ABSTRACT

An evolutionary developed eukaryotic proteins for transformation of extracellular signal into intracellular targets are receptors. There are known receptors integrated to intracellular organelle membranes e.g. inositol-3-phosphate receptor (Inositol-3-phoshpate receptor; IP3R) specific for endoplasmic reticulum or nuclear receptors e.g. receptors for gonad hormones transforming the intracellular signals, however the vast majority of receptors are present on plasmatic membrane and recognize an extracellular signals. High percentage of these receptors are included in family of proteins called G-protein coupled receptors, (G-protein coupled receptors, GPCRs) due to their signal mediators heterotrimeric G-proteins. Each receptor of this family has the preference to specific type of G-protein influencing the intracellular concentrations of Ca^{2+}, cyclic adenosinmonophosphate or inositol-3-phosphate. Some of them are able to signalise by dual manner or through G-protein independent pathways.

Independently on cellular target membrane, GPCRs integration into membranes is enables by the content of GPRCs-specific 7-transmembrane domain (7-transmembrane domain, 7TM) which is consist of hydrophobic amino acids. The relation between the structure and functionality is very tightly related. Many pathological conditions and rare diseases are caused by mutations or mistakes in transcription in one of receptor essential domains. These mistakes may have influence on intracellular receptor targeting (mutations in signal, export/retention sequence), inability of signal transformation (intracellular sequence of receptor, intracellular loops), membrane instability (interaction domains for intracellular proteins in C-terminal sequence) or inability to be recognized by signal molecule (N-terminal sequence or extracellular loops). Together with increasing knowledge about molecular aspects of GPCR structure, functionality and activity regulation increases the chances of causal pharmacotherapy development which would bring the benefit for patient suffering from disease.

This thesis describing the results of basic research of two GPCRs which are expressed in mammalian central nervous system. As was already discussed, signalisation of GPCRs is complex and may be processed by G-proteins or by G-protein independent way through activation of specific MAP kinases. Moreover, in the last decades, it was discovered that receptor characteristic might not be strictly limited only by primary structure of receptor, however could be largely inluenced by quarter structure of receptor. The first described dimerization was confirmed in case of GPCR family A Rhodopsin-like receptors member, β_2-adrenergic receptor and then observed among the other receptor families e.g. family C member mGluR5 (Hebert, 1996; Romano, 1996). The phenomenon in the last decade has been also observed across the receptor types and families. Our laboratory is focused on research of family C GPCRs metabotropic glutamate receptors (mGluRs). The eight described members mGluR1-mGluR8 are stratified according to pharmacology and localization into I-III. Subfamilies. Subfamily I (mGluR1 and mGluR5) are localized postsynaptically and mediates regulation of the fast excitatory synaptic

transmission, retrograde cannabinoid signalling included. Both receptors found to be in form of homodimers connected through covalent interaction of cysteines in receptor protomers´ domains. Four years ago, the intra- and intermolecular movements during the homodimer mGluR1 activation as well as functional consequence has been firstly described by one of our collaborators Hlavackova (Hlavackova, 2012). Although the partial activation of receptor has been recorded when one protomer in homodimer accepted the molecule of agonist, publication postulated that receptor is fully activated when both N-terminal so called Venus Fly Trap domains are occupied by agonist. This receptor is expressed in several isoforms. The longest and the most studied mGluR1a is followed by shorter mGluR1b, mGluR1c and mGluR1d splice variants which differ in lenght of their C-terminus while the N-terminal sequences are identical. In contrast to mGluR1a, other izoforms are poorly studied despite the presence of dimerization domain and different C-terminus composition which leads to hypothesis that may serve as "buffer" molecules of mGluR1a with possible functional consequence. Our research further described in this thesis has been focused on the hypothesis that mGluR1a forms a heterodimer with mGluR1b *in vitro* and *in vivo*.

Postsynaptic mGluR I. signal transduction increases the phospholipid transformation into endocannabinoid formation: Anandamide and 2-arachidonoylglycerol (2-arachidonoylglycerol, 2-AG). These lipophilic substances are involved in retrograde, neuroprotective cannabinoid signalling of neurons. Once the presynaptically localized cannabinoid receptor 1 (Cannabinoid receptor 1, CB1R) are activated, the neurotransmitter synthesis as well as fusion of presynaptic vesicles with membrane is silenced. Retrograde neuronal signalling might be also activated by exogenous agonist Δ^9-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol, Δ^9-THC), the psychoactive substance of *Cannabis sativa*. The psychoactive effects of this plant are well known however cannabinoid pathway found to be modulating memory formation, reaction to stress conditions as well as appetite and energy homeostasis. In the second project, we found and confirmed the new interaction of CB1R with SGIP1 (Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1, SGIP1), adaptor protein previously found to be connected in direct correlation with obesity occurrence in rodent and human (Trevaskis, 2005; Dergai, 2010; Cummings, 2012).