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PhD Thesis

COMPLEX INVESTIGATION OF PAIN MECHANISMS USING IN VIVO ANIMAL MODELS

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INTRODUCTION

The International Association for the Study of Pain (IASP) defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. In animal experiments we can examine only the activation of the nociceptors or the nocifensive behaviour which occurs by one of the noxious stimuli. Nociceptors are sensory nerve endings reacting for specifically harmful stimuli, and they can be grouped upon several points of view. Based on the nociceptors sensitivity they can be either unimodal (activated only by thermal or mechanical stimuli), or polimodal (besides thermal and mechanical stimuli they are also sensitive to chemical substances). Nociceptors can be also classified based on the their myelinization of axons: they can be either thinly-myelinated fast-conducting (12–30 m/s) Aδ, or unmyelinated, slowly-conducting (0.5–2 m/s) C nociceptors.

Capsaicin-sensitive sensory nerve-endings

A huge number of the peripheral nociceptors (50-70%) produces the capsaicin-sensitive sensory-nerves (Holtzer et al 1991). Their name came from the alkaloid of hot chilli pepper, since its receptor, the Transient Receptor Potential Vanilloid 1 (TRPV1) ion channel is located in the membrane of these nerve-endings. These nerve-endings have uniquely a three-fold functions: On the one part the classical afferent function is activation by stimuli depolarisation and transmission of information to the central nervous system, thus develops the pain perception (nociception). Pro-inflammatory sensory neuropeptides (calcitonine-gene-related-peptide (CGRP), tachykinins (substance P and neurokinin-A) released from the activated sensory nerve-endings inducing vasodilation, plasma protein extravasation and activation of pro-inflammatory cells which leads to the neurogenic inflammation (Szolcsányi 1984 a, b, 1988). This is the second part named locally efferent function. The neurogenic inflammation plays a crucial role in pathomechanism of numerous clinical states, however none of the currently available pharmaceutical drugs can inhibited its neurogen component (Helyes et al 2003). The third part is the systemic efferent function: besides the pro-infammatory neuropeptides anti-inflammatory and analgesic agents (e.g. somatostatin, pituitary-adenylate-cyclase- activating polypeptide (PACAP) are also released from the activated sensory nerve-endings and exert systemic anti-inflammatory and analgesic actions (Szolcsányi et al 1998 a, b).

Structure and function of Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) ion channels

Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) expressed on the polimodal nociceptive primer sensory neurons of the dorsal horn and trigeminal ganglia. Both receptors play a relevant role due to activation of sensory nerves in mechanisms of pain (Fernandes et al 2011) and neurogenic inflammation (Geppetti et al 2008). In the membrain 97% of TRPA1 expressing sensory nerves presented also TRPV1 ion channels, while 30% of the TRPV1 expressed neurons coexpressed TRPA1 receptors (Story et al 2003). TRPV1 receptor is a molecular integrator of physical and chemical stimuli (Tominaga et al 1998).The presence of the receptor supposed to by Prof.

Szolcsányi in 1975 (Szolcsányi et al 1975). A number of exogeneous plant-derived pungent vanilloid agents (resiniferatoxin (RTX), piperin, gingerol) (Pingle et al 2007, Szállási 2007) can stimulate the receptor. Therefore its name was originally vanilloid receptor 1 (VR1), which was altered to TRPV1 because of structural similarity of other transient receptor potential mediated ion channels (Gunthorpe et al 2002). Besides capsaicin noxious heat (above 43°C) can stimuli the receptor, as well as changes of the pH, the endovanilloids (e.g.anandamid), arachidicacid-metabolites, or essential oils (Pingle et al 2007, Szállási 2007). Pro-inflammatory mediators, such as bradykinin, prostaglandins, adenosine-triphosphate (ATP) protease-activating receptors (PAR 1, 2 ,4), tumor necrosis factor-alfa (TNF-alfa), nerve growth factor (NGF) also can sensitize the receptor by phosphorylation. On the effects of these mediators the pore proteins are allosteric modified due to increasing the heat, protons and/or capsaicin induced receptor activation (Moriyama et al. 2005, Szállási et al. 2007). Exogeneous agonists of TRPA1 receptor are remarkable the plant-derived irritant: allyl isothiocyanate (AITC) from mustard oil (Jorgt et al 2004, Bandell et al 2004, Bautista et al 2006), allicin from garlic (Macpherson et al 2005), cinnamaldehyde (Macpherson et al 2006), as well as compounds of toxique gases and smoking (e.g. acrolein) (Bautista et al 2006). Endogenous agonists are the reactive oxigenenous radical (Bessac et al 2008b), the 4-hydroxi-nonenal (Trevisani et al 2007), lipide peroxydase products (Taylor-Clark et al 2008). Endogeneous modulators of TRPA1 are agents released from inflammatory processes e.g. bradykinin (Bandell et al 2004), PAR-2 agonists (Dai et al 2007), and hydrogen-sulfide (H_2S) (Miyamoto et al 2011). Zinc, copper and cadmium ions also can stimuli the TRPA1 receptors (Hu et al 2009, Gu és Lin 2010).

The involvement of TRPA1 receptors in cold sensation are contradictory. Numerous different results can be found in the literature, there are argues in favour (Story et al 2003, Obata et al 2005, Kwan et al 2006, Sawada et al 2008), and there are some against (Jordt et al 2004, Nagata et al 2005, Bautista et al 2006). It is supposed to that besides other mechanisms TRPA1 ion channel can contribute the sensation of temperature below 17 °C.

Significance of developing novel analgesic drugs

There was no breakthrough in the development of analgesic drugs acting on novel target in the last century. Pain killers with the same mechanism of action are used for decades, which can be divided into two main groups: the non-steroidal anti-inflammarory drugs (NSAID) and the opioids. Although these drugs have high efficacy in several clinical states, they are uneffective in some specific pain condition (e.g. neuropathic pain). In the treatment of these diseases adjuvant analgetics are necessary. However the analgesic effect of these drugs is not always sufficient, and the therapeutic importance is decreased by the occasionaly serious side effects. Due to of these, it is highly relevant to identify novel targets acting selectively on the peripheral nociceptors, in order to minimalize the classical side effects of analgesic drugs.

AIMS

We investigated the activation or inhibition mechanisms of TRP channels located on capsaicin-sensitive sensory nerve-endings as well as their involvements in pain and inflammation using different pharmacological compounds or gene-deleted animals.

Our goals were the following:

I. Investigation of the mechanisms of desensitisation of the capsaicin-sensitive sensory nerve-endings and the TRPV1 receptors using noxious heat or cold threshold determination technique.

II. Comparison of three novel TRPV1 receptor antagonists, SB705498, BCTC and AMG9810 developed by different pharmaceutical companies, in TRPV1 agonist induced-, mild heat injury induced-, and plantar incision-evoked thermal hyperalgesia models using an increasing temperature water bath suitable for noxious heat threshold determination. The effects of these antagonists on the RTX-induced heat hyperalgesia were also investigated by measurement of the paw withdrawal latency using a plantar test apparatus in order to compare the sensitivity of the newly established method of heat threshold determination.

III. We aimed to adopt the increasing temperature water bath to be suitable for measuring the noxious heat thresholds on the murine tail. Since the roles of TRPV1 and TRPA1 in heat and mechanical perceptions are contradictory, therefore we investigated these receptor functions with noxious threshold determining techniques using gene-deficient mice.

IV. We aimed to establish and validate a well-functioning passive-transfer trauma murine model for the Complex Regional Pain Syndrome to confirm a role for pathogenic autoantibodies with a collaboration with Walton Pain Centre, University of Liverpool (dr. Andreas Goebel).

I. INVESTIGATION OF THE DESENSITISATION AND ANTINOCICEPTION EFFECTS OF TRPV1 RECEPTOR AGONISTS MEASURING THE NOXIOUS HEAT AND COLD THRESHOLDS IN RATS

The investigation of the roles of TRPV1 receptor-expressing polymodal nociceptors started with experiments on capsaicin's selective excitatory and consequent blocking effects. Resiniferatoxin presenting naturelly in Morrocan *Euphorbia resinifera* plants can activate the TRPV1 receptor. It is 100 fold potent than capsaicin, which means indicating the same effect as a defined concentration of capsaicin: 100 fold lower concentration of RTX is sufficient (Szolcsányi 1990, Szállási 1999). The N-oleyldopamin is an activator of TRPV1 receptor, but its affinity is lower than capsaicin (Chu et al 2003, Szolcsányi et al 2004).

During the activation of TRPV1 ion channel it is permeable to Na^+ ,Ca^{2+} and K^+ ions. If the receptor is stimulated permanently and freqvently, the concentrate of intracellular cations are increased, which leads to swelling of cytoplasma and mithocondria, the energy consumption of the cells are decreased, then the nerve-ending became inefficient. This process is the desensibilization, which has 2 forms.

With lower concentration or applied for shortly periods only the response to capsaicin and other TRPV1 receptor agonists is diminished suggesting desensitization of the TRPV1 receptor. Higher concentrations and more prolonged exposure lead to a reduced responsiveness to all stimuli (heat, mechanical and chemical) activating the polimodal nociceptors. This form of desenstisation is suggested the impairment nociception of the whole sensory nerve terminal expressing TRPV1 receptors and its background supposed to be dose-dependent ultrastructural or robust morphological differences (Szolcsányi et al 1975, 1987, Szállási et al 1989, Bevan and Szolcsányi 1990, Szolcsányi 1993).

METHODS

1. Examination of the desensitization of the sensory nerve-endings: 100 µl capsaicin (3.3 nmol – 1 µmol), RTX (0.016 – 0.5 nmol) or OLDA (5nmol – 1.25 µmol) was injected into both of the rat's hindpaws. To investige the noxious heat thresholds increasing temperature hot plate was used every day, at that timepoint till the significantly differences compared to the controls are disappeared. The noxious heat and cold thresholds were examined in a parallel manner: both thresholds were determined in the same group of rats before bilater i.pl. capsaicin (0.1 and 1 µmol i.pl.) or RTX (0.16 and 0.5 nmol i.pl.) administration and subsequently daily for a week.

2. Examination of the desensitization of the TRPV1 receptors: To investigate the homologue desensitization of the TRPV1 receptor, intraplantarly applied RTX (0.016 nmol, 50 µl i.pl.) - or OLDA (250 nmol, 50 µl i.pl.) -evoked acute responses such as induction of nocifensive behaviour (time spent with paw shaking, licking or linching) and decrease of the noxious heat threshold were assessed. 3 hours later the heat thresholds were determined and subsequently the same dose of RTX (0.016 nmol, 50 µl i.pl.) either OLDA (5 nmol, 50 µl i.pl.) or its vehicles was injected into the previously treated paw. To examine the specifity of the desensitization intraplantarly applied RTX (0.016 nmol) or its vehicle was injected 3 hours before, then one of the hindpaws was treated with 1% formalin to investigate the effects of pretreatment. To observe the possible cross-desensitization between OLDA and RTX animals were treated with OLDA (250 nmol, 50µl i.pl.) or its solvent. 3 hour later RTX (0.016 nmol, 50µl i.pl.) was injected in the previously treated hindpaw, then the latency of the nocifensive reaction and drop of the thermonociceptive thresholds were detected.

RESULTS

1. Examination of the desensitization of the sensory nerve-endings: Administration intraplantarly capsaicin or RTX increased the thermonociceptive thresholds in a dose-dependent manner measuring on the consecutive days. The minimum effective doses (defined as the lowest dose causing a significant increase of the heat threshold at any time point of measurement) were 10 and 0.05 nmol, respectively. The maximum threshold elevation was 2.3 ± 0.5 °C for capsaicin and 2.8 ± 0.5 °C for RTX within the investigated dose. However the effects of the agonists were dose-dependent, neither of

OLDA concentration elevated significantly the heat thresholds. Measuring parallel the heat and cold thresholds only the higher doses of capsaicin (0.1 and 1 μmol i.pl.) or RTX (0.16 and 0.5 nmol i.pl.) induced significant decrease of cold thresholds, which are returned to the control levels within 2-4 days.

2. Examination of the desensitization of the TRPV1 receptors: The intraplantar injection of 0.016 nmol RTX and 5 nmol OLDA elicited acute nocifensive reactions which disappeared within 5 and 10 min, respectively. On the effects of both agonist indicated a robust drop of heat thresholds (8-10 °C), which dissapeared after 30 min. To determinate the desensibilization of TRPV1 ion channels to chemical stimulation, in the previously treated hindpaw was injected with the same dose of RTX (0.016 nmol) or OLDA (5 nmol). The 3 hours later giving second injection significantly decreased both the latency of the nocifensive behaviour and the thermal hyperalgesia compared to the effects evoked by the first injection. In contrast, the same RTX pretreatment (0.016 nmol i.pl. for 3 h) failed to alter the nocifensive reaction evoked by a subsequent i.pl. injection into the RTX-treated hindpaw of formalin (TRPA1 agonist) solution (1%). Pretreatment with OLDA (250 nmol i.pl.) significantly diminished the acute effects of 3 hours later i.pl. injected RTX (0.016 nmol) compared to the vehicle-treated animals, which may indicated a cross-desensitization between the two TRPV1 receptor agonists.

CONCLUSION

In our experiments the locally (intraplantarly) applied capsaicin and RTX evoked a long-lasting elevation of the noxious heat thresholds indicating the thermal antinociception. The latency of the effects induced by capsaicin or RTX was dose-dependent: the effects lasted with lower concentration to 2-5 day, while with higher concentration they could be observed for more than a week, up to 11 days. The background of long-lasted threshold elevation is the functional elevation, that is the impairment of the TRPV1 expressed polimodal nociceptors renders them less to all stimuli.

Intraplantarly injection of RTX or OLDA induced acute nocifensive reaction and markedly reduced the heat thresholds. These were almost abolished by i.pl. pretreatment with the same TRPV1 receptor agonist at doses that failed to evoke a lasting elevation of the noxious heat threshold suggests that desensitization of the TRPV1 receptor and not the whole sensory nerve ending occurred.

Interpretation of the drop of cold thresholds induced by TRPV1 agonists is the impairment of the cold-sensitive nociceptors, which molecular mechanisms are not clear yet. The observation, that the cold threshold values recovered faster to the control levels, than the heat thresholds can be normalized suggesting the response to noxious heat or cold stimuli mediated –at least partly –by different fibres.

II. INVESTIGATION OF THE EFFECTS OF TRPV1 ANTAGONISTS IN DIFFERENT THERMAL HYPERALGESIA MODELS

During preclinical investigations numerous molecular mechanisms have been identified which are involved in development and maintaining of pain. Due to the large number of pro-nociceptive endogenous activators/sensitizers of TRPV1, this receptor arose as a promising target for the development of novel analgesic drugs which act directly on peripheral nociceptors by blocking TRPV1 (Brederson and Szállási 2013).

METHODS

1. RTX-induced drop of noxious heat threshold: After measuring the controls with increasing temperature water bath TRPV1 receptor antagonist drugs or its vehicles were administered orally (0.5 ml/100 g). 1 h later RTX (0.01 μg) was administered intraplantarly into one of the hindpaws. Then the noxious heat thresholds were determined 5, 10, 15 and 20 min after RTX treatment.

2. Mild heat injury-induced thermal hyperalgesia: After control heat threshold measurements rats were anaesthetized with halothane and one of the hind paws was immersed in a 51 °C hot water bath for 20 s. TRPV1 antagonists were administered intraperitoneally (i.p., 0.5 ml/100 g) after the 20-min measurement which was followed by repeated heat threshold measurements at 40, 50 and 60 min after heat injury.

3. Plantar incision-induced thermal hyperalgesia: Following control threshold measurements heat threshold drop was induced by a standardized surgical incision performed on the plantar surface of the hindpaw (Füredi et al 2009). Rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.) and a 1 cm long midline incision was made starting 0.5 cm from the heel involving skin, fascia and muscle. 24 h after surgery, two heat threshold measurements were performed and TRPV1 blockers were administered per os (p.o., 0.5 ml/100 g). Heat threshold measurements were repeated 1, 2, 3 and 4 h after treatment.

4. Paw withdrawal latency measurement with a plantar test apparatus to assess RTX-induced thermal hyperalgesia: A separate series of experiments were performed to assess the effect of the same TRPV1 antagonists on the RTX-induced thermal hyperalgesia using a Plantar Test apparatus for measurement of paw withdrawal latency. After control measurements, RTX was administered intraplantarly (0.06 μg) and paw withdrawal latency measurement was repeated 10 min later. One half of the group was treated with the drug and the other with its solvent employing oral administration (0.5 ml 100 g) 1 h prior to RTX treatment.

RESULTS

1. Inhibition of the resiniferatoxin (RTX)-induced thermal hyperalgesia: The control noxious heat threshold of rats was 43.2 ± 0.4 °C (n=36). After the RTX treatment a robust drop of heat threshold

(8–10 °C) was measured with the increasing temperature water bath which was maintained during the 20 min of experiment. Pre-treatment with any of the three TRPV1 receptor antagonist compounds dose-dependently inhibited the RTX-induced heat threshold drop at all time points in the dose range of 1–30 mg/kg p.o. The minimum effective dose was 1 mg/kg for each compound. The highest applied dose of SB705498 (10 mg/kg) completely abolished the drop of heat threshold, while BCTC (30 mg/kg) and AMG9810 (10 mg/kg) produced maximal inhibition of 74.5% and 66.2%, respectively.

2. Inhibition of the heat injury-induced thermal hyperalgesia: All TRPV1 antagonist compounds injected i.p. as a posttreatment after the 20-min measurement significantly reversed the heat injury-induced drop of heat threshold. The minimum effective i.p. doses were as follows: SB705498 10 mg/kg, BCTC 3 mg/kg and AMG9810 1 mg/kg. The dose–response curves of SB705498 and BCTC slightly declined at the maximum applied dose (30 mg/kg) producing their maximal inhibitory effect at the 10 mg/kg dose (54.1% and 74.2%, respectively) whereas AMG9810 had a maximal inhibitory effect of 60.3% at the 30 mg/kg dose.

3. Inhibition of the surgical incision-induced thermal hyperalgesia: All antagonist compounds applied p.o. after the confirmation of thermal hyperalgesia dose-dependently diminished the postoperative drop of the noxious heat threshold in the dose range of 3–30 mg/kg. The minimum effective doses were as follows: SB705498 10 mg/kg, BCTC 3 mg/kg and AMG9810 3 mg/kg. The maximal inhibitory effects were 40.5% for SB705498, 52.9% for BCTC and 84.4% for AMG9810.

4. Effects of TRPV1 receptor antagonists on the RTX-induced thermal hyperalgesia assessed by paw withdrawal latency measurement: Baseline paw withdrawal latency was 11.03 ± 0.3 s, which decreased to 4.38 ± 0.3 s after intraplantar RTX (0.06 μg) injection. The antihyperalgesic effect of either SB705498, BCTC or AMG9810 pretreatment (p.o., 1 h prior to RTX injection,), observed as a prolongation of paw withdrawal latency, was only significant at the highest applied dose (30 mg/kg), with the percentage inhibition values being 43%, 38% and 37%, respectively.

CONCLUSIONS

We were the first who published comparing data about TRPV1 receptor antagonists developed by different pharmaceutical companies using methods based on a novel approach of noxious heat threshold measurement and a traditional technique of latency measurements. Our results demonstrate that the heat threshold drop evoked by direct TRPV1 receptor activation, mild heat injury or surgical incision are appropriate thermal hyperalgesia models that display a remarkably high sensitivity to TRPV1 receptor antagonists. Comparison of the sensitivity to TRPV1 antagonism of our heat threshold measurement approach with that of the conventional paw withdrawal latency determination (Hargreaves et al 1988) was performed in the RTX hyperalgesia model. It has been shown that the heat threshold measurement is much more sensitive as it resulted in considerably (30 times) lower minimum effective dose for each TRPV1 antagonist. These results confirmed the role of the increasing temperature water bath as a novel reliable preclinical testing method.

III. INVESTIGATION OF THE ROLES OF TRPV1 AND TRPA1 RECEPTORS IN PAIN MODELS USING GENE-DELETED MICE

Cloning the TRPV1 receptor opened a new way to study its structure. With the help of this emerged that the receptor can be directly activated by noxious heat and low pH, furthermore the receptor also plays a central role in the integration of painful stimuli (Caterina et al 1997; Tominaga et al 1998). The detailed investigation of the involvement of the receptor in inflammatory or pain processes was permitted after generating gene-deleted mice in 2000 (Caterina et al 2000; Davis et al 2000).

Altrough TRPA1 receptor has been already cloned in 1999 (Jaquemar et al 1999), its expression on the neurons was published only in 2003 (Story et al 2003), by whom, the receptor has been identified as a sensor of noxious cold. After the first few experiments using gene-deficient mice it was considered, that the receptor essential in thermal hyperalgesia (Davis et al 2000), but its role in basic heat sensation is unclear (Caterina et al 2000). It is confirmed by previous results of us, that the nociceptive heat thresholds of TRPV1 gene-deleted mice were not differ from the thresholds of their wildtypes (Almási et al 2003). However the thermal and mechanical hyperalgesia induced by mild heat injury was significantly lower in mice lacking TRPV1 gene. (Bölcskei et al 2005).

Mustard oil was considered as a selective exogeneous agonist of TRPA1 receptor (Jorgt et al 2004), but its selectivity is ambigous, since it is supposed to also involving in the activation of TRPV1 receptors (Gees et al 2013, Everaerts et al 2011).

Nociceptive thermal threshold determinations on the murine hindpaw is not known in the literature. Because of mice are not tolerated well handling by the investigator and the size of the murine paw is relatively small, we focused on the investigation of murine tail. During our experiments we adopt the increasing temperature water bath developed in our department to be suitable for measuring the noxious heat thresholds of the murine tail with the help of a narrow plastic tube.

METHODS

1. Preliminary measurements and determination of the basal (control) noxious heat threshold: Preliminary measurements were performed on male CD1 mice (25-35g), while during further experiments we used male TRPV1 and TRPA1 gene-deleted animals and their wildtype counterparts. In order to eliminate the difference resulting from the handling of the investigator, we innovated a restrainer (from narrow plastic tube with holes on its walls) for holding the animals during the measurements. After putting the animals into the restainers, they were hung over the equipment to let the murine tail being immersed into the water bath deep enough. After few minutes habituation, noxious heat thresholds were determined on the tails of the animals every 10 minutes during 1 hour on two consequetive days. The noxious heat thresholds of their littermates holding in hands by the investigator were detected for comparison.

2. Investigation of mustard oil-evoked thermal hyperalgesia: Mustard oil-induced drop of noxious heat threshold was determined both on the tail and on the paw with increasing temperature water bath or increasing temperature hot plate, respectively. After the control measurements, either the tail or the paw was dipped into 1% mustard oil, dissolved in 30% DMSO for 30 or 60 sec, respectively. Heat threshold measurements were repeated every 10 minutes for 1 hour after application of mustard oil.

3. Investigation of mustard oil-evoked mechanical hyperalgesia: The mechanical touch sensitivity of the plantar surface of the murine paws were determined with dynamic plantar aesthesiometer. After control measurements one of the hindpaws of the mice was immersed into 1% mustard oil for 60 sec. Measurements were assessed 30, 60, 120, 180 minutes after the application.

4. Investigation of the latency of the mustard oil-induced nocifensive behaviour: The murine tail or one of the hindpaws was immersed into 1% mustard oil, and the latency of the nocifensive behaviour was detected. Strongly tail or paw shaking was accepted as nocifensive behaviour. The maximal time of the latency was 3 minutes.

RESULTS

1. Preliminary measurements and determination of the basal (control) noxiuos heat threshold: Between closing the animals in restainers or holding them in hands during the measurements highly reproducible threshold values without significant alterations were obtained. However, application of restainers contributes to the semi-automation of the process, allowing less time-consuming measurements. Despite of the TRPA1 gene-deleted mice, the basal noxious heat thresholds of the tails of TRPV1 gene-deficient mice were significantly higher ($TRPV1^{-/-}$: 45.42 $\pm$ 0.34 °C), compared to their wildtypes ($TRPV1^{+/+}$: 42.98 $\pm$ 0.4 °C), but these differences were not detectable on the paws neither investigated murine strains.

2. Investigation of the effects of mustard oil-evoked thermal hyperalgesia: The average control noxious heat threshold of $TRPV1^{+/+}$ animals was 43.53 $\pm$ 0.33 °C measured on the murine tails. 10 minutes after mustard oil application this was decreased to 35.96 $\pm$ 1.23 °C. This drop of heat thresholds was significantly reduced in TRPV1 gene-deleted animals (from 46.05 $\pm$ 0.35 °C to 40.6 $\pm$ 0.6 °C), and this distinction (4-6°C) levelled out during the total time of the measurement. Drop of the noxious heat thresholds measuring on the tail was not statistically significantly in presence or failure of TRPA1 receptor. The average of the noxious heat thresholds of $TRPV1^{+/+}$ and $TRPA1^{+/+}$ wildtype animals (44.8 $\pm$ 0.4°C és 45.0 $\pm$ 0.2 °C, respectively) decreased 12-14°C after 20 min the mustard oil application. This decrease maintaining at the end of the experiment was significantly lower in TRPV1 gene-deleted mice compared to their wildtypes, but not in TRPA1 gene-deficient animals.

3. Investigation of the effects of mustard oil-evoked mechanical hyperalgesia: 30 minutes after the mustard oil application a markedly drop of mechnonociceptive thresholds (45-55%) was observed. This decrease maintaining at the end of the experiment was significantly lower (around 25%) in

TRPV1 gene-deleted animals. The mechanical hyperalgesia was similar in TRPA1 gene deficient mice comparing their wildtypes.

4. Investigation of mustard oil-evoked nocifensive behaviour: Measuring the latency of appearing of the nocifensive behaviour was 103 ± 14 s in mustard oil not-containing solution on the tail of $TRPV1^{+/+}$ animals, which was significantly higher, than the total time spent in 1% mustard oil containing solution (48 ± 5 s). Total time of $TRPV1^{-/-}$ animals dipping into 1% mustard oil was significantly higher (89 ± 13 s). $TRPA1^{+/+}$ animals spent 63 + 8 s in mustard oil not-containing solution, while this was significantly lower (26 ± 4 s) in 1% mustard oil. Time spent in mustard oil until nocifensive reaction was significantly higher (82 ± 13 s) measuring on the tail of TRPA1 gene deleted animals.

CONCLUSIONS

With the help of adopting increasing temperature water bath for mice we had the opportunity to measure the noxious heat threshold of the murine tail. Using the restainer measurements were less time-consuming, and eliminated the differences resulting from the handling of the investigator. This modified increasing temperature water bath is suitable to be a novel reliable preclinical testing method and with the application of increasing temperature hot plate it is appropiate for comparing the heat thresholds of both the murine tail and paws, simultanously. With the help of these methods, we provided the first evidence that the tail heat thresholds of TRPV1 gene-deleted mice were significantly higher compared to their wildtypes, while there was no difference between the heat thresholds of the paws. These results suggest, that the murine tail may have a greater relevance in heat sensation compared to the paw and body regions can be characterized with different receptor density.

Our inflammatory hyperalgesia model has been considered as a well-established model, however mustard oil-induced hyperalgesia was similar both on the tail and the paw of $TRPA1^{-/-}$ animals. These results suggest, that mustard oil can activate TRPA1 receptors in various processes, in our experiments they played a role only in mustard oil-induced nocifensive reaction. Mustard oil-evoked inflammatory (thermal and mechanical) hyperalgesia independently from the examined body regions was significantly smaller in $TRPV1^{-/-}$ animals compared to their wildtypes. This may be due to other unidentified mechanisms may involve the development of its mechanisms as well as, that mustard oil can be also activate the TRPV1 receptor besides TRPA1.

IV. THE PASSIVE TRANSFER-TRAUMA MODEL OF COMPLEX REGIONAL PAIN SYNDROME (CRPS)

The mechanisms of the neurogenic inflammation play a crucial role in numerous inflammatory disease with severe persistent pain condition (e.g. rheumatoid arthritis, Levine et al 1986), as well as in the development of Complex Regional Pain Syndrom (CRPS), presumably. The disease usually occurs

after a minor limb injury, however neither its aetiology nor its pathophysiological processes are clear yet. Overreaction of the immune system againts antigens released from sensory nerves and complex neuro-immune interactions are suggested to be involved in its mechanisms (Blaes et al 2007).

The symptoms can be healed spontanously, but they can be relapsed and developing a monthly or yearly longstanding severe pain condition in their higher proportion of the cases. The previously applied plasmapheresis treatments were successful, suggesting the possible role of autoantibodies mediated immun reactions (Goebel et al 2010, 2011, Kohr et al 2011, Marinus et al 2011). Diseases due to pathogenic autoantibodies can sometimes be transferred to rodents by intra-peritoneal injection of patients' serum-IgG ('passive transfer').

METHODS

Experimental design and protocol: We investigated the IgG fractions purified from blood sera of 6 CRPS patients and 6 healthy volunteers sent by Dr. Goebel, using in the experimental paradigm of the murine model elaborated by us. All the patients fulfilled the following diagnostic criteria (Harden et al 2010): they had suffered from CRPS more than 1 year, but no other significant pains or medical disorders, their pain intensity was 5 or higher on a 11 point numeric rating scale (0-10), they had been seen at the study centre within the year before enrollment. Healthy volunteers were matched by age ($\pm$ 10 years) and gender to the patients, and had no chronic pain problems, their first degree relatives were not suffering from autoimmune disorders.

Mice (n=5-7 per group) were treated with the IgG fractions obtained from the 6 CRPS patients. IgG fractions obtained from healthy volunteers as well as saline-treated groups served for controls. The IgG concentrations from CRPS or healthy volunteers were same. Mice were treated intraperitoneally twice per day with serum IgG, or saline on days -1, 0, and 5, 6. 'Day 0' was the day of the plantar skin and muscle incision injury adapted for use in mice (Banick et al 2006, Pogatzki-Zahn et al 2007). We examined mechanical sensitivity (pain threshold) with dynamic plantar aesthesiometry, the paw volume with plethysmometry, heat and cold sensitivity, the spontanous weight distribution with incapacitance tester, the spontaneous locomotor activity with open field test, motoric coordination with Rotarod, the temperature of the plantar surface with contact thermometer, as well as the changes of body weight during the 8 days of the experiment. On day 8 the animals were sacrificed, their limbs were removed including the tibiotarsal joint and stored frozen at -80°C for later analysis of tissue neuropeptides and cytokines.

1. Investigation of touch sensitivity: Pain and mechanical hyperalgesia are the most common positive sensory signs in CRPS. The touch sensitivity measurements were performed before the passive-transfer treatment and on experimental days 1, 2, 3, 7 and 8.

2. Paw swelling: The other characteristic clinical symptom of CRPS-affected limbs is swelling. The paw volumes measuring with plethysmometry were investigated before passive transfer experiments (baseline), and on days 1, 2 ,3 , 7 and 8 of the experimental period.

3. Heat and cold sensitivity: Both heat and cold allodynia/hyperalgesia are common in CRPS affecting a third of patients. We measured the thermonociceptive threshold of the paw on days 1, 2, 3, 7 and 8 with an increasing temperature hot plate. Cold sensitivity was determined by the withdrawal latency after immersing the affected paw in 0 ^{o}C icy water at baseline, and on days 3, 7 and 8.

4. Spontaneous weight distribution: Spontaneous weight bearing on the hindlimbs was determined with the incapacitance tester and the measurements were performed on days 7 and 8.

5. Spontaneous locomotor activity and motoric coordination: The locomotor activity was assessed in a minimally anxiogenic open field test on days 0 and 6. Motoric function and coordination was investigated with RotaRod apparatus on days 0 and 6. The speed of the rotating wheel in the first 10 seconds was constant, then increased from 4 to 40 rounds per minute.

6. Determination of paw temperature and body weight monitoring: The temperature of the plantar surface of the hindpaws was measured with contact thermometer on day 7. Body weight of the animals was detected every day at the same time.

7. Determination of inflammatory neuropeptides and cytokines in tissue homogenates

After all functional testing had been completed on day 8, all animals were deeply anaesthetized , then sacrificed by cervical dislocation. The paws were excised, including the tibiotarsal joint, then the toes were removed and the samples were homogenized in sterile phosphate buffer. CGRP- and SP-like immunoreactivities were determined with the help of specific and sensitive radioimmunoassays, while tumor necrosis factor- alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1-beta (IL-1β) were simultaneously detected with Luminex® 100™ xMAP system according to protocol described in the user manual of the kit.

RESULTS

1. Mechanical hyperalgesia: The preoperative mechanonociceptive thresholds of the affected limbs were similar. The plantar skin-muscle incision decreased these thresholds by 45-50% in all three groups, one day after surgery. In the saline-treated control group the mechanical hyperalgesia recovered to 16 ± 2.2 % on day 3, and was thereafter maintained at that level. On day 7 hyperalgesia in the CRPS IgG-injected groups was significantly greater when compared to the healthy IgG-injected or saline treated groups.

2. Paw oedema: The preoperative control paw volumes of saline, healthy IgG and CRPS IgG-treated mice were similar. Plantar incision induced a 20.56 ± 2.52 % oedema formation relative to the preoperative volume, in the saline-treated control group one day after the surgical procedure, which gradually decreased to 10.52 ± 1.83 % by day 7. The greatest increase (32.3 ± 1.8%) in paw volume in

the CRPS group was seen on day 2, this was 45 % relative increase compared to the healthy IgG-treated group.

3. Heat and cold hyperalgesia: Measuring the thermonociceptive thresholds there were only minor changes in response to injury without significant differences between groups. Therefore we decided testing cold hyperalgesia in the further experiments. The cold sensitivity developed from the beginning of the experiments till day 8 in all groups, but without any significant differences.

4. **Spontanous weight distribution**: There was a mild reduction in weight bearing on the injured side in all groups with a peak on day 3, but no significant differences between the groups, testing was therefore discontinued.

5. Spontanous locomotor activity and motoric coordination: The spontaneous locomotor activity as determined in the open field test by the number of fields crossed and number of rearings, as well as time spent with moving, spent in the central regions and spent with grooming on days 0 and 6 did not differ significantly in all groups. There was also no significant difference in rotarod performance measuring on day 0 between groups.

6. Changes of paw temperature and body weight: There were no differences between CRPS-IgG, and saline or healthy-IgG groups in either the absolute paw temperatures of the injured limbs, or in the mean absolute temperature differences between the respective injured- and non-injured paws on days 1, 5 and 7. The weight did not change significantly during the 8 days of the experiment.

7. Determination of inflammatory neuropeptides and cytokines in tissue homogenates: Incision significantly increased SP concentration in all groups, but this increase was significantly greater in CRPS IgG-injected mice as compared to healthy IgG treatments. In contrast, CGRP-immunoractivity did not change either in response to injury, or IgG treatments. IL-1β, IL-6 and TNF-α concentrations were no different between groups.

CONCLUSIONS

We were the first who described, that the most prominent clinical signs (persistent pain/hyperalgesia, oedema) of CRPS occuring after minor limb injury can be reproduced by passive transfer human IgG to mice. Although most patients improve quickly, those whose condition becomes chronic (about 15%, Birklein et al 2004, de MM et al 2009) have persistent pain, whereas their initial limb signs, such as limb swelling often improve. The characteristics of our model resemble this pattern. These results suggest a possible role for pathogenetic autoantibodies in patients with long-standing CRPS. However in cold hyperalgesia significantly differences were not detected between the groups, mechanical hyperalgesia was severe in the CRPS IgG-treated groups. It has been suggested that central sensitization processes may contribute to development of mechanical hyperalgesia. An additional important finding paralleling human disease (Weber et al 2001) is the significantly increased concentration of the inflammatory neuropeptide SP in the injured paws of CRPS-IgG-injected animals.

The major strength of our results is the first delivery of a good reproducible model in which both limb injury and variant human condition (specific IgG serum-autoantibodies) are necessary elements, as in the clinical disease. With the help of human serum-IgG from patients with longstanding CRPS we induced the most prominent clinical (pain, oedema) and laboratory features (SP identified as a relevant pathophysiological factor) of the human disease. Our results should be important to clinical research, because they suggest the value of considering autoantibody-removing therapies for longstanding CRPS. The model is suitable to investigate the precise mechanisms of the disease, to identify the key mediators and target molecules, which may open new perspectives in pharmaceutical research.

SUMMARY OF THE NOVEL FINDINGS

1. Using exact measurement of threshold temperatures which elicit nocifensive reactions in rats both in the hot and cold range revealed that intraplantar injection of the TRPV1 receptor agonist capsaicin or RTX impairs noxious thermosensation in both ranges for several days albeit the recovery of the cold threshold is faster. These alterations indicate a functional desensitization of peripheral terminals of TRPV1-expressing sensory neurons responsible for noxious heat and cold responsiveness which could be differentiated from desensitization of then TRPV1 receptor by low doses of RTX or OLDA. Both types of desensitization have relevance for development of novel analgesics with a peripheral site of action. Nerve-ending desensitization is already exploited for analgesia in form of topically applied TRPV1 receptor agonists, but the employed experimental paradigms based on measurement of the noxious thermal thresholds may serve as novel in vivo preclinical screening methods.

2. We have firstly provided comparing data about TRPV1 receptor antagonists using methods based on a novel approach of noxious heat threshold measurement and a traditional technique of latency measurements. Our results demonstrate that the heat threshold drop evoked by direct TRPV1 receptor activation, mild heat injury or surgical incision are appropriate thermal hyperalgesia models that display a remarkably high sensitivity to TRPV1 receptor antagonists. The increasing temperature water bath is suitable to be a novel reliable preclinical testing method.

3. We provided evidence that the heat thresholds of TRPV1 gene-deleted mice were significantly higher compared to their wildtypes when measuring the tail, but not the paw. Despite of the literature TRPV1 receptors play a role in noxious heat sensation, at least on the murine tail. In TRPV1 gene-deficient mice the mustard oil-induced inflammatory thermal hyperalgesia decreased significantly in both examined body regions, but the mechanical hyperalgesia diminished only on the paws. There were no differences in both investigated body regions by in case of failuring TRPA1 receptor. By investigation of the latency of appearing the acute nocifensive reaction both TRPV1 and TRPA1 gene-deleted mice showed significantly later the nocifensive reaction compared to their wildtype counterparts. This is suggesting, that the TRPA1 agonist mustard oil is not selective to TRPA1, but it

can also activate TRPV1 receptor. This latter mechanism may mediate the inflammatory hyperalgesia and may have an involvement of nocifensive reaction.

4. We were the first who described, that the most prominent clinical signs (persistent pain/hyperalgesia, oedema) of CRPS occuring after minor limb injury can be reproduced by passive transfer human IgG to mice. Our results should be important to clinical research, because they suggest the value of considering autoantibody-removing therapies for longstanding CRPS. The model is suitable to investigate the precise mechanisms of the disease, to identify the key mediators and target molecules, which may open new perspectives in pharmaceutical research.

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REFERENCES

Almási R, Pethö G, Bölcskei K, Szolcsányi J. (2003).. *Br. J. Pharmacol.* 139, 49–58.

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. (2004). *Neuron* 41: 849–857.

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. (2006.) *Cell*. 24;124(6):1269-82.

Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, Jordt SE. (2008b). *J Clin Invest* 118: 1899–1910.

Bevan S, Szolcsányi J. (1990). *Trends in Pharmacological Sciences* 11: 330-333.

Birklein F, Riedl B, Sieweke N, Weber M, Neundorfer B. (2000/4). *Acta NeurolScand*; 101(4):262-269.

Blaes F, Tschernatsch M, Braeu ME, Matz O, Schmitz K, Nascimento D, Kaps M, Birklein F. (2007). *Ann N Y Acad Sci. 1107:168-73.*

Brederson JD, Kym PR, Szallasi A. (2013). *Eur J Pharmacol.* 716 (1-3):61-76.

Bölcskei K, Helyes Z, Szabó A, Sándor K, Elekes K, Németh J, Almási R, Pintér E, Pethő G, Szolcsányi J. (2005 *Pain.* 117(3):368-376.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. (1997). *Nature*. 389: 816-824.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. (2000). *Science.* 288, 306–313.

Chu CJ, Huang SM, De Petrocellis L, Bisogno T, Ewing SA, Miller JD, Zipkin RE, Daddario N, Appendino G, Di Marzo V, Walker JM. (2003). *J Biol Chem*. 278:13633–9.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latchman J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PA, Rogers DC, Bingham S, Randall A, Sheardown AS. (2000). *Nature.* 405, 183–187.

de M Mos, de Bruijn AG, Huygen FJ, Dieleman JP, Stricker BH, Sturkenboom MC. (2007). *Pain*. 129:12–20.

Everaerts W, Gees M, Alpizar YA, Farre R, Leten C, Apetrei A, Dewachter I, van Leuven F, Vennekens R, De Ridder D, Nilius B, Voets T, Talavera K. (2011). *Curr Biol.* 21(4):316-21.

Fernandes ES, Russell FA, Spina D, McDougall JJ, Graepel R, Gentry C, Staniland AA, Mountford DM, Keeble JE, Malcangio M, Bevan S, Brain SD. (2011). . *Arthritis Rheum.* (3):819-29.

Füredi R, Bölcskei K, Szolcsányi J, Pethő G. (2009). *Eur. J. Pharmacol*. 605, 63–67.

Gees M1, Alpizar YA, Boonen B, Sanchez A, Everaerts W, Segal A, Xue F, Janssens A, Owsianik G, Nilius B, Voets T, Talavera K. (2013). *Mol Pharmacol.* 84(3):325-34. doi: 10.1124/mol.113.085548.

Geppetti P, Nassini R, Materazzi S, Benemei S. (2008). *BJU Int*.;101 Suppl 3:2-6.

Goebel A, Baranowski AP, Maurer K, Ghiai A, McCabe C, Ambler G. (2010). *AnnInternMed.* 152(3):152-158.

Goebel A, Leite MI, Yang L, Deacon R, Cendan CM, Fox-Lewis A, Vincent A. (2011). *Eur J Pain* 15(5):504 e501-506.

Gu Q, Lin RL. (2010). *J Appl Physiol* 108: 891–897.

Gunthorpe MJ, Benham CD, Randall A, Davis JB. (2002). *Trends Pharmacol Sci.* 23: 183-191.

Harden RN, Bruehl S, Perez RS, Birklein F, Marinus J, Maihofner C, Lubenow T, Buvanendran A, Mackey S, Graciosa J, Mogilevski M, Ramsden C, Chont M, Vatine JJ. (2010). *Pain*. 8; 150 (2):268-274.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. (1988).. *Pain* 32, 77–88.

Helyes Zs, Pintér E, Németh J, Szolcsányi J. (2003). *Curr. Med. Chem*. 2: 191-218.

Holzer P. (1991). *Pharmacol. Rev*. 43: 143-201.

Hu H, Bandell M, Petrus MJ, Zhu MX, Patapoutian A. (2009). *Nat Chem Biol* 5: 183–190.

Jaquemar D, Schenker T, Trueb B. (1999). *J Biol Chem.* 274(11):7325-33.

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED, Meng ID, Julius D. (2004). *Nature* 427: 260–265.

Kohr D, Singh P, Tschernatsch M, Kaps M, Pouokam E, Diener M, Kummer W, Birklein F, Vincent A, Goebel A, Wallukat G, Blaes F. (2011). *Pain.* 152(12):2690-2700.

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP. (2006). *Neuron.* 20;50(2):277-89.

Levine JD, Collier DH, Basbaum AI, Moskowitz MA, Helms CA. (1986). *J Rheumatol* 12: 406-11.

Macpherson LJ, Hwang SW, Miyamoto T, Dubin AE, Patapoutian A, Story GM. (2006). *Mol. Cell. Neurosci.* 32:335–343.

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A. (2005). *Curr. Biol.* 15: 929–934.

Marinus J, Moseley GL, Birklein F, Baron R, Maihofner C, Kingery WS, Van Hilten JJ. (2011). *Lancet Neurol.*10(7):637-648.

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM. (2007). *Proc. Natl. Acad. Sci. USA.* 104(33):13525-30.

Miyamoto R, Otsuguro K, Ito S. (2011). *Neurosci Lett*. 499(2):137-42.

Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M. (2005). *Mol. Pain* 1, 3.

Nagata K, Duggan A, Kumar G, García-Añoveros J. (2005). *J. Neurosci.* 25(16):4052-61.

Obata K, Katsura H, Mizushima T, Yamanaka H, Kobayashi K, Dai Y, Fukuoka T, Tokunaga A, Tominaga M, Noguchi K. (2005). *J. Clin. Invest*. 115(9):2393-401.

Pingle SC, Matta JA, Ahern GP. (2007). *Handbook of Experimental Pharmacolog*. 179:155-171.

Sawada Y, Hosokawa H, Matsumura K, Kobayashi S. (2008). *Eur J Neurosci.* 27(5):1131-42.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, 0Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A. (2003). Cell. 112:819–29.

Szallasi A, Szabó T, Bíró T, Modarres S, Blumberg PM, Krause JE, Cortright DN, Appendino G. (1999). Br J Pharmacol. 128(2):428-34.

Szallasi A, Joo F, Blumberg PM. (1989). *Brain Res*. 503(1):68-72.

Szolcsányi J, Sándor Z, Petho G, Varga A, Bölcskei K, Almási R, Riedl Z, Hajos G, Czéh G. (2004). *Neurosci Letter*. 361:155–8.

Szolcsányi J, Helyes Zs, Oroszi G, Németh J, Pintér E. (1998a). *British Journal of Pharmacology*. 123: 936-942.

Szolcsányi J, Pintér E, Helyes Zs, Oroszi G, Németh J. (1998b). *British Journal of Pharmacology* 125: 916-922.

Szolcsányi J. (1993). *London: Academic Press*. p. 1–26.

Szolcsányi J. (1988). *Agents Actions*. 23: 4-11.

Szolcsányi J. (1987). *J Physiol (Lond).* 388:9–23.

Szolcsányi J. (1984a.) pp. 7-26, *Akadémiai Kiadó*, Budapest.

Szolcsányi J. (1984b). eds: Chahl LA, Szolcsányi J, Lembeck F), pp. 27-53, *Akadémiai Kiadó*, Budapest.

Szolcsányi J, Jancsó-Gábor A. (1975). *Arzneimittelforschung*. 25: 1877-1881.

Taylor-Clark TE, Undem BJ, Macglashan DW Jr, Ghatta S, Carr MJ, McAlexander MA. (2008). *Mol Pharmacol* 73: 274–281.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann, B.E., Basbaum, A.I., Julius, D. (1998). *Neuron*. 21:531-543.

Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andrè E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW,Julius D, Geppetti P. (2007). *Proc. Natl. Acad. Sci. USA* 104:13519–13524.

Weber M, Birklein F, Neundorfer B, Schmelz M. (2001). *Pain*. 91(3):251-257.

PUBLICATIONS LIST

Full-length articles related to the present thesis

Valéria Tékus, Zsófia Hajna, Éva Borbély, Adrienn Markovics, Teréz Bagoly, János Szolcsányi, Victoria Thompson, Ágnes Kemény, Zsuzsanna Helyes, Andreas Goebel: A CRPS-IgG-transfer-trauma model reproducing inflammatory and positive sensory signs associated with Complex Regional Pain Syndrome.
Pain. 2014 Feb; 155 (2):299-308. doi: 10.1016/j.pain.2013.10.011. Epub 2013 Oct 18. (**IF:5,644**)

Tékus V., Bölcskei K., Kis-Varga A., Dézsi L., Szentirmay E., Visegrády A., Horváth C., Szolcsányi J., Pethő G.: Effect of transient receptor potential vanilloid 1 (TRPV1) receptor antagonist compounds SB705498, BCTC and AMG9810 in rat models of thermal hyperalgesia measured with an increasing-temperature water bath.
Eur. J. Pharmacol. 641(2-3), 2010, 135-141. **(IF: 2.737 IC:4)**

Bölcskei K., **Tékus V**., Dézsi L., Szolcsányi J., Pethő G.: Antinociceptive desensitizing actions of TRPV1 receptor agonists capsaicin, resiniferatoxin and N-oleoyldopamine as measured by determination of the noxious heat and cold thresholds in the rat.
Eur. J. Pain 14 (5), 2010, 480-486. **(IF:3.819 IC:3)** (**contributing equally**)

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Oral and poster presentations related to the present thesis

Valéria Tékus, Hajna Zsófia, Borbély Éva, Markovics Adrienn, Bagoly Teréz, Szolcsányi János, Thompson Victoria, Kemény Ágnes, Andreas Goebel, Zsuzsanna Helyes: Passive- transfer-trauma model for the Complex Regional Pain Syndrome (CRPS) in mice *II. Pécs-Oklahoma Szimpózium, Pécs, 2013.* (oral)

Hajna Zsófia, **Valéria Tékus**, Borbély Éva, Markovics Adrienn, Bagoly Teréz, Szolcsányi János, Thompson Victoria, Kemény Ágnes, Andreas Goebel, Zsuzsanna Helyes: Passive- transfer-trauma model for the Complex Regional Pain Syndrome (CRPS) in mice *Neuroinflammation Crongess, Prága, 2013.* (poster)

Valéria Tékus, Hajna Zsófia, Borbély Éva, Markovics Adrienn, Bagoly Teréz, Szolcsányi János, Thompson Victoria, Kemény Ágnes, Andreas Goebel, Zsuzsanna Helyes: Passive- transfer-trauma model for the Complex Regional Pain Syndrome (CRPS) in mice. *From Bionics to Medicine 1st European Ph.D. Conference Budapest, 2013.* (poster)

Tékus Valéria, Kóger Tamás, Scheich Bálint, Hajna Zsófia Pintér, Erika, Szolcsányi János, Helyes Zsuzsanna: A Tranziens Receptor Potenciál Ankyrin 1 (TRPA1) ioncsatorna szerepe fájdalomérző folyamatokban
Magyar Élettani Társaság, a Magyar Anatómusok Társasága, a Magyar Biofizikai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Kongresszusa, Budapest, 2013. (oral)

Zsuzsanna Helyes, **Valéria Tékus**, Hajna Zsófia, Borbély Éva, Markovics Adrienn, Bagoly Teréz, Szolcsányi János, Thompson Victoria, Kemény Ágnes, Andreas Goebel: A komplex regionális fájdalom szindróma (CRPS) passzív transzfer-trauma egérmodellje. *Magyar Élettani Társaság, a Magyar Anatómusok Társasága, a Magyar Biofizikai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Kongresszusa, Budapest, 2013.* (poster)

Valéria Tékus, Tamás Kóger, Bálint Scheich, Zsófia Hajna, Éva Borbély, Erika Pintér, János Szolcsányi, Zsuzsanna Helyes: Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel in nociceptive processes. *10th Joint Meeting of the European Neuropeptide Club and the Summer Neuropeptide Conference, Gdynia 2013.* (poster)

Valéria Tékus, Bálint Scheich, Tamás Kóger, Ádám Horváth, Zsófia Hajna, Éva Borbély, Erika Pintér, János Szolcsányi, Zsuzsanna Helyes: Role of Transient Receptor Ankyrin 1 (TRPA1) ion channel in nociceptive processes. *Magyar Idegtudományi Társaság XIV. Konferenciája, 2013. Budapest* (poster)

Tékus V., Helyes Z., Hajna Z., Horváth Á,, Kun J., Bölcskei K., Pintér E., Szolcsányi J.: Transient Receptor Potential Vanilloid (TRPV1), but not Ankyrin 1 (TRPA1) ion channels mediate mustard oil-induced hyperalgesia in mice. *I. International Doctoral Workshop of Natural Sciences 2012. Pécs* (oral)

Tékus, V, Hajna, Z, Horváth, A, Kun J, Bölcskei, K, Szolcsányi, J and Helyes Z. : Role of the Transient Receptor Potential Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) ion channels in thermonociception in mice. *IBRO International Workshop 2012. Szeged, Hungary* (poster)

Tékus, V, Helyes Z., Hajna, Z, Horváth, A, Kun J, Bölcskei, K, Pintér E. Szolcsányi, J: Involvement of Transient Receptor Vanilloid 1 (TRPV1) but not Ankyrin 1 (TRPA1) ion channels in mustard oil-induced hyperalgesia in the mouse. *9th Joint Meeting of the European Neuropeptide Club and the Summer Neuropeptide Conference, London 2012.* (poster)

Bölcskei Kata, **Tékus Valéria**, Pethő Gábor, Szolcsányi János: A termonocicepció új állatkísérletes vizsgálómódszerei: a magatartási nociceptív hőküszöb mérése. *Magyar Élettani Társaság, a Magyar Anatómusok Társasága, a Magyar Biofizikai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Kongresszusa, Debrecen, 2012.* (oral)

Valéria Tékus, Kata Bölcskei, Ágnes Kis-Varga, László Dézsi, Csilla Horváth, Gábor Pethő, János Szolcsányi: Antihyperalgesic effect of Transient Receptor Potential Vanilloid 1 (TRPV1) receptor antagonists SB705498, BCTC, AMG9810 in the rat measured with an increasing-temperature water bath .*IBRO International Workshop, Pécs, 2010.* (poster)

Gábor Pethő, Kata Bölcskei, **Valéria Tékus**, Ágnes Kis-Varga, László Dézsi, Csilla Horváth, János Szolcsányi: Models for screening in rats the effects of TRPV1 receptor antagonists on thermal hyperalgesia and thermoregulation. *WorldPharma, Copenhagen, 2010.* (poster)

Valéria Tékus, Kata Bölcskei, László Dézsi, János Szolcsányi: Effects of the TRPV1 receptor antagonists SB705498, BCTC, AMG9810 in rat models of thermal hyperalgesia using noxious heat threshold measurements *12th World Congress of Pain, Glasgow, 2008* (poszter)

Dézsi László, Bölcskei Kata, **Tékus Valéria**, Szolcsányi János: Kapszaicin (TRPV1) receptor blokkolók hatása a nociceptív hőküszöbre patkány termális hiperalgézia modellekben
Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium, 2008. Zalakaros (oral)

Dézsi László, Bölcskei Kata, Sándor Zoltán, **Tékus Valéria,** Szőke Éva, Tóth Dániel Márton és Szolcsányi János: Kísérletes mérési módszerek alkalmazása a fájdalomcsillapítók kutatásában
In: Proc. of BUDAMED '08 14. MATE, 5 MEDING Orvostechnikai Konferencia, 2008. Budapest (oral)

Valéria Tékus, Kata Bölcskei, László Dézsi, János Szolcsányi: Effects of the TRPV1 receptor antagonists SB705498, BCTC, AMG9810 in rat models of thermal hyperalgesia using noxious heat threshold measurements *Richter Gedeon Kutatási Fórum, Visegrád 2008.* (poster)

Tékus Valéria, Dézsi László, Bölcskei Kata, Szolcsányi János: TRPV1 receptor antagonisták hatása a fájdalmas hőküszöbre patkány termális hiperalgézia modellekben *MKKFT-MÉT Vándorgyűlés, Debrecen 2008.* (poster)

Tékus Valéria, Bölcskei Kata, Tóth Dániel Márton, Dézsi László, Szolcsányi János: Az SB705498 TRPV1 receptor antagonista vegyület hatásai patkány in vivo nociceptív modellekben. *Magyarországi Fájdalom Társaság Konferenciája, Kecskemét, 2007.* (poster)

Dézsi László, Bölcskei Kata, **Tékus Valéria**, Tóth Dániel Márton, Horváth Csilla,Pethő Gábor, Szolcsányi János: Kísérleti módszerek kapszaicin érzékenyreceptorok által közvetített fájdalom vizsgálatára.
Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium, Eger, 2007. (oral)

Dézsi László, **Tékus Valéria**, Tóth Dániel Márton, Bölcskei Kata, Horváth Csilla, Pethő Gábor és Szolcsányi János: Nocicepció vizsgálata patkány in vivo modelleken a PTE-RG Analgetikum Kutatólaboratóriumban
Richter Gedeon Kutatási Fórum, Dobogókő, 2007. (oral)

Articles not related to the thesis

de Oliveira C, Garami A, Lehto SG, Pakai E, **Tekus V**, Pohoczky K, Youngblood BD, Wang W, Kort ME, Kym PR, Pinter E, Gavva NR, Romanovsky AA.: Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents.
J. Neurosci. 2014 Mar 26;34 (13):4445-52. doi: 10.1523/JNEUROSCI.5387-13.2014**. (IF: 7,271; IC:0)**

Helyes Zs., Sándor K., Borbély É., **Tékus V.,** Pintér E., Elekes K., Tóth D.M., Szolcsányi J., J.J. McDougall: Involvement of Transient Receptor Potential Vanilloid 1 receptors in Protease-Activated Receptor 2-induced joint inflammation and nociception. *Eur. J. Pain* 14(4), 2010, 351-358. **(IF: 3.819; IC: 7)**

Elekes K., Helyes Zs., Kereskai L., Sándor K., Pintér E., Pozsgai G., **Tékus V.,** Bánvölgyi Á., Németh J., Szűts T., Kéri Gy., Szolcsányi J.: Inhibitory effects of synthetic somatostatin receptor subtype 4 agonists on acute and chronic airway inflammation and hyperreactivity in the mouse.
Eur. J. Pharmacol. **578(2-3),** 2008, 313-322. **(IF:2,787, IC:14)**

Abstract published in cited journals:

Tékus, V, Hajna, Z, Horváth, A, Kun J, Bölcskei, K, Szolcsányi, J and Helyes Z.: Role of the Transient Receptor Potential Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) ion channels in thermonociception in mice *Clinical Neuroscience* 2012; 65: (1). pp.68 **(IF:1.247)**

Scheich B, Kormos V, **Tékus V**, Hajna Z, Gaszner B, Pintér E, Szolcsányi J, Helyes Z: Somatostatin receptor subtype 4 (sst4) plays an inhibitory role in anxiety and depression-like behaviours of mice. *Clinical Neuroscience* 2012; 65: (1). pp.57 (**IF:1.247)**

Pethő G, Bölcskei K, **Tékus V,** Dézsi L, Szolcsányi J. Antinociceptive desensitizing actions of TRPV1 receptor agonists capsaicin, resiniferatoxin and N-oleoyldopamine as measured by determination of the noxious heat and cold thresholds in the rat. *Acta Physiol Hung* 2010; 97:(1) pp. 129-130 **(IF: 0.453)**

Helyes Z, Sandor K, **Tekus V**, Pinter E, Elekes K, Toth DM, Szolcsanyi J, McDougall JJ**.** Involvement of TRPV1 receptors in protease-activated receptor 2-induced joint inflammation and nociception *NEUROPEPTIDES* 43: (5)*451* (2009) **(IF:2.036)**

Helyes Zs., Sándor K., **Tékus V.,** Pintér E., Elekes K., Tóth D.M., Szolcsányi J., J.J. McDougall: Involvement of TRPV1 receptors in protease-activated receptor 2-induced joint inflammation and nociception. Joint Meeting of the European Neuropeptide Club and Summer Neuropeptide Conference, Salzburg, Austria, 2009 - absztrakt: *Neuropeptides* 43(5), p.451., 2009. **(IF:2.438)**

Tékus V, Dézsi L, Bölcskei K, Szolcsányi J.: Effects of TRPV1 receptor antagonists on the noxious heat threshold in rat thermal hyperalgesia models. *Acta Physiol Hung* 96:(1), 2009. p. 68. **(IF: 0.75**)

Tóth DM, Szőke É, Bölcskei K, **Tékus V**, Dézsi L, Sándor Z, Szolcsányi J; Inhibition of the expression of the rat TRPV1 receptor by RNA interference. A Magyar Kísérletes és Klinikai Farmakológiai Társaság és a MÉT LXXII. Vándorgyűlése, Debrecen, Magyarország, 2008 (poszter). absztrakt: *Acta Physiol Hung* 2009, 96:(1): p.138 **(IF: 0.75)**

Dézsi L, Laszy J, Kocsis P, Bölcskei K, **Tékus V**, Tarnawa I, Gyertyán I, Szolcsányi J. Hippocampus-dependent learning and cortical spreading depression unaffected by TRPV1 receptor. Joint Meeting of the European Neuropeptide Club and Summer Neuropeptide Conference, Salzburg, Austria, 2009 - absztrakt: Neuropeptides 2009; 43:(5) pp. 450-451 **(IF: 2.036)**

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