Evaluation of the Doctoral Thesis

Exploring the role of opioid signaling in modulation of microglial function

by **Akash Shivling Mali, MSc.**

The doctoral thesis by Akash Mali, MSc. was elaborated at the Department of Physiology, Faculty of Science CU under the supervision of doc. RNDr. Jiří Novotný, DSc. The dissertation is based on two original research papers, of which he is the first author.

The main objective of this work was to investigate the effects of activation of all three subtypes of opioid receptors (μ, δ, κ) by selective agonists (DAMGO, DADLE, and U-50488, respectively) in C8-B4 microglial cells. Opioid ligands tested were found to exert cyto-protective effects via a mechanism affecting redox balance, NADPH synthesis and glucose uptake. Furthermore, it was found that activation of opioid receptors can suppress lipopolysaccharide (LPS)-induced M1 polarization and promote M2 polarization of microglia, and it prevents the decrease in mitochondrial respiration and ATP content observed in LPS-treated ells. The results of the present dissertation thus contribute to a better understanding the relationship between the modulatory effects of opioids, metabolic states, and inflammatory responses in microglia.

The dissertation consists of all the classic chapters, including an introduction, aims, materials and methods, results, discussion and conclusion. The introductory section (literature review) describes all essential aspects related to the dissertation. There are four illustrative images that make the text easier to understand. The aims and specific objectives of the thesis are clearly formulated. The methods and results are described in sufficient detail and the discussion provides an appropriate interpretation of the results in the context of current state of knowledge. The data presented are well illustrated. The only point of criticism could be that the author used mostly the same figures as in his publications and has not even bothered to change the legends. In the conclusion section, the main findings of the thesis are summarized and the author suggests considering the idea regarding the potential use of selected opioid receptor agonists for the treatment of neurodegenerative diseases. However, this is rather bold idea that would require a lot of further research. In general, the dissertation is well readable and carefully worked out, which is documented by almost no typographical errors.

In conclusion, the dissertation of Mr. Mali represents an interesting and important contribution to studying the effects of opioids on microglia. I believe that despite some minor objections, the dissertation meets the necessary formal requirements and is worthy of being defended before the dissertation defense committee. Mr. Mali has fully demonstrated that he is capable od conducting independent scientific investigations using state-of-the-art methods and discussing the results of his research comprehensively.

Questions to be discussed:

Question 1: Is there an explanation for how opioid agonists and activation of opioid receptors can prevent the decrease in mitochondrial respiration and ATP production observed when microglia are treated with LPS? Do opioid ligands affect mitochondrial functions and ATP levels in the absence of LPS?

Question 2: It appears that all opioid ligands tested exerted similar effects on microglia. Some difference was noted between DAMGO compared to DADLE and U-50488 when the effects on IL-6 and COX-2 were examined in LPS-treated cells. Is there an explanation for the observed differences and the possible involvement of some specific post-receptor signaling pathways?

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