In this presentation the results of the co-operation between the School of Arts and the Faculty of Veterinary Medicine to develop a haptic interface to practice rectal examination will be shown.

The goal of the project was to develop a device that can be used between the classes of our regular course of topographical anatomy and the rectal examination in the clinical phase of the veterinary school teaching program. The major learning goal was to achieve the 3D orientation in the cow. The result is the Sensa cow which has wax elements with sensors. After evaluation it appears that Sensa is very useful in learning the first 3D orientation in the cow.

DIFFERENCES IN SKIN COMPONENTS INSIDE REPTILIAN AND AMPHIBIAN GROUPS

Malan Štrbenc
Institute for Anatomy, Histology and Embryology, Veterinary Faculty, University of Ljubljana, Slovenia
E-mail: malan.strbenc@vf.uni-lj.si

Reptiles and amphibians became increasingly popular pets. In recent years the knowledge on medicine of these animals is improving; however there is a gap between general knowledge on morphology and detailed studies of certain organs on selected species. Comparative study of integument of conventionally kept species, dissected at student practical work was performed. Both reptiles and amphibians demonstrate skin shedding or slough and commonly histology slides show the upper, shedding layer of skin.

While amphibians in terrestrial phase show slightly more prominent keratinisation, the skin is very thin. In dermis there are prominent poison (serous) and mucous glands, and secreting Leydig cells are occasionally encountered in epidermis. While axolotl was not known to posses toxic or irritating skin secretion, we found prominent poison glands. Buffonidae are supposed to have poison glands concentrated on warts. We did find numerous poison glands also on other parts of the body but the size of them increased from abdomen through legs and was greatest at warts on dorsum. In Ranidae the size of poison and mucous glands was approximately the same. In aquatic species Leydig cells were more numerous.

While snakes have similar strength and distribution of scales, in lizards seemingly the skin toughness varies a lot. However, the epidermis on flank skin (excluding ornamental scales) was only twice as thick in green iguana (or tortoise red-eared slider) compared to leopard chamelon, tocke gecko and leopard gecko. The main difference is in dermis. Geckos are colloquially known as scaleless lizards, nevertheless, typical overlaps and hinges were also found.

The black and white subcutaneous glandsî that students found on the neck region in tocke gecko and Rana frog turned out to be at least in part lymphatic tissue.

EPIDERMAL SHEETS - PREPARATION, QUALITY CONTROL, IMUNOHISTOCHEMISTRY AND VISUALISATION BY CONFOCAL MICROSCOPY

Kirsti Witter
Histology and Embryology, Department of Pathobiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria
E-mail: kirsti.witter@vu-wien.ac.at

Epidermal sheets are often used for studies regarding exclusively the outer skin layer. They can be prepared with inorganic salt solutions or enzymes and used subsequently e.g. for tissue culture and immunohistochemistry. The aim of this study was to test selected methods for preparation of epidermal sheets and to assess conservation of histological structure, stainability including immunohistochemical staining and the possibility of visualisation of staining results by confocal microscopy. Punch necropsy samples (diameter 3 mm) of shaven neck skin of an eight days old piglet euthanised for another study were taken and stored in a moist chamber at 4°C before processing. Epidermal sheet preparation was attempted after incubation with 2 M CaCl2 solution (20 min, 37°C), with 20 mmol/l EDTA-solution (3 hours, 37°C), or with 0.1% trypsin solution (30-120 min, 37°C). Whole mount immunohistochemistry and/or nuclear staining with DAPI was performed without permeabilisation of the sheets, after permeabilisation with chilled acetone, or after permeabilisation with 0.1% Triton X-100. Staining results were visualised using a laser scanning confocal microscope. For quality control, selected samples were embedded in paraffin and epoxy resin for light and electron microscopy, respectively. The easiest and least time consuming method for epidermal sheet preparation was incubation in a CaCl2 solution. The epidermis was firm enough to handle and peeled of the corium without difficulties, including epithelial root sheaths of hairs. Preparation of epidermal sheets with trypsin was unsuccessful, even after prolonged incubation. Only a 0.5†mm margin of the epidermis could be detached from corium, both corium and epidermis were very brittle. CaCl2 as well as EDTA sheets stained well without differences regarding different pretreatment methods. Morphology of epithelium and corium was conserved satisfyingly in all samples. Interestingly, basement membrane material (laminin, PAS-positive material) could be found on both epithelium and corium, indicating a splitting of the membrane itself during preparation. Connective tissue did never remain on epidermal sheets. If the basement membrane was split incompletely during preparation, basal epithelial cells remained on the surface of corium. Confocal microscopy could be used successfully to visualise individual cell layers of the epidermal sheets. However, epithelial root sheaths of hairs caused a wavy appearance of the epidermis and impaired the assessment of e.g. cell numbers and stratification. In conclusion, skin epidermis can be easily detached from corium after incubation in a CaCl2 solution. Whole mount immunohistochemical staining as well as routine histology and staining of sections are possible without disruption of the sheets. For hairy skin removal of root sheaths from the epidermis should be attempted for confocal microscopy.

CONFOCAL MICROSCOPY – A TOOL TO STUDY 7TM RECEPTOR CHIMERAS OF GHRELIN RECEPTOR WITH GABAB RECEPTOR TAIL-SWAP

Valentina Kubale[1,2], Azra Pogačnik1, Birgitte Holst[2], Thue W. Schwartz[2,3], Milka Vrecl[1]
[1]Institute for Anatomy, Histology and Embryology, Veterinary Faculty of University of Ljubljana, Gerbičeva 60, SI-1115 Ljubljana, Slovenia; [2]Laboratory for Molecular Pharmacology, Department of Pharmacology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200, Copenhagen, Denmark; [3]7TM Pharma, Fremtidsvej 3, DK-2970, Hørsholm, Denmark

Seven transmembrane receptors (7TM-Rs) also designated as G protein-coupled receptors (GPCRs), were traditionally thought

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

Žužek Monika Cecilija, Frangež Robert
Veterinary Faculty, Institute for Physiology, Pharmacology and Toxicology, University of Ljubljana, Slovenia

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

Alenka Hrovat, Robert Frangež, Azra Pogačnik, Milka Vrecl
Veterinary Faculty, University of Ljubljana, Slovenia

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.

Acknowledgements

This study was supported by the Slovenian Ministry of Higher Education, Science and Technology (grant number 0406-007).

DEMONSTRATION OF CONNEXINS IN CELL CULTURES OF BOVINE PLACENTA

Christiane Pfarrer[1], Philip S. Bridger[2], Rudolf Leiser[2]
[1]Dept. of Obstetrics and Gynecology, [2]Dept. of Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University Giessen, Germany

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