

Evaluation of the present PhD Thesis

The doctoral thesis entitled "The study of functional and pharmacological properties of glutamate receptors" by author Bohdan Kysilov is a summary of the results of his scientific work carried out at the Dept. of Cellular Neurophysiology Institute of Physiology AS CR.

The thesis is well-structured with respect to traditional form and content commonly used in doctoral theses, *i.e.* abstract, theoretical introduction, its objectives, followed by the chapters entitled Material and methods and Results, appropriately structured discussion, summary, references and an overview of the author's publications. In terms of language, the text is well-written with a bare minimum of misprints.

The aim of the thesis is to present his study of the relationship between the structure of neuroactive steroids and their activity on the NMDA receptor. In terms of methodology, the research involved the use of electrophysiological and molecular-biological methods in combination with computer modeling. The research work is focused on neuroactive steroids that positively modulate the NMDA receptor. Compared to negative modulators, these substances are neglected in pharmacological research. The main contribution of the study is the fact that it focuses on the identification of the NMDAr binding site of these substances.

The experiments performed in the second part of the thesis attempt to understand the role of hGluN2B and hGluN1 gene mutations in the function and expression of NMDAr and therefore in the role of selected mutations in the development of the neuropsychiatric diseases. The results show the key influence of the chain length at the C3 of pregnanes on the mode of NMDAr modulation. Only substances with a shorter chain have a negative effect. The author explains this by the planarity of the structure of the studied substances.

Further experiments, made it possible to identify the binding site and describe the potentiation mechanism of action at molecular level. In experiments focused on the effect of selected mutations, not only the impact on NMDAr functionality was demonstrated. At the same time, it was shown that neurosteroid positive modulators are able to compensate for hypofunctionality of NMDAR within some parameters. But it should equally be noted that the effect of neuroactive steroids differed considerably among certain mutations.

It is obvious that PhD candidate succeeded in achieving the objectives and aims of the doctoral thesis. At the same time, it is clear that the achieved results offer an opportunity for further research and new CNS drugs development.

Questions:

- Why, in your opinion, are positive NMDAr modulators neglected in the pharmacological research? What behavioral profile and side effects can be expected after their administration?
- In several places in the text, it is rather unclearly and vaguely stated that pregnenolone sulfate is similar in effect to pregnanolone butyrate. However, the results clearly show that both substances have different binding sites and the different effect on the mutated receptors. It could be argued that both substances belong to the same category of "dis-use" modulators only, and the specific mechanism of action makes both substances different and unique. So could you elaborate on how the mechanism of action is similar?
- It is concluded that both "bent" and "planar" neurosteroids can potentiate responses to NMDAr. So what are the structural prerequisites for inhibition of the NMDAr response by neurosteroids?


- Conclusion:

It is perfectly clear that the student demonstrates a high degree of the creative activity, the ability to study scientific literature, to plan and to conduct scientific experiments. He successfully had the results of his study published in scientific literature. These publications appearing in

journals with IF, forming the basis of the doctoral thesis, meet the requirements set out of the rules of Third Faculty of Medicine of Charles University

In conclusion, the doctoral thesis clearly demonstrates that the PhD candidate has all the prerequisites for carrying out of an independent creative scientific work. The thesis reviewed fulfills all the requirements imposed on the doctoral theses. Therefore, I strongly recommend that the candidate be given the opportunity to defend his thesis and be awarded the title of "Ph.D."

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