zymo Research, Irvine, CA, USA) and quantified by spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies Inc., Wilmington DE, USA). 1 µg purified RNA was reverse transcribed into complementary DNA (cDNA) using Maxima™ First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific, Waltham, MA, USA). qPCR was conducted by Stratagene Mx3000P QPCR System (Agilent Technologies, Santa Clara, CA, USA) using glycerinaldehyde-3-phosphate-dehydrogenase (GAPDH) as a reference gene. Each reaction contained 20 ng cDNA, 1x Luminaris HiGreen Low ROX qPCR Master Mix (Thermo Scientific) and 0.3 µM from each primer. qPCR cycle conditions were as follows: 95°C 10 min, followed by 40 cycles of 95 °C 30 sec, 60 °C 30 sec then 72 °C 1 min. All real-time PCR experiments were carried out in triplicate and the mean value was used for the determination of messenger RNA (mRNA) expression levels. Measurements included a dissociation curve analysis to verify the amplification specificity. The relative gene expression ratios were calculated according to the comparative ΔΔCt method using samples of untreated animals as calibrator. When calculating gene expression ratios primer efficiencies were taken into account (Pfaffl 2001). Neurons were incubated with 100 pM or 1 nM E2 overnight at 37 °C in a humidified atmosphere with 5 % CO_2, or were untreated controls.

5. Measurement of the mechano- and thermonociceptive thresholds

The mechanonociceptive threshold of the plantar surface of the paw was determined by dynamic plantar aesthesiometry (Ugo Basile 37400, Comerio, Italy). After a 45-min habituation period, the withdrawal thresholds for mechanical stimulation were recorded in grams (Borbély et al. 2016).

The thermonociceptive threshold of the paw was measured on an increasing temperature hot plate (IITC Life Sciences, Woodland Hills, CA, USA) by the temperature when the mice showed nocifensive reactions (lifting, licking, shaking). The cut-off value was 53 °C (Almási et al. 2003). These thresholds of WT and TRPV1$^{-/-}$ male and female mice (n=8 in each proup) were investigated during 14 consecutive days, every day at the same time in the morning. Then the estrous cycle phase was determined by vaginal smear as described earlier (Byers et al. 2012, Bölcskei et al. 2005).

6. Evaluation of estrus cycle

Vaginal smears were taken every morning, 1 h before the nociceptive testing. Vaginal material was stained by 0.1% aqueous solution of methylene blue and it was examined under the microscope to evaluate the stage of estrus cycle (Byers et al. 2012). The threshold values were compared in the estrous and proestrous stages.

7. Investigation of inflammatory hyperalgesia induced by the TRPV1 agonist RTX

Acute neurogenic inflammation and consequent hyperalgesia (drop of the mechano-and thermonociceptive thresholds) were induced in the right hindpaw by intraplantar administration of the TRPV1 agonist RTX (0.01 μg/ml). The thermonociceptive thresholds were determined before and 10 min, 30 min, 1 h and 2 h after the stimulation. In a separate group, E2 treatment (33 ng/g s.c.) was performed before RTX administration. In our previous studies, 33 ng/g E2 rapidly induced CREB phosphorylation (within 15 min) in the central nervous system (Ábrahám et al. 2003), therefore, measurements were performed 10 min, 30 min, 1 h and 2 h after and intraplantar RTX administration.

8. Drugs and chemicals

Capsaicin (Sigma, St. Louis, MO, USA) was dissolved in DMSO to obtain 10 mM stock solution. Further dilutions were made with Krebs or ECS solution to reach final concentrations of 100 nM or 330 nM, respectively. AITC (Sigma, St. Louis, MO, USA) was dissolved in DMSO to obtain 10 mM stock solution further dilutions were made with Krebs or ECS solution to reach final concentrations of 100 μM or 200 μM, respectively. HC 030031 (Tocris) was dissolved in DMSO obtain 10 mM stock solution, and further diluted in ECS to achieve a final concentration of 10 μM, respectively. DMEM, horse serum, foetal bovine albumin and newborn calf serum were purchased from Gibco (Grand Island, N.Y. USA). Collagenase, deoxyribonuclease I, poly-D-lysine, NGF were purchased from Sigma (St. Louis, MO, USA). SZV-1287 was synthesized on Semmelweis University at the Department of Organic Chemistry (Mátyus et al. 2010, WO 2010/039379 A1), LJP 1207 was synthesized at Semmelweis University at the Department of Organic Chemistry (Wang et al. 2006). SZV-1287 was dissolved in DMSO, Tween 80 and distilled water (1:1:8) to obtain 10 mM stock solution, LJP 1207 was dissolved in distilled water to obtain 10 mM stock solution. Further dilutions were made with ECS or Krebs solution to reach final concentrations of 100, 500 and 1000 nM, respectively. D-MEM, horse serum, foetal bovine albumin and newborn calf serum were purchased from Gibco (Grand Island, N.Y. USA). Collagenase, deoxyribonuclease I, poly-D-lysine, NGF, RTX, AG879 and E2 were purchased from Sigma (Sigma, Budapest, Hungary).

9. Statistical analysis

GraphPad Prism 5.0 (GraphPad, La Jolla, CA) was used for statistical analyses. In case of microfluorimectric investigation of the SZV-1287 and LJP 1207 Fisher's exact-test was used as statistical. In isotipic $^{45}Ca^{2+}$ uptake measurement one-way ANOVA with Dunnett's post hoc test was performed. For the evaluation of RIA measurement results Student t-test was performed.

For evaluating gene expression, analysis was processed by one-way ANOVA followed by Dunnett's multiple comparison. Calcium imaging and qPCR data are expressed as means±S.E.M. of three independent experiments. Statistical analysis was performed by one-way ANOVA with Bonferroni's post hoc test.

Results of the *in vivo* investigations were evaluated with one-way ANOVA with Bonferroni's multiple comparison post hoc tests and shown as means±SEM of n=8/group. Differences between groups were assessed by two-way ANOVA with Bonferroni's multiple comparison post hoc tests, significance levels were *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

VI. RESULTS

The effects of SZV-1287 on TRPA1 and TRPV1 ionchannel activation

1. SZV-1287 inhibits TRPA1 receptor-mediated Ca^{2+}-influx in cultured TRG neurones

In the first series of experiments, the percentage of responding neurones to the TRPA1 agonist AITC (200 μM) was determined in control plates by measuring fluorescence Ca^{2+}-influx. The response to AITC (30 sec) had a longer duration and developed after a longer latency (30 sec). To confirm whether TRPA1 is involved in generation of Ca^{2+}-influx, a TRPA1 selective antagonist HC030031 was used in 10 μM concentration. Co-administration of HC030031 with AITC resulted in significant decrease of response.

SZV-1287 significantly and concentration-dependently decreased the Ca^{2+}-influx in the cytoplasm of TRG neurones. Similar reduction in the number of activated cells to AITC was detected 60 min after the pre-incubation of the plates with SZV-1287.

In the second series of experiments, LJP 1207 a reference SSAO inhibitor with different structure was used to reveal whether the effect of SZV-1287 on TRPA1 activation is related to its SSAO inhibitory action. Despite SZV-1287, LJP 1207 had no effect on TRPA1 activation.

The vehicles of SZV-1287 and LJP 1207 did not cause any change in Ca^{2+}-influx.

2. SZV-1287 inhibits TRPV1 receptor-mediated Ca^{2+}-influx in cultured TRG neurones

In contrast to the longer-lasting AITC-induced Ca^{2+}-influx, there was a short response 10 sec after 330 nM capsaicin administration. It had a transient peak followed by a prolonged plateau in some neurones.

The TRPA1 selective antagonist HC030031 had no effect on capsaicin-induced calcium response. Concentration-dependent diminution in capsaicin-evoked calcium response was detected after co-administration of capsaicin and 100, 500 nM and 1000 nM concentration of SZV-1287. After pre-incubation for 60 min with the same concentrations of SZV-1287, the intracellular Ca^{2+}-influx was reduced in a concentration-dependent manner.

Similarly to what was observed for TRPA1 stimulation, LJP 1207 had no effect on TRPV1 activation. The vehicles of SZV-1287 and LJP 1207 did not cause any change in Ca^{2+} -influx.

3. SZV-1287 decreases TRPA1-induced CGRP release from peripheral sensory nerve terminals

SZV-1287 significantly and concentration-dependently inhibited the stimulation of AITC-evoked CGRP release in the higher concentrations. The release of CGRP decreased after using 100, 500 and 1000 nM SZV-1287. The solvent of SZV-1287 alone affected CGRP release evoked by AITC. Furthermore, SZV-1287 or its solvent did not influence the basal, non-stimulated peptide outflow. LJP 1207 had no significant effect on TRPA1 activation-induced CGRP release.

4. SZV-1287 inhibits AITC- and capsaicin-induced Ca^{2+}-influx in CHO cells expressing the cloned TRPA1 and TRPV1 receptor

In the first series of experiments ratiometric technique of Ca^{2+} measurement was performed. In control circumstances 200 μM AITC-evoked Ca^{2+}-influx was detected on TRPA1 receptor-expressing cells. After pre-incubation for 60 min with 1, 5 and 10 μM of SZV-1287, the intracellular Ca^{2+}-influx was diminished in a concentration-dependent manner.

In second series in control plates 330 nM capsaicin-evoked Ca^{2+}-influx was detected on TRPV1 receptor-expressing cells. After pre-incubation for 60 min with the same concentrations of SZV-1287, the intracellular Ca^{2+}-influx was significantly reduced. There were no changes in AITC- and capsaicin-induced Ca^{2+}-influx after pre-incubation with the vehicle of the highest concentration of SZV-1287.

In the presence of nM concentrations of SZV-1287 the intracellular Ca^{2+}-influx induced by 200 μM AITC and 330 nM capsaicin was significantly diminished in a concentration-dependent manner, as measured by the radioactive $^{45}Ca^{2+}$ uptake method.

The IC_{50} values of SZV-1287 were 2.39 lM and 8.64 lM on TRPA1 and TRPV1 receptor-expressing cell lines, respectively. SZV-1287 itself did not cause changes in CPM values. Capsaicin and AITC did not induce $^{45}Ca^{2+}$ uptake in control CHO cells without the TRPV1 and TRPA1 receptors.

The effect of E2 on TRPV1 ionchannel activation

1. Sex hormone-dependent differences in mechano- and thermonociceptive thresholds in mice

In order to evaluate the sex difference in pain sensation the mechanonociceptive and thermonociceptive thresholds were detected in both sexes.

Mechano- and thermonociceptive thresholds of the plantar surface of the paw of female C57BL/6J WT mice in proestrus were significantly lower than those of the males

To examine the effect of normal physiological conditions of estrogen exposure on pain sensation, we compared the mechanonociceptive and thermonociceptive thresholds of OVX mice with intact females at different stage of estrus. Mechanonociceptive and thermonociceptive threshold values were higher in OVX mice compared to mice in estrus or proestrus stage. The threshold values were higher in estrus stage that in proestrus stage.

2. Lack of sex hormone-dependent differences in nociceptive parameters in TRPV1 receptor-deleted mice

To evaluate the role of TRPV1 receptor we examined the mechano- and thermonociceptive thresholds in TRPV1$^{-/-}$ female and male mice as well as in OVX and intact TRPV1$^{-/-}$ mice with different stage of estrus cycle.

The mechano- and thermonociceptive thresholds of TRPV1$^{-/-}$ mice and WT mice were similar in males and females in estrus stage. Interestingly, there was no difference between thermonociceptive parameters of TRPV1$^{-/-}$ female mice irrespective of their estrous stage. However TRPV1$^{-/-}$ males showed higher mechanonociceptive thresholds.

3. TRPV1 activation-induced mechanical hyperalgesia is potentiated by E2

To examine the effect of E2 on TRPV1 receptor activation TRPV1 receptor agonist RTX was applied to E2 primed OVX mice. Significant mechanical hyperalgesia developed in response to intraplantar administration the TRPV1 receptor agonists RTX (0.01 μg/ml) both in OVX and E2-primed OVX mice. This reached a maximum of 52.8% after 10 min, which gradually decreased during the 120 min examination period. E2 treatment significantly increased the nociceptive response from the 30 min as shown by shifting down the hyperalgesia curve suggesting an E2-induced TRPV1 sensitisation.

4. ERα, ERβ, GPER1 and TRPV1 mRNA are expressed in cultured primary sensory neurons

In order to clarify whether the primary sensory neurons express ERs we examined the presence of ERα, ERβ and GPER1 mRNAs in DRG cultures obtained from WT mice. All of the three receptor messages were detected in these cells (n=3 independent cell cultures). Overnight incubation with 100 pM, but not with 1 nM concentration of E2 caused a significant, 15-fold increase in TRPV1 mRNA expression compared to the untreated controls. In contrast, short, 10 min treatments did not remarkably alter TRPV1 expression.

5. E2 enhances capsaicin-induced TRPV1 receptor activation in cultured primary sensory neurons

To examine the effect of E2 on TRPV1 receptor activation ratiometric calcium imaging was applied on capsaicin treated DRG neurons. The TRPV1 agonist capsaicin (330 nM) alone caused a robust and transient Ca^{2+}-influx into the neurons (53%; the mean of R values of the first capsaicin was 0.59±0.06) followed by desensitization (R=0.31±0.05 after the second capsaicin application). The vehicle control alone did not cause Ca^{2+}-influx. Overnight pretreatment of DRG neurons with 100 pM E2 abolished the capsaicin-induced TRPV1 receptor desensitization.

In the following experiments five repeated treatment of capsaicin on the same cultured sensory neurons were performed for 50 min. First, application of 330 nM capsaicin for 10 second induced transient Ca^{2+}-accumulation in the cytosol as detected by the magnitude of the fluorescence response R=0.55±0.06, then capsaicin stimulus was repeated after a 10 min washout period. The second and third responses were gradually smaller due to TRPV1 desensitization. A short (10 min) E2 treatment after the third capsaicin administration prevented

the desensitization, but the following capsaicin stimulation without E2 again resulted in a significantly smaller response.
In a third series of experiments the effect of the TrkA receptor inhibitor AG879 was investigated. Ten min E2 and AG879 co-treatment were used after the second capsaicin administration. In this case the third capsaicin induced smaller response showing TRPV1 desensitization.

VII. DISCUSSION

The antagonist effect of SZV-1287 on TRPV1 and TRPA1 receptors

There is a great need to learn more about the operational features, activation mechanisms and characteristics of TRPA1 and TRPV1 channels even regarding drug development, since there are no peripherally acting analgesic drugs which could directly inhibit nociceptor activation resulting in pain relief (Szolcsányi 2002, 2008, Szállási et al. 2007, Gavva 2008, Gunthorpe and Chizh 2009). Therefore, in this study we investigated the effects of our novel 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanal oxime compound SZV-1287 originally developed for inhibiting the SSAO enzyme on TRPA1 and TRPV1 activation on native sensory neuronal cell bodies, peripheral nerve terminals and TRPA1 and TRPV1 receptor-expressing CHO cell lines. We provide here the first evidence that SZV-1287 exerts potent antagonistic actions at both cation channels. It significantly and concentration-dependently decreased AITC- and capsaicin-induced Ca^{2+}-influx in trigeminal neurons and receptor-expressing cell lines but had no effect on voltage-gated calcium currents. The reference SSAO inhibitor LJP 1207 with a different structure did not alter TRPA1 and TRPV1 activation showing that these actions of SZV-1287 are independent of the SSAO inhibition.
The trachea is an excellent model system to investigate the activation of peripheral nerve terminals. It is densely innervated by these fibers, the nerve endings are close to the surface and can be easily activated by different chemicals, and neuropeptide release is a perfect indicator of their activation (Helyes et al. 2001). Barabas and co-workers described that only 22% of the AITC sensitive cell were CGRP-positive in DRG neurons (Barabas et al. 2012). Nevertheless, we are able to detect CGRP-release in fmol concentration after AITC administration (Helyes et al. 1997, 2001, Németh et al. 1998, Pozsgai et al. 2012, Sághy et al. 2015). We described earlier the inhibitory effect of the commercially available TRPA1 antagonist HC030031 on AITC-induced CGRP release (Pozsgai et al. 2012). Furthermore, the capsaicin-induced CGRP release could be prevented by the TRPV1 antagonist CZP (Jakab et al. 2005). SZV-1287 significantly inhibited the TRPA1 receptor activation-induced CGRP release in a concentrationdependent manner, but in contrast to what was found on the cell bodies, this inhibition was not significant in case of TRPV1 receptor activation. Although further investigations are needed to reveal the precise molecular mechanisms of the differences between the TRPV1 receptor activation properties on the neuronal cell bodies and terminals, we hypothesize that in the cell body capsaicin induces a small Ca^{2+}-influx, but the endoplasmic reticulum rapidly accumulates the free Ca^{2+} from the cytoplasm. Meanwhile, the same extent of receptor activation on the nerve terminal might be sufficient to induce CGRP release, since there are no endoplasmic reticulum or Golgi apparatus present (Messlinger 1996). These findings and concept are supported by our earlier experiments, where differences in TRPV1 gating mechanisms were described in these

structures (Szőke et al. 2010). Some data indicated a potential link between COX activity, prostaglandins and TRPA1 activation (Moilanen et al. 2012). Due to its chemical structure, the COX inhibitor oxaprozin can be a metabolite of SZV-1287 in vivo particularly after oral administration. Although we cannot rule out that decreased production of COX-derived prostanoids is involved in the TRPA1/V1 inhibitory actions of SZV-1287 or metabolite(s), such an action is unlikely in our in vitro experimental system after short (1 min) exposure.

Most of the TRPA1 antagonists covalently modify the cysteine residues on the amino-terminal domain of the channel (Cebi and Koert 2007). Four classes of TRPA1 antagonist have been described: (a.) the oxime related A-967079 (from Abott; Chen et al. 2011), (b.) a series of trichlorosulfide antagonists, for example AMG7160 (from Amgen), (c.) a diaminohexane derivative (from Patapoutian lab), and (d.) a series of purine acetamides, exemplified by HC030031 (from Hydra Biosciences). We don't know the exact mechanism of action for these antagonists, it is possible that compounds such as AP18 and AMG7160 are capable of covalently modifying TRPA1, but are unable to cause a conformational change leading to the channel activation. DeFalco and co-workers examined the structure–activity relationships of 19 oximes related to AP18, as modulators of TRPA1 (DeFalco et al. 2010). Replacement of chloro group with hydrogen atom resulted in antagonist activity. However weak agonist activity at TRPA1 was detected when the chloro group of AP18 was replaced with a methoxy substituent and the agonist activity was even more potent when the para-chloro group of AP18 was replaced with a meta-methyl substituent. Deleting one or more methyl groups in AP18 also caused TRPA1 agonist activity. DeFalco and coworkers suggested that it behaves as a covalent modifier of TRPA1 channels and a related AP18 derivative, 3-methyl-4-phenylbut-3-en-2-one oxime acts as TRPA1 antagonist (DeFalco et al. 2010). Massspectrometry measurements elucidated the presence of covalently modified cysteine residues. This method could reveal the covalent or non-covalent mechanism by which AP18 and the oxime agonists and antagonists modulate TRPA1 activity (DeFalco et al. 2010). Another study revealed that the binding regions of AP18 to the TRPA1 are located in the fifth transmembrane domain (TM5) (Xiao et al. 2008). Furthermore, two specific amino acid residues located also within the putative TM5 domain were involved in the inhibitory action of A-967079 (Nakatsuka et al. 2013). We are planning in silico modeling to reveal the potential binding sites of SZV-1287 on TRPA1 and TRPV1 ion channels.

AP18 was shown to inhibit the human and mouse TRPA1 receptor activation, but failed to block the TRPV1, TRPV2, TRPV3, TRPV4 and TRPM8 receptors (Petrus et al. 2007). Abott Laboratories used (heteroaryl) alkenone oxime derivatives (which structure are similar to that of (Z)-4-(4-chlorophenyl)-3-methylbut-3-en-2-oxime) as TRPA1 antagonists in several disease models (Perner et al. 2009). Orally administered A-967079 exhibit analgesic efficacy in osteoarthritic pain and allyl-isothiocyanate-induced nocifensive response in rats (Chen et al. 2011). AP18, reverses CFA-induced mechanical hyperalgesia in normal but not in TRPA1-deficient mice, inhibits the AITC-induced muscle nociception and cold hyperalgesia (Petrus et al. 2007). TRPA1 as a target and TRPA1 antagonists as potential candidates for drug development have been extensively reviewed. Role of TRPA1 ion channel in numerous disease models has been documented. TRPA1 as a target for analgesic drugs has been described (Garrison and Stucky 2011) the role of TRPA1 in chemotherapyinduced neuropathic pain or in diabetic neuropathy has been investigated (Vander Jagt 2008). Garrison and Stucky reported

the involvement of TRPA1 receptor in the development of chronic pain in animal model of arthritis induced by complete Freund's adjuvant (Garrison and Stucky 2014). Moreover TRPA1 has a role in asthma and chronic obstructive pulmonary disease (Nesuashvili et al. 2013), migraine (Benemei et al. 2013), colitis (Yang et al. 2008) and itch (Lieu et al. 2014). Unlike TRPV1 or TRPM8, TRPA1 is not involved in body temperature regulation at basal level or under cold challenge (Chen et al. 2011). Therefore, TRPA1 antagonists might be free of the side effects associated with TRPV1 or TRPM8 antagonists (hyperthermia). The therapeutic potential of TRPA1 has been proven in pain, respiratory, itch, and other diseases, therefore, it might be the new target for novel analgesics instead of TRPV1 (Chen and Hackos 2015). Several studies described that TRPA1 is highly coexpressed with TRPV1 in trigeminal sensory neurons, in a subpopulation of dural afferents and also in non-neuronal tissues, and numerous studies suggest that TRPV1 and TRPA1 play an integrative role in regulating nociceptor function (Akopian et al. 2007, Salas et al. 2009., Nilius and Szallasi 2014). This synergistic function accounts for the investigation of both ion channels in the same model systems.

As a summary, our novel oxime compound SZV-1287 proved to be a potent TRPA1 and TRPV1 antagonist on primary sensory neurons. Therefore, it might be a promising new candidate for drug development focusing on neuropathic pain, migraine and arthritis.

The role of estradiol in pain

We present here the first data that E2 sensitizes the TRPV1 receptor via direct and indirect mechanisms through the TrkA pathway, as well as upregulates its expression. These findings explain the sex differences in nociception and hormonal cycle-dependent pain sensitivity alterations.

It is well-established that TRPV1 has a key role in pain signaling (Pogatzki-Zahn et al. 2005, Nilius and Szallasi 2014, Chen et al. 2004) and some data have suggested interactions between E2 and TRPV1. They mostly showed increased TRPV1 activation after E2 administration, but this crosstalk was not investigated by a comprehensive experimental approach and the mechanism was not clear.

Electrophysiological data demonstrated the E2 facilitates TRPV1-transmitted ion currents in rat sensory neurons (Lu et al. 2009). TRPV1-evoked nocifensive responses were more pronounced in female rats via E2-mediated processes (Martins et al. 2015). These are supported by the present results showing significantly reduced RTX-evoked hyperalgesia in OVX mice, which is reversed by exogenous E2 administration. In agreement with these experimental findings, clinical data also described that women feel capsaicin-evoked pain more intensively than men presumably by differential modulation of the TRPV1 receptor by sex steroids (Gazerani et al. 2005). E2 application increased nociceptor excitability, reduced action potential thresholds and facilitated TRPV1 activation in sensory neurons (Flake et al. 2005, Diogenes et al. 2006). Not only the sensitivity, but also the expression of TRPV1 were significantly increased by overnight incubation of primary sensory neuronal cultures with E2, similarly to the endometrium cells (Pohóczky et al. 2016). These in vitro results are supported by the E2-induced increased temporomandibular joint pain and TRPV1 upregulation in the synovial membrane explaining the dominant representation of this disease in females (Puri et al. 2011). Similarly, exogenous E2 upregulated TRPV1 expression in sensory neurons of OVX rats and

the lack of E2-effect in ERαKO and ERβKO mice resulted in TRPV1 downregulation (Cho and Chaban 2012).

Potential mechanism of action

In agreement with previous studies (Dun et al. 2009, Lu et al. 2013) our results demonstrated the expression of all the three 3 estrogen receptors (ERα, ERβ and GPER1) in primary sensory neurons. Overnight E2 treatment abolished the capsaicin-induced TRPV1 desensitization and sensitization developed within a short, 10 min period. Therefore, the effect of E2 on TRPV1 is likely to involve the classical genomic and the rapid non-classical GPER1 mediated action via PKCs. The GPER1-mediated fast sensitization is supported by our result demonstrating TRPV1 activation-induced mechanical hyperalgesia increase 30 min after E2 administration, as well as recent data showing mechanical hyperalgesia 20 min following exogenous E2 in OVX rats (An et al. 2014). It is also established that E2 level regulates GPER1 expression, it is down-regulated in sensory neurons after OVX in rats and recovers by E2 replacement (Takanami et al. 2010).

E2-evoked GPER1 activation results in rapid intracellular cAMP and Ca^{2+} increase (within minutes and even seconds) in sensory neurons (Craft 2007, Mannino et al. 2007, Dennis et al. 2009, Hucho et al. 2006, Hucho and Levine 2007, Kuhn et al. 2008). Intracellular Ca^{2+} elevation stimulates protein kinase Cε (PKCε) and evokes pain sensitization supporting GPER1-induced E2 actions (Goswami et al. 2004, 2007). The target of PKCε phosphorylation is serine 800 at the C-terminus of TRPV1. Goswami and co-workers suggest the C-terminus of TRPV1 to be a signaling intermediate downstream of estrogen and PKCε, regulating microtubule-stability and microtubule-dependent pain sensitization (Goswami et al. 2011).

Meanwhile, E2-induced nociceptor excitability and TRPV1 sensitization in sensory neurons (Flake et al. 2005, Diogenes et al. 2006) is similar to the effects of NGF acting through the TrkA receptor. This activates a signaling pathway in which phosphoinositide-3 kinase plays a crucial early role, with Src kinase as the downstream element phosphorylating the TRPV1. Phosphorylation at a single tyrosine residue explains most of the rapid effects of NGF on TRPV1. A major effect of NGF is causing a translocation of TRPV1 to the cell surface membrane. Ten min NGF treatment induced a 1.6-fold increase in membrane TRPV1 expression on HEK293 cells transfected with both TrkA and TRPV1 (Zhang et al. 2005). Our results that the TrkA inhibitor abolished the E2-induced TRPV1 sensitization in sensory neuronal cultures provided evidence that a NGF-like mechanism can be suggested for E2. This is likely to explain the higher pain sensitivity in females and be related to the molecular mechanism of E2-induced enhanced TRPV1 activation in sensory neurons.

This mechanism cannot only be proposed for the primary sensory neurons and their peripheral terminals, but also the non-neural cells the central nervous system. In primary cultured synoviocytes, TRPV1 is upregulated by E2 and NGF, and NGF antibodies fully blocks E2-induced TRPV1 increase (Jezierski et al. 2001). The hippocampus plays an important role in sex-based differences of pain perception. Hippocampal NGF, as well as synovial NGF and TRPV1 were upregulated in case of temporomandibular inflammation in the rat, which was further potentiated by E2. Blocking this central NGF and its TrkA pathway partially reversed the allodynia of the inflamed temporomandibular joint (Wu et al. 2010). Therefore, besides a direct action, E2-evoked TRPV1 sensitization might involve a NGF-mediated mechanism.

Clinical significance

Sex differences in chronic pain pathologies are well-established (Ruau et al. 2012). Most data describe that women are overrepresented in chronic pain conditions compared until the menopause (Hurley and Adams 2008, Von Korff et al. 1988, Riley et al. 1998). In general, females have higher prevalence of musculoskeletal degenerative diseases accompanied by severe pain, such as discopathies and low back pain. However, it was shown to further increase in the post-menopausal period with lower estrogen levels (Ganderton et al. 2016, Wáng et al. 2016) which virtually contradicts the E2-induced pain sensitization concept. This finding is easily explained by the lack of E2-evoked protective effect on the bones and the musculosceletal system and the postmenopausal greater degenerative process obviously causes more intensive pain. Since extensive clinical studies with a variety of multiple complicating factors (psychological, geographical, genetic and epigenetic background, education, physical exercise, etc.) are not appropriate to draw conclusions for E2-induced pain modulation (Kozinoga et al. 2015, Frange et al. 2016), systematic experimental investigations are need to reveal the mechanisms.

This is the first comprehensive approach to provide in vivo and in vitro evidence for E2-induced TRPV1 receptor upregulation and sensitization, both via the classical genomic and rapid, non-genomic estrogen action mediated by TrkA. E2-induced TRPV1 sensitization and upregulation in sensory neurons can explain the greater pain sensitivity in females.

VIII. REFERENCES

Ábrahám et al. Estrogen receptor beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. J Neurosci. 2003;23(13):5771–5777.

Akopian et al. 2007. Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J. Physiol. 583, 175-93.

Almási et al. Effect of resiniferatoxin on the noxious heat threshold temperature in the rat: a novel heat allodyniamodel sensitive to analgesics. Br J Pharmacol. 2003;139(1): 49–58.

An et al. Estrogen rapidly enhances incisional pain of ovariectomized rats primarily through the G protein-coupled estrogen receptor. Int J Mol Sci 2014; 15(6):10479-91.

Andersson et al. 2008. Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. J. Neurosci. 28, 2485–2494.

Bandell et al. 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron.41(6), 849-57.

Barabas et al. 2012.TRPA1 is functionally expressed primarily by IB4-binding, non-peptidergic mouse and rat sensory neurons. PLoS One. 7(10):e47988.

Baraldi et al. 2010. Transient receptor potential ankyrin 1 (TRPA1) channel as emerging target for novel analgesics and anti-inflammatory agents. J. Med. Chem. 53(14), 5085-107.

Bautista et al. 2005. Pungent products from garlic activate the sensory ion channel TRPA1. Proc. Natl. Acad. Sci. U S A. 102(34), 12248-52.

Benemei et al. 2013. TRPA1 and other TRP channels in migraine. J Headache Pain 14:71.

Bereiter et al. Oestrogen receptor-immunoreactive neurons in the trigeminal sensory system of male and cycling female rats. Arch Oral Biol. 2005;50(11):971–979.

Bianchi et al. 2006. Modulation of human TRPV1 receptor activity by extracellular protons and host cell expression system. Eur. J. Pharmacol. 537(1-3), 20-30.

Borbély et al. Role of capsaicin-sensitive nerves and tachykinins in mast cell tryptase-induced inflammation of murine knees. Inflamm Res. 2016;65(9):725–736.

Bölcskei et al. Investigation of the role of TRPV1 receptors in acute and chronic nociceptive processes using gene-deficient mice. Pain. 2005;117(3):368 376.

Buffoni and Ignesti 2000. The copper-containing amine oxidases: biochemical aspects and functional role. Mol. Genet. Metab. 71, 559–564.

Byers et al. Mouse estrous cycle identification tool and images. PLoS One. 2012;7(4):e35538.

Caterina et al. 2006. TRPV1, A polymodal sensor in the nociceptor terminal. In: Oh, U. (Ed.) The nociceptive membrane. Academic Press, San Diego, pp. 114–150.

Cebi and Koert, 2007. Reactivity recognition by TRPA1 channels. Chembiochem. 8(9), 979-80.

Chen and Hackos. 2015. TRPA1 as a drug target–promise and challenges. Naunyn Schmiedebergs Arch Pharmacol 88 (4):451–463.

Chen et al. 2011. Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. Pain 2(5):1165–1172.

Chen et al. Competitive inhibition of the capsaicin receptor-mediated current by dehydroepiandrosterone in rat dorsal root ganglion neurons. J Pharmacol Exp Ther. 2004;311(2):529–536.

Cho and Chaban. Expression of P2X3 and TRPV1 receptors in primary sensory neurons from estrogen receptors-a and estrogen receptor-b knockout mice. Neuroreport. 2012;23(9):530–534.

Corey et al. 2004. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature. 432(7018), 723-30.

Craft. Modulation of pain by estrogens. Pain 2007; 132 Suppl 1:S3-12.

DeFalco et al. 2010. Oxime derivatives related to AP18: agonists and antagonists of the TRPA1 receptor. Bioorg. Med. Chem. 20, 276–279.

Dennis et al. In vivo effects of a GPR30 antagonist. Nat Chem Biol 2009; 5(6):421-7.

Diogenes et al. Prolactin modulates TRPV1 in female rat trigeminal sensory neurons. J Neurosci. 2006;26(31): 8126–8136.

Dun et al. Expression of estrogen receptor GPR30 in the rat spinal cord and in autonomic and sensory ganglia. J Neurosci Res. 2009;87(7):1610–1619.

Eid et al. 2008. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol. Pain. 4:48.

Enzsöly, et al. 2011. Preliminary studies of the effects of vascular adhesion protein-1 inhibitors on experimental corneal neovascularization. J. Neural. Transm. 118(7), 1065-9.

Flake et al. Estrogen and inflammation increase the excitability of rat temporomandibular joint afferent neurons. J Neurophysiol. 2005;93(3):1585–1597.

Frange et al. Musculoskeletal pain and the reproductive life stage in women: is there a relationship? Climacteric 2016; 19(3):279-84.

Ganderton et al. Does menopausal hormone therapy (MHT), exercise or a combination of both, improve pain and function in post menopausal women with greater trochanteric pain syndrome (GTPS)? A randomised controlled trial. BMC Womens Health 2016; 16(1):32.

Gavva 2008. Body-temperature maintenance as the predominant function of the vanilloid receptor TRPV1.Trends Pharmacol. Sci. 29(11), 550-7.

Gazerani et al. A human experimental capsaicin model for trigeminal sensitization: gender-specific differences. Pain. 2005;118(1-2):155–163.

Goswami et al. Identification and characterisation of novel tubulin-binding motifs located within the Cterminus of TRPV1. J Neurochem 2007; 101: 250–262.

Goswami et al. Identification and characterization of a Ca2+ -sensitive interaction of the vanilloid receptor TRPV1 with tubulin. J Neurochem 2004; 91:1092–1103.

Goswami et al. Oestrogen destabilizes microtubules through an ion-conductivity independent TRPV1 pathway. J of Neurochemistry 2011; 117(6):995-1008.

Gunthorpe and Chizh 2009. Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. Drug. Discov. Today. 14(1-2), 56-67.

Helyes et al. 1997. Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. Br. J. Pharmacol. 121(4), 613-5.

Helyes et al. 2001. Anti-inflammatory effect of synthetic somatostatin analogues in the rat. Br. J. Pharmacol. 134(7), 1571-9.

Holdcroft and Berkley. Sex and gender differences in pain and its relief. In: McMahon SB, Koltzenburg M, Walk PD, and Melzaks R, eds. Walk and Melzak's Textbook of Pain. 5thed. Edinburgh, Scotland: Elsevier Churchill Livingstone; 2006: 1181–1197.

Holzer 2008. The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nocisensor. Br. J. Pharmacol. 155(8), 1145-62.

Hucho and Levine. Signaling pathways in sensitization: Toward a nociceptor cell biology. Neuron 2007; 55:365–376.

Hucho et al. Estrogen controls PKCε dependent mechanical hyperalgesia through direct action on nociceptive neurons. Eur J Neurosci 2006; 24:527–534.

Hurley and Adams. Sex, gender, and pain: an overview of a complex field. Anesth Analg. 2008;107(1):309–317.

Jakab et al. 2005. Pharmacological characterization of the TRPV1 receptor antagonist JYL1421 (SC0030) in vitro and in vivo in the rat. Eur J Pharmacol 517(1–2):35–44.

Jezierski et al. NGF stimulation increases JNK2 phosphorylation and reduces caspase-3 activity in the olfactory bulb of estrogen-replaced animals. Endocrinology 2001; 142(6):2401.

Jordt et al. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature. 427(6971), 260-5.

Kozinoga et al. Low back pain in women before and after menopause. Prz Menopauzalny 2015; 14(3):203-7

Kuhn et al. GPR30 estrogen receptor agonists induce mechanical hyperalgesia in the rat. Eur J Neurosci 2008; 27:1700–1709.

Kumar et al. Contribution of estrogen receptor subtypes, ERa, ERb, andGPER1 in rapid estradiolmediated enhancement of hippocampal synaptic transmission in mice. Hippocampus. 2015;25(12):1556–1566.

Lacroix-Fralish et al. Progesterone mediates gonadal hormone differences in tactile and thermal hypersensitivity following L5 nerve root ligation in female rats. Neuroscience. 2006;138(2):601–608.

Lu et al. 17b-Estradiol mediates the sex difference in19 capsaicin-induced nociception in rats. J Pharmacol Exp Ther. 2009;331:1104–1110.

Lu et al. 17b-estradiol rapidly attenuates P2X3 receptor-mediated peripheral pain signal transduction via ERa and GPR30. Endocrinology. 2013; 154(7):2421–2433.

Lyles 1996. Mammalian Plasma and Tissue-Bound SemicarbazideSensitive Amine Oxidase: Biochemical, Pharmacological and Toxicological Aspects. Int. J. Biochem. Cell. Biol. 28, 259-274.

Macpherson et al. 2005. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr. Biol. 15(10):929-34.

Macpherson et al. 2007. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature. 445(7127), 541-5.

Mannino et al. Estradiol replacement in ovariectomized rats is antihyperalgesic in the formalin test. J Pain 2007; 8(4):334-42.

Martins et al. Effects of gonadal steroids and of estrogen receptor agonists on the expression of estrogen receptor alpha in the medial preoptic nucleus of female rats. Neuroscience. 2015;310:63–72.

Mátyus et al. Compounds for inhibiting semicarbazide-sensitive amine oxidase (SSAO)/vascular adhesion protein-1 (VAP-1) and uses thereof for treatment and prevention of diseases. WO 2010/029379,2010.

Messlinger K. 1996 Functional morphology of nociceptive and other fine sensory endings (free nerve endings) in different tissues. Prog Brain Res 113:273–298.

Moilanen et al. 2012. TRPA1 contributes to the acute inflammatory response and mediates carrageenan-induced paw edema in the mouse. Sci Rep 2:380.

Myers et al. 2008. A yeast genetic screen reveals a critical role for the pore helix domain in TRP channel gating. Neuron. 58, 362-373.

Nakatsuka et al. 2013. Identification of molecular determinants for a potent mammalian TRPA1 antagonist by utilizing species differences. J Mol Neurosci 3:754–762

Németh et al. 1996. Development of somatostatin radioimmunoassay for the measurement of plasma and tissue contents of hormone. Acta Physiol. Hung. 84(3), 313-5.

Németh et al. 1998. Development of a new sensitive CGRP radioimmunoassay for neuropharmacological research. Neurobiology (Bp). 6(4), 473-5.

Nesuashvili et al. 2013. Sensory nerve terminal mitochondrial dysfunction activates airway sensory nerves via transient receptor potential (TRP) channels. Mol Pharmacol 83(5):1007–1019.

Nilius and Szallasi. 2014. Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. Pharmacol Rev 66(3):676–814.

Noda et al. 2009. Vascular adhesion protein-1 regulates leukocyte transmigration rate in the retina during diabetes. Exp. Eye Res. 89(5), 774-81.

O'Sullivan et al. 2004. Semicarbazide-sensitive amine oxidases: enzymes with quite a lot to do. Neurotoxicology. 25(1-2), 303-15.

Papka and Storey-Workley. Estrogen receptor-alpha and -beta coexist in a subpopulation of sensory neurons of female rat dorsal root ganglia. Neurosci Lett. 2002;319(2):71–74.

Perner et al. 2009. (Heteroaryl)alkenone Oxime Derivatives as TRPA1 Antagonists Useful in the Treatment of Various Diseases. WO 2009089083.

Petrus, M., Peier, A.M., Bandell, M., Hwang, S.W., Huynh, T., Olney, N., Jegla, T., Patapoutian, A., 2007. A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. Mol. Pain. 3:40.

Pfaffl. Anew mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.

Pogatzki-Zahn et al. Heat hyperalgesia after incision requires TRPV1 and is distinct frompure inflammatory pain. Pain. 2005;115(3):296–307.

Pohóczky et al. Estrogen-dependent up regulation of TRPA1 and TRPV1 receptor proteins in the rat endometrium. J Mol Endocrinol. 2016; 56(2):135–149.

Pozsgai et al. 2012. The role of transient receptor potential ankyrin 1 (TRPA1) receptor activation in hydrogen-sulphide-induced CGRP-release and vasodilation. Eur J Pharmacol. 689(1-3):56-64.

Puri et al. Estrogen in cycling rats alters gene expression in the temporomandibular joint, trigeminal ganglia and trigeminal subnucleus caudalis/upper cervical cord junction. J Cell Physiol. 2011;226(12):3169–3180.

Raisinghani et al. 2005. Activation of transient receptor potential vanilloid 1 (TRPV1) by resiniferatoxin. J. Physiol. 567, 771-86.

Riley et al. Orofacial pain symptom prevalence: selective sex differences in the elderly? Pain 1998b; 76(1-2):97-104.

Ruau et al. Sex differences in reported pain across 11,000 patients captured in electronic medical records. J Pain. 2012;13(3):228–234.

Sághy et al. 2015. Evidence for the role of lipid rafts and sphingomyelin in Ca2+-gating of Transient Receptor Potential channels in trigeminal sensory neurons and peripheral nerve terminals. Pharmacol. Res. 100: 101-116.

Salas et al. 2009. TRPA1-mediated responses in trigeminal sensory neurons: interaction between TRPA1 and TRPV1. Eur. J. Neurosci. 29(8), 1568-78.

Salmi et al. 1993. Induction and function of vascular adhesion protein-1 at sites of inflammation. J. Exp. Med. 178(6), 2255–2260.

Salter-Cid et al. 2005. Anti-inflammatory effects of inhibiting the amine oxidase activity of semicarbazide-sensitive amine oxidase. J. Pharmacol. Exp. Ther. 315(2), 553-62.

Sándor et al. 2005. Construction of a stable cell line uniformly expressing the rat TRPV1 receptor. Cell. Mol. Biol. Lett. 10: 499-514.

Smart et al. 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br. J. Pharmacol. 129(2), 227-30.

Story et al. 2003. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell. 112(6), 819-29.

Szállási et al. 2007. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat. Rev. Drug. Discov. 6(5), 357-72.

Szolcsányi 2002. Capsaicin receptor as target molecule on nociceptors for development of novel analgesic agents. In: Kéri G., Tóth I. (Eds.) Molecular pathomechanisms and new trends in drug research. Taylor and Francis, London, pp. 319-333.

Szolcsányi 2008. Hot target on nociceptors: perspectives, caveats and unique features. Br. J. Pharmacol. 155(8), 1142-4.

Szőke et al. 2000. Interacting effects of capsaicin and anandamide on intracellular calcium in sensory neurones. Neuroreport. 11(9), 1949-52.

Szőke et al. 2010. Effect of lipid raft disruption on TRPV1 receptor activation of trigeminal sensory neurons and transfected cell line. Eur. J. Pharmacol. 628(1-3), 67-74.

Tábi et al. 2013. Study on SSAO enzyme activity and anti-inflammatory effect of SSAO inhibitors in animal model of inflammation. J. Neural. Transm. 120(6), 963-7.

Takanami et al. Expression of Gprotein-coupled receptor 30 in the spinal somatosensory system. BrainRes. 2010;1310:17–28.

Trevisani et al. 2007. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc. Natl. Acad. Sci. U S A. 104(33), 13519-24.

Vander Jagt 2008. Methylglyoxal, diabetes mellitus and diabetic complications. Drug Metabol Drug Interact 23(1–2):93–124.

Von Korff et al. 1988. An epidemiologic comparison of pain complaints. Pain 32(2):173-83.

Wang et al. 2006. Design, synthesis, and biological evaluation of semicarbazide-sensitive amine oxidase (SSAO) inhibitors with anti-inflammatory activity. J. Med. Chem. 49(7), 2166-73.

Wang et al. 2008. Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. Brain 131, 1241–1251.

Wáng et al. 2016. Increased low back pain prevalence in females than in males after menopause age: evidences based on synthetic literature review. Quant Imaging Med Surg; 6(2):199-206.

Welch et al. 2000. The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. Proc. Natl. Acad. Sci. U S A. 97(25), 13889-94.

Wu et al. 2010. 17-Beta-estradiol enhanced allodynia of inflammatory temporomandibular joint through upregulation of hippocampal TRPV1 in ovariectomized rats. J Neurosci; 30(26):8710-9.

Xiao et al. 2008. Identification of transmembrane domain 5 as a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels. J Neurosci 28(39):9640–9651.

Yang et al. 2008. Transient receptor potential ankyrin-1 participates in visceral hyperalgesia following experimental colitis. Neurosci Lett 440(3):237–241.

Zhang et al. 2005. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBOJ; 24(24):4211–4223.

IX. LIST OF PUBLICATIONS

Articles related to thesis

Payrits M, Sághy É, Mátyus P, Czompa A, Ludmerczki R, Deme R, Sándor Z, Helyes Z, Szőke É. (2016). *A novel 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanal oxime compound is a potent Transient Receptor Potential Ankyrin 1 and Vanilloid 1 (TRPA1 and V1) receptor antagonist.* Neuroscience. 324:151-162. doi: 10.1016/j.neuroscience.2016.02.049.
IF: 3,277

Payrits M, Sághy É, Szolcsányi J, Pohóczky K, Csekő K, Bölcskei K, Barabás K, Ernszt D, Ábrahám I, Helyes Z, Szőke É. (2017). *Estradiol sensitises the Transient Receptor Potential Vanilloid 1 channel in pain responses.* Endocrinology. 158(10):3249-3258. doi.org/10.1210/en.2017-00101.
IF: 4,286

Articles not related to the thesis

Sághy É, Szőke É, **Payrits M,** Helyes Z, Börzsei R, Erostyák J, Jánosi TZ, Sétáló G. Jr., Szolcsányi J. (2015). *Evidence for the role of lipid rafts and sphingomyelin in Ca^{2+}-gating of Transient Receptor Potential channels in trigeminal sensory neurons and peripheral nerve terminals.* Pharmacol. Res. 100: 101-116. doi: 10.1016/j.neuroscience.2015.08.043. IF: 4.816

Sághy É, **Payrits M,** Helyes Z, Reglődi D, Bánki E, Tóth G, Couvineau A, Szőke É. (2015). *Stimulatory effect of pituitary adenylate cyclase-activating polypeptide 6-38, M65 and vasoactive intestinal polypeptide 6-28 on trigeminal sensory neurons.* Neuroscience. 308: 144-156. IF: 3.357

Pohóczky K, Kun J, Szalontai B, Szőke É, Sághy É, **Payrits M,** Kajtár B, Kovács K, Környei JL, Garai J, Garami A, Perkecz A, Czeglédi L, Helyes Z. (2016). *Estrogen-dependent up-regulation of TRPA1 and TRPV1 receptor proteins in the rat endometrium.* J. Mol. Endocrinol. 56: 135-149. doi: 10.1530/JME-15-0184. IF: 3.993

Hajna Z, Sághy É, **Payrits M,** Aubdool AA, Szőke É, Pozsgai G, Bátai IZ, Nagy L, Filotás D, Helyes Z, Brain SD, Pintér E. (2016). *Capsaicin-sensitive sensory nerves mediate the cellular and microvascular effects of H_2S via TRPA1 receptor activation and neuropeptide release*. **Journal of Molecular** Neuroscience. 60(2):157-70. doi: 10.1007/s12031-016-0802-z. IF: 2.229

Pozsgai G, **Payrits M**, Sághy É, Sebestyén-Bátai R, Steen E, Szőke É, Sándor Z, Solymár M, Garami A, Orvos P, Tálosi L, Helyes Z, Pintér E. (2017). *Analgesic effect of dimethyl trisulfide in mice is mediated by TRPA1 and sst4 receptors.* Nitric Oxide. 65:10-21. doi: 10.1016/j.niox.2017.01.012. IF:4.181

Bölcskei K, Kriszta G, Sághy É, **Payrits M**, Sipos É, Vranesics A, Berente Z, Ábrahám H, Ács P, Komoly S, Pintér E (2018). *Behavioural alterations and morphological changes are attenuated by the lack of TRPA1 receptors in the cuprizone-induced demyelination model in mice*. Journal of Neuroimmunology (accepted for publication 26.03.2018.)
IF:2.720

Cumulative impact factor of the publications related to the thesis: **7.563**
Cumulative impact factor of all papers: **28.859**

X. First Authored Posters and presentations

M. Payrits, É. Szőke, É. Sághy, T. Bagoly, Zs. Helyes, J. Szolcsányi: *Effect of resolvin D1 and resolvin D2 on TRP ion channel activation.*
International Brain Research Organization Workshop, Debrecen, Hungary 2014. P12, 33.

Payrits M., Sághy É., Szőke É., Bagoly T., Helyes Zs., Szolcsányi J. *Inhibition of transient receptor potential ion channels by resolvins.* A Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Farmakológiai szekciójának VIII. szimpoziuma és az MBKE Gyógyszerbiokémiai Szakosztály XXVIII. Mukaértekezlete, Velence, Hungary, 2014.

M. Payrits, É. Szőke, É. Sághy, T. Bagoly, Zs. Helyes, J. Szolcsányi: *Inhibition of transient receptor potential ion channels by endogenous lipid mediators.* Joint Meeting of the Federation of European Physiological Societies (FEPS) and Hungarian Physiological Society, Budapest, 2014.

Payrits M., Sághy É., Szőke É., Bagoly T., Helyes Zs., Szolcsányi J. *Tranziens Receptor Potenciál ioncsatornák gátlása resolvinnal.* A Magyarországi Fájdalomtársaság Kongresszusa és a IV. Neurostimulációs Szimpózium a Magyar Neurológiai Társaság Részvételével, Pécs, Hungary, 2014.

Payrits M., Sághy É., Bagoly T., Szolcsányi J., Helyes Zs., Mátyus P., Deme R., Szőke É.: *Egy új SSAO gátló vegyület hatása a Tranziens Receptor Potenciál ioncsatornák aktivációjára.* Idegtudományi Centrum/Szentágothai János Kutatóközpont PhD és TDK konferencia, Pécs.

M. Payrits, É. Sághy, P. Mátyus, A. Czompa, R. Ludmerczki, R. Deme, Zs. Helyes, É. Szőke: Egy *új oxim vegyület antagonista hatásának jellemzése Tranziens Receptor Potenciál ioncsatornákon.* A Magyar Kísérlete és Klinikai Farmakológiai Társaság Experimentális Farmakológiai szekciójának IX. szimpóziuma, Velence. P-013, 20.

M. Payrits, É. Sághy, P. Mátyus, A. Czompa, R. Ludmerczki, R. Deme, Zs. Helyes, É. Szőke. *Egy új Tranziens Receptor Potenciál ioncsatorna antagonista vegyület farmakológiai jellemzése szenzoros neuronokon*. Élettani Társaság 79. Vándogyűlése, Szeged, 2015.

M. Payrits, É. Szőke, Zs. Helyes, I.M. Ábrahám, E. Pintér: *The role of Transient Receptor Potential Ankyrin1 receptors in β-amyloid$_{1-42}$ –induced cholinergic neurodegeneration in the basal forebrain.* Neuropeptides 2015. Scotland, Aberdeen. P12.

M. Payrits, É. Szőke, Zs. Helyes, I.M. Ábrahám, E. Pintér: *The role of Transient Receptor Potential Ankyrin1 receptor in β-amyloid$_{1-42}$-induced Alzheimer's disease.* IBRO, Budapest. P2/54.

Payrits M., Sághy É., Szolcsányi J., Pohóczky K., Csekő K., Bölcskei K., Ernszt D., Barabás K., Ábrahám I., Helyes Zs., Szőke É. *Evidence for the role of eastradiol on gating on the Transient Receptor Potential Vanilloid 1 channels in trigeminal sensory neurons and in in vivo animal models*. IBRO Workshop, Budapest, Hungary, 2016.

Payrits Maja, Borbély Éva, Szőke Éva, Helyes Zsuzsanna, Ábrahám M. István, Pintér Erika: *A Tranziens Receptor Potenciál Ankyrin 1 receptor szerepe β-amyloid$_{1-42}$-indukált kolinerg sejtpusztulásban in vivo.* FAMÉ2016, Pécs P3.107

Payrits M, Borbély É, Szőke É, Helyes Z, Ábrahám I and Pintér E*: The role of Transient Receptor Potential Ankyrin 1 receptor in β-amyloid$_{1-42}$-induced Alzheimer's disease*. RegPep2016, Rouen, France 2016.

Payrits Maja, Borbély Éva, Szőke Éva, Helyes Zsuzsanna, Ábrahám M. István, Pintér Erika: *A Tranziens Receptor Potenciál Ankyrin 1 és Vanilloid 1 receptor szerepe demenciában*. MFT Gyógyszerinnovációs Kongresszusa. Velence 2017.

Payrits M, Borbély É, Szőke É, Helyes Zs, Barabás K, Ábrahám IM, Pintér E. *The role of Transinet Receptor Potential Ankyrin1 Receptor in B-amyloid$_{1-42}$-induced Alzheimer's disease.* FENS Regional Meeting, Pécs, Hungary, 20–23. September 2017.

Payrits Maja, Éva Sághy, Kata Csekő, Krisztina Pohóczky, Kata Bölcskei, Dávid Ernszt, Klaudia Barabás, János Szolcsányi, István Ábrahám, Zsuzsanna Helyes, Éva Szőke. *Estradiol sensitises the Transient Receptor Potential Vanilloid 1 channel in pain responses.* 13th International Symposium on VIP, PACAP and Related Peptides. Hong Kong, China 2017.

Maja Payrits, Éva Borbély, Klaudia Barabás, Éva Szőke, Zsuzsanna Helyes, Klaudia Barabás, István M. Ábrahám, Erika Pintér: *The Transient Receptor Potential Ankyrin1 receptor influences the β-amyloid$_{1-42}$-induced Alzheimer's disease and demencia* Advances in Alzheimer's and Parkinson's Therapies an ATT-AD/PD focus meeting. Torino, Italy. 2018.

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