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G-protein coupled receptors, neurotransmitters, cannabinoid receptor

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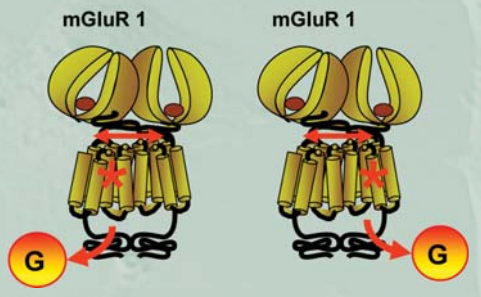
Research topics

We aim to describe principles of activation of G-protein-coupled receptors (GPCRs) for major neurotransmitters. The research is focused on the structure-function relationships of these receptors and molecular machinery that regulates their signalling properties. The metabotropic glutamate (mGlu) receptors that belong to family 3 GPCRs are composed of two identical subunits. The relevance of dimerization of these receptors in respect to activation of the transmembrane heptahelical domain (HD) of each subunit is of our particular interest. Using the mutagenesis approach combined with a functional expression system we showed that within the homodimeric structure only one HD reaches active state. Interestingly, this situation is very similar to that observed in GABAb receptor. Within the GABAb receptor that is composed of two different proteins, only one of them activates G-proteins. The activation process of these family 3 GPCRs is thus asymmetrical. Currently, we take use of this observation to reveal the mechanism of action of allosteric modulators on these receptors. To this aim we analyse energy transfer deviations upon activation and/or modulation of the receptors tagged with different fluorochromes at distinct portions of the receptors.

Regulation of the receptor activity on the cell surface is examined by search for associated proteins that interact mainly with the intracellular C-termini. This project is focused on signalling of cannabinoid receptor 1. It is approached by molecular biology means combined with biochemical tools including yeast two-hybrid technology, *in vivo* introduction of tagged "bites" into living animal brains followed up by the pull-down method for isolation of the interactors and their successive identification.

Class C GPCR activation process is assymetrical

In heterodimeric GABAb receptor (gamma-aminobutyric acid receptor-type b) only the GB2 subunit is capable of G-protein activation, while the ligand-binding site is located within the extracellular domain of GB1 subunit.



In our studies we showed that in metabotropic glutamate receptors, upon competitive agonist binding within both extracellular „venus-fly trap-like" domains only one of two identical subunit's transmembrane heptahelical domains reaches activated state.

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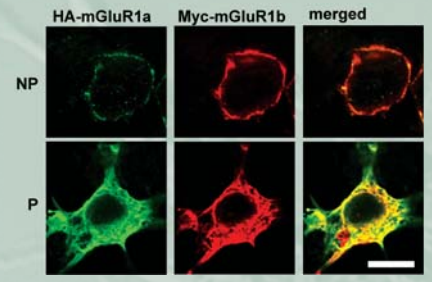
Selected recent papers

1. Bertaso F, Lill Y, Airas JM, Espeut J, Blahos J, Bockaert J, Fagni L, Betz H, El-Far O. MacMARCKS interacts with the metabotropic glutamate receptor type 7 and modulates G protein-mediated constitutive inhibition of calcium channels. **J Neurochem.** 2006;99:288-298.
2. Kumpost J, Syrova Z, Kulihova L, Frankova D, Bologna JC, Hlavackova V, Prezeau L, Kralikova M, Hruskova B, Pin JP, Blahos J. Surface expression of metabotropic glutamate receptor variants mGluR1a and mGluR1b in transfected HEK293 cells. **Neuropharmacology.** 2008;55:409-418.



Distribution of mGluR1a and mGluR1b splice variants in transfected HEK293 cells
Cells were transfected with the mGluR1a or mGluR1b coding plasmids. Paraformaldehyde-fixed cells transfected with the indicated plasmids were permeabilized (P) or not (NP) and stained with rabbit anti-HA (for HA-mGluR1a) or mouse anti-c-Myc (for Myc-mGluR1b) antibodies and visualized with corresponding secondary antibodies labelled with FITC or Cy3, respectively. Staining was analysed by confocal microscopy with co-localized fluorescence being shown in yellow. Bar equals 10 μm in vivo. This together with other results suggests that splicing within intracellular C-terminus of mGlu1 receptor does not encode targeting signals into distinct cell compartments.