Abstract

Activation of thyrotropin-releasing hormone receptor (TRH-R) signaling has an irreplaceable role in a number of cellular processes. Thyroliberin (TRH) plays an important role in the regulation of secretion of other hormones and there are also mentions of its possible antiapoptotic and neuroprotective effects. On the other hand, TRH is quickly degraded and also its other properties, such as cardiac and endocrine side effects and low lipophilicity, disadvantage the therapeutic applications of this hormone compared to its various analogues. Due to their effects on the central nervous systém, TRH and its analogues represent a potential possibility for the treatment of various neurological diseasses, including neurodegenerative disorders. The molecular mechanisms responsible for the beneficial effects of TRH and its analogues have not yet been fully elucidated. So far, little is known about the involvement of TRH, or its analogues, in the regulation of energy metabolism or impact on the cellular phosphoproteome.

In the first part of the thesis, we focused on the study of the effect of TRH and interacting partners of TRH-R on the lateral mobility of this receptor in the membrane of the TRY-1 cell line stably expressing TRH-R labeled with yellow fluorescent protein (YFP) at the C-terminus. The results of these experiments show that not only ligand stimulation but also the presence of binding partners contribute to the modulation of the lateral mobility parameters of the receptor.

The second part of the thesis was devoted to an extensive analysis of the functional state of TRH-R. In particular, the effect of individual signaling molecules on the levels of intracellular second messengers, such as calcium ions, inositol-1,4,5-triphosphate (IP_3) and cyclic adenosine monophosphate (cAMP), after TRH-R activation, was studied. It has been shown that, in addition to its action via the $G_{q/11}\alpha$ protein, this receptor can, under certain conditions, also be coupled to $G_s\alpha$ or $G_i\alpha$ proteins, thus regulating various cellular processes. The effect of TRH and its analogue taltirelin (TAL) on the phosphorylation of ERK1/2 and Akt kinases has also been demonstrated.

In the next part of the thesis we monitored the effect of TRH-R activation on levels of free radicals, on the processes of cellular bioenergetics, on the function of the mitochondrial respiratory chain. The application of both TRH and TAL revealed certain antioxidant properties of these substances and a mild protective effect against oxidative stress induced by *tert*-butyl hydroperoxide (*t*-BHP). None of the ligands showed any effect on oxidative phosphorylation processes or on oxygen consumption in the GH1 cell line.

In the last part of this thesis, we investigated the effect of β-arrestin2 on the phosphoproteome in the GH1 cell line after TRH-R stimulation. Activation of TRH-R by TRH or TAL, as well as decreased expression of β-arrestin2, revealed extensive changes in phosphorylation of proteins related to the signaling pathways of small GTPases, mitogen-activated protein kinases (MAPKs), serine/threonine and tyrosine protein kinases, Wnt/βcatenin and proteins from other signaling pathways.

In addition, TRH and TAL showed many differences in the phosphorylation of a large number of proteins, which could indicate the ability of these two ligands to act as biased agonists at TRH-R.