Laboratory of Molecular Pharmacology

G protein-coupled receptors, GPCR, neurotransmitter, glutamate signalling, cannabinoid signalling

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In mammalian brain the major excitatory neurotransmitter glutamate activates two types of receptors: ligand-operated ion channels (NMDA, AMPA and kainate receptors) and G protein-coupled receptors (GPCRs). There are eight genes that code for the metabotropic glutamate (mGlu) receptors in mammals. These receptors diverge in location within brain regions and cellular compartments and have distinct functions. As such, they constitute possible promising targets for treatment of several neurological diseases. Our research is focused on the structure-function relationships of these receptors and molecular machinery that regulates their signalling properties. The mGlu receptors belong to family C GPCRs and are traditionally viewed as composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their homodimeric complexes only one HD reaches active state. Our recent data using dynamic FRET approach are in accord with this notion. The activation process of these family 3 GPCRs is thus asymmetrical. Recent data suggest that the mGluRs can also form heterodimers. We published data suggesting that heterodimerization between distinct splice variants of the mGlu1 receptor, the mGluR1a and mGluR1b, results in novel receptor complexes with altered function and trafficking properties in tranfected heterologous cells. Now we aim at revealing the situation in brain using a set of splice variant-specific antibodies we developed.

The cannabinoid receptor project is focused on regulation of signalling of the CB1 receptor 1. It is approached by molecular biology techniques combined with biochemical tools including yeast two-hybrid screen. Currently, several hits obtained by the latter technique are being analysed. One highly promising lead is investigated in deep detail. The mechanism of regulation of cell-surface stability of the CB1 receptor by endocytosis machinery proteins shows possible novel player(s). The mechanism of internalization of the CB1 receptor and its regulation is therefore currently under heavy investigation in our lab.

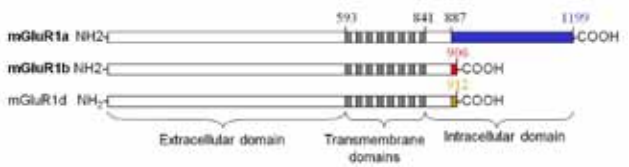

Fig. 1. Splicing of rat metabotropic glutamate receptor 1 (mGluR1) gene results in expression of long and short forms. Following the heptahelical domain and short sequence including RRKK motive (Endoplasmic Reticulum retention signal), the long form mGluR1a has a unique sequence of 312 aa, short forms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

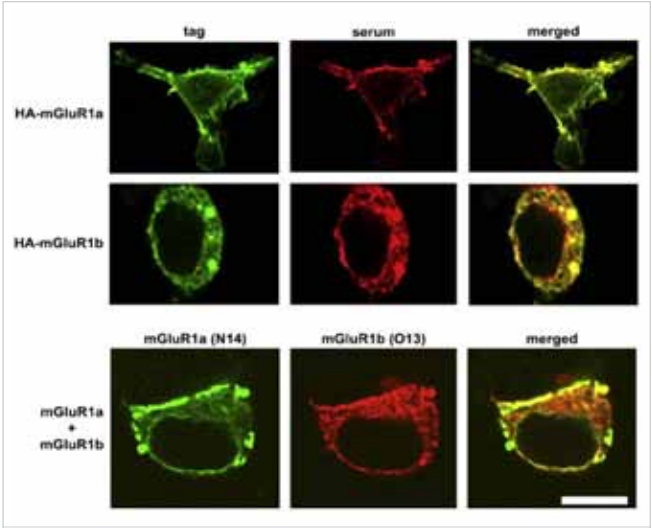

Fig. 2. For immunocytochemistry cells were transfected with HA-mGluR1a and stained with monoclonal anti-HA antibodies (secondary antibodies labelled with FITC) and our N14 antibodies (secondary anti-rabbit antibodies labelled with Cy3). c-Myc mGluR1b-expressing cells were labelled with anti-c-Myc antibodies and guinea pig anti-mGluR1b antibodies (O13) 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 to 10 μm *in vivo*.

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- GA CR, GA303/08/1591 – Study of glutamate receptors conformational changes using novel fluorescent techniques, 2008-2012, J. Blahoš
- Ministry of Education, Youth and Sports of the Czech Republic, LC06063 – Fluorescence Microscopy in Biological and Medical Research, 2006-2011, P. Hozák, J. Blahoš

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