

Annotation

Modulation of a synaptic transmission in the spinal cord dorsal horn plays a key role in nociceptive signalling, especially in states of pathological pain. The goal of this study was to develop a method for calcium imaging in spinal cord slices *in vitro*. This method allowed us to record changes of intracellular free calcium ions concentration (iCa^{2+}), that are a major mediator of neuronal plasticity. In this work, we have focused on application of this method in a conventional fluorescence microscope and on the role of different neuromodulators of synaptic activity. Changes of iCa^{2+} induced by dorsal root electrical stimulation were recorded altogether in 744 dorsal horn (lamina I and II) neurons.

In the first series of experiments, stimulation protocols activating preferentially A and A + C dorsal root fibers were used and long-term stability of the calcium responses was verified. The dorsal root stimulation induced in the neurons fast and delayed type of calcium response.

Application of AMPA and NMDA receptors antagonists, CNQX (50 μ M) and MK801 (45 μ M), reduced the calcium response amplitude and confirmed the importance of glutamate receptors in synaptic activation. In several experiments the effect of capsaicin a TRPV1 receptors agonist, application was tested. Application of even low concentrations of capsaicin (30nM) significantly reduced the calcium response, compared to the control values. These results suggest a probable inactivation of presynaptic endings expressing TRPV1 receptors by capsaicin. In preliminary experiments, substances which are known as modulators of nociceptive synaptic transmission (TNF α , PMA, and bradykinin) were also tested.

Our results demonstrate that this *in vitro* technique allows monitoring synaptic activation of neuronal populations in the spinal cord slices. Further study of nociceptive transmission modulation mechanisms may contribute to new therapeutic approaches in the therapy of pathological pain states.