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Capsaicin-sensitive sensory nerves exert complex regulatory functions in the serum-transfer mouse model of autoimmune arthritis

Éva Borbély [a,b,c,1], Bálint Botz [a,b,c,1], Kata Bölcskei [a,b,c], Tibor Kenyér [a], László Kereskai [d], Tamás Kiss [b], János Szolcsányi [a,b,c,f], Erika Pintér [a,b,c,f], Janka Zsófia Csepregi [e], Attila Mócsai [e], Zsuzsanna Helyes [a,b,c,f,g,*]

[a] Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Pécs, Hungary
[b] János Szentágothai Research Centre, Molecular Pharmacology Research Team, University of Pécs, Pécs, Hungary
[c] Centre for Neuroscience, University of Pécs, Medical School, Pécs, Hungary
[d] Department of Pathology, University of Pécs, Medical School, Pécs, Hungary
[e] Department of Physiology, and MTA-SE "Lendület" Inflammation Physiology Research Group, Semmelweis University, School of Medicine, Budapest, Hungary
[f] PharmInVivo Ltd, Pécs, Hungary
[g] MTA-PTE NAP B Pain Research Group, Hungary

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ABSTRACT

Objective: The K/BxN serum-transfer arthritis is a widely-used translational mouse model of rheumatoid arthritis, in which the immunological components have thoroughly been investigated. In contrast, little is known about the role of sensory neural factors and the complexity of neuro–immune interactions. Therefore, we analyzed the involvement of capsaicin-sensitive peptidergic sensory nerves in autoantibody-induced arthritis with integrative methodology.

Methods: Arthritogenic K/BxN or control serum was injected to non-pretreated mice or resiniferatoxin (RTX)-pretreated animals where capsaicin-sensitive nerves were inactivated. Edema, touch sensitivity, noxious heat threshold, joint function, body weight and clinical arthritis severity scores were determined repeatedly throughout two weeks. Micro-CT and *in vivo* optical imaging to determine matrix-metalloproteinase (MMP) and neutrophil-derived myeloperoxidase (MPO) activities, semiquantitative histopathological scoring and radioimmunoassay to measure somatostatin in the joint homogenates were also performed.

Results: In RTX-pretreated mice, the autoantibody-induced joint swelling, arthritis severity score, MMP and MPO activities, as well as histopathological alterations were significantly greater compared to non-pretreated animals. Self-control quantification of the bone mass revealed decreased values in intact female mice, but significantly greater arthritis-induced pathological bone formation after RTX-pretreatment. In contrast, mechanical hyperalgesia from day 10 was smaller after inactivating capsaicin-sensitive afferents. Although thermal hyperalgesia did not develop, noxious heat threshold was significantly higher following RTX pretreatment. Somatostatin-like immunoreactivity elevated in the tibiotarsal joints in non-pretreated, which was significantly less in RTX-pretreated mice.

Conclusions: Although capsaicin-sensitive sensory nerves mediate mechanical hyperalgesia in the later phase of autoantibody-induced chronic arthritis, they play important anti-inflammatory roles at least partially through somatostatin release.

1. Introduction

Rheumatoid arthritis (RA) is chronic autoimmune disease characterized by the destruction and deformation of the joints leading to persistent pain, movement disability and decreased life quality. It is a great public health problem worldwide due to its high incidence and prevalence, unsatisfactory therapeutic outcomes and unfavorable life expectancy (Kourilovitch et al., 2014; Jones et al., 2003). Despite promising novel drugs introduced recently in its pharmacotherapy, we still have to cope with several resistant cases and severe drug-induced adverse effects (Schett and Gravallese, 2012; Alarcón, 2000). Although our knowledge about the immunological aspects of the pathophysiological mechanisms has

* Corresponding author at: Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Szigeti u. 12., H-7624 Pécs, Hungary. Tel.: +36 72 536 000/35591; fax: +36 72 536 218.
E-mail address: zsuzsanna.helyes@aok.pte.hu (Z. Helyes).
[1] These authors made equal contributions to this work.

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extensively increased in the last decade, the regulatory role of sensory nerves and the complexity of neuro–immune interactions in this condition are still not understood (Levine et al., 2006; Pongratz and Straub, 2010; Meinel et al., 2013; Stangenberg et al., 2014).

Capsaicin-sensitive peptidergic sensory nerves densely innervate the joint capsule and the synovium, which do not only mediate pain (classical afferent function), but also play an important role in inflammation via sensory neuropeptide release (efferent function). The Transient Receptor Potential Vanilloid 1 (TRPV1) non-selective cation channel located on these nerves is activated and sensitized by a variety of exogenous irritants, such as capsaicin, and resiniferatoxin (RTX), as well as endogenous molecules like protons, bradykinin, prostanoids, tumor-necrosis factor-α, nerve growth factor, gasotransmitters or lipid peroxidase products (Yoo et al., 2014). Many of these are crucial participants of inflammatory processes in RA. As a result of activation of the capsaicin-sensitive nerve terminals, sensory neuropeptides are released, such as the proinflammatory tachykinins (substance P, neurokinin A) and calcitonin-gene related peptide (CGRP) responsible for vasodilation and inflammatory cell recruitment (neurogenic inflammation) (Maggi, 1995; Szolcsanyi, 1996), as well as somatostatin, which is a potent antiinflammatory and antinociceptive agent. We have provided several lines of evidence in a variety of inflammation models that the overall role of these fibers depends on the functional significances of the simultaneously released pro- and antiinflammatory peptides in the respective pathophysiological processes (Pintér et al., 2014). We have also shown that sensory nerve-derived somatostatin is an important endogenous inhibitor in the adjuvant-induced arthritis model of the rat (Helyes et al., 2004).

The pathophysiological relevance of these peptides in humans is beyond doubt, since increased proinflammatory and decreased antiinflammatory neuropeptide levels have been demonstrated in the serum and/or synovial fluid of RA patients (Anichini et al., 1997; Larsson et al., 1991; Denko and Malemud, 2004).

Investigating rheumatoid arthritis mechanisms in animals is difficult; therefore there are many different rodent models which can more or less mimic the main symptoms of the disease (Bevaart et al., 2010; Zhang et al., 2012; Boettger et al., 2010). The K/BxN serum-transfer arthritis is a widely-used translational mouse model of RA, it shares a lot of similarities to the human disease, e.g. swelling of distal joints of all the paws with erosive synovitis, caused by the activation of neutrophils, macrophages, complement system which play a pivotal role in the induction and maintenance of arthritis (Kouskoff et al., 1996; Korganow et al., 1999; Fukushima et al., 2010). The immunological components of this model have thoroughly been investigated (Németh et al., 2010; Hickman-Brecks et al., 2011), but nothing is known about the role of sensory neural factors and the complexity of neuro–immune interactions. Therefore, we analyzed the involvement of capsaicin-sensitive peptidergic sensory nerves in autoantibody-induced arthritis with integrative methodology after the functional impairment of these fibers with high dose RTX pretreatment (desensitization).

2. Material and methods

2.1. Ethics statement

Experiments 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), complied with the recommendations of IASP, and approved by the Ethics Committee on Animal Research of University of Pécs (licence: BA 02/2000-2/2012).

2.2. Experimental animals

Male and female C57Bl/6 mice (10–12-week-old; 25–30 g) bred and kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs at 24–25 °C under a 12-h light–dark cycle were used in all studies. Standard mouse chow and water were provided *ad libitum*.

2.3. Resiniferatoxin pretreatment

Pretreatment with the ultrapotent TRPV1 agonist resiniferatoxin (RTX, Sigma–Aldrich; 30, 70, 100 μg/kg s.c. on 3 consecutive days) leads to long-lasting defunctionalization of capsaicin-sensitive nerves (desensitization) (Szolcsanyi et al., 1990). Two weeks later the success of the pretreatment was verified by the lack of eye-wiping after capsaicin drops (50 μl, 0.1%) (Helyes et al., 2004).

2.4. Induction of arthritis

Chronic arthritis of male and female C57Bl/6 mice was induced by intraperitoneal (i.p.) injection of 150–150 μl of K/BxN serum on the days 0 and 3. Control groups of intact animals were treated with BxN (not arthritogenic/control) serum following the same protocol.

2.5. Assessment of arthritis severity and paw edema

Hind paw volume was measured by plethysmometry (Ugo Basile 7140, Comerio, Italy) (Helyes et al., 2004; Szabó et al., 2005). Arthritic changes were semiquantitatively scored using a grading scale of 0–10 (0–0.5: no change, 10: maximal inflammation) by evaluating edema and hyperemia (Németh et al., 2010). Volumes and scores were assessed before serum injection and every day during the 2-week period.

2.6. Measurement of mechanical and thermal hyperalgesia

Mechanonociceptive threshold of the paw was determined by dynamic plantar aesthesiometry (Ugo Basile 37400, Comerio, Italy) before and after serum administration. Mechanical hyperalgesia was expressed as % of initial, control mechanonociceptive thresholds (Helyes et al., 2004; Szabó et al., 2005). The thermonociceptive threshold of the paw was determined on increasing temperature hot plate (IITC Life Sciences, Woodland Hills, CA, USA) by nocifensive reactions (lifting, licking, shaking) or reaching the maximum value (53 °C) (Almási et al., 2003).

2.7. Assessment of joint function (grid test)

An easy and reproducible method to determine grasping ability correlating with joint function. Mice were placed on a horizontal wire-grid, then it was turned over and the latency to fall was determined (Németh et al., 2010).

2.8. Measurement of arthritis induced weight loss

As a typical sign of systemic effect of arthritis, mice lost weight after serum administration. Weight measurements were performed daily and weight loss was expressed in % of control values.

2.9. In vivo bioluminescence imaging of myeloperoxidase-activity

Luminol bioluminescence (BLI; 5-amino-2,3-dihydro-1,4-phthalazine-dione) correlates with neutrophil myeloperoxidase activity in arthritis *in vivo* (Chen et al., 2004; Gross et al., 2009). Na–luminol (150 mg/kg i.p., Sigma–Aldrich) dissolved in PBS

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