Investigation of TRP channel- mediated effects of hydrogen sulphide in animal models of acute and chronic arthritis

The main original findings of the project:

1.The transient receptor potential ankyrin 1 (TRPA1) is a calcium-permeable cation channel that is expressed on capsaicin-sensitive sensory neurons, endothelial and inflammatory cells. It is activated by a variety of inflammatory mediators, such as methylglyoxal, formaldehyde and hydrogen sulphide. Since only few data are available about the role of TRPA1 in arthritis and related pain, we investigated its involvement in inflammation models of different mechanisms.
Chronic arthritis was induced by complete Freund's adjuvant (CFA), knee osteoarthritis by monosodium iodoacetate (MIA) in TRPA1 knockout (KO) mice and C57Bl/6 wild-type mice. For comparison, carrageenan- and CFA-evoked acute paw and knee inflammatory changes were investigated. Thermonociception was determined on a hot plate, cold tolerance in icy water, mechanonociception by aesthesiometry, paw volume by plethysmometry, knee diameter by micrometry, weight distribution with incapacitance tester, neutrophil myeloperoxidase activity and vascular leakage by in vivo optical imaging, and histopathological alterations by semiquantitative scoring.
CFA-induced chronic mechanical hypersensitivity, tibiotarsal joint swelling and histopathological alterations, as well as myeloperoxidase activity in the early phase (day 2), and vascular leakage in the later stage (day 7), were significantly reduced in TRPA1 KO mice. Heat and cold sensitivities did not change in this model. Although in TRPA1 KO animals MIA-evoked knee swelling and histopathological destruction were not altered, hypersensitivity and impaired weight bearing on the osteoarthritic limb were significantly decreased. In contrast, carrageenan- and CFA-induced acute inflammation and pain behaviours were not modified by TRPA1 deletion.
Reference: Ádám Horváth, Valéria Tékus, Melinda Boros, Gábor Pozsgai, Bálint Botz, Éva Borbély, János Szolcsányi, Erika Pintér and Zsuzsanna Helyes: Transient receptor potential ankyrin 1 (TRPA1) receptor is involved in chronic arthritis: in vivo study using TRPA1-deficient mice, Arthritis Research & Therapy, 2016

2. It is supposed that TRPA1 receptor can be activated by hydrogen sulphide (H2S). Here, we have investigated the role of TRPA1 receptor in H2S-induced [Ca(2+)]i increase in trigeminal ganglia (TRG) neurons, and the involvement of capsaicin-sensitive sensory nerves in 2S-evoked cutaneous vasodilatation. [Ca(2+)]i was measured with ratiometric technique on TRG neurons of TRPA1(+/+) and TRPA1(-/-) mice after NaHS, Na2S, allylisothiocyanate (AITC) or KCl treatment. Microcirculatory changes in the ear were detected by laser Doppler imaging in response to topical NaHS, AITC, NaOH, NaSO3 or NaCl. Mice were either treated with resiniferatoxin (RTX), or CGRP antagonist BIBN4096, or NK1 receptor antagonist CP99994, or K(+) ATP channel blocker glibenclamide. Alpha-CGRP(-/-) and NK1 (-/-) mice were also investigated.
NaHS and Na2S increased [Ca(2+)]i in TRG neurons derived from TRPA(+/+) but not from TRPA1(-/-) mice. NaHS increased cutaneous blood flow, while NaOH, NaSO3 and NaCl did not cause significant changes. NaHS-induced vasodilatation was reduced in RTX-treated animals, as well as by pre-treatment with BIBN4096 or CP99994 alone or in combination. NaHS-induced vasodilatation was significantly smaller in alpha-CGRP(-/-) or NK1 (-/-) mice compared to wild-types. H2S activates capsaicin-sensitive sensory nerves through TRPA1 receptors and the resultant vasodilatation is mediated by the release of vasoactive sensory neuropeptides CGRP and substance P.
Reference: Hajna Z, Saghy E, Payrits M, Aubdool AA, Szoke E, Pozsgai G, Batai IZ, Nagy L, Filotas D, Helyes Z, Brain SD, Pinter E: Capsaicin-Sensitive Sensory Nerves Mediate the Cellular and Microvascular Effects of H2S via TRPA1 Receptor Activation and Neuropeptide Release., JOURNAL OF MOLECULAR NEUROSCIENCE 60:(2) pp. 157-170. 2016

3. TRPA1 receptors are calcium-permeable ligand-gated channels expressed in primary sensory neurons and involved in inflammation and pain. Activation of these neurons might have analgesic effect. Suggested mechanism of analgesic effect mediated by TRPA1 activation is the release of somatostatin (SOM) and its action on sst4 receptors. In the present study analgesic effect of TRPA1 activation on primary sensory neurons by organic trisulfide compound dimethyl trisulfide (DMTS) presumably leading to SOM release was investigated. Opening of TRPA1 by DMTS in CHO cells was examined by patch-clamp and fluorescent Ca2+ detection. Ca2+ influx upon DMTS administration in trigeminal ganglion (TRG) neurons of TRPA1 receptor wild-type (WT) and knockout (KO) mice was detected by ratiometric Ca2+ imaging. SOM release from sensory nerves of murine skin was assessed by radioimmunoassay. Analgesic effect of DMTS in mild heat injury-induced mechanical hyperalgesia was examined by dynamic plantar aesthesiometry. Regulatory role of DMTS on deep body temperature (Tb) was measured by thermocouple thermometry with respirometry and by telemetric thermometry.
DMTS produced TRPA1-mediated currents and elevated [Ca2+]i in CHO cells. Similar data were obtained in TRG neurons. DMTS released SOM from murine sensory neurons TRPA1-dependently. DMTS exerted analgesic effect mediated by TRPA1 and sst4 receptors. DMTS-evoked hypothermia and hypokinesis were attenuated in freely-moving TRPA1 KO animals. Our study has presented original evidence regarding analgesic action of DMTS which might be due to TRPA1-mediated SOM release from sensory neurons and activation of sst4 receptors. DMTS could be a novel analgesic drug candidate.
Reference: Pozsgai G, Payrits M, Saghy E, Sebestyen-Batai R, Steen E, Szoke E, Sandor Z, Solymar M, Garami A, Orvos P, Talosi L, Helyes Z, Pinter E: Analgesic effect of dimethyl trisulfide in mice is mediated by TRPA1 and sst4 receptors, NITRIC OXIDE-BIOLOGY AND CHEMISTRY, 2017

4. In the frame of the study, we set out to compare the participation of this mechanism in antinociceptive and anti-inflammatory effects of inorganic sodium POLY and DMTS in carrageenan-evoked hind-paw inflammation. Inflammation of murine hind paws was induced by intraplantar injection of carrageenan (3% in 30 µL saline). Animals were treated i.p. with POLY (17 µmol/kg) or DMTS (250 µmol/kg) or their respective vehicles 30 min prior paw challenge and six times afterward every 60 min. Mechanical pain threshold and swelling of the paws were measured by dynamic plantar aesthesiometry and plethysmometry at 2, 4, and 6 h after initiation of inflammation. Myeloperoxidase (MPO) activity in the hind paws were detected 6 h after challenge by luminescent imaging. Mice genetically lacking TRPA1 ion channels, sst4 receptors and their wild-type counterparts were used to examine the participation of these proteins in POLY and DMTS effects.
POLY counteracted carrageenan-evoked mechanical hyperalgesia in a TRPA1 and sst4 receptor-dependent manner. POLY did not influence paw swelling and MPO activity. DMTS ameliorated all examined inflammatory parameters. Mitigation of mechanical hyperalgesia and paw swelling by DMTS were mediated through sst4 receptors. These effects were present in TRPA1 knockout animals, too. DMTS inhibited MPO activity with no participation of the sensory neuron-SOM axis. While antinociceptive effects of POLY are transmitted by activation of peptidergic nerves via TRPA1, release of SOM and its effect on sst4 receptors, those of DMTS partially rely on SOM release triggered by other routes. SOM is responsible for the inhibition of paw swelling by DMTS, but TRPA1 does not contribute to its release. Modulation of MPO activity by DMTS is independent of TRPA1 and sst4.
Reference: Bátai IZ, Horváth Á, Pintér E, Helyes Z, Pozsgai G: Role of transient receptor potential ankyrin 1 ion channel and somatostatin sst4 receptor in the antinociceptive and anti-inflammatory effects of sodium polysulfide and dimethyl trisulfide, FRONTIERS IN ENDOCRINOLOGY, 2018

5. Hydrogen sulfide (H2S) influences nociception and inflammation in rheumatoid arthritis (RA). One target molecule of H2S is the neuronal TRPA1 ion channel. The present study investigates the effect of H2S from slow-release donor GYY4137 regarding the interaction with TRPA1 and sst4 in K/BxN serum-transfer arthritis. TRPA1 wild-type (WT), ss and knockout (KO) mice were injected i.p. with K/BxN or control BxN serum. Mice were treated daily with GYY4137 (50 mg/kg, i.p.). Severity of hind paw inflammation, grip stamina, mechanonociceptive threshold and swelling of the hind feet were assessed on days 3, 5 and 7. Myeloperoxidase (MPO) activity and plasma extravasation in the hind paws were registered on days 2 and 6 by luminescent and fluorescent imaging. On day 3 inflammatory mediators (IL-1β, KC, MIP-1α and MIP-2) were detected from the subcutaneous flushing fluid of hind feet by MILLIPLEX Assay. On day 14 and tibiotarsal joint samples underwent histological scoring of cartilage destruction, synovial hyperplasia, mononuclear cell infiltration and number of fibroblasts together with collagen deposition. H2S aggravated mechanical hyperalgesia in TRPA1 KO mice, but ameliorated it in WT ones. Arthritis score was lowered by H2S in TRPA1 WT animals. Increased MPO activity, plasma extravasation and MIP-2 levels were detected in TRPA1 KO mice upon H2S treatment. H2S did not affect body weight, hind paw swelling, concentration of IL-1β, KC, MIP-1α and histopathological changes. We did not detected any significant differences of the measured parameters in the sst4 wild-type a KO animals. According to our data, protective effect of H2S is mediated by TRPA1 while detrimental effects are independent of the ion channel in the K/BxN serum-transfer arthritis model in mice.

Reference: István Z. BÁTAI, Cecília PÁPAINÉ SAÁR, Ádám HORVÁTH, Éva BORBÉLY, Ágnes KEMÉNY, Zsuzsanna HELYES, Anikó PERKECZ, Attila MÓCSAI, Gábor POZSGAI, Erika PINTÉR: TRPA1 ion channel determines beneficial and detrimental effects of hydrogen sulfide in murine serum-transfer arthritis (manuscript in preparation)

Transient receptor potential canonical 5 (TRPC5) is functionally expressed on a range of cells including fibroblast-like synoviocytes, which play an important role in arthritis. A role for TRPC5 in inflammation has not been previously shown in vivo. We investigated the contribution of TRPC5 in arthritis. Male wild-type and TRPC5 knockout (KO) mice were used in a complete Freund's adjuvant (CFA)-induced unilateral arthritis model, assessed over 14 days. Arthritis was determined by measurement of knee joint diameter, hindlimb weightbearing asymmetry and pain behaviour. Separate studies involved chronic pharmacological antagonism of TRPC5 channels. Synovium from human postmortem control and inflammatory arthritis samples were investigated for TRPC5 gene expression. At baseline, no differences were observed. CFA-induced arthritis resulted in increased synovitis in TRPC5 KO mice assessed by histology. Additionally, TRPC5 KO mice demonstrated reduced ispilateral weightbearing and nociceptive thresholds (thermal and mechanical) following CFA-induced arthritis. This was associated with increased mRNA expression of inflammatory mediators in the ipsilateral synovium and increased concentration of cytokines in synovial lavage fluid. Chronic treatment with ML204, a TRPC5 antagonist, augmented weightbearing asymmetry, secondary hyperalgesia and cytokine concentrations in the synovial lavage fluid. Synovia from human inflammatory arthritis demonstrated a reduction in TRPC5 mRNA expression Genetic deletion or pharmacological blockade of TRPC5 results in an enhancement in joint inflammation and hyperalgesia. Our results suggest that activation of TRPC5 may be associated with an endogenous anti-inflammatory/analgesic pathway in inflammatory joint conditions.

Reference: Alawi et al. Transient receptor potential canonical 5 (TRPC5) protects against pain and vascular inflammation in arthritis and joint inflammation. ANN RHEUM DIS, 2017

Additional experimental results

Besides the main focus of the project we have also executed and published original findings concerning the supposed modulatory role of TRPA1 receptor in psoriasis. The fruitful cooperation between the Hungarian and British groups resulted in original findings in the psoriasis research. The OTKA grant supported the exchange of young investigators with short visits and provided running costs for the research. The mutual published paper (Kemény et al. 2018) has facilitated the planning and preparation of a subsequent successful four year OTKA grant application of the Hungarian research group in the 2018 round.

Reference: Kemény A, Kodji X, Horváth Sz, Komlódi R, Szőke E, Sándor Z, Perkecz A, Gyömörei Cs, Sétáló Gy, Kelemen B, Bíró T, Tóth B I, Brain S D, Pintér E, Gyulai R: TRPA1 acts in a protective manner in imiquimod-induced psoriasiform dermatitis in mice, JOURNAL OF INVESTIGATIVE DERMATOLOGY, 2018

We also published papers about the modulatory role of TRPV1 and TRPA1 receptors in oral lichen planus and nasal polyps using human samples.

References: Kun J, Perkecz A, Knie L, Setalo G Jr, Tornoczky T, Pinter E, Ban A: TRPA1 receptor is upregulated in human oral lichen planus, ORAL DISEASES, 2016

Toth E, Tornoczky T, Kneif J, Perkecz A, Katona K, Piski Z, Kemeny A, Gerlinger I, Szolcsanyi J, Kun J, Pinter E: Upregulation of extraneuronal TRPV1 expression in chronic rhinosinusitis with nasal polyps., RHINOLOGY, 2018

Furthermore, we have examined the anti-inflammatory effect of a Hungarian sulphur containing thermal water in murine models of the rheumatoid- and osteoarthritis. Our experimental data show that Heviz thermal water attenuates the pain and oedema in murine osteoarthritis.

Reference: V Tékus, É Borbély, T Kiss, A Perkecz, Á Kemény, J Horváth A Kvarda, E Pintér: Investigation of Lake Hévíz Mineral Water Balneotherapy and Hévíz Mud Treatment in Murine Osteoarthritis and Rheumatoid Arthritis Models, EVIDENCE-BASED COMPLEMENTARY AND ALTERNATIVE MEDICINE, 2018