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„Vliv deplece cholesterolu na signální dráhu iniciovanou receptory spřaženými s G proteiny třídy G_q/G_{11}"

Submitted by Mgr. Pavel Ostašov

"Oponentský posudek disertační práce"

The PhD. Thesis entitled „Vliv deplece cholesterolu na signální dráhu iniciovanou receptory spřaženými s G proteiny třídy G_q/G_{11}" written by Mgr. Pavel Ostašov has three separate parts, each of them describing a different aspect of the TRH receptor functioning. In the first section, the effect of cholesterol depletion on the TRH-receptor and its ability to activate G-proteins is considered. The results show that the binding of the TRH analogue is not affected by the cholesterol depletion, whereas the ability of TRH-R to activate G-proteins is impaired. The second part deals with the TRH-R desensitation. It is shown that the intact membrane is necessary for the proper termination of the G-protein signalling pathways. The final third part considers the spatial distribution and diffusion of the GFP labelled TRH-receptor in the intact and cyclodextrin treated membranes. By means of fluorescence microscopy techniques, it is proved that TRH-receptor possesses two populations in the plasma membrane. TRH-receptor molecules can exchange between these populations and the rate of exchange is strongly altered upon the cholesterol depletion. It is concluded that TRH-receptor is a part of a supra-molecular structure or a membrane domain, which is present in the functional form only in the intact cells.

I found the topic of the investigation of the receptor functioning both on the living cells and on the plasma membrane extracts extremely demanding, scientifically relevant and up-to-date. Mgr. Pavel Ostasov chose one particular G-protein coupled receptor and decided to explore its functioning from three different point of views in the normal and modified plasma membranes. This brought a detailed insight into the mechanism how and to what extent cholesterol content regulates the signalling and the distribution of TRH-receptor. I

appreciated that Mgr. Ostašov used a large number of the experimental approaches combining the biochemical methods with the advanced methods of the fluorescence microscopy. Moreover, the interpretation of the gained data is decent and prove that Pavel Ostašov has a deep view both into the investigated system and the experiments.

As far as the formal aspects of the thesis are concerned, the text is nicely written and is understandable even for the beginner to the field. The chapters are logically sectioned and the graphical arrangement is on a high level.

I have following questions to answer:

1. Does the TRH-R contain any specific recognition sites, or binding sites for cholesterol? If so, can the cholesterol depletion have any influence on the occupation of these binding sites?
2. Some GPCRs needs (de)palmitoylation for their proper functioning. Is it also valid for the TRH-R? If so, do you think that the degree of palmitoylation can affect the exchange of TRH-R between the two populations or desensitation?
3. The question concerns figure 27 on page 84. Do you have any explanation why the apparent diffusion coefficient of TRH-R-eGFP is lower for the depleted membrane than for the intact membrane in the case of the smallest bleached area (4 μm^2). Intuitively, the mobility of the TRH-R should be comparable or higher in the cholesterol depleted membrane as it is true for the other two sizes of the bleached spot (9 and 36 μm^2).

In conclusion, the presented work is excellent both from the scientific and formal point of view. Moreover, Mgr. Ostašov is the first author of two articles published in the impacted scientific journals, and another one is submitted at the moment. Therefore, I highly recommend the PhD. thesis elaborated by Mgr. Pavel Ostašov for the defense.

In Prague, 9.12. 2010

Martin Hof