Abstract

The G-protein-coupled receptor (GPCR) family represents the largest family of cell surface receptors. GPCRs are activated by endogenous or exogenous ligands, and are targets for more than a quarter of currently used drugs. Activation of receptors initiates intracellular signaling pathways. This way the membrane receptors transfer information from the outside environment into the cell. Based on the signal the cell can respond to the changes of the environment. Key observation important for this thesis is interplay of cannabinoid and opioid signaling *in vivo*, which can have significant physiological effects[1].

Cannabinoid receptor 1 (CB1R) and μ opioid receptor (MOR) belong to the rhodopsin family of receptors, and both are coupled with $G_{\alpha i/o}$ proteins[2]. Both are located in certain areas in central nervous system (CNS) and share a lot of important features. Activation of both of the receptors leads to inhibition of adenylyl cyclase, thus decreasing the level of cyclic adenosine monophosphate in the cell, and modulates extracellular regulated kinase 1 and 2 (ERK1/2)[2]. In view of the numerous anatomical, biochemical and pharmacological evidence supporting the existence of the functional interaction between opioid[3] and cannabinoid receptor systems this topic became interesting for our research.

In our previous study we described interaction between SGIP1 and cannabinoid receptor 1 (CB1R)[4]. After agonist stimulation, SGIP1 significantly inhibits internalization and alters signalization of CB1R[4]. In case the of MOR, inhibition of the receptor internalization by protein SGIP1 does not occur. From these results we can deduce that internalization of CB1R is influenced by protein SGIP1 specifically, whereas MOR internalization is not affected by SGIP1 at all – presumably because of a different interaction of SGIP1 with these receptors.

In this thesis, we were observing effects of SGIP1 on signaling of both receptors (CB1R and MOR) and their created of chimera versions. Cloning of functional of expression chimeras vectors by combination of CB1R and MOR will be used for mapping of interaction places of the receptors with SGIP1. In newly created receptors, we observed influence of their function via protein SGIP1 by the signal pathway ERK1/2. Our results indicate that SGIP1 is influencing the level of phosphorylation in ERK1/2 not by signaling of receptor CB1, but also MOR. Signalization of ERK1/2 is activated by both studied receptors or their chimeras and is influenced by protein SGIP1 nonspecifically.

Key words: GPCR; signaling cascade; Cannabinoid receptor 1; SGIP1; ERK1/2; μ-opioid receptor