Laboratory of Molecular Pharmacology

G-protein-coupled receptors, neurotransmitters, metabotropic glutamate receptors, Cannabinoid receptors

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Our research is focused on the structure-function relationship of metabotropic glutamate receptors (mGluRs) and Cannabinoid Receptor 1 (CB1R) signalling. The mGluRs belong to Class C-G-protein Coupled Receptors (GPCRs) and were traditionally viewed as homodimers, composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their dimeric complexes only one subunit reaches the active state. The activation process of mGluRs is initiated by agonist binding that causes conformational changes of the extracellular ligand-binding domains. This is followed by relative movement of the transmembrane regions of the two subunits, and finally a conformational change within one of the heptahelical transmembrane domain can be transmitted to the intracellular signalling machinery. Recently resolved crystal structure of the mGluR1 is in accord with our model of activation mechanism being asymmetrical, as suggested by our functional data.
Moreover, we showed that splice variants mGluR1a and mGluR1b form heterodimers in vivo. The functional relevance of the splice variant combinations in the dimeric mGluR1 complexes in vivo are now under investigation using genetically modified mice.
Cannabinoid receptors are located predominantly pre-synaptically, where the receptors show modest internalization upon agonist stimulation, while the CB1R expressed in heterologous systems are readily internalized. We detected novel interacting partner of CB1R that modulates internalization of CB1R and also signalling of the receptor in biased manner. Overexpression of SGIP1 in animals is associated with obesity. Functional significance of the SGIP1 protein on CB1R signalling is studied both on level of signalling-pathway specificity, as well as in animal models for energy homeostasis regulation.

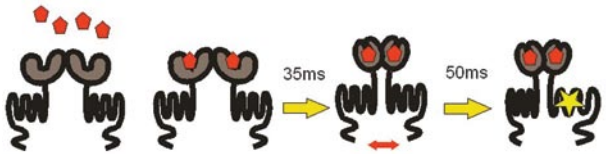

Fig. 1. Activation of Class C GPCR schematically Together with our collaborators we brought evidence that activation of metabotropic glutamate receptors following agonist binding (red pentagons) within extracellular binding sites (also known as Venus fly-trap like domains) results in the change in relative position of the two subunits of transmembrane (heptahelical) domains followed by a conformational change within one of the two heptahelical regions. This active state (yellow star) of a single heptahelical domain then may activate intracellular G-protein signalling and possibly other pathways. [**EMBO J 2005** 24(3): 499-509 and **Science Signal 2012** 5(237): ra59]

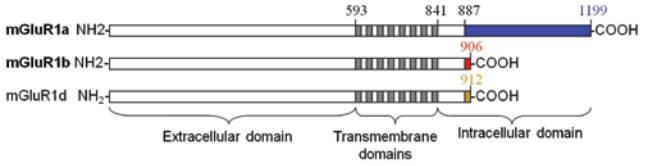

Fig. 2. Long and short variants of metabotropic glutamate receptor 1 Splicing of metabotropic glutamate receptor 1 (mGluR1) gene results in expression of long and short forms. Following the heptahelical domain and short sequence including RRKK motive (Endoplasmic Reticulum retention signal), the long form mGluR1a has unique sequence of 312 aa, short forms are termed mGluR1b and d. The mGluR1b unique sequence is 19 aa long following the splicing site.

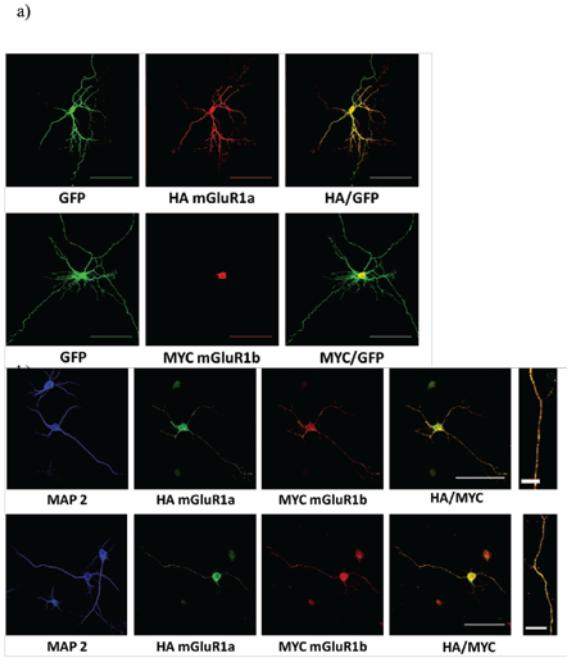

Fig. 3 Splice variants mGluR1a or mGluR1b combine in mGluR1a/b dimers Cells from primary prefrontal cortical neurons were transfected a) with GFP and corresponding single tagged subunits and stained 72 hours later against HA and MYC epitopes or visualized using GFP fluorescence. UPER ROW: Left panel: GFP; middle panel: HA-mGluR1a detected using anti-HA antibodies; right panel-merge. LOWER ROW: left panel: GFP; middle panel MYC-mGluR1b detected using anti-MYC antibodies, right panel: merge; b) Two primary cortical neurons co-transfected with HA-mGluR1a (Green) and MYC-mGluR1b (Red); right panel: merge; dendritic marker MAP2 shown in left panel in blue. Co-localization HA-mGluR1a and MYC-mGluR1b in MAP2 positive distal dendrites is shown in extreme right in detail as merge for green and red channels (HA-mGluR1a and MYC-mGluR1b, respectively). Scale bar is 20 μm.

- GACR, GAP303/12/2408 - Functional Consequences of Metabotropic Glutamate Receptor 1a and 1b Splice Variants Assembly in Heterodimeric Complexes, 2012-2016, J. Blahoš

1. Techlovská S, Chambers JN, Dvořáková M, Petralia RS, Wang YX, Hájková A, Nová A, Franková D, Prezeau L, Blahos J: Metabotropic glutamate receptor 1 splice variants mGluR1a and mGluR1b combine in mGluR1a/b dimers in vivo. **Neuropharmacology 2014** 86: 329-36.

From the left: Šárka Techlovská, MSc / PhD Student, Michaela Dvořáková / Diploma Student, Assoc Prof Jaroslav Blahoš, MD, PhD / Head of Laboratory, Alena Hájková, MSc / PhD Student, Daniela Franková / Technician,

Not in the picture: Pavla Hubálková (until 2014), Jayne Nicole Rafferty, PhD / Postdoc