ABSTRACT

The first nociceptive synapse in the spinal cord dorsal horn represents an important site, where nociceptive synaptic transmission can be modulated under pathological conditions. One of the modulatory mechanism involves activation of the transient receptor potential vanilloid 1 (TRPV1) that is expressed on central terminals of primary nociceptive neurons, where it regulates release of neurotransmitters and neuromodulators. Previous studies suggested that changes in TRPV1 activity may be related to effects of chemokine CCL2 (C-C motif ligand 2) and may be also involved in synaptic transmission modulation after μ-opioid receptors (MOP-R) activation. Because CCL2 receptors CCR2 often co-localize with TRPV1 and MOP-R, the goal of this work was to study possible interactions of these receptors on the pre-synaptic endings of primary afferents in the spinal cord dorsal horn and their role in nociceptive signalling under pathological conditions. The presented thesis focused on the effect of CCL2 during peripheral neuropathy and its interference with μ-opioid receptor activation.

To study synaptic transmission at the spinal cord level, patch-clamp recordings of excitatory post-synaptic currents (EPSC) in superficial spinal cord dorsal horn neurons in acute lumbar spinal cord slices from rats was used. TRPV1-expressing afferents were confirmed by increased mEPSC frequency after capsaicin application in most of the neurons. In the first block of experiments, the recordings were made in neurons isolated from animals with peripheral neuropathy. We observed an increase in mEPSC frequency after application of TRPV1 agonist OLDA in a low, normally ineffective concentration (0.2 uM). This indicated that spinal TRPV1 receptors are sensitized during peripheral neuropathy. In neurons from neuropathic animals incubated with CCL2, we observed higher basal EPSC frequency and amplitude, as well as the response to OLDA that was, however, very diversified. The second block of experiments was conducted on neurons from naïve animals and the effect of MOP-R agonist (D-Ala2,N-Me-Phe4,Gly-ol 5)-enkephalin (DAMGO) application on synaptic transmission was tested. In the control group, we observed a long-lasting inhibitory effect of MOP-R activation that influenced the frequency but not the amplitude of mEPSC. In slices pre-incubated with CCL2, the inhibitory effect of DAMGO was partially diminished, suggesting a possible interaction between the receptors. Acute application of CCL2 before DAMGO had a much smaller effect. Our data indicate that TRPV1, CCL2 and MOP-R may interact at the first nociceptive synapse in the spinal cord dorsal horn, possibly through activation of distinct intracellular pathways, and may influence nociceptive transmission under pathological conditions.

Key words: TRPV1, CCL2, μ-opioid receptor, OLDA, DAMGO, spinal cord dorsal horn, nociception