Laboratory of Molecular Pharmacology

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

Jaroslav Blahoš

jaroslav.blahos@img.cas.cz

www.img.cas.cz/research-groups/jaroslav-blahos

Glutamate is a major excitatory neurotransmitter that activates two types of receptors in mammalian brains: ligand-operated ion channels (NMDA, AMPA and kainate receptors) and metabotropic G-protein-coupled receptors (GPCRs). There are eight genes that code for the metabotropic glutamate (mGlu) receptors in mammals. These diverge in the location within brain regions and cellular compartments and have distinct functional properties. As such, they constitute a promising target for treatment of certain neurological diseases.

Our research is focused on the structure-function relationship of these receptors. The mGlu receptors belong to family C GPCRs and are traditionally viewed as homodimers composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their homodimeric complexes only one subunit reaches the active state. Our recent data using the dynamic FRET approach are in accord with this notion. The activation process of these family C GPCRs is initiated by agonist binding that causes conformational changes of the extracellular ligand-binding domain. 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. The active state of these receptors is thus asymmetrical.

Recent data from our laboratory suggest that splice variants of the mGluRs can also form heterodimers. We have obtained data suggesting that heterodimerization between distinct splice variants of the mGlu1 receptor, mGluR1a and mGluR1b, results in novel receptor complexes with modified trafficking properties in transfected heterologous cells and primary neurons. This observation about the combination of distinct splice variants within the dimeric receptor complexes are being investigated from the point of functional relevance in vivo.

Fig. 1. Activation of Class C GPCR schematically
Together with our collaborators we brought evidence [**EMBO J 2005** 24(3): 499-509 and **Science Signal 2012** 5(237): ra59] that activation of metabotropic glutamate receptors after agonist binding (red rectangle) 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.

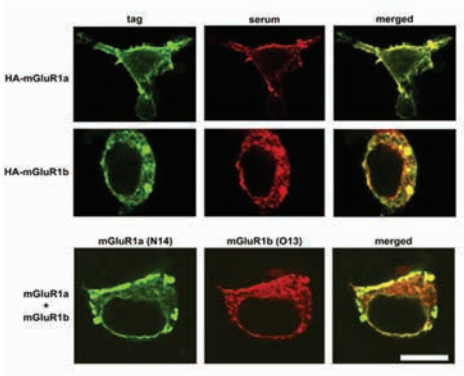

Fig. 2. For immunocytochemistry cells were transfected with HA-mGluR1a and stained with monoclonal anti-HA antibodies (secondary antibodies labelled with FITC) and our N14 antibodies (secondary anti-rabbit antibodies labelled with Cy3). c-Myc mGluR1b-expressing cells were labelled with anti-c-Myc antibodies and guinea pig anti-mGluR1b antibodies (013) and detected with secondary antibodies (FITC, Cy3, respectively). Their patterns confirm specificity of the novel antibodies by overlap of corresponding anti-tag antibodies and staining with the subunit-specific sera. Bar equals 10 mm *in vivo*.

- GA CR, GA303/08/1591 - Study of glutamate receptors conformational changes using novel fluorescent techniques, 2008-2012, J. Blahoš
- GA CR, GAP303/12/2408 - Functional consequences of metabotropic glutamate receptor 1a and 1b splice variants assembly in heterodimeric complexes in vivo, 2012-2016, J. Blahoš

1. Hlavackova V, Zabel U, Frankova D, Bätz J, Hoffmann C, Prezeau L, Blahos J, Lohse MJ. Sequential inter- and intrasubunit rearrangements during activation of dimeric metabotropic glutamate receptor 1. **Science Signal 2012** 5(237): ra59.

From the left:
Michaela Dvořáková / Diploma Student · Daniela Franková / Technician · Assoc Prof Jaroslav Blahoš, MD, PhD / Head of Laboratory · Šárka Techlovská, MSc / PhD Student · Jayne Nicole Rafferty, PhD / Postdoc

Not in the picture:
Alena Hájková, MSc / PhD Student