resolution over conventional fluorescence microscopy, optical sectioning of examined samples, 3-D images reconstruction and multi channels acquisition enabled widespred use of confocal microscopy in the cell biology imaging.

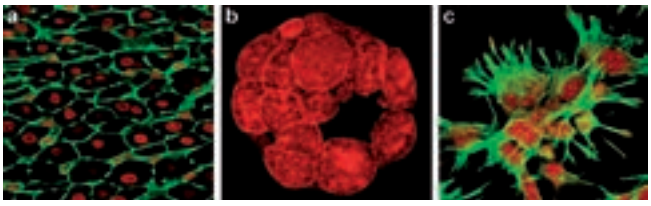

Figure 1: Confocal images of actin and microtubules cytoskeleton organisation. (a) confocal image of liver tissue section shows cortical actin organisation of rat hepatocytes (green signal) (b) actin cytoskeleton distribution in blastomeres of three days old rabbit embryo shown in red (c) cultured HEK-293 cells showing normal distribution of microtubule filaments (green signal). TO-PRO-3 iodide stained nuclei (a, c) are shown in red.

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MITOCHONDRIAL TRIGGERING OF CELL DEATH AND CONFOCAL MICROSCOPY

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The laser scanning confocal microscope detects images of multiple labelled fluorescent samples. One can follow intracellular distribution of the protein under investigation by tracing the location of fluorescently labelled protein or fluorescent antibodies directed against the protein under investigation and of marker proteins for cellular compartments. This is useful to localize the protein under investigation and even more to follow the movements of a particular protein within the living cells. Here we present an example of procaspase-9 movements during the early stages after triggering apoptosis, before its activation can be detected by other biochemical methods.

Apoptosis is a process that controls the number of cells and their quality. Procaspase-9 is the inactive form of one of the main apoptotic initiators, caspase-9. It is activated as a consequence of mitochondrial damage and can be also activated directly or indirectly by other initiator caspases. There were contrasting reports that caspase-9 is in different cellular compartments, i.e. in the cytosol, the nucleus and in the mitochondria. We have determined that procaspase-9 is located in the cytoplasm in physiological conditions in rat neurocrine cells and rat hepatocytes, by transfecting the cells with DNA encoding the fluorescent fusion protein between the caspase-9 and enhanced green fluorescent protein (EGFP) and by immunocytochemistry. However, upon the induction of apoptosis, procaspase-9 is translocated to mitochondria. This shift depends on an activated caspase, other than caspase-9. The colocalization signal of caspase-9 and of mitochondria observed under the confocal microscope does not tell us whether the caspase-9 is associated with mitochondria or it is located closely to the mitochondrial outer membrane. Through biochemical methods, like cellular fractionations, in vitro import of proteins into mitochondria, mitochondrial fractionations and protease treatments of mitochondrial membranes, we determined that procaspase-9 is attached to the outer surface of the mitochondrial outer membrane shortly after the initiation of apoptosis.

MEDCIAL IMAGE ANALYSIS

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Over the last decades, medical imaging has witnessed a diversification of image formation methods, which has led to a rich palette of modalities providing information on many aspects of human anatomy, physiology, and pathology. In order to use the vast amount of available image information efficiently, the relevant image content needs to be extracted, analyzed, and interpreted. For a human operator, it is by no means trivial to interpret the images accurately in a limited amount of time. In addition, such an interpretation is subjective and generally irreproducible. Accordingly, a number of image analysis techniques have been introduced to assist the human expert in a broad variety of tasks, such as image restoration, image segmentation, image registration, motion tracking and change detection, and measurement of anatomical and physiological parameters. Image analysis techniques, which have expanded the role of medical imaging beyond mere visualization, are nowadays used increasingly throughout the clinical track of events, not only within diagnostic settings, but also prominently in the areas of planning, performing, and evaluating surgical and radiotherapeutical procedures.

Oral presentations: abstracts – Predavanja: izvlečki

COMPARATIVE MORPHOFUNCTIONAL ORGANIZATION OF THE ENTERIC NERVOUS SYSTEM IN MAMMALS

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The gastrointestinal (GI) tract fulfils a variety of functions such as transport of luminal content, secretion, absorption of ions, water and nutrients, blood flow, defence against pathogens and elimination of waste material. The enteric division of the autonomic nervous system (i.e., the enteric nervous system (ENS), the brain in the gut, the small brain) organizes and coordinates these activities in a dynamic way through interaction with different cell systems, including the interstitial cells of Cajal, the enteric glia, the smooth musculature, and the vascular, immune and mucosal epithelial systems. The ENS is composed of enteric neurons and glial cells which arise from vagal and sacral precursors cells of the neural crest line. The ENS extends along the entire GI tract and contains an estimated 108 neurons which are situated between two major layers in two interconnected ganglionated plexuses: the myenteric plexus

(MP), between the longitudinal and circular muscle, and the submucosal plexus (SP) associated with the mucosal epithelium. Both plexuses are composed of ganglia (contain neurons and glial cells) and interconnecting nerve fibre strands, which consist of the axons of myenteric neurons, the axons of extrinsic neurons that project to the gut wall and glial cells. Over the last decades, several studies dealing with the ENS of different species have revealed that the architecture of the enteric plexuses is more complex in larger animals, including man, than in small animals. The MP forms a continuous network that is continuous around the circumference and extends from the upper oesophagus to the anal sphincter. Its texture and ganglionic density show regional differences in the same individual, and differences between species. The submucous plexus exhibits a limited number of neurons in the oesophagus and gastric compartments, with a more complex intramural structural organization in the ruminant forestomach, and a continuous plexus in the intestine, that is situated on one plane in small animals, and multilayered and functionally distinct in large animals.

GI neurons release a plethora of substances that are chemically different but only partially have been identified functionally.

Combined morphological, electrophysiological, pharmacological, neurochemical and retrograde labelling, has led to identification of GI neurons into different functional classes, i.e., sensory neurons, interneurons, excitatory and inhibitory motor neurons. These neurons are interconnected by chemical synapses into intrinsic neuronal circuits that generate functional reflexes: they are partly independent of the central nervous system (CNS). In the intestine reflex functions arise even if the segment has been isolated from the body.

FLOURESCENT IMMUNOCYTOCHEMISTRY – A METHOD FOR STUDYING GENE EXPRESSION IN A MOUSE BRAIN

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Fluorescent microscopy techniques are excellent tool for studying cell identity and micro-circuitry in the brain. We are using Steroidogenic factor 1 knockout mice (SF-1 KO) and mice expressing GFP under the influence of SF-1 promotor as models to study neuroendocrine brain development. In the SF-1 KO mice, a very specific disorganization of the ventromedial hypothalamic nucleus (VMH) occurs, with all other parts of the brain being intact.

For studying gene expression in the mouse brain, immunocytochemistry on free-floating sections is used. To obtain brain tissue, mice are perfused with 0.05M PBS and 4% paraformaldehyde. 50?m thick coronal brain sections are cut on an Integraslice vibrotome (Campden instruments) and further processed for immunocytochemistry. For the present study, primary antibodies against calbindin D-28k raised in mouse, estrogen receptor alpha and green fluorescent protein both raised in rabbits, were used. For fluorescent detection of bound antibodies, sections were incubated with secondary antibodies conjugated with Cy2 or Cy3 fluorophores. Bound Cy2 and Cy3 flourophores were visualized under specified excitation wavelengths using confocal microscope. Primary mouse and rabbit antibodies (anti GFP/anti calbindin, anti ERalpha/anti calbindin) were used simultaneously while labelling of GFP/ER alpha coexpressing cells was performed by sequential incubation with each antibodies.

Immunocytochemistry with all three antibodies produced a strong fluorescent signal. Examination of sequential sections revealed that calbindin and ER-alpha are expressed in the same cells both in WT and SF-1 KO mice, even though the location of these cells is altered in SF-1 KO mice, while GFP cells (SF-1 expressing cells) do not co-express either ER-alpha or calbindin.

THE INFLUENCE THROUGH FEEDING ON THE FAT PADS IN THE BOVINE DIGITAL CUSHION

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The effect of an intensive respectively extensive feed on the fat content and the fatty acid profile of the bovine digital cushion was tested by examining the claws of 32 feedlot animals. In addition, it was examined if, respectively how the fatty acid profiles can affect the claw health. Samples from subcutaneous adipose tissue and the claws of 9 cows served as comparison. Furthermore, the microscopic structure of the fat pads was analyzed and the results were compared with those from previous studies.

The fat pads as well as the subcutaneous adipose tissue showed obvious differences in the fat content and the fatty acid profile between the two different feeding-groups. The fat pads of the intensive fed animals contained a lot less fat and noticeable more omega-6-fatty acids, above all Linoleic and Arachidonic acid. In addition, these animals showed the highest proportion of Eicosapentaenoic acid (EPA) and Docosahexapentaenoic acid (DHA), two omega-3-fatty acids. The extensive fed animals contained more omega-3-fatty acids, mainly α- Linoleic acid. Also the subcutaneous adipose tissue of the intensive fed animals showed a much higher proportion of omega-6-fatty acids, whereas the extensive fed group had a higher proportion of omega-3-fatty acids. The differences in the fatty acid profile are for sure due to the different composition of the feeds.

The claws of the intensive fed animals showed post mortem a significant better claw health than the extensive fed group.

THE SENSA COW, A HAPTIC MODEL FOR RECTAL EXPLORATION TO BRIDGE THE GAP BETWEEN ANATOMY AND CLINICAL WORK: PRESENTATION OF THE WORK OF THE COWBOYS EMMA PROJECT GROUP

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