to act as monomers, but this idea has been challenged over the past several years by accumulating pharmacological and biochemical data about the association of many GPCR types into higher-ordered oligomers. Many different approaches were employed e.g. co-immunoprecipitation of differentially-tagged expressed receptors, sucrose density gradient fractionation, Western blot, functional complementation of two inactive mutant receptors, atomic force microscopy, and proximity assessment of receptor proteins in cell membranes using bioluminescence and fluorescence resonance energy transfer (BRET, FRET) techniques to show that 7TM-R can form either homo- or heterodimers.

Heterodimerization in family C 7TM-R has been most extensively studied and demonstrated. Therefore, these receptors represent a good model for studying the functional relevance of 7TM-R dimerization. GABAB receptor, which is a member of class C 7TM-Rs, is an obligatory heterodimer composed of two distinct subunits, $GABA_{B1a}$ (GB1a) and $GABA_{B2}$ (GB2). During evolution, a system has been developed to ensure that only the functional heterodimer reaches the cell surface. GB1 subunit contains an endoplasmic reticulum (ER) retention signal in its intracellular tail, preventing it from reaching the cell surface as a monomer. Only when associated with GB2, this subunit can reach the cell surface and function. Although no covalent linkage between the subunits has been observed, these dimers are likely to be very stable due to the coiled-coil interaction. Consequently, our approach to study dimerization of family A member ghrelin receptor (ghR) was based on engineering ghrelin receptor (ghR) constructs with swapped GB1a (ghR-$GB_{1a}$) or GB2 (ghR-$GB_2$) C-terminal tails, which should selectively lead to formation of heterodimers. Constructs were tested with the classical pharmacological tools and the results confirmed by the means of confocal microscopy. To detect cellular localization of ghR-$GB_{1a}$ chimera indirect immunofluorescent staining in non-permeabilized and permeabilized cells was employed. Co-localization experiments with an ER resident chaperone protein calnexin were employed to detect distribution pattern of the chimeric protein.

On the basis of obtained results, it could be suggested that the ghR-GB1a chimeric construct was not completely retained in the ER in the absence of the ghR-GB2 chimeric construct. On the contrary this chimera was capable of targeting to the cell surface, binding, and signaling. Therefore this system cannot be considered for studying dimerization of the ghR, a member of family A of 7TM-Rs, or adapted to other families of 7TM-Rs for which the functional significance of dimer formation is still unknown.

## LASER SCANNING CONFOCAL MICROSCOPY IN CELL CYTOSKELETON AND APOPTOSIS STUDIES

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Fluorescence-based imaging including laser scanning confocal microscopy (LSCM) is extensively used in the field of biomedical research. In our laboratory effects of some toxic substances on cytoskeleton organisation and apoptosis have been studied by LSCM. Rabbit embryos and whole embryo cultures were examined with a multispectral laser scanning confocal microscope (Leica), using an Argon ion laser beam of wavelength 488 nm and a helium–neon laser with wavelengths of 543 and 633 nm. Immunofluorescence and fluorescence methods were used to stain microtubules, actin filaments and nucleic acids. Additionally, apoptotic cells (programmed cell death) based on the TUNEL method were determined by LSCM. Applications of LSCM and procedures that have been used to stain and visualize the cytoskeleton in rabbit embryos, embryo cell cultures and apoptosis will be introduced.

## EVALUATION OF G PROTEIN-MEDIATED ACTIN CYTOSKELETON REARRANGEMENT PATTERN USING CONFOCAL MICROSCOPY

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Reorganization of the actin cytoskeleton could coincide with the activation of several G protein coupled receptors (GPCRs). The small GTPase RhoA plays a central role in GPCR stimulated actin polymerization and stress fiber formation. RhoA is activated through various GPCRs and it has been well established that G proteins of the $G\alpha_{12}$ and $G\alpha_{13}$ family can link GPCRs to RhoA. However, several controversies exist as to the exact role of $G\alpha_{q/11}$ and $G\alpha_s$ in this process. While several reports clearly demonstrate the exact role of $G\alpha_{q/11}$ in this process others show no such involvement. The role of $G\alpha_s$ is still under debate.

Therefore the aim of our study was to examine the changes in actin cytoskeleton rearrangement pattern in cells after the activation of the $G\alpha_{q/11}$- and $G\alpha_s$-coupled GPCRs. We have also monitored the status of actin cytoskeleton in cells expressing different constitutively active mutants of G-protein α-subunits.

To study the role of different G-proteins in actin cytoskeleton rearrangement autofluorescently-tagged β-actin (pEYFP-actin) was co-expressed together with receptor constructs (neurokinin type 1 receptor (NK1-R) and $\beta_2$-adrenergic receptor $\beta_2$-AR)) or constitutively active mutants of $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, $G\alpha_{13}$ and $G\alpha_s$ in the HEK 293 cells. Evaluation of the autofluorescently-labeled actin filaments was performed with the use of confocal microscope.

Our findings show that the $G\alpha_{q/11}$-coupled NK1-R activation as well as the expression of different constitutively active mutants of $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$ and $G\alpha_{13}$ caused changes in cell morphology, enhancement in the cortical actin signal and stress fiber formation. In contrast, neither the $\beta_2$-AR activation nor constitutively active mutant of $G\alpha_s$ caused any apparent changes in actin cytoskeleton status in the HEK-293 cells. Based on these findings it could be assumed that only $G\alpha_{q/11}$-coupled receptors activation coincides with the robust changes in the actin cytoskeleton organization.

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## DEMONSTRATION OF CONNEXINS IN CELL CULTURES OF BOVINE PLACENTA

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Connexins (cx) are the subunits of hexagonal connexons which form gap junctions when docking to each other. Signals may not