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RAPID COMMUNICATION

Parvalbumin and TRPV1 Receptor Expression in Dorsal Root Ganglion Neurons after Acute Peripheral Inflammation

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Summary

Expression of parvalbumin (PV) and transient receptor potential vanilloid (TRPV1) receptors in the lumbar dorsal root ganglion neurons (DRG) was evaluated in control animals and in rats after acute carageenan-induced knee joint inflammation. PV is a calcium binding protein that acts as a calcium buffer, affects intracellular calcium homeostasis and may thus influence signal transduction and synaptic transmission. TRPV1 receptors are viewed as molecular integrators of nociceptive stimuli and modulate spinal cord synaptic transmission beside their function in the peripheral nerve endings. In naive rats, 13 % of the L4 DRG neurons had PV immunopositivity (PV+) and 36 % expressed TRPV1 receptors (TRPV1+). The soma of the PV+ neurons was of medium to large size, while the TRPV1 receptors were expressed in small diameter neurons. The co-localization of the PV and TRPV1 immunoreactivity was minimal (0.2 %). There was no significant change in the PV+ (11 %), TRPV1+ (42 %) and PV+TRPV1+ (0.25 %) expression, or shift in the neuronal size distribution 28 h after the unilateral peripheral inflammation, both when compared to controls and when ipsilateral to contralateral sides were evaluated. Thus under the given experimental conditions, no change in somatic TRPV1 receptors and PV expression in L4 DRG neurons was found.

Key words

Pain • Arthritis • DRG • Caregeenan

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Modulation of synaptic activity at the spinal cord level is thought to be one of the underlying mechanisms of pathological pain states characterized by allodynia and hyperalgesia (Willis 2001). While number of molecules and receptors were suggested to play a role in this process, the changes in intracellular calcium concentration followed by activation of second messenger systems seem to be critical to this process. Parvalbumin (PV), a calcium-binding protein, acts as an intracellular calcium buffer (Neher and Augustine 1992) and was shown to affect significantly synaptic transmission (Caillard *et al.* 2000, Muller *et al.* 2007). It was shown previously that PV is expressed in medium and large sized lumbar DRG neurons that project to the spinal cord dorsal horn (Carr *et al.* 1989, Honda 1995, Jamieson *et al.* 2005).

The transient receptor potential vanilloid (TRPV1) receptors are expressed in small size DRG neurons (Caterina *et al.* 1999) and are transported to both peripheral and central branches of primary afferents (Guo *et al.* 1999). TRPV1 receptors viewed as a molecular integrator of chemical and physical nociceptive stimuli, have undisputable role in peripheral nociception (Szallasi *et al.* 2007) and recently their significance in modulation of nociceptive pain transmission at the spinal cord level has emerged (Špicarová and Paleček 2008). Experiments using TRPV1 knockout animals confirmed their importance for the development of inflammatory pain (Catarina *et al.* 2000). It was reported that acute peripheral inflammation leads to increased TRPV1 receptor expression in DRG neurons (Ji *et al.* 2002, Amaya *et al.* 2003) or to a shift in size distribution of the

TRPV1 expressing neurons from small to medium size (Luo *et al.* 2004), while others did not find any significant change (Bar *et al.* 2004, Zhou *et al.* 2003).

In our previous study (Zachařová *et al.* 2009), we have found decreased expression of PV in the spinal cord superficial dorsal horn after acute peripheral inflammation. This change could be due to decreased PV expression in GABA interneurons (Antal *et al.* 1991, Laing *et al.* 1994) and/or in projecting primary afferents, as slightly decreased PV staining in spinal dorsal horn was observed after unilateral ganglionectomy (Ren and Ruda 1994). Therefore in the present study, parvalbumin and TRPV1 receptor expression and co-expression in DRG neurons were evaluated in order to determine if carrageenan-induced peripheral inflammation changes the number or the size distribution of these neurons.

All procedures used in these experiments were reviewed and approved by the Institutional Animal Care and Use Committee and were consistent with the guidelines of the International Association for the Study of Pain for the care and use of laboratory animals. Adult male Wistar rats (250-350 g) were kept in plastic cages with soft bedding, free access to food and water and were maintained on a 12 h light, 12 h dark cycle. In total, 10 animals were used in this study.

Experimental arthritis was induced by unilateral intra-articular knee injection of a 3 % mixture of kaolin and carrageenan in saline solution (0.1 ml) under ether anesthesia. The rats were deeply anesthetized with ketamine (120 mg/kg i.p., Narkamon, Zentiva) and xylazine (10 mg/kg i.m., Rometar, Zentiva) 28 h after the arthritis induction and transcardially fixed with 4 % paraformaldehyde. The bilateral L4 dorsal root ganglia (DRG) were postfixed, cryo-protected and transversely serially sectioned at 15 μm thickness with cryocut (Leica). Every third serial section was used, so the sections were separated by at least 30 μm of tissue thickness. Sections were then double-immunostained with specific antibody to parvalbumin (monoclonal, Sigma P3088) and TRPV1 receptor (polyclonal, AB5370, Chemicon). Sections were blocked in 3 % donkey serum, incubated overnight with primary antibody and then with corresponding secondary antibody (Cy2, Texas Red, Jackson Immuno Research). Sections were counterstained with fluorescent nuclear dye Hoechst 33342 (Fluka) for nuclei identification. No positive staining was observed on control sections, when primary antibody was omitted in the process. The histological slides were digitized using fluorescent microscope (Olympus AX). Ten randomly selected sections from the control rats (no inflammation) and 20-25 sections from the experimental rats were evaluated from each DRG. Neuronal profiles were measured (area) and counted using morphometric Ellipse software (ViDiTo, Slovakia). Only profiles with visible nuclei (identified by nuclear stain Hoechst) were evaluated for immunopositivity for PV (PV+) and TRPV1 receptors (TRPV1+). On average, about 110 neurons were analyzed on one section. In each DRG the number of evaluated neurons was averaged and the means ± S.E.M. were then calculated for each evaluated group. Statistical significance was tested using ANOVA.

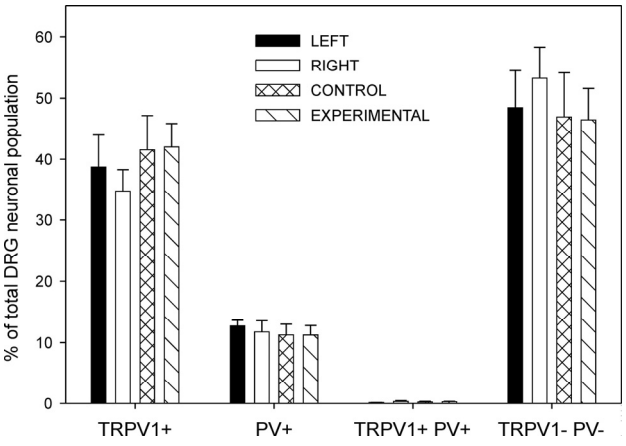

Fig. 1. There were no significant differences in expression of PV and TRPV1 receptors in the naive animals (left, right) and after acute unilateral inflammation (control, experimental) in L4 DRG neurons.

The injected knee joints and the surrounding tissues showed robust signs of inflammation, such as increased volume, redness and leg guarding behavior, at the time of the perfusion in the experimental group of animals. Altogether 7509 L4 DRG neuronal profiles were measured in the intact control rats (n=3) and 35176 L4 DRG profiles in the experimental animals (n=7). In the controls this represented altogether 2847 TRPV1+, 963 PV+, 12 TRPV1+PV+ and 3687 TRPV1-PV– neurons. In the experimental group of animals 16145 TRPV1+, 4376 PV+, 54 TRPV1+PV+ and 14601 TRPV1-PV– neurons were identified and measured. The number of the DRG neurons in each subgroup evaluated as percent of the total number of neurons measured in each ganglion is shown in Fig. 1. There was no significant difference between the control and experimental groups. It was also evident that there was only a minimal neuronal population (0.1-0.3 %) that expressed both PV and TRPV1 receptors.

The average cross-section areas of the evaluated DRG neuronal profiles in the experimental animals after

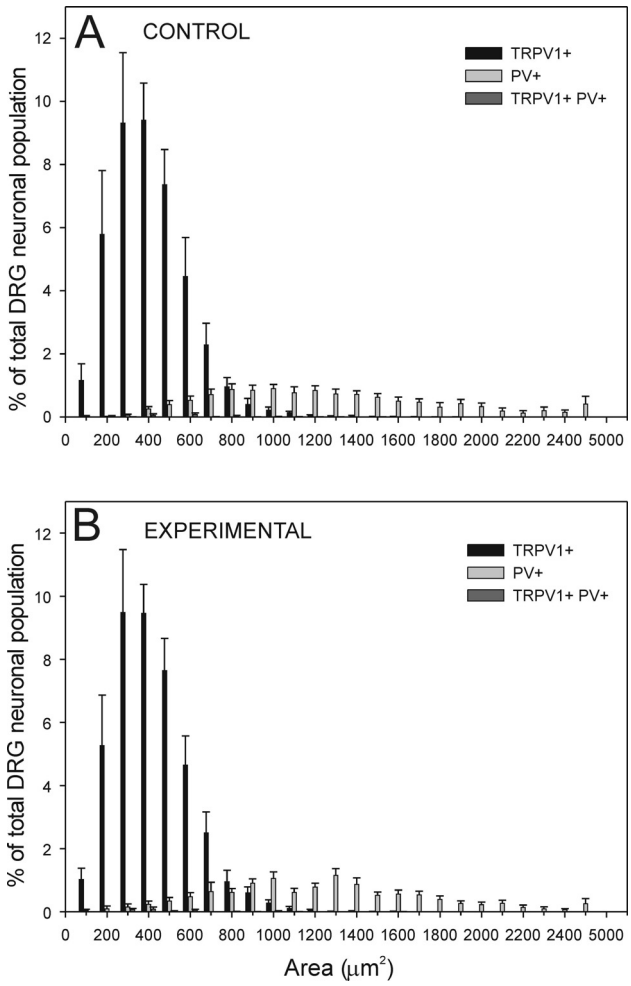

Fig. 2. The somatic size distribution of L4 DRG neurons expressing TRPV1 receptors and PV on the control (contralateral, A) and experimental (ipsilateral, B) side after acute unilateral inflammation.

peripheral inflammation are shown on a detailed histogram (Fig. 2). The values represent the average number of neurons in each size category in percent of the total number of evaluated neurons in each ganglion, with the negative (TRPV1– PV–) cells omitted from the histogram for better clarity. The profiles of the TRPV1+ neurons had small area between 100 and 1400 μm^2 with average value 470±31 μm^2 on the control and 482±32 μm^2 on the experimental side. The PV+ neuronal profiles were much bigger, the average area was 1277±90 μm^2 on the control and 1270±86 μm^2 on the experimental side. The statistical analysis did not show any significant difference of the TRPV1+ and PV+ DRG neuronal populations on the control (contralateral to inflammation) and experimental (ipsilateral) sides. The population of TRPV1+ PV+ neurons had profiles area between 100 and 1700 mm^2 on both sides. The number of these neurons was very limited – 0.2 % of the neurons on the control and 0.3 % on the experimental side. There was a higher number of these neurons bilaterally in the animals after inflammation when compared to the intact animals (7.7 vs. 4.0), but this difference did not reach statistical significance.

Our experiments demonstrated that there are only very few L4-DRG neurons that co-express TRPV1 receptors and PV, both under control conditions and after acute inflammation. The unilateral knee inflammation did not also affect PV or TRPV1 receptor expression in the L4-DRG neurons under the given experimental conditions. Although the duration of knee inflammation used in this study was short, changes in TRPV1 receptors (Luo *et al.* 2004) and PV (Zachařová *et al.* 2009) expression were described after 1 day of inflammation.

The proportion of PV positive profiles in the experimental (11 %) and control (12-13 %) animals was similar to the published data (14 % in L4, Carr *et al.* 1989; 11 % pooled L1-S1, 20 % L4, 14 % L5, Honda 1995; 14 % L5, Jamieson *et al.* 2005). The expression of PV in predominantly large DRG neurons corresponds to generally acknowledged view that PV+ DRG neurons function primarily as muscle proprioceptors (Honda 1995, Ichikawa *et al.* 1999). This is in contrast to the rat trigeminal ganglion neurons, where coexistence of PV with calcitonin gene related protein was found in small but significant population of primary neurons innervating the tooth pulp (Ichikawa *et al.* 1995, Ichikawa and Sugimoto 1997) and also co-expression of PV with the high threshold noxious heat activated TRPV2 receptor was found (Ichikawa and Sugimoto 2000), suggesting that subset of PV+ trigeminal primary neurons may be involved in nociception.

The acute inflammation significantly decreased expression of PV in the superficial spinal dorsal horn region (Zachařová *et al.* 2009). However, using the same experimental model in this study we did not observe any effect on PV expression in the DRG neurons. This suggests that the decrease in PV expression in the dorsal horn was preferentially in spinal, presumably GABA interneurons. This corresponds to study, where dorsal rhizotomy did not affect PV immunoreactivity in the spinal cord dorsal horn (Yamamoto *et al.* 1989), while slight decrease was observed after ganglionectomy (Ren and Ruda 1994).

The proportion and size distribution of L4-DRG TRPV1 receptors expressing neurons in our experiments fits well with values reported in other studies (Caterina *et al.* 1999, Zhou *et al.* 2003, Bar *et al.* 2004, Fernihough *et*

al. 2005, Hensellek *et al.* 2007). Published data regarding expression of TRPV1 receptors in DRG neurons after peripheral inflammation are contradictory. Similar to our study, Bar *et al.* (2004) did not find change in proportion of lumbar (pooled L1-L5) DRG neurons in acute (3 days) or chronic (21 days) antigen-induced knee joint arthritis and after acute carrageenan or chronic CFA inflammation of the hindpaw, no change was found in the number of TRPV1 expressing L5-DRG neurons (Zhou *et al.* 2003). On the contrary, increased TRPV1 immunoreactivity on ipsilateral side was reported 2 days after peripheral inflammation induced by plantar injection of Freund's complete adjuvant in L4 and L5 lumbar DRG (Ji *et al.* 2002, Amaya *et al.* 2003), while no change (Ji *et al.* 2002) or decrease (Tohda *et al.* 2001) in the level of TRPV1 mRNA in the DRG was reported.

In our experiments minimal co-localization of PV with TRPV1 receptor was found in L4-DRG neurons in control rats which corresponds well to the expression of TRPV1 receptors predominantly in small size DRG neurons and PV presence preferentially in medium and large size DRG cells (Guo *et al.* 1999, Caterina *et al.* 1999, Jamieson *et al.* 2005). It was demonstrated previously that unilateral peripheral inflammation can induce shift in size distribution of the TRPV1 expressing neurons from small to medium size (Luo *et al.* 2004). In our experiments we did not find significant shift in the size distribution of the TRPV1 expressing DRG neurons and the proportion of PV+ cells which co-expressed TRPV1 did not change significantly after the experimental knee arthritis. However, we cannot exclude the possibility that in our study the possible change in expression may have occurred only in a small restricted subpopulation of TRPV1+ or PV+ neurons, similar to what was found in the iodoacetate arthritis model (Fernihough *et al.* 2005).

Our study did not show any significant changes in the number or in the size distribution of L4 DRG neurons expressing and co-expressing PV and TRPV1 receptors after acute carageenan peripheral inflammation.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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