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 β_2-adrenergic receptor β_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 β_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

only be transduced through gap junctions but also through the mere presence of connexons or even connexins. The expression of cx differs according to their location and function. In human and rodent reproduction, deficiencies in cx expression lead to inadequate embryo implantation and trophoblast invasion and also characterize stages of malignancy in endometrial carcinogenesis. Therefore, cell-cell communication via gap junctional cx may be essential for the restricted trophoblast invasion performed by migrating trophoblast giant cells (TGC) in the synepitheliochorial placenta of the cow. TGC fuse with single caruncular epithelial cells forming mostly trinucleated feto-maternal hybrid cells which deliver hormonal products to the maternal compartment.

To study the potential role of connexins during placentomal development we localized cx26, cx32 and cx43 in frozen sections from day 90-210 of pregnancy and primary cell cultures by immunofluorescence and confocal laser scanning microscopy. The presence of the corresponding cx proteins was confirmed by Western blot analysis.

Although cx26 was present in tissue sections (trophoblast cells) it could not be detected in placentomal cell cultures. Surprisingly cx32 was localized in nuclei of cultured caruncular epithelial cells, whereas in placentomal sections it was found in caruncular stroma and caruncular epithelium specifically at the tips of maternal septa. In contrast to in vivo material, only cultured fibroblastoid cells were positive for cx43. In tissue sections also mononuclear trophoblast cells showed an apical-lateral cell membrane associated cx43 expression. In TGC, cx43 signals differed depending on the localization within the placentome. In the centre of the placentome cx43 was associated to the cell membranes whereas at the base of the fetal villi TGC additionally showed cytoplasmatic cx43 specific fluorescence. In contrast, TGC which were about to fuse with uterine epithelial cells and hybrid cells were negative.

We may conclude that apical cx43 localization supports the hypothesis that cx43 connexons may be involved in the regulation of cell proliferation without forming gap junctions. The correlation of TGC invasion with the loss of cx43 suggests that cx43 plays an important role for the differentiation and migration of TGC. The unexpected finding of cx32 in nuclei of cultured caruncular epithelial cells cannot be explained up to date. The loss of cx26 and cx43 during cell culture of epitheloid cells may be due to suboptimal culture conditions. Funded by the German Research Foundation (DFG).

APPLICATION OF COMPUTER ASSISTED THREE-DIMENSIONAL VISUALIZATION TECHNIQUES IN HISTOLOGY, MEDICAL COMPUTER TOMOGRAPHY AND NUCLEAR MAGNETIC RESONANCE IMAGING

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Over the last years, three-dimensional imaging has become more and more prevalent in biomedical and material science studies. For almost every highly specialized application, different specific 3D-software solutions have been designed. Although the 3D visualization package amira ResolveRTTM has primarily been developed for confocal microscopy, it has also proven to be a valuable instrument in different other applications. In recent studies performed at our department, this stand-alone software pack turned out to be a rewarding tool in morphometrical examinations of the equine semicircular canals starting from medical CT images, ex-vivo haemodynamic studies in porcine and human livers observed by MRI and micro-CT, and histological investigations on the developing digestive system in sea bass larvae and juveniles. The major benefits of this versatile application include the fact that image segmentation is not necessarily based on pixel value thresholds, its ability to deal with unaligned or lost slices and its capacity to render different types of real 3D stereo images or movies, starting from a wide range of input data types. Although the user can easily intervene in almost every automated process such as image alignment or labelling, many of these manual corrections and adaptations are rather time-consuming. Another inconvenience is that the broad potential and complexity of the program causes a substantial load of the internal and graphical memory of the system. Notwithstanding these disadvantages, investment in this software is certainly paying off as it can offer unparalleled representations of complex structures as a basis for the development of new insights in various morphological domains.

Poster presentations: summaries Povzetki posterjev

ELEMENTS OF THE ANATOMY OF TWO STURGEONS (ACIPENSER STELLATUS PALLAS, 1771 AND *ACIPENSER BAERII* BRANDT, 1869): OSTEOLOGY AND RADIOLOGY

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Most of the studies†that have been carried out on†the Acipenseridae concern evolution, phylogeny, diversity, conservation and reproduction, notably concerning their economic importance for caviar production (Billard, 2002). However, information about the peculiar osteology of this family†is relatively rare. The only osteologic data that can be found in the literature (Grassé, 1958 ; Findeis, 1997) are difficult to use in the diagnosis of the bone remains discovered on archaeological sites and are only known for a few species (Radu, 2003). Consequently, a study associating dissections and radiology observations has been attempted in order to gather anatomical information.

Two individuals of two different species have been studied here: a Siberian sturgeon Acipenser baerii and a stellate sturgeon Acipenser stellatus. The first species is potamodromous (migration only in fresh water) while the second is diadromous (migration between salt water and fresh water).

This work is a preliminary contribution to the knowledge of the osteology of these two species of sturgeons and we hope that it will be developed in a near future.

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