Reviewer report on doctoral thesis of Monica Pontearso named

Modulation of nociceptive signalling on a spinal cord level under normal and pathological conditions

The present doctoral thesis is devoted to the study of several interconnected aspects of neuropathic pain and its pharmacologic treatment. The thesis is written in English on 101 pages and is supported by two publications of author. The structure of the thesis is standard and list of references contains 195 items. The theoretical part summarizes detailed information on the mechanisms and structures involved in the generation and propagation of nociceptive signals in dorsal roots, ganglia and spinal cord. Special chapters are devoted to neuroinflammation, neuropathic pain and immune system involvement and modulation of pain signalling.

The objectives of the thesis are clearly stated.

Experimental methods include histochemical, behavioural, electrophysiological and biochemical procedures. Surgical procedures include chronical constriction injury (CCI) of the sciatic nerve. Spinal cord , dorsal root ganglion and sciatic nerve slices were the primary experimental objects on which macrophage infiltration was determined by immunohistochemical detection of CD 68 and CD 206 antigens. In addition, the expression level of pro-inflammatory markers was quantified in the slices and mRNA was determined by quantitative polymerase chain reaction (qPCR) methods. Mechanical and temperature sensitivity of experimental mice was used to quantify experimentally induced hypersensitivity associated with peripheral neuropathy. The patch-clamp method was used to study synaptic activity of dorsal horn neurons in acute spinal cord slices. The experimental methods are appropriately chosen and are clearly described.

The results can be divided thematically and methodically into several chapters. The model of chronic constriction injury of sciatic nerve (CCI) induced neuropathy was chosen to induce number of different symptoms involving decrease of threshold for mechanical hypersensitivity, reduced time interval for thermal hypersensitivity, change in the balance between excitatory and inhibitory synaptic transmission in spinal cord dorsal horn (increased frequency of excitatory postsynaptic currents and decreased frequency of inhibitory postsynaptic currents), increased infiltration of macrophages to different parts of sciatic nerve and DRG. The mechanism connecting together all these changes is involvement of proinflammatory cytokine Macrophage migration inhibitory factor (MIF). This linking step was interrupted by using its specific inhibitor ISO-1. ISO-1 was injected intraperitoneally into experimental animals and was shown to be effective in counteracting many of the effects of CCI. An interesting and potentially

important finding was that the effects of ISO-1 on some changes were significantly stronger in males than in females.

The second major theme was the effects of the endogenous cannabinoid anandamide on synaptic activity in the spinal cord. The effects of anandamide on CB1 receptors and TRPV1 receptors were studied in the spinal cord dorsal horn synaptic activity. The use of appropriate inhibitors and modulators suggested the possibility of enhancing the role of anandamide in pain control.

The discussion of the results obtained is appropriate and their interpretation is reasonable and justified by comparison with the literary sources.

The value of the results obtained and the adequacy of the methods used are evidenced by the fact that they have already been published and passed a rigorous peer-review process in international scientific journals, which has undoubtedly verified their quality and factual accuracy. The goals of the thesis have been fulfilled.

The author has mastered a number of challenging experimental methods and demonstrated the ability of independent creative scientific work. Therefore, I recommend the thesis for defense and recommend that the author be awarded the degree of Ph.D.

Prague, 4 June 2024

RNDr. Jan Krůšek, CSc.

I have following questions:

1) Is the Chronic constriction model reversible? Is it possible to remove ligature after some time and observe signs of recovery?

2) Could you explain why markers CD 68 and CD 206 were chosen to quantify infiltration of macrophages to studied tissues? Would it be possible to use more specific markers of M1/M2 macrophages?

3) Can you compare the results obtained in the mouse and rat models?