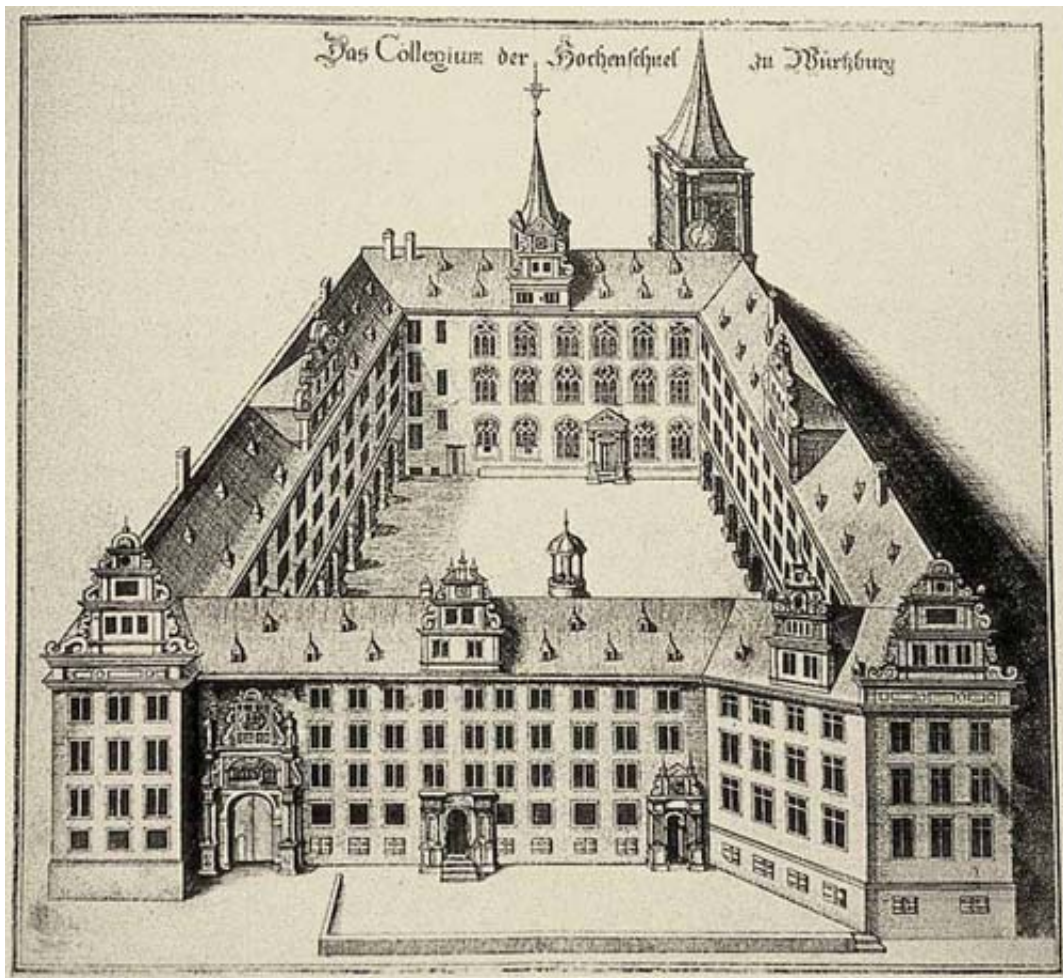


**Zusammenfassung aller Vortrags- und
Posterabstracts der 24. Arbeitstagung der
Anatomischen Gesellschaft in Würzburg vom
26.09. bis 28.09.2007**



- 1. Vortragsabstracts**
- 2. Posterabstracts**

Vortrag 1

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:MAST CELL-DEFICIENT MICE DISPLAY INCREASED BRAIN INFLAMMATION, NEURODEGENERATION AND ASTROGLIOSIS

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Abstract:

Mast cells play a key role in the development and severity of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis. However, the role of mast cells in the wound-healing response after traumatic brain injury is unknown. Here, we compared the CNS wound-healing response in mast cell-deficient W/W^v-mice and wildtype controls after entorhinal cortex lesion by immunohistochemistry, microarray analysis and multiplexed immunoassays. Microarray analysis revealed 1076 significantly regulated gene products after ECL in wildtype mice, as well as 1617 differentially regulated gene products, when comparing lesioned brain tissue of wildtype and mast cell-deficient mice.

To characterize the wound-healing response on the protein level, we analyzed the immunoreactivity patterns of 7/4 (neutrophils), CD3 and CD4 (T cells), IBA-1 (macrophages & microglia), GFAP (astrocyte activation), MBP (myelin), Ki-67 (proliferation), and FluoroJade B (neurodegeneration) after injury.

In the early phase of the CNS wound-healing response, mast cell-deficient W/W^v-mice showed a decrease of neutrophil invasion, however, in later phases a significant increase in invading T cells, macrophages/microglia and activated astrocytes was evident. The number of proliferating astrocytes and macrophages/microglia was dramatically increased. Finally, at day 4 after lesion, there were a significantly higher number of FluoroJade B-positive neurons in mast cell-deficient mice, suggesting increased neurodegeneration. The levels of endothelin-1, a classical substrate for mast cell proteases, were significantly upregulated in the brains of MC-deficient mice. These data suggest that MCs protect from brain inflammation, neurodegeneration and astrogliosis, possibly mediated by the degradation of endogenous endothelin-1.

Kategorie: Vortrag

Vortrag 2

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:INDUCTION OF ANTIMICROBIAL PEPTIDE rCRAMP BY BACTERIAL COMPONENTS IN GLIAL CELLS

Autoren: Brandenburg L.(1),Varoga D.(2),Nicolaeva N.(1),Leib S.(3),Wilms H.(4),Podschun R.(5),Pufe T.(1),Deuschl G.(4),Wruck C.(1),Sievers J.(1),Lucius R.(1),

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Abstract:

Antimicrobial peptides are a part of the innate immune system at epithelial surface, and may also have important functions in the brain. However, little is known about the expression of antimicrobial peptides in the CNS and whether neural cells can secrete these peptides. We have used cell cultures, real-time PCR, immunohistochemistry, ELISA and an animal model to get more information about the role of antimicrobial peptides in the CNS. In detail, we have investigated the expression of the antimicrobial peptide rCRAMP (homologe of the human LL-37) in rat glial cells (astrocytes and microglia) after incubation with bacterial components. Furthermore, we used cerebrospinal fluid (CSF) and serum from patients with bacterial meningitis to detect LL-37 and other antimicrobial peptides. Finally, we investigated the occurrence of rCRAMP in an animal model of bacterial meningitis. We here demonstrate (i) not only the expression but also secretion of biological active rCRAMP in glial cells, and (ii) the occurrence of antimicrobial peptides in the cerebrospinal fluid of meningitis patients. Moreover, we could show an involvement of rCRAMP in the rat meningitis model pointing to a role of rCRAMP in the pathogenesis of this disease. Our results suggest that rCRAMP respectively LL-37 is an important part of the innate immunity in the brain against bacterial pathogens.

Kategorie: Vortrag

Vortrag 3

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:MUCIN SUGARS ARE FINE-TUNED TO BACTERIA

Autoren: Berry M.(1),Nelson S.(2),Corfield T.(3),

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Abstract:

Purpose The components of tears reflect competing needs for transparency, stability, hydration, and protection of the ocular surface. Oligosaccharides on many glycoconjugates, including mucins, might represent expandable binding ligands that affect microbial binding and invasiveness. The structure of O-linked glycans derived from human ocular mucins provided the basis for exploring mucin and lectin effects on *Pseudomonas aeruginosa* invasion of human ocular epithelial cells in vitro.

Methods Human ocular mucins were purified; their O-glycans released by hydrazinolysis, and analysed with a combination of HPLC, exoglycosidase digestions and LC-MS/MS. Adherence and invasiveness of *P. aeruginosa* transfected with a plasmid incorporating *Photobacterium luminescens lux* gene was monitored by the decrease in luminescence after antibiotic treatment and saponification, respectively. Purified mucin and lectins were added with the bacterial inoculum.

Results The oligosaccharides were short: most commonly tetra-, tri- or disaccharides. Most of the 12 different O-glycans were negatively charged and terminated in sialic acid, in α -2-3 or α -2-6 linkage. Treatment of cultured cells with cognate lectins for these terminal sugars had no effect on bacterial adherence. Addition of mucins decreased adherent bacteria and was synergistic with the lectins. However, neither lectins nor mucins could block *P. aeruginosa* invasion on permissive cells.

Conclusions Glycan expression is related to microbial and environmental challenges. The inability of lectins to block bacterial binding is indicative of the combinatorial mixtures of ligands at the cellular surface.

Kategorie: Vortrag

Vortrag 4

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel: DETECTION OF ALL FOUR SURFACTANT PROTEINS IN HUMAN TEAR FLUID AND THE HUMAN LACRIMAL SYSTEM

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Abstract:

To evaluate the expression and presence of the surfactant proteins (SP) A, B, C and D in the lacrimal apparatus, at the ocular surface, in tears and aqueous humor. Expression of mRNA for SP-A, -B, -C and -D was analyzed by RT-PCR in healthy lacrimal gland, conjunctiva, cornea and nasolacrimal ducts. Deposition of all surfactant proteins was determined by Western blot and immunohistochemistry in healthy tissues, in tears and aqueous humor. Additionally, expression of SP-A and SP-D was observed for immortalized human corneal and conjunctival epithelial cells lines. The presence of both SP-A, -B, -C and -D on mRNA and protein level was evidenced in healthy lacrimal gland, conjunctiva, cornea and nasolacrimal duct samples. Moreover, both proteins were present in tears but were absent in aqueous humor. Immunohistochemistry revealed production of the four peptides by acinar epithelial cells of the lacrimal gland as well as epithelial cells of the conjunctiva and nasolacrimal ducts. Healthy cornea revealed only weak reactivity on epithelial surface cells for SP-A and SP-D but no reactivity for SP-B and SP-C. Our results show that, in addition to SP-D, SP-A, SP-B and SP-C are peptides of the ocular surface and the human lacrimal apparatus. Based on the known direct and indirect antimicrobial effects of collectins, the surfactant-associated proteins A and D seem to be involved in several ocular surface diseases, whereas SP-B and SP-C might support surface tension features of the tear film.

Kategorie: Vortrag

Vortrag 5

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:TREATMENT WITH THE ADJUVANT ALUM ALTERS THE LOCALIZATION OF THE DISEASE CAUSING B CELLS IN MICE WITH EPIDERMOLYSIS BULLOSA ACQUISITA (EBA)

Autoren: Bieber K.(1),Sitaru C.(2),Zillikens D.(2),Westermann J.(1),

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Abstract:

Lymphocytes can react against self-components which can lead to the establishment of autoimmune diseases. An example is the deposition of auto-antibodies against collagen type VII at the dermal-epidermal junction which leads to blistering and epidermolysis bullosa acquisita (EBA). In EBA a dominance of CD4 T cells is described which preferentially produce IFN-g (T-helper1 cells; Th1). In the present study we addressed the question, whether a shift from Th1 to Th2 cells (preferentially producing IL-4) would ameliorate the course of the disease. In a mouse model of EBA Th2 cells were induced in vivo by using Alum as an adjuvant. Three major observations were made:

- 1) The course of the disease was improved as shown by the reduction of the clinical score.
- 2) In the blood the concentration of collagen-specific antibodies of the Th1 (IgG2a) and the Th2 subtype (IgG1) were identical among the groups.
- 3) In Alum treated mice the number of antigen specific B cells in the spleen was increased in the red pulp and decreased in the follicles.

Our results indicate that the affinity of the antibodies in the alum treated group is reduced thereby causing a milder disease. This conclusion is supported by the observation that the localization of the antibody producing cells is altered in the treated animals. It is known that the affinity of antibodies is influenced by the localization of the antibody producing cell. Thus, further studies will characterize in detail the distribution of antigen-specific B cells and directly demonstrate the reduced affinity of anti-collagen antibodies.

Kategorie: Vortrag

Vortrag 6

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:EXPRESSION OF INTERLEUKINS IN HUMAN PANCREATIC ISLETS EXPOSED IN VITRO TO A TYPE 2 DIABETES MILIEU

Autoren: Eppler E.(1),Ehses J.(2),Ellingsgaard H.(2),Donath M.(3),Reinecke M.(1),

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Abstract:

Pancreatic islet pathology in patients with type 2 diabetes is characterised not only by reduced beta-cell mass and function but also by increased alpha-cell mass and dysfunction. Cytokines are presently recognized as factors involved in this pathology. The present study investigates the presence of IL-1beta, IL-6 and IL-8 in human islets isolated from pancreata of organ donors under different experimental conditions. The islets were cultured in the presence of low (5 mM), high (33 mM) glucose, or palmitate (0.5 mM). Islets from 4 separate isolations were investigated by immunocytochemistry (semi-thin sections) and double-immunogold-labeling at the ultrastructural level using antisera specific for IL-1beta, IL-6, IL-8, glucagon, and insulin. IL-1beta was mainly confined to beta-cells where it was found within the insulin-containing granules and especially expressed under high glucose. In contrast, IL-6 and IL-8 occurred in alpha-cells but not in beta-cells or delta-cells, suggesting that islet IL-6 and IL-8 are alpha-cell-derived. By electron microscopy, IL-6 and IL-8 were detected in alpha-cell granules together with glucagon. Pre-treatment of the human islets with high glucose strongly enhanced the IL-6 content of the alpha-cells. Under treatment with high glucose and 0.5 mM palmitate, also IL-8 was elevated. In summary, all interleukins investigated occurred in untreated isolated human islets with a differential localization pattern. Challenge with high glucose +/- hyperlipid treatment elevated IL-6 and IL-8 peptide contents within the alpha-cells. The data support the concept of endocrine derived production of inflammatory factors which may contribute to islet failure in the pathogenesis of type-2 diabetes.

Kategorie: Vortrag

Vortrag 7

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:INTRAVITAL TWO-PHOTON MICROSCOPY OF MURINE PEYER'S PATCHES: DYNAMICS OF LYMPHOCYTE TRAFFICKING IN M-CELL POCKETS

Autoren: Gebert A.(1),von Smolinski D.(1),Orzekowsky R.(2),Koop N.(2),Hüttmann G.(2),Klinger A.(1),

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Abstract:

Tissue micro-environments are likely to exert profound impacts on cell behaviour, yet most studies have relied on cultured cells or fixed tissues that fail to convincingly reflect the complexity and dynamics of tissue micro-compartments. Using two-photon laser scanning microscopy based on NADPH fluorescence, we established a novel experimental setup that allows intravital multi-dimensional imaging of the murine intestinal mucosa to be performed over several hours. In optical sections about 1 micron in thickness, we identified membranous (M) cells in the undisturbed physiological environment of the Peyer's patch dome epithelium, and three-dimensionally analysed their association with intraepithelial lymphocytes and dendritic cells. Time-lapse series of optical sections across the dome epithelium showed that the contained lymphoid cells are in vigorous movement and typically migrate at a speed of 5–15 microns/min. While these intraepithelial lymphocytes frequently move from one M cell cluster to the next, entry into or out of the dome epithelium are relatively rare events. Time-lapse sequences further revealed that the lymphocyte clusters beneath M cells are highly dynamic structures which may form or disappear within less than 20 min. These data provide a level of perception previously unattainable, and, for the first time, depict the in vivo interactions of epithelial and lymphoid cells going on in mucosa-associated lymphoid tissues. Our novel approach will enable us to study time-dependent cell-cell interactions and immunological processes during the initiation of immune responses.

Kategorie: Vortrag

Vortrag 8

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:TH2-CYTOKINES MODULATE THE EXPRESSION OF CELL MATRIX RECEPTORS AND COLLAGEN TYPE I IN TENOCYTES

Autoren: Schulze-Tanzil G.(1),Jammrath J.(1),Müller R.(1),Kohl B.(1),John T.(1),

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Abstract:

More and more evidence arose that the immunoregulatory Th2 cytokines interleukin(IL)-6 and IL-10 contribute to extracellular matrix remodelling of connective tissues involved in processes such as wound healing. Since their role in tendon remains unclear, the aim of the present study was to investigate the effect of these cytokines on key parameters of tendon homeostasis such as cell proliferation, synthesis of extracellular matrix and cell matrix receptors, essential for tendon rupture healing.

Human tenocyte cultures were investigated for endogen IL-10 production and IL-10 receptor-alpha expression as a prerequisite for IL-10 signalling using flow cytometry. Serum starved tenocytes were treated with IL-6, IL-10 or the pro-inflammatory cytokine TNFalpha (10 or 50 ng/mL, 48 h). Expression of the main extracellular matrix component collagen type I, cell matrix signal transduction receptor beta1-integrin and the cytoskeletal signal protein vinculin was studied using western blot analysis and immunohistochemistry. Cell proliferation was evaluated using a CFDA-SE proliferation assay.

The tenocytes produced IL-10 and expressed the respective IL-10 receptor-alpha. IL-6 as well as IL-10 exhibited a stimulatory effect on tenocyte proliferation. In contrast, cell proliferation was inhibited by TNFalpha. Additionally, IL-6 increased beta1-integrin and collagen type I expression. TNFalpha had a slightly stimulatory effect on integrin expression but decreased collagen type I. The synthesis of cytoskeletal protein vinculin remained unaffected by these cytokines.

We conclude that the Th2 cytokines investigated in the study are involved in the regulation of cell-matrix homeostasis and proliferation of tenocytes and might be implicated in processes such as tendon healing.

Kategorie: Vortrag

Vortrag 9

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:ON THE WAY TO THE DENERVATED EYE AS A KEY FOR UNDERSTANDING THE INTRINSIC CHOROIDAL INNERVATION: FIRST RESULTS

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email:falk.schroedl@anatomie1.med.uni-erlangen; (2)Rome|Dip. Biologia Cellulare e dello Sviluppo|Rome|I; (3)New England College of Optometry||Boston, MA|USA

Abstract:

Choroidal innervation controls blood flow, hence intraocular pressure (IOP). It also mediates choroidal thickness, which is relevant in experimentally induced myopia.

Aim of the study was to eliminate extrinsic autonomic innervation of the eye.

In chickens, transection of the sympathetic pathway (via superior cervical ganglion) and both parasympathetic pathways (via ciliary ganglion and pterygopalatine ganglion; PPG) was performed. In birds, only preganglionic transection of the PPG can be performed. IOP was measured in both eyes. 9 days after transections immunohistochemistry for nNOS, VIP, CGRP, Somatostatin (SOM), ChAT and TH was performed in choroids and PPG.

In both eyes reduced IOP was measured after surgery. 3 days pOP, IOP was down to R= -5 mmHg and L= -2 mmHg compared to the preexperimental value. Until day 9 pOP, this is followed by a rise in IOP in both eyes to R= -2mmHg compared to preexperimental value, and to L= normal. Immunohistochemistry shows almost complete absence of SOM and TH in the ipsilateral choroid, while ChAT remains in nasal parts. In ipsilateral ciliary nerves, increased CGRP immunoreactivity is detectable. Intrinsic choroidal neurons (VIP/nNOS) remain, as do neurons of the PPG. Within this ganglion, SOM positive boutons are absent.

Reduction of IOP in both eyes indicates a contralateral effect. Ipsilateral upregulation of IOP might be mediated by intrinsic choroidal neurons or primary afferent fibres (via CGRP). The role of „surviving“ neurons of the PPG (ChAT in nasal parts of choroid) is unclear. SOM boutons in the PPG originate in the ipsilateral ciliary ganglion, enabling interganglionic signal transduction.

Kategorie: Vortrag

Vortrag 10

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:PERIOCULOMOTOR CELL GROUPS IN MONKEY AND MAN: A REAPPRAISAL OF THE EDINGER-WESTPHAL NUCLEUS.

Autoren: Horn A.(1),Härtig W.(2),Büttner-Ennever J.(1),

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(2)Universität Leipzig|Paul-Flechsig Institut|Leipzig|Deutschland

Abstract:

The periculomotor area around the oculomotor nucleus (nIII) contains functional cell groups which cannot be identified by their cytoarchitecture alone: parasympathetic preganglionic neurons of the ciliary ganglion, eye muscle motoneurons of multiply-innervated muscle fibres (MIF) and urocortin-positive neurons. Traditionally the parasympathetic preganglionic neurons are referred to as lying in the Edinger-Westphal nucleus (EW).

To outline the periculomotor groups in monkey and human, midbrain sections were immunostained for choline-acetyltransferase (ChAT), cytochrome oxidase (CytOx), non-phosphorylated neurofilaments (NP-NF), chondroitinsulfate proteoglycan (CSPG) and urocortin (UCN). For the first time the MIF-motoneurons were identified in human - as small ChAT-positive neurons lacking CSPG-immunoreactivity. In monkey, preganglionic neurons were characterized by their ChAT-, NP-NF- and strong CytOx-immunoreactivity, and their lack of UCN- and CSPG-immunostaining. They lie within the EW medial to the separate UCN-positive group. However, in human the UCN-positive neurons occupy the traditional EW, which did not contain ChAT-positive neurons. In fact based on their staining properties in monkey, the presumed human preganglionic neurons were found as an inconspicuous ChAT- and CytOx-positive group dorsal to the UCN-positive population.

For a comparison between species, a new nomenclature is proposed, which designates periculomotor groups (pIII) in terms of their function and histochemistry. The name Edinger-Westphal nucleus is kept for the cytoarchitectural entity, but the addition of "PG" and "U" specifies its main population. Accordingly, in monkey it is termed EWPG and in human EWU. These results provide the basis for addressing the appropriate functional cell groups in correlative clinico-pathological studies for example of Alzheimer disease.

(DFG-Projekt Ho-1639/4-1/4-2)

Kategorie: Vortrag

Vortrag 11

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:LAYER-SPECIFIC EXPRESSION OF MULTIPLE CADHERINS IN THE DEVELOPING VISUAL CORTEX OF THE FERRET

Autoren: Kumar. K.(1),Nuernberger M.(1),Weth F.(2),Redies C.(1),

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Schiller-Universität Jena|Nachwuchsgruppe Neurogenetik|Jena|Deutschland

Abstract:

Cadherins are superfamily of Ca²⁺-dependent transmembrane glycoproteins with more than 100 members. They play a role in a wide variety of developmental mechanisms, including cell proliferation, cell differentiation, cell-cell recognition, neurite outgrowth and synaptogenesis. The development of the mammalian cerebral cortex is a highly orchestrated process that comprises many of the above-mentioned mechanisms. Therefore, studying the expression of cadherins can help us to understand the molecular cues regulating corticogenesis.

We cloned 16 novel members of the classic cadherin (Cdh) and delta-protocadherin (Pcdh) subgroups from ferret brain. Their expression patterns were investigated by in situ hybridization in the primary visual cortex (V1) of the ferret, a model system for cortical development, at various stages of development (from E23 to the adult stage). Our results demonstrate that all 16 cadherins are expressed in a spatiotemporally restricted fashion throughout development. Generally, the expression of Pcdhs sets in at early embryonic stages, whereas that of Cdhs begins in late gestation or following birth. Expression of Pcdhs is more widespread while expression of Cdhs is more restricted to specific layers. Each layer of V1 can be characterized by the combinatorial expression of a subset of cadherins at any given developmental stage. A few cadherins are expressed by subsets of neurons dispersed throughout all cortical layers.

In conclusion, our results suggest that cadherins provide a potentially adhesive code during layer formation in V1. The persistence of expression in the adult suggests a functional role also in the mature cortex.

Kategorie: Vortrag

Vortrag 12

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:AXONAL PATHWAY CHOICE IN THE OPTIC SYSTEM OF TUPAIA
BELANGERI

Autoren: Knabe W.(1),Washausen S.(1),Happel N.(2),Kuhn H.(1),

Adressen:(1)Georg August University|Department of Anatomy and Embryology|Göttingen|Germany; email:wknabe@gwdg.de; (2)Georg August University|Institute for Biochemistry and Molecular Cell Biology|Göttingen|Germany

Abstract:

According to the current view, different mechanisms underlie pathway choice of ipsilaterally projecting ganglion cell axons in the optic chiasm of marsupials (Metatheria) and placental mammals (Eutheria), respectively. In all marsupials studied, ipsilaterally and contralaterally projecting axons segregate already in the prechiasmatic optic nerve. In contrast, permanent ipsilaterally projecting axons turn close to the chiasmatic midline in mice, rats, and ferrets. We wished to learn, whether interactions in the chiasmatic midline are also required to establish the pathways of ipsilaterally projecting axons in *Tupaia belangeri* (Scandentia, Eutheria). Routinely as well as immunohistochemically stained serial sections from embryonic day 18 (E18) to birth (around E43) were studied. We found that ipsilaterally projecting ganglion cell axons made their turns in the prechiasmatic optic nerve, thus, at a distance to the chiasmatic midline. Most probably, these ipsilateral turns depended on glial guide post cells which transiently existed at the border between the optic nerve and chiasm. Thus, contrary to expectations, highly similar mechanisms appear to underlie pathway choice of ipsilaterally projecting ganglion cell axons in *Tupaia belangeri* and marsupials. Clinical observations suggest that ipsilaterally projecting axons are also confined to lateral parts of the human optic chiasm. Hence, ongoing studies in *Tupaia belangeri* may contribute to a better understanding of the mechanisms which regulate axon pathway choice in primates.

Kategorie: Vortrag

Vortrag 13

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:SCLEROTOMAL ORIGIN OF SMOOTH MUSCLE CELLS IN THE WALL OF THE AVIAN DORSAL AORTA

Autoren: Wiegrefe C.(1),Christ B.(1),Huang R.(1),Scaal M.(1),

Adressen:(1)Freiburg|Institut für Anatomie und Zellbiologie, Abteilung Molekulare Embryologie|Freiburg|Germany; email:martin.scaal@anat.uni-freiburg.de

Abstract:

The dorsal aorta is the earliest formed intraembryonic blood vessel. It is composed of an inner lining consisting of endothelial cells and an outer wall consisting of smooth muscle cells (SMCs) and fibrocytes. Aortic SMCs have been suggested to arise from several developmental lineages. Cephalic neural crest provides SMCs of the proximal part of the aorta, and SMCs of the distal part are derived from the paraxial mesoderm. Here, we show by using quail-chick chimerization that in the avian embryo SMCs in the wall of the dorsal aorta at trunk level arise from the sclerotome. Our findings indicate a two-step process of aortic wall formation. First, non-paraxial mesoderm-derived mural cells accumulate at the floor of the aorta. We refer to these cells as primary SMCs. Second, SMCs from the sclerotome are recruited to the roof and sides of the aorta, eventually replacing the primary SMCs in the aortic floor.

Kategorie: Vortrag

Vortrag 14

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE CHEMOKINE SDF-1 CONTROLS MULTIPLE STEPS OF MYOGENESIS THROUGH ATYPICAL PKC α ;

Autoren: Ödemis V.(1),Boosmann K.(1),Engele J.(1),

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Abstract:

We previously observed that the chemokine stromal cell derived factor-1 (SDF-1) and its receptor CXCR4 are highly expressed in the developing mouse limb and further demonstrated that disrupting CXCR4 expression results in a massive impairment of limb myogenesis. To further clarify the role of this chemokine in muscle development, we have now analyzed the effect of SDF-1 on specific steps of myogenesis of C2C12 myoblasts and characterized the signalling cascades employed by SDF-1/CXCR4 to control myogenesis. We found that SDF-1 stimulates proliferation and induces migration of C2C12 cells with a potency similar to that of FGF-2 and HGF/SH. In addition, SDF-1 inhibited myogenic differentiation in both C2C12 cells as well as primary myoblast as assessed by MyoD, myosin heavy chain, and/or myogenin expression. Furthermore SDF-1 activated Erk and PKC α ; in C2C12 cells, whereas even after prolonged treatment, levels of activated Akt, p38, and PKC β ; remained unaffected. Blocking of the Map kinase cascade with the Erk-inhibitor, UO126, abolished SDF-1-induced proliferation and migration of C2C12 cells, but not the inhibitory action of SDF-1 on myogenic differentiation. Moreover, the effects of SDF-1 on proliferation, migration, and differentiation of C2C12 cells were all abrogated in the presence of myristoylated PKC α ; peptide pseudosubstrate and/or upon cellular depletion of PKC α ; by RNA interference. Collectively these findings unravel a previously unknown broad role of CXCR4-PKC α ; signaling in myogenesis and the control of secondary muscle growth.

Kategorie: Vortrag

Vortrag 15

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:SPECIFICATION OF THE SCAPULA PRECURSORS TAKES PLACE AFTER THE EPITHELIOMESENCHYMAL TRANSITION OF THE DERMOMYOTOME

Autoren: De D.(1),Wang B.(1),Proels F.(1),Christ B.(1),Scaal M.(1),Huang R.(1),

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Abstract:

The dermomyotome, the dorsal somite compartment, gives rise to different cell lineages like angiogenic, myogenic, dermogenic and chondrogenic cells to form blood vessels, skeletal and smooth muscles, back dermis as well as scapula cartilage. The epithelial cells of the dermomyotome express Pax3. Cells of the central part of the dermomyotome undergo an epitheliomesenchymal transition (EMT) to form the subectodermal mesenchyme. In this mesenchyme Pax3 is down-regulated. A part of this mesenchyme in the hypaxial domain begins to express Pax1 and differentiates into the scapula cartilage. In this study we determined whether the scapula precursors are specified in the epithelial dermomyotome prior to EMT. Using quail-chick chimerisation, the epaxial part of the dermomyotomal epithelium and dermomyotome-derived mesenchyme was replaced by the hypaxial one prior and after EMT, respectively. Snail2 was used as a marker for EMT. After a reincubation period of 6 days the operated embryo was stained for cartilage. When the transplantation was carried out before EMT, the grafted dermomyotome epithelium developed according to the local cues. However, if the tissue was taken out after EMT, the transplant from the lateral dermomyotome could form ectopic scapula-like cartilage in the epaxial domain. These results indicate that cells of the dermomyotomal epithelium are still naive before EMT. The scapula precursors are determined after going through EMT. This provides evidence for the theory that the epithelial status represses cell specification and maintains the multipotency of the dermomyotome cells.

Kategorie: Vortrag

Vortrag 16

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:ALTERED BONE MORPHOLOGY IN CLOCK GENE MUTANT MICE

Autoren: Maronde E.(1),Schilling A.(2),van der Horst G.(3),Amling M.(2),Albrecht U.(4),

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Abstract:

Clock genes and their protein products regulate circadian rhythms in mammals but have also been shown to exert profound effects on various features. We investigated overall skeletal morphology in femur and vertebral spine of single (Per-2, Cry-2) and double clock gene (Per-2/Cry-2) knockout mice using von Kossa staining (for analysis of mineralized bone) and calcein green labeling (for analysis of bone turnover activity) as well as analysis of the circulating bone marker osteocalcin. We found an increase in cortical and spongiosal density in both Per2- and Cry-2 mutant mice by von Kossa staining. These changes depended on animal age with vertebral spine density being significantly different between wildtype and mutant mice at the age of 3, 12 and 48 weeks, but not at 24 weeks. These changes in bone density seem to involve both anabolic and catabolic processes as we see differences in both mineralized bone content pointing to osteoblastic contribution and calcein green staining suggesting osteoclastic contribution. Some of the observed changes in bone features were compensated in double clock gene mutant animals, e.g. Per2/Cry2 mice. It has been described before that in these Per2/Cry2 double mutant mice some circadian clock gene related deficits that occur in the single mutants are compensated in the double mutant. Thus, clock genes and their protein products seem to have additional roles next to generation of circadian rhythms and may also add a circadian component to bone turnover processes related to osteoblast and osteoclast activity cycles.

Kategorie: Vortrag

Vortrag 17

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:CORRELATION BETWEEN MINERALISATION AND STRENGTH IN LUMBAR VERTEBRAE BODIES MEASURED BY CT –OSTEOABSORPTIOMETRY (CTOAM) AND INDENTATION TEST.

Autoren: Ercan Y.(1),Mühlhofer H.(1),Drews S.(1),Matsuura M.(1),Linsenmaier U.(2),Putz R.(1),Müller-Gerbel M.(1),

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Abstract:

Background: Intervertebral disc prothesis used for implantation in present time are controversial discussed, especially the orthopaedic experts are discordant about the way of fixation. Therefore studies are needed to figure out the exact position of implantation due to physiological strength in vertebral body. Measurement of CTOAM point out the distribution of mineralisation on the edge of vertebral body. The aim of this study was to determine that there is a correlation between mineralisation and strength in lumbar vertebral bodies.

Material and Methods: Seventeen lumbar vertebral bodies were used. The cadaveric specimens were fixated with formalin solution. After CTOAM analysis was made, both sides of the vertebral bodies were mechanically tested with an indenter after the intervertebral disc was removed. The coefficient of determination, correlation and the t-test were performed with $p < 0,05$ being significant.

Results: The linear regression of the mineralization density and the maximal mechanical strength to penetrate both side of the vertebral bodies were determined. High maxima of strength in the edge of the vertebral bodies were registered. The maximum of correlation between mineralisation and strength was found in anterior and posterior edges of the lumbar vertebral body.

Conclusion: The correlation between mineralisation and strength in the edge of lumbar vertebral bodies were shown. This study defines the region of interest for positioning intervertebral prothesis.

Kategorie: Vortrag

Vortrag 18

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:VIRTUAL REALTIME SONOGRAPHY TO GUIDE SACROILIAC JOINT (SIJ) INJECTION: FEASIBILITY STUDY [MACHBARKEITSSTUDIE ZUR ANWENDUNG DER VIRTUELLEN SONOGRAPHIE FÜR DIE GEZIELTE INJEKTION DES ILIOSAKRALGELENKS]

Autoren: B. Moriggl¹, G. Feuchtner², E. Mur³, F. Frauscher², L. Pallwein², M. Schirmer⁴, A. Klauser² B.(1),

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Abstract:

Purpose: To assess the feasibility of virtual realtime sonography by using navigator technology with a CT data set to guide needle insertion sonographically for intraarticular injection of the SIJ.

Methods and Materials: Ten SIJ of five human cadavers were investigated. Sonography was performed with a 2.5-6.0 MHz curved array transducer (Technos MPX, Esaote Biomedica, Genoa, Italy) on cadavers in prone position, using a fusion imaging software and a tracking system. CT (Sensation Siemens, Erlangen, Germany) of the SIJ was performed with a slice thickness of 0.75 mm to obtain a volumetric CT data set, which was transferred into the US system. Image fusion of the CT data set with real-time sonographic scanning of the lower back was used to guide needle insertion at the dorsocaudal SIJ. CT was repeated to prove intraarticular needle placement. Time to perform SIJ injection with virtual realtime sonography was calculated.

Results: Using virtual sonography, intraarticular needle placement was successful and confirmed by CT in all 10 SIJ (100%). Time to perform SIJ injection with virtual realtime sonography was 11.9 min (± 9.3), including both sides.

Conclusion: Needle insertion for intraarticular SIJ injection using virtual sonography on the basis of image fusion of volumetric CT data is feasible, and after an initial learning curve, quick to perform. Image fusion using a CT data set acquired once for multiple injections could be valuable, especially in young spondyloarthritis patients, where limited radiation exposure is desired.

Kategorie: Vortrag

Vortrag 19

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:CORRELATION OF MINERALIZATION AND MECHANICAL STRENGTH OF THE SUBCHONDRAL BONE PLATE OF THE INFERIOR TIBIAL FACIES

Autoren: Mühlhofer H.(1),Ercan Y.(1),Drews S.(1),matsuura m.(1),Linsenmaier U.(2),Putz R.(1),Müller-Gerbl M.(1),

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Radiologie|München|Deutschland

Abstract:

Background/Aims:

The implantation of total ankle prosthesis is one of the most challenging operation in orthopaedic surgery. The main problem surgeons are facing is the fixation of the total ankle prosthesis on the tibial side. The subchondral bone plate of the distal tibia is considered to be the strongest region on the inferior tibial facies. Based on information on the mineralization of the subchondral bone plate, conclusions concerning the mechanical stress, age-related changes, post surgical biomechanical situations and regions of fixation can be drawn. The aim of this study was to determine the correlation between the mineralization of the subchondral bone plate and the local mechanical strength.

Material and Methods:

After CT osteoabsorptiometry analyses, the mineralization of the subchondral bone plate in 18 distal tibiae was investigated. After removal of the cartilage of the facies articularis inferior, the mechanical strength of the joint surface was measured with an indenter. The linear regression of the mineralization density and the maximal mechanical strength to penetrate the subchondral bone plate was determined.

Results:

Our data showed a coefficient of determination between 0.75 – 0.97 and coefficient of correlation between 0.86 – 0.97 ($p < 0.05$). Furthermore we demonstrated a bicentric distribution of mineralization patterns. The maximal mineralization was found venteromedial and mediolateral on the joint surface.

Conclusion:

Our study showed good correlation in mineralization and mechanical property of the inferior tibial facies, therefore the results could be useful for the development of new fixation methods for total ankle prosthesis.

Kategorie: Vortrag

Vortrag 20

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:DEGENERATIVE MORPHOLOGICAL CHANGES AND SUBCHONDRAL BONE MINERALIZATION IN HUMAN TARSOMETATARSAL JOINTS

Autoren: Ebel C.(1),Mühlhofer H.(1),Ercan Y.(1),Linsenmeier U.(2),Putz R.(1),

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Abstract:

Degenerative morphological changes and subchondral bone mineralization in human tarsometatarsal joints

Ch. Ebel, H. Mühlhofer, Y.Ercan, U. Linsenmaier, R. Putz

Background/aims:

The distribution of OA in european people aged between 55-70 years occurs in 70% at the hand, 40% at the foot, 10% at the knee and 3% at the hip.The diagnosis “unknown foot pain” is a topissue in the orthopedic field, but sparse the information about degenerative changes in the midfoot (DMC). In this study the occurrence of DMCs and the subchondral bone mineralization in tarsometatarsal joints was investigated.

Materials/Methods:

37 formalin-fixed cadaver feet were observed in CT- osteoabsorbtiometries. After dissection, ink- colouring and surface area subdivision into quadrants, DMCs location and severity were graded into the scale of Collins.

Results:

The lowest occurrence of DMCs in the proximal midfoot was in the Os cuneiforme lat. (30%) , the maximum value in the Cuboid and Os cuneiforme intermed. (40%) . The summation of the degeneration score points of each joint surface generated a severity-maximum with 170 (Os cuboideum), a minimum with 96 points (Os.cun.med.). The subchondral mineralization distribution corresponds to the pattern of DMC in every joint, especially the location of mineralisation maxima.

Conclusion:

The loading transmission is distributed all over the midtarsal joints. The Prevalence of DMC ranges between 3% and 45% in the population. DMCs are provable in each midfoot-stream. Unknown Metatarsalgia should be considered as an OA. CT-OAM can be used as an excellent tool for localising the problem zones.

Kategorie: Vortrag

Vortrag 21

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:DEGENERATIVE CHANGES ON JOINT SURFACES OF THE FOOT

Autoren: Mühlhofer H.(1),Ebel C.(1),Ercan Y.(1),Linsenmaier U.(2),Putz R.(1),Müller-Gerbl M.(1),

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(2)Ludwig-Maximilians-Universität München|Chirurgische Klinik München/
Radiologie|München|Deutschland

Abstract:

Aims:

Maldeformations of the foot such as flat, splayfeet, hallux valgus as well as changes in the static of the foot are known as to be prearthrotic changes. The diagnosis “unknown foot pain” could be often referred to degenerative changes like osteoarthritis in the joints of the foot. The aim of this study was to investigate the prevalence of degenerative changes in the joint surfaces of feet with and without maldeformations.

Material/Methods:

After X-ray investigation of 37 formalin fixed cadavers feet (1295 joint surfaces), the articular surfaces of the upper and lower ankle joints, the Chopart-joint as well as the tarso-metatarsal joint (I-V) and the metatarso-phalangeal joints (I-V) were investigated. For each joint surface the patterns of distribution and degree of degenerative changes (Collins1-4) were compiled.

Results:

34% of the joint surfaces of the foot were affected by degenerative changes. The joint mostly affected was the first metatarsophalangeal joint (70 %). The mostly unaffected joint was the dorsal compartment of the lower ankle joint (17.5%). We demonstrated a correlation between a hallux valgus and changes in the first metatarsal joint as well as the existence of degenerative changes without pathological findings in radiographs.

Conclusions:

This study points out the high prevalence of degenerative changes in the joints of the foot in general and especially in feet with maldeformations. Furthermore it shows the appearance of degenerative changes without radiological correlation leading to the conclusion that degenerative changes play an important role in cases of patients with the diagnosis “unknown foot pain”.

Kategorie: Vortrag

Vortrag 22

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:GLUTAMATE RECEPTORS 2 & 3 (GLUR 2/3) ON MYENTERIC NEURONS IN THE MOUSE ESOPHAGUS –TARGETS OF GLUTAMATE RELEASED FROM IGLES?

Autoren: Raab M.(1),König S.(1),Neuhuber W.(1),

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Abstract:

Intraganglionic laminar endings (IGLEs) are the most frequent vagal afferent endings in the gut and contain the vesicular glutamate transporter 2 (VGLUT2) in both rat and mouse esophagus (Raab & Neuhuber, 2003; Raab & Neuhuber, 2004). They represent one important though not the only source of glutamate in myenteric ganglia since enteric neurons as well as glia also contain glutamate, VGLUT1 and glutamine synthase (Kato et al., 1990; Liu et al. 1997; Kraus et al. 2007). In this study we analyzed the distribution of ionotropic non-NMDA receptors GluR2/3, one possible target for ganglionic glutamate. Immunohistochemistry in esophageal wholemounts showed GluR2/3-immunoreactivity (-ir) in nearly 30% of myenteric neurons. To determine the neuronal subpopulations which express GluR2/3 receptors, we performed NADPh-diaphorase histochemistry and combined GluR2/3 with nNOS- and ChAT- immunohistochemistry, respectively. Seventy-three % of myenteric neurons were nitrergic, 22% cholinergic and 5% non-nitrergic-non-cholinergic. Interestingly, GluR2/3 receptors were mostly found on the cholinergic subpopulation, of which 85,5% were GluR2/3 positive, while only 20,5% of nitrergic neurons contained GluR2/3-ir. Double and triple channel confocal imaging further revealed GluR2/3-ir within the ganglionic neuropil, to some extent also in close relation to, but not within IGLEs and in motor endplate areas. Thus, IGLEs, via glutamate release, might be involved in regulation of esophageal motility mediated by GluR2/3.(Supported by Johannes und Frieda Marohn-Stiftung, Erlangen)

Kategorie: Vortrag

Vortrag 23

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:Enteric co-innervation of striated muscle fibers in human esophagus

Autoren: Wörl J.(1),Kallmünzer B.(1),Sörensen B.(1),Neuhuber W.(1),

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Abstract:

Striated esophageal muscle receives dual innervation from both vagal motor fibers originating in the brain stem and enteric nerve fibers originating in the myenteric plexus (Neuhuber et al., Cell Tissue Res, 1994; Wörl et al., J Auton Nerv Syst, 1994). This peculiarity of the esophageal muscle, termed enteric co-innervation, was demonstrated during the last decade in several animal species, but not in human. The purpose of this study was to analyze enteric co-innervation of striated muscle in human esophagus by applying morphological techniques used in animal studies (for review see Wörl and Neuhuber, Histochem Cell Biol, 2005). In addition, the composition of the tunica muscularis was studied semiquantitatively. Contrary to the descriptions in textbooks the upper half of the esophagus was built up of both muscle types with predominance of striated, the lower half of smooth muscle. The majority of motor endplates was compact and ovoid. Enteric nerve fibers on motor endplates stained for nitric oxide synthase, vasoactive intestinal polypeptide, galanin and neuropeptide Y and were separated from vagal cholinergic terminals. Myenteric neurons in entirely or predominantly striated areas stained for all substances given above and were highly colocalized with NADPH-diaphorase. From these and recent functional results we conclude that this new innervation component of the esophagus exists also in human and might represent a mechanism for local modulation of esophageal peristalsis (Supported by Johannes und Frieda Marohn-Stiftung and DFG).

Kategorie: Vortrag

Vortrag 24

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:EXPRESSION OF THE MUSCLE SPECIFIC CAVEOLIN-ISOFORM, CAV-3, IN MOUSE SENSORY NEURONS

Autoren: Krasteva G.(1),Filip A.(1),Pfeil U.(2),Kummer W.(1),

Adressen:(1)Justus-Liebig-Universität Giessen|Institut für Anatomie und Zellbiologie|Giessen|Deutschland; email:Gabriela.Krasteva@anatomie.med.uni-giessen.de; (2)Institut für Anatomie und Zellbiologie|Justus-Liebig-Universität Giessen|Giessen|Deutschland

Abstract:

Nitric oxide (NO) production in dorsal root ganglia (DRG) is stimulated under hypoxic conditions and thereby promotes cellular survival. The activity of the endothelial NO synthesizing enzyme (eNOS) is regulated by binding to caveolae. In neurons, no caveolae are present. Previously we have shown that eNOS is located in the juxtamitochondrial ER in DRG neurons (Henrich et al., 2002). In ciliated epithelial cells, one structural protein of caveolae, caveolin (cav)-3, is located in an intracellular membrane compartment where it interacts with eNOS (Krasteva et al., 2007). Thus, we set out to investigate the expression and distribution of cav-3 in sensory (trigeminal and DRG) neurons.

We detected cav-3 in mouse primary afferent neurons. Laser-assisted microdissection followed by RT-PCR provided further proof that mRNAs for cav-3 and eNOS are expressed in DRG and trigeminal sensory neurons. Using Western blotting we detected an immunoreactive protein of appropriate size.

Immunohistochemistry on cryosections demonstrated cav-3-immunoreactivity in the cell bodies of the sensory neurons, in the same region as its interacting protein eNOS. This labelling could be abolished by preabsorption. Particularly intense labelling was observed in small to medium-sized perikarya. In addition, cav-3-immunoreactivity was observed in satellite cells and in some axons. We co-immunoprecipitated cav-3 with eNOS from DRG and trigeminal ganglion extracts, indicating that both proteins indeed interact in sensory neurons. Cav-3 immunolabelling did not colocalize with mitochondrial labelling.

Our data indicate a functional role of cav-3 in intracellular sorting to ER and in regulation of eNOS in sensory neurons

Kategorie: Vortrag

Vortrag 25

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:END-TO-END- VERSUS END-TO-SIDE-ANASTOMOSIS: HISTOLOGICAL AND MORPHOMETRICAL EVALUATION OF AXONAL REGENERATION IN TRANSECTED PERIPHERAL NERVES IN A RAT MODEL

Autoren: Joswig H.(1), Samii M.(2), Grothe C.(3), Haastert K.(4),

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Abstract:

End-to-side neurorrhaphy is a potent surgical technique for transected peripheral nerve repair with no available proximal nerve end or not feasible tension free end-to-end neurorrhaphy. Although axonal and functional regeneration has been confirmed experimentally and clinical reports show encouraging results, the cellular mechanism behind this remained unclear. Therefore, we investigated collateral sprouting in recipient tibial nerve and donor nerve morbidity (tibial or fibular nerve, respectively) in an experimental rat sciatic nerve model by comparing end-to-end to end-to-side neurorrhaphy, both through a perineurial window. Eight weeks after surgery, immunocytochemical staining of the coaptation site verified regenerated axons in both groups. Morphometrical quantification of all myelinated axons was done in semithin sections at different levels in epon-embedded donor and recipient nerves and compared to healthy non-operated nerve samples. The results demonstrate significant higher numbers of regenerated myelinated axons distal to end-to-end as compared to end-to-side repair. However, axonal sprouting of regenerating axons is located in the donor nerve proximal to the end-to-side coaptation site. Furthermore, regarding quantitative parameters such as axon diameter, axon area, fibre density and g-ratio-index, impairment of the donor nerve is not evident. Thus, end-to-side neurorrhaphy represents a valuable alternative to end-to-end neurorrhaphy. To confirm the theory of collateral sprouting retrograde tracing of regenerated axons was performed and is under current investigation.

Kategorie: Vortrag

Vortrag 26

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:MICE LACKING FGF-2 SHOW ENHANCED REMYELINATION AND SENSORY FUNCTION RECOVERY AFTER SCIATIC NERVE LESION

Autoren: Jungnickel J.(1),Grzybek M.(1),Lipokatic-Takacs E.(1),Claus P.(1),Grothe C.(1),

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Abstract:

Neurotrophic factors have been shown to stimulate and support peripheral nerve repair. One of these factors is basic fibroblast growth factor (FGF-2), which is up-regulated after peripheral nerve injury and influences early sciatic nerve regeneration by regulating Schwann cell proliferation (Jungnickel et al., 2006). Our previous study on FGF-2 deficient mice suggested that FGF-2 is important for axonal maturation and remyelination (Jungnickel et al., 2004). However, the functional impact of these effects on sensory and motor fibers was not clear. Furthermore, the mechanisms by which FGF-2 regulates myelin thickness were not explored. To elucidate the role of FGF-2 on structural and functional recovery, we analyzed FGF-2 deficient mice and wild-type littermates 2 and 4 weeks after sciatic nerve crush. Two weeks after peripheral nerve injury, regenerating fibers of mutant mice showed both significantly increased axon and myelin size, but no difference in number of myelinated and unmyelinated fibers. Molecular analysis indicated that the expression level of myelin protein zero and myelin basic protein were significantly enhanced in lesioned nerves of these mice. In addition to morphological and biochemical changes, we found a delay of sensory and motor function recovery in mutant mice after performing pinch test and walking track analysis. These results indicate that FGF-2 promotes functional recovery and inhibits remyelination by regulating transcription of myelin genes.

Kategorie: Vortrag

Vortrag 27

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:PROMOTION OF NEURITE OUTGROWTH BY FGFR1 OVEREXPRESSION IN PC12 CELLS AND ADULT SENSORY NEURONS

Autoren: Hausott B.(1),Schlick B.(2),Valant N.(1),Klimaschewski L.(1),

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Abstract:

FGF-2 (basic fibroblast growth factor) is involved in development and repair of the nervous system including peripheral nerve regeneration. It is strongly and rapidly up-regulated in response to a nerve lesion and promotes axonal elongation of prelesioned neurons. FGF-2 binds to fibroblast growth factor receptor 1 (FGFR1) and induces axonal growth and differentiation through a sustained Ras/MAP kinase activation. We analyzed the effects of FGFR1-overexpression combined with lysosomal inhibition of receptor degradation by PC12 cells and adult sensory neurons. Ligand-induced degradation of FGFR1 was measured in PC12 cell cultures by detection of fluorescence intensities of cells over-expressing FGFR1 fused to EGFP. FGF-2 induced FGFR1 degradation which was inhibited by the lysosomal inhibitor leupeptin and the proteasomal inhibitor lactacystin. FGFR1 overexpression resulted in flattened morphology, enhanced neurite outgrowth with elevated neurite length and activation of ERK and AKT in response to treatment with FGF-2. In adult sensory neurons, FGFR1 overexpression enhanced FGF-2-induced axon growth which was further increased by co-treatment with leupeptin. Our data indicate that lysosomal inhibition of receptor degradation concomitant with ligand stimulation of neurons overexpressing FGFR1 represents a new mechanism of tyrosine kinase receptor mediated promotion of axon regeneration in the peripheral nervous system in vitro.

Kategorie: Vortrag

Vortrag 28

Rubrik: Zellbiologie

Abstract Nr: 4

Titel: GLUCOCORTICOID MEDIATED GENE INDUCTION IN THE BLOOD BRAIN BARRIER

Autoren: N. Harke¹, D. Drenckhahn¹, C. Forster¹

Adressen: ¹Institute of Anatomy and Cell Biology, University of Würzburg, Germany

Abstract:

Tight junctions play an important role in the development and maintenance of the blood-brain-barrier. Occludin and the claudins are known to be integral membrane proteins forming these tight junctions, but the regulatory mechanisms of the gene induction are still not clear.

We could already show in former projects that treatment of cerebral endothelial cells building the blood-brain-barrier with glucocorticoids leads to increased barrier functions. We proved for example that expression of several genes depends on the presence of the glucocorticoid receptor and its binding to specific binding sites on the gene promoter. We therefore tried to elucidate target genes and the putative binding sites of glucocorticoids on the gene promoters. We further attempted to elucidate the molecular mode of binding of the glucocorticoid receptor to the DNA.

Kategorie: Vortrag

Vortrag 29

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:NEURONAL ACTIVITY REGULATES THE DYNAMICS OF MATURE, SPINE APPARATUS CONTAINING SPINES

Autoren: Fester L.(1),Labitzke J.(1),Disteldorf E.(1),Zhou L.(1),Rune G.(1),

Adressen:(1)Universitätsklinikum Hamburg-Eppendorf|Institut für Anatomie I, Zelluläre Neurobiologie|Hamburg|Germany; email:lfester@uke.uni-hamburg.de

Abstract:

The spine apparatus (SA) is a spine-specific cell organelle, which is present in 80% of mature spines and is presumed to function as a calcium store. In hippocampal neurons, synaptopodin (Synpo) has been shown to be specifically associated with the spine apparatus (Deller et al. 2003). Here, we studied the dynamics of mature spines in response to various glutamatergic agonists in order to find out whether neuronal activity plays a regulatory role on the SA. Application of NMDA, kainate and AMPA downregulated the percentages of spines, which contain a SA, as determined in ultrathin sections of hippocampal slice cultures, and downregulated immunoreactivity of Synpo in dissociated neurons. MK801, a blocker of glutamate receptors, and TTX induced opposite effects. Notably, in these cultures, aromatase, the final enzyme of estrogen synthesis, and estradiol content in the medium, as measured using a RIA, were significantly downregulated. Since neuronal glutamatergic input induces a calcium induced calcium release (CIC) and aromatase gets inactivated after calcium release from intracellular stores, we assumed that Ca²⁺-release from the SA downregulates aromatase activity in spines. In fact, testosterone which activates aromatase activity abolished glutamate-induced downregulation of synpo and aromatase inhibition by letrozole was paralleled by a decrease in the percentage of SA containing spines. Moreover, in the aromatase knock-out mouse we found a loss of spines containing a SA and a downregulation of Synpo in dendritic layers of the hippocampus, which may confirm our hypothesis.

Kategorie: Vortrag

Vortrag 30

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE BALANCE OF ESTROGEN RECEPTOR β ; AND ESTROGEN RECEPTOR β ; MAINTAINS MATURE DENDRITIC SPINES

Autoren: Prange-Kiel J.(1),Hinz R.(1),Disteldorf E.(1),Hareghas S.(1),Zhou L.(1),Rune G.(1),

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Abstract:

Mature spines are characterized by the presence of a spine apparatus (SA), a potential spine-specific calcium store. Since inhibition of aromatase, results in loss of preferentially mature spines and in a decrease in immunoreactivity of Synpopodin (Synpo), a SA marker, we studied estrogen signalling downstream of aromatase inhibition. DPN, a specific agonist of estrogen receptor (ER) β , downregulated Synpo, whereas the protein was upregulated in response to PPT, a specific agonist of ER α . After application of ICI 182 780, an ER receptor blocker, no response was seen, neither on Synpo immunoreactivity nor on the density of mature spines in electron micrographs. Being ligand-inducible transcription factors, we found ER α to be downregulated and ER β to be upregulated in response to letrozole, an aromatase inhibitor. Conversely, activation of aromatase by testosterone or cholesterol resulted in an upregulation of ER α , whereas no effect was found with ER β . Our results indicate that the maintenance of mature spines is balanced by the activity of aromatase, which either activates spinogenesis-promoting ER α or spinogenesis-inhibiting ER β .

Kategorie: Vortrag

Vortrag 31

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:CALEB/NGC INDUCES DENDRITE AND SPINE MORPHOGENESIS VIA DIFFERENT SIGNALING PATHWAYS

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Abstract:

We previously showed that the neural EGF family member CALEB/NGC mediates dendritic tree and spine complexity in vitro and in vivo. However, it was not clear which intracellular signaling pathways are important for this effect to occur. The phosphatidylinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) signaling pathway has gained attention in neuroscience since it was highlighted to be implicated in dendritic arborization and synaptic plasticity. We now examine the importance of this pathway for CALEB/NGC-mediated dendritic tree and spine differentiation. We find that either inhibition of PI3K, Akt, or mTOR blocks the effect of CALEB/NGC on dendritic branching. In contrast, CALEB/NGC still induces spine differentiation totally independent of PI3K-Akt-mTOR. However, both dendrite and spine morphogenesis induced by CALEB/NGC are inhibited by interfering with protein kinase C (PKC) function. We find that the extracellular EGF-like domain of CALEB/NGC is necessary and sufficient to drive both dendritic tree and spine morphogenesis. In contrast, different intracellular peptide segments of CALEB/NGC are necessary to stimulate dendrite or spine morphogenesis. An identified intracellular interaction partner of CALEB/NGC, which is known to inhibit Akt signalling, interferes with CALEB/NGC-mediated dendritic tree but not spine complexity. Together, our findings reveal a novel switch of specificity in signaling leading to neuronal process differentiation in consecutive developmental events.

Lit.: Brandt et al. (2007). EMBO J 26:2371-86. Supported by DFG (SFB 665-A2 to S.S. and R.N.).

Kategorie: Vortrag

Vortrag 32

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE SPINE APPARATUS CONTRIBUTES TO CA²⁺ TRANSIENTS IN DENDRITIC SPINES: TWO PHOTON MICROSCOPY-BASED CA²⁺ IMAGING AT IDENTIFIED SYNAPSES IN ORGANOTYPIC SLICE CULTURES.

Autoren: Drakew A.(1),Klatt N.(1),Tippmann A.(1),Hoffmann T.(1),Vida I.(2),Deller T.(3),Frotscher M.(1),

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Abstract:

Transient changes in the concentration of free cytoplasmic Ca²⁺ are assumed to link synaptic excitation to intracellular mechanisms underlying synaptic plasticity. Large spines of forebrain neurons often contain a specific organelle, the spine apparatus, which may serve as a postsynaptic Ca²⁺ store. We have recently shown that synaptopodin knock-out mice lack the spine apparatus accompanied by deficits in synaptic plasticity as well as spatial learning (Deller et al., 2003). Here, we tested the hypothesis that the spine apparatus modifies Ca²⁺ transients in dendritic spines and thereby contributes to synaptic plasticity. We analyzed Ca²⁺ dynamics in individual spines and dendrites of patch-clamped hilar mossy cells in organotypic hippocampal slice cultures of synaptopodin knock-out and wild-type mice by means of two-photon microscopy. Since Ca²⁺ release from intracellular stores is supposed to be a self-enhancing process triggered by Ca²⁺, we used back-propagating action potentials in order to elevate the free Ca²⁺ concentration by the activation of voltage-gated Ca²⁺ channels. Indeed, trains of action potentials resulted in Ca²⁺ transients in complex spines. These transients were recorded as relative changes in fluorescence intensity of a Ca²⁺-sensitive dye. We found a significant reduction of the amplitude of Ca²⁺ transients in large spines, but not dendritic shafts, of synaptopodin knock-out mice. These findings strongly support the hypothesis that the spine apparatus serves as a Ca²⁺ store that can rapidly release Ca²⁺ into the spine. Thus, the spine apparatus may play an important role in modulation of Ca²⁺ transients directly at the synapse and thereby regulate synaptic plasticity.

Kategorie: Vortrag

Vortrag 33

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:IDENTIFICATION OF A PRESYNAPTIC PROTEIN LOCALIZED TO SUBSETS OF SYNAPSES

Autoren: Dresbach T.(1),Kremer T.(1),Nawrotzki R.(1),Kirsch J.(1),

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Abstract:

Presynaptic nerve terminals contain scaffolding proteins that orchestrate neurotransmitter release at active zones. These scaffolding proteins form a molecular web, or cytomatrix, that is a general feature of active zones and has been implicated in synapse assembly, synapse maintenance, and in local anchoring of diffusible proteins. Here, we describe Mover, a non-transmembrane protein which is targeted to presynaptic terminals when overexpressed in cultured neurons. Confocal immunomicroscopy revealed that Mover colocalizes with presynaptic markers in distinct regions of the brain. In the hippocampus, Mover localizes to mossy fibre terminals but is absent from inhibitory nerve terminals. By contrast, Mover localizes to inhibitory terminals throughout the cerebellar cortex, but does not colocalize with excitatory synapse markers in this region of the brain. Our results suggest that Mover may act in concert with generally expressed scaffolding proteins in distinct sets of presynaptic terminals.

Kategorie: Vortrag

Vortrag 34

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:NEW WAYS OF VISUALIZING MOSSY FIBER SYNAPSES IN THE HIPPOCAMPUS

Autoren: Frotscher M.(1),Zhao S.(1),Graber W.(2),Drakew A.(1),Studer D.(2),

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Anatomie|Bern|Schweiz

Abstract:

Our image of a synapse in the central nervous system is based on electron microscopic studies of tissue fixed by perfusion using solutions of paraformaldehyde and glutaraldehyde. However, this chemical fixation as well as subsequent dehydration in ethanol result in uncontrolled shrinkage of tissue components. Thus, the dimensions and precise locations of cell organelles are difficult to estimate. Moreover, attempts to monitor subtle changes in synaptic ultrastructure associated with functional changes such as long-term potentiation (LTP) or long-term depression (LTD) may fail due to alterations of the tissue during fixation and dehydration. One way to overcome these disadvantages of chemical fixation is by high-pressure freezing (HPF) of tissue samples. With this procedure, the tissue is shock-frozen in less than a second in the absence of aldehyde solutions. As a result, the ultrastructure of tissue components is seen in unsurpassed detail. Electron microscopy does not allow one to study living synapses over time. A method will be presented by which presynaptic mossy fiber boutons in the hippocampus are stained with a green fluorescent dye (Alexa 488), and the postsynaptic pyramidal cell with another (red) fluorescent dye (Alexa 594). With this approach identified mossy fiber synapses in slice cultures can be monitored for extended periods of time. High-pressure freezing and double labeling of pre- and postsynaptic elements are useful tools to characterize the ultrastructure and plasticity of mossy fiber synapses in the hippocampus.

Kategorie: Vortrag

Vortrag 35

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:LAPSER1 INTERACTS WITH SPAR1 VIA ITS C-TERMINAL FEZ1 DOMAIN

Autoren: Schmeisser M.(1),Grabrucker A.(1),Boeckers T.(1),

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Abstract:

The postsynaptic density (PSD) is an electron-dense structure underneath the postsynaptic membrane of excitatory synapses in the central nervous system. Scaffolding molecules within the PSD are known to anchor and cluster membrane-bound receptors, cell adhesion molecules, components of signal cascades and cytoskeletal elements. Recently, we have identified a novel family of postsynaptic scaffolding proteins, the "Fezzins", consisting of four molecules (ProSAP1, PSD-Zip70, LAPSER1 & Nedd4bP3) all coding for a so-called C-terminal Fez1 domain. In this study, we have characterized the "Fezzin" family member LAPSER1 which shows a wide distribution in rat brain. LAPSER1 interacts with ProSAP/Shank scaffolding molecules via its C-terminal PDZ domain binding motif. This interaction could be confirmed by in vitro transfection-, yeast two hybrid assays as well as by immunoprecipitation experiments using rat brain homogenate. To characterize the function of the highly conserved Fez1 domain, we subcloned this protein region as bait and screened a rat brain prey library in yeast. We pulled out several C-terminal clones of the spine-associated RAP-GAP domain protein SPAR1 that is capable of altering spine morphology via the regulation of small GTPases. Further analysis confirmed that the Fez1 domain of all "Fezzins" is a specific protein-protein interaction domain for SPAR1's C-terminal coiled coil domain region. Results demonstrate that this novel family of PSD proteins is able to effectively localize SPAR1 molecules at postsynaptic sites and might therefore be involved in plasticity events at spines/PSDs.

Kategorie: Vortrag

Vortrag 36

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:REDUCED GABA-ERGIC INHIBITION IN THE HIPPOCAMPUS OF NEUROLIGIN-2 DEFICIENT MICE IN VIVO

Autoren: Schwarzacher S.(1),Jedlicka P.(1),Hoon M.(2),Brose N.(2),Deller T.(1),Varoqueaux F.(2),

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Abstract:

Hippocampal network activity and plasticity can be studied in transgenic mice under in vivo conditions using extracellular recording techniques. Neuroligins (NLs) interact with presynaptic neurexins. While NL-1 is associated with glutamatergic excitatory synapses, NL-2 is selectively localized at inhibitory synapses. Deletion of all NLs induces impairment of glutamatergic, GABAergic and glycinergic synaptic transmission (Varoqueaux et al., Neuron (2006) 51:741-754).

Here we studied network activity in the dentate gyrus of urethane-anesthetized NL-2 deficient mice following perforant-path stimulation. We found strongly enhanced input-output relationship between stimulus intensity and population spikes. In addition, paired-pulse inhibition of the population spike, a measure for GABAergic feedback inhibition, was severely impaired. Furthermore, threshold frequency for the induction of epileptiform discharges was significantly decreased. These data suggest a strong increase of network excitability in the dentate gyrus of NL2-deficient mice. In contrast, we found no changes in input-output curves of evoked field excitatory postsynaptic potentials (fEPSPs) paired-pulse facilitation of fEPSPs suggesting normal excitatory synaptic transmission at perforant path-granule cell synapses in mutant mice.

We investigated the anatomical substrates underlying these alterations. Immunostaining for presynaptic markers evaluated the integrity/accuracy of the perforant path input and of local interneurons network onto dentate gyrus neurons. Furthermore, detection of landmark postsynaptic proteins (receptors, adhesion and scaffold molecules) contributed to assess the composition of excitatory and inhibitory synapses in the NL-2 deletion-mutant mouse as compared to wildtype.

Our findings provide the first electrophysiological evidence that the lack of NL-2 leads to a pronounced impairment of GABAergic inhibition under adult in vivo conditions.

Kategorie: Vortrag

Vortrag 37

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:RIBEYE, A MAJOR COMPONENT OF SYNAPTIC RIBBONS, IS A NADH-REGULATED LYSOPHOSPHATIDIC ACID-ACYLTRANSFERASE

Autoren: Schwarz K.(1),Schmitz F.(1),

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Abstract:

Ribbon synapses are specialized chemical synapses built by certain non-spiking neurons in sensory organs, e.g. retinal photoreceptor- and bipolar cells and inner ear hair cells. These synapses are capable of dynamically transmitting synaptic signals covering a broad range of stimulus intensities. Morphologically, ribbon synapses are characterized by the presence of unique presynaptic organelles, the synaptic ribbons, which are associated with large numbers of synaptic vesicles. Synaptic ribbons are crucial for exocytosis in ribbon synapses, however the precise physiological function(s) and molecular activities of ribbons are still unknown. We have previously identified a novel protein, "Ribeye", as a unique and major component of synaptic ribbons (Schmitz et al., 2000) in all vertebrate species. Ribeye is found along the entire extension of the synaptic. Thus it is likely that Ribeye is a key determinant of ribbon function.

In the present study we show that Ribeye is a NADH-regulated LPA-acyltransferase (LPAAT) that converts lysophosphosphatidic acid (LPA) into phosphatidic acid (PA) using palmitoyl-CoA as cofactor. Binding of NADH stimulates LPAAT activity of Ribeye, whereas NAD⁺ does not. NADH-stimulation of LPAAT activity can be mimicked by addition of beta-mercaptoethanol suggesting conformational changes due to reduction of cysteine disulfid bridges. Our study shows that Ribeye and synaptic ribbons restructure phospholipids at the active zone. We provide first evidence that generation of the lipid messenger PA plays a central role in ribbon function and demonstrate how synaptic ribbons work at a molecular level.

Deutscher Titel: 'Ribeye, eine Hauptkomponente synaptischer Bänder, ist eine NADH-regulierte Lysophosphatidat-Acyltransferase'

Kategorie: Vortrag

Vortrag 38

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:MATURATION OF SYNAPTIC CONTACTS IN DIFFERENTIATING NEURAL STEM CELLS (NSCS)

Autoren: Liebau S.(1),Böckers T.(1),

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Abstract:

Clonogenic neural stem cells (NSC) are self-renewing cells that maintain the capacity to differentiate into brain-specific cell types and retain the capacity to generate both glial cells and neurons. Nevertheless it is unclear whether these cells are still capable of gaining full functionality, which would be one of the prerequisites for the replacement or repair of diseased brain tissue. The ability to establish and maintain polarized excitatory synaptic contacts that localize key molecules of functional synapses is one of the basic requirements for intercellular communication and a functional integration into a neuronal network. In primary hippocampal cultures it has already been shown that synaptogenesis is characterized by a time dependent targeting and recruitment of pre- and postsynaptic proteins with versatile structural and functional assignments. In this study we investigated the expression and localization of important pre- and postsynaptic proteins including Bassoon and synaptophysin as well as proteins of the ProSAP/Shank family in differentiating neural stem cells. Moreover, we characterized ultrastructural features of cell-cell contacts during synaptogenesis. Here we show that murine fetal neural stem cells express and localize cytoskeletal and scaffolding molecules of the pre- postsynaptic specialization in a defined temporal order, leading to excitatory synaptic contacts after about 14 days of differentiation. Our results demonstrate that NSCs are capable of creating a neuronal network based on the expression and localization of synaptic proteins according to a clear temporal and spatial pattern. Therefore NSCs seem to be well equipped to potentially compensate for lost or injured brain tissue.

Kategorie: Vortrag

Vortrag 39

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:FUNCTIONAL ANALYSIS OF LATROPHILINS, A GROUP OF PUTATIVE G-PROTEIN COUPLED RECEPTORS, IN CAENORHABDITIS ELEGANS

Autoren: Langenhan T.(1),Petkova V.(1),Mestek L.(1),Prömel S.(1),Esmaeili B.(1),Russ A.(1),

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Abstract:

Latrophilins belong to class B of G-protein coupled receptors, a group that comprises some 30 members. Latrophilins are best-known for their capacity to cluster alpha-latrotoxin, a component of Black widow spider toxin, at cell membranes thereby inflicting massive exocytotic release, a property that has been extensively used for neurophysiological and endocrinological research. However, it is still unclear, which endogenous function latrophilins exert and in which physiological context they operate. We are currently investigating latrophilin functions using the nematode *Caenorhabditis elegans* and its extensive toolkit.

lat-1, one of two *C. elegans* latrophilin homologs, displays a widespread expression profile within various tissues including neurons, epidermal, muscle, intestinal and gland cells, somatic parts of the worm gonad and spermathecae. The early onset of expression in neuroblasts and epidermal precursors implicate LAT-1 protein in morphogenetic events in the early embryo. Reporter fusion transgenes localize LAT-1 to neuronal membranes and the luminal surface of pharyngeal muscle cells throughout and after development.

A lat-1 null allele causes tight larval lethality in homozygous carriers at the first larval stage after hatching. Although no apparent morphological defects are present, lat-1^{-/-} larvae exhibit severe pharyngeal pumping defects preventing sufficient food intake.

This pumping defect is constituted by a specific loss of fast pharyngeal pumping rates. Thus, we speculate that LAT-1 is implicated in the neuromuscular control of the pharynx of *C. elegans*. Ongoing experiments address the cellular and developmental requirements of LAT-1 within this tightly controlled organ system and a detailed structure-function analysis of this B-GPCR.

Kategorie: Vortrag

Vortrag 40

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE 4TH TRANSMEMBRANE HELIX IS PART OF THE BINDING POCKET OF HUMAN ORGANIC CATION TRANSPORTER 3

Autoren: Volk C.(1),Gorboulev V.(1),Semlanski C.(1),Koepsell H.(1),

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Abstract:

Organic cation transporters OCT1-3 translocate structurally different organic cations. Great effort was made to understand the molecular basis of polyspecific substrate binding. Using mutational analysis we identified three positions in the 10th transmembrane helix (TMH) which play a critical role for the affinity of interacting compounds, e.g. corticosterone and were considered to be part of the substrate binding pocket. However, additional regions of the protein must be involved in binding, since human and rat OCT3 are 100% identical in this domain but show significant differences in affinities for corticosterone, which binds with 20 times higher affinity to hOCT3 than to rOCT3. Therefore, we tried to identify amino acids responsible for this difference. Alignment of the protein sequences showed differences in seven TMHs. Single amino acids were introduced from hOCT3 into rOCT3 and mutants were analyzed. Employing uptake measurements with radiolabelled substrates we showed, that exchange of phenylalanine to tyrosine in position 222 in the 4th TMH increased the affinity to corticosterone 10fold while mutations in other TMHs showed no effect. Previously, we observed that this position is also involved in substrate binding of rat OCT1. There, in contrast to our present observation with corticosterone, the introduction of tyrosine significantly decreased the binding affinity of tetraalkylammonium compounds.

Taken together, our findings demonstrate that the 4th transmembrane domain is critically involved in substrate binding of OCTs. The binding pocket appears rather complex, since several positions in different TMHs are involved in binding and mutations in these positions influence binding of diverse substrates differently.

Kategorie: Vortrag

Vortrag 41

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:POST-TRANSCRIPTIONAL INHIBITION OF THE HUMAN NA⁺-D-GLUCOSE COTRANSPORTER HSGLT1 BY TRANSPORTER REGULATOR HRS1 IS MODULATED BY PROTEIN 14-3-3

Autoren: Veyhl M.(1),Vernaleken A.(1),Kipp H.(1),Gorboulev V.(1),Koepsell H.(1),

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Abstract:

The intracellular 67 kDa-protein hRS1 (RSC1A1) is a transcriptional and posttranscriptional down-regulator of hSGLT1. We reported that posttranscriptional down-regulation by hRS1 occurs at the trans-Golgi-network, is stimulated by PKC and is modulated by intracellular D-glucose (Veyhl et al., Am J Physiol Renal Physiol 291, 2006). Employing oocytes of *Xenopus laevis* as expression system we identified two tripeptides (Gln-Cys-Pro (QCP) and Gln-Ser-Pro (QSP)) in hRS1 that mediate high affinity post-transcriptional down-regulation of hSGLT1. Similar to total hRS1 protein down-regulation by QCP and QSP is glucose dependent. QCP is located in the middle of the hRS1 sequence and QSP is located NH₂-terminal. QSP is framed by two consensus sequences for binding of regulator protein 14-3-3 that is involved in posttranscriptional regulation of various membrane proteins. Within this NH₂-terminal domain of hRS1 (a.a. 16-98) we identified the octapeptide Ser-Asp-Ser-Asp-Arg-Ile-Glu-Pro (SDSDRIEP) that is also capable to down-regulate hSGLT1 with nanomolar affinity. Posttranscriptional down-regulation of hSGLT1 by aa. 16-98 of hRS1 was enhanced after stimulation of PKC and when the intracellular concentration of 14-3-3-proteins was reduced, however, the down-regulation was not glucose dependent. Our data suggest that hRS1 contains a complex regulatory interaction surface (RIS) that is modulated by binding of 14-3-3-proteins to the RIS, by phosphorylation of the RIS or a distant site by PKC, and by binding of a glucose-binding protein to hRS1. Detailed knowledge about the RS1-mediated regulation of hSGLT1 will help to develop drugs to down-regulate glucose uptake in small intestine or glucose reabsorption in kidney during obesity or diabetes.

Kategorie: Vortrag

Vortrag 42

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE ROLE OF VASP IN THE REGULATION OF cAMP- AND Rac 1-MEDIATED ENDOTHELIAL BARRIER STABILIZATION

Autoren: Schlegel N.(1),Burger S.(1),Drenckhahn D.(1),Waschke J.(1),

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Abstract:

Regulation of actin dynamics is critical for endothelial barrier functions. We provide evidence that the actin-binding protein vasodilator-stimulated phosphoprotein (VASP) is required for endothelial barrier maintenance. Baseline permeability was significantly increased in VASP-deficient (VASP $-/-$) microvascular myocardial endothelial cells (MyEnd) in the absence of discernible alterations of adherens and tight junctions. We tested whether VASP is involved in the endothelium-stabilizing effects of cAMP or Rac 1. Forskolin and rolipram (F/R) to increase cAMP and cytotoxic necrotizing factor 1 (CNF-1) to activate Rac 1 were equally efficient to stabilize barrier functions in VASP $-/-$ and in wild-type (wt) cells. In wt, VASP was phosphorylated in response to F/R but did not localize to intercellular junctions. In contrast, Rac 1-activation induced translocation of VASP to cell borders in wt cells and increased the peripheral actin belt in both cell lines. In VASP $-/-$ cells, Rac 1 activity was significantly reduced compared to wt levels in controls and increased about 20-fold in response to CNF-1 compared to 7-fold activation in wt cells. These data demonstrate that VASP is not required for endothelial barrier stabilization mediated by cAMP and Rac 1. However, from the present data we conclude that VASP may be involved in the regulation of Rac 1 activity.

Kategorie: Vortrag

Vortrag 43

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A INDUCES ONLY A TEMPORARY INCREASE OF P21 PROTEIN EXPRESSION IN BREAST CANCER CELLS

Autoren: Krusche C.(1),Hennes-Mades S.(2),Bonn B.(2),Lindemann-Docter K.(3),

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(3)RWTH Aachen|Institut für Pathologie|Aachen|Deutschland

Abstract:

In vitro, predominantly short term effects of histone deacetylase (HDAC) inhibitors on gene expression are assessed. However, in phase I clinical trials long term administration of HDAC inhibitors are used.

Therefore, we analysed the effects of the HDAC inhibitor TSA on the protein expression of p21(WAF1/CIP1), a key inhibitor of proliferation, and the estrogen receptor (ER) alpha after different treatment times (12 –96 hours) and after administration of different doses. Two breast cancer cell lines were used. Analyses were performed by Western Blot experiments and immunohistochemistry.

Time course experiments demonstrated that in MCF7 cells and MDA-MB-231 cells p21 protein expression was elevated 12-24 hours after the onset of TSA treatment. Thereafter, despite ongoing TSA administration, in both cell lines p21 expression declined to basal levels or even below in case of MCF7 cells. The p21 protein was detected in the nuclei of both cell lines.

In MCF7 cells, ERalpha protein expression was regulated conversely to p21 expression: TSA induced a strong decrease of ERalpha protein expression at 12 and 24 hours. However, after 48 hours ERalpha expression normalized to and remained at the pretreatment level.

Our data demonstrate that p21 and ERalpha protein expression are just temporarily affected by TSA. This implies that compensatory mechanisms are activated to balance the increase of target protein acetylation induced by the HDAC inhibitor. Further studies are needed to unravel these mechanisms. However, this observation has to be taken into account in clinical HDAC inhibitor application.

Kategorie: Vortrag

Vortrag 44

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:LOCALIZATION AND FUNCTION OF ACETYLCHOLINE CLEAVING ENZYMES IN THE MOUSE TRACHEA

Autoren: Lips K.(1),Freidhof J.(1),Faulhammer P.(1),Papdakis T.(1),Bodenbenner M.(1),Wiegand S.(1),Paroanu L.(2),Layer P.(2),Wessler I.(3),Li B.(4),Duysen E.(4),Lockridge O.(4),Kummer W.(1),

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Abstract:

Acetylcholine (ACh) is an important neurotransmitter whose synaptic action is rapidly terminated through enzymatic cleavage by acetylcholinesterase (AChE).

Butyrylcholinesterase (BChE), abundantly present in blood plasma, is an additional ACh cleaving enzyme with broader specificity. Here, we analysed the distribution and function of these enzymes in the mouse trachea where ACh is produced and released both from nerve fibres and from non-neuronal sources. Enzyme histochemistry, RT-PCR, AChE- and BChE-activity assay, and analyses of the mucociliar transport capacity were performed in AChE- and BChE-knockout and wild-type mice.

Enzyme histochemistry revealed BChE in the tracheal smooth muscle and AChE in nerve fibres innervating it, whereas neither AChE nor BChE were found in the airway epithelium. Only minimal amounts of AChE- and BChE-mRNA were detected by RT-PCR in the tracheal epithelium, consistent with the observation that AChE- and BChE-activity assays revealed no specific epithelial AChE- and BChE-activity.

Neither acute inhibition of AChE by eserine in tracheas of wild-type mice nor genetic ablation of the AChE and BChE genes had any impact on the stimulatory effect of ACh on particle transport speed at the tracheal surface, a measure for mucociliary clearance.

In conclusion, these results show that, in the trachea, AChE and BChE are localized at the site of neuronal cholinergic transmission from parasympathetic axons to smooth muscle whereas these enzymes are missing in the epithelium where ACh is also produced. This lack of epithelial AChE and BChE allows for the auto-/paracrine effects of the small amount of ACh released by the epithelial cells.

Kategorie: Vortrag

Vortrag 45

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:CHARACTERIZATION OF THE CEACAM EXPRESSION IN THE PULMONARY EPITHELIAL CELLS

Autoren: Singer B.(1),Scheffrahn I.(1),Kammerer R.(2),Lucka L.(3),Slevogt H.(4),Ergün S.(1),

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Pulmonary Medicine|Berlin|Deutschland

Abstract:

CEACAM1 has been implicated in cancer development. Based on its diminished expression in colon tumors CEACAM1 is believed to act as tumor suppressor. However, CEACAM1 was found to be overexpressed in lung carcinomas but to be absent in normal lung tissue. These data indicate that CEACAM-1 might promote lung cancer progression. To analyze this hypothesis we studied the human alveolar epithelial cell line A549. Our examinations revealed no CEACAM1 expression in A549 cells if kept in proliferating culture condition as tested by FACScan, ELISA and western blotting. Additionally, there was no expression of CEA, CEACAM6 and CEACAM7, which are molecules known to be co-expressed with CEACAM1 in human epithelial cells. Interestingly, A549 cells did express high amounts of CEACAM1 on the cell surface after becoming confluent. However, there was no CEA expression and only a fraction of A549 cells did express CEACAM6. Interestingly, we found high amounts of CEA and CEACAM6 in the cell lysate leading to the assumption, that CEA and CEACAM6 were synthesized but not expressed on the cell surface. In contrast to the transmembrane bound CEACAM1, CEA and CEACAM6 are linked to membrane via a GPI-anchor. Therefore we speculated that A549 cells lack functional GPI-transferases for the post-transcriptional modification. Transfection with CEA revealed, that A549 cells are able to express GPI-linked CEA on their cell surface. Our data highlight a complex expression pattern and regulation of CEACAMs in alveolar epithelial cells and further analyses may help to explain the seemingly contradictory functional role of CEACAMs in different epithelium.

Kategorie: Vortrag

Vortrag 46

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:NEW ASPECTS OF THE ROLE OF VASOPRESSIN IN RENAL CONCENTRATING MECHANISM

Autoren: Mutig K.(1),Paliege A.(1),Kahl T.(1),Böhlick A.(1),Müller-Esterl W.(2),Bachmann S.(1),

Adressen:(1)Charité|Institut für Vegetative Anatomie|Berlin|Deutschland; email:kerim.mutig@charite.de; (2)Universitätsklinikum Frankfurt|Institut für Biochemie II|Frankfurt|Deutschland

Abstract:

Vasopressin (AVP) influences epithelial salt and water transport in the kidney chiefly via the V2 receptor (V2R). V2R-mediated signaling in the loop of Henle, although functionally relevant, is less well established than in the collecting duct. We hypothesize a major role for the V2R-mediated activation of the Na,K,Cl-cotransporter 2 (NKCC2) in the medullary thick ascending limb (MTAL). Two aspects are analyzed: (I) renal expression of V2R across species; (II) downstream effects of V2R activation on NKCC2 including its phosphorylation, expression, and trafficking via an integration of NKCC2 into lipid rafts. Kidneys from Brattleboro rats (DI), C57 mice, and humans, and cultured rbTAL cells were studied. Short term AVP treatment with dDAVP was applied in experimental setups. Cellular distribution and expression of V2R, NKCC2, and phospho-NKCC2 were analyzed using immunohistochemistry, in situ hybridization, Western blot, and PCR. Floating assays for analysis of lipid raft fractions were performed. V2R expression was strong in MTAL across species, and in rbTAL cells. dDAVP administration to DI rats induced increases of phospho-NKCC2 predominantly in kidney medulla (+198%), along with markedly enhanced luminal insertion of NKCC2 in MTAL. These data were confirmed in mice and in rbTAL cells. There was enhanced partitioning of NKCC2 into lipid rafts upon dDAVP (>+25%). Association of NKCC2 with lipid rafts was essential for apical trafficking and surface expression. In sum, AVP plays a major role in MTAL transepithelial transport via V2R signaling. NKCC2 becomes activated via lipid raft-dependent surface expression.

Kategorie: Vortrag

Vortrag 47

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:DRAMATIC ENLARGEMENT OF THE KIDNEY IN RESPONSE TO VEGF OVEREXPRESSION IN RENAL TUBULES

Autoren: Hakroush S.(1),Moeller M.(2),Koesters R.(3),Akeson A.(4),Hosser H.(1),Hähnel B.(1),Sijmonsma T.(3),Kriz W.(1),

Adressen:(1)Ruprecht-Karls-Universität Heidelberg|Institut für Anatomie und Zellbiologie|Heidelberg|Deutschland; email:hakrosam@gmx.de; (2)RWTH Aachen|Medizinische Klinik II - Nephrologie und Klinische Immunologie|Aachen|Deutschland; (3)Ruprecht-Karls-Universität Heidelberg|Deutsches Krebsforschungszentrum (DKFZ)|Heidelberg|Deutschland; (4)University of Cincinnati College of Medicine|Divisions of Pulmonary Biology and Neonatology|Cincinnati|USA

Abstract:

The multiple effects of VEGF in the kidney are insufficiently understood. We overexpressed VEGF₁₆₄ in renal tubules by crossing the following two transgenic lines: Pax8-rtTA-M2, a novel line that expresses the tetracycline-inducible transactivator within the entire tubular system and (tetO)₇VEGF, that expresses VEGF under the control of a tetracycline responsive promoter. Bitransgenic mice were induced with doxycycline for 7, 14 or 28 days.

Results: The hematocrit was significantly decreased. The kidneys underwent dramatic hypertrophy: after 28 days of treatment, the kidney weight increased to 5,3 % of body weight compared to 2.1 % in controls. In the tubulo-interstitium, new capillaries were formed together with a vivid proliferation of interstitial cells and matrix deposition leading to a dramatic increase of the peritubular interstitium with hypercapillarisation. Focally, the glomerular capillary phenotype with open pores lacking a diaphragm was encountered around tubules. This finding supports the notion that high-levels of VEGF are necessary to induce pore formation in the glomerular endothelium. In the glomerulus, profound architectural rearrangements were seen consisting of a numerical decrease of capillaries but instead of an increase in calibre. Podocyte cell bodies decreased in size, endothelial cells increased in number and their cell bodies were frequently found in unusual peripheral locations; mesangial cells were focally clustered.

Conclusion: VEGF stimulated the formation of new peritubular capillaries and changed their phenotype. Most surprising, it initiated profound architectural changes within the glomerular tuft.

Kategorie: Vortrag

Vortrag 48

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:INVASIVE CAPACITY OF EXTRAVILLOUS TROPHOBLAST CELLS IS MODULATED BY IL-11 IN AN EXPLANT CULTURE MODEL

Autoren: von Rango U.(1),Classen-Linke I.(1),Seelis-Schmidt D.(1),Krusche C.(1),

Adressen:(1)RWTH University of Aachen|Dept. of Anatomy and Reproductive Biology|Aachen|Deutschland; email:uvonrango@ukaachen.de

Abstract:

IL-11 is discussed to be involved in the regulation of extravillous trophoblast (EVT) invasion. Defects within the IL-11 system in the mouse result in EVT-over-invasion of maternal decidua. However, there are no functional studies on the influence of IL-11 on human primary EVT cells.

Therefore, trophoblast villi explants were cultured in a dual chamber system on inserts coated with collagen matrix. The cultures were supplemented with estradiol (E2; 10⁻⁸ mol/l) and medroxyprogesterone acetate (MPA; 10⁻⁶ mol/l). Explants were cultured with 0, 20, 50, 100 and 200 microgram/ml IL-11 for three days. Trophoblast invasion was monitored by light-microscopy and studied on the histological level after fixation and embedding in paraffin. Invading cells were identified as trophoblast cells by cytokeratin and c-erbB2 expression. Trophoblast proliferation (MIB) and apoptosis (M30) were assessed and IL-11 signal transduction was shown by P-STAT3 immunohistochemistry.

Cultured trophoblast established cell columns, invaded the collagen matrix and showed a proliferation pattern comparable to the in vivo situation. Extensive apoptosis was not detected. Invading EVT and the cell columns stained for P-STAT3. Whereas adherence and cell column formation were comparable between the treatment groups, EVT invasion was significantly decreased when cultures were treated with 100 and 200 microgram/ml IL-11.

These data suggest that IL-11 may limit invasive capacity of EVT cells without affecting adherence of EVT or column formation. This is consistent with our in vivo data on IL-11 expression during the 1st trimester of pregnancy and the reduced IL-11 expression found in ectopic tubal pregnancy characterized by EVT over-invasion.

Kategorie: Vortrag

Vortrag 49

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:THE PRO-ANGIOGENIC PROTEIN CYR61 IS REGULATED BY PRE-MENSTRUAL SIGNALING FACTORS

Autoren: Gashaw I.(1),Stiller S.(1),Winterhager E.(1),

Adressen:(1)Duisburg-Essen|Anatomie|Essen|DE; email:isabella.gashaw@uk-essen.de

Abstract:

The menstrual breakdown of the functionalis is triggered by cytokines, prostaglandins as well as hypoxic conditions in endometrium. The pro-angiogenic factor CYR61 is heavily expressed in endometrium with maximal levels in menstrual tissues. To identify factors responsible for this increase, we investigated the regulation of CYR61 expression by TNFalpha, PGE2 and hypoxia, which are associated with tissue remodeling immediately before menstruation.

The benign endometrial epithelial cell line HES was incubated under normoxic (20% O₂) and hypoxic (1% O₂) conditions for up to 24h. Hypoxia transiently induced CYR61 mRNA levels after 2h of incubation but not the cellular protein. However, enhanced secretion of the CYR61 protein into the medium was detected already after 1h under low oxygen. These effects were accompanied by a permanent stabilization of HIF1alpha protein. Activation of HIF-1 via dimethylxalylglycine increased the levels of CYR61 mRNA and protein secretion as well, whereas application of HIF1alpha siRNA led to decreased CYR61 levels. TNFalpha and the prostaglandin PGE2 elevated significantly CYR61 transcript levels as well as cellular and secreted CYR61 protein amounts under normoxic conditions. Moreover, PGE2 and TNFalpha combined with hypoxic conditions, respectively, prolonged the induction of CYR61 mRNA expression in the cells and increased the amount of secreted CYR61 protein.

The regulation of CYR61 by hypoxia via HIF1alpha as well as TNFalpha and prostaglandin represents possible inducers for CYR61 upregulation in premenstrual and menstrual endometrium. Increased secretion of CYR61 protein might induce paracrine effects in the tissue such as endometrial remodeling in the course of premenstrual changes.

Kategorie: Vortrag

Vortrag 50

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:SELECTIVE TRANSDUCTION OF ADULT NEURAL PROGENITOR CELLS IN THE MOUSE DENTATE GYRUS BY ADENOVIRAL VECTORS

Autoren: Haas S.(1),Schmidt A.(2),Pützer B.(2),Wree A.(1),

Adressen:(1)University of Rostock|Institute of Anatomy|Rostock|Germany;
(2)University of Rostock|Department of Vectorology & Experimental Gene Therapy|Rostock|Germany

Abstract:

The dentate gyrus (DG) of the hippocampus is a region of ongoing neurogenesis throughout life in rodents, primates and human species. Neural progenitor cells (NPC) of this region engineered to promote lineage-specific differentiation or to express therapeutic genes might be used in investigations to study their differentiation behaviour or their potential for restorative therapies. Here we report the identification of two NPC specific ligands from phage display peptide libraries by using a mouse hippocampal neurosphere cultivation and differentiation system. Injection of fluorescent-protein encoding adenoviral (Ad) vectors engineered to bind these NPC specific peptide ligands into the DG of pNestin-GFP transgenic or C57BL/6 mice, resulted in a highly specific infection of NPC of the dentate gyrus. This was demonstrated by the radial glia like morphology of infected Type-1 or B-cells, and by co-labelling of infected cells with the progenitor markers nestin or doublecortin. Quantitative analysis of infected cells in the brain revealed for the peptide tagged viruses $83.5\pm 9.4\%$ and $85.6\pm 4.4\%$ double labelled cells, respectively, whereas only $15.5\pm 1\%$ or $8.6\pm 1.7\%$ were determined for wild-type vector or virus containing an unspecific control peptide. The Ad vector related immunologic response of these animals shown by Iba1-immunohistochemistry, was similar as compared to saline injected controls. This novel approach of selective and safe targeting NPC now opens the way for direct in situ manipulation (e.g. differentiation, therapeutic genetic engineering) of newborn stem cells in the adult mouse brain. Work was supported by grant 01ZZ0108 from BMBF and the Medical Faculty of Rostock University to B.M.P.

Kategorie: Vortrag

Vortrag 51

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:BEHAVIOURAL AND CELL BIOLOGICAL INVESTIGATIONS OF THE CIRCADIAN SYSTEM OF THE LANCELET, BRANCHIOSTOMA LANCEOLATUM

Autoren: Schomerus C.(1),Laedtke E.(1),Zhang Q.(1),Korf H.(1),Wicht H.(1),

Adressen:(1)Johann Wolfgang Goethe-Universität Frankfurt|Dr. Senckenbergische Anatomie, Institut für Anatomie II|Frankfurt/Main|Deutschland;
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Abstract:

Lancelets are the closest living invertebrate relatives of craniates. Knowledge about its behaviour is sparse, because adult animals are filter-feeders and spend their entire lifetime buried in the sediment. In particular, it is unknown whether they display any rhythmic activity. We developed a method to quantify locomotor activity of Branchiostoma within the sediment by counting \"new holes dug per hour\" and investigated their activity under different light regimes. Lancelets are clearly diurnal, with peak activities in the dark. Their rhythms are entrained by light: the onset of the dark phase determines the onset of locomotor activity. They are also circadian: rhythmic activity persists in constant darkness. Thus, they probably have an endogenous oscillator. In mammals, this oscillator is located in the hypothalamic suprachiasmatic nuclei. In order to localize this oscillator in Branchiostoma, we cloned partial sequences of amphi-per, amphi-bmal, and amphi-cry, homologues of genes known to play key roles in the oscillator of craniates. Using in situ hybridization we located the mRNA of amphi-per in a restricted region of the anterior neural tube. The amphi-per mRNA is expressed rhythmically with peak levels in the first half of the light phase. The neurons expressing amphi-per mRNA are located in the posterior part of the cerebral vesicle, a putative homologue of the craniate diencephalon. They lie in close vicinity to a number of photoreceptors, some of which have been homologized to the lateral eyes of craniates. Thus, these neurons may be homologous to the suprachiasmatic nucleus of craniates.

Kategorie: Vortrag

Vortrag 52

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:F-SPONDIN REGULATES NEURONAL SURVIVAL IN THE CHICKEN CILIARY GANGLION

Autoren: Peterziel H.(1),Paech T.(1),Klar A.(2),Unsicker K.(1),

Adressen:(1)University of Heidelberg|Department of Anatomy and Cell Biology III|D-69120 Heidelberg|Germany; (2)Hebrew University-Hadassah Medical School|Department of Anatomy and Cell Biology|Jerusalem 91120|Israel

Abstract:

The extracellular membrane (ECM)-associated protein F-spondin is expressed in the floor plate during embryonic development and plays an important role in patterning the axonal trajectory in the spinal cord. We found that F-spondin is expressed in non-neuronal cells in the chicken ciliary ganglion (CG) and efficiently promotes survival and neurite outgrowth of these neurons in culture. Treatment of CG neurons with F-spondin induces rapid phosphorylation of the intracellular adaptor molecule disabled-1, and protein kinase B/Akt. Using deletion constructs of F-spondin we found that the N-terminal Reelin/Spondin (R/S) domain is sufficient to mediate downstream activation of the intracellular signalling pathways. However, the survival promoting effect requires the thrombospondin type 1 repeat (TSR) 6, a domain which mediates TGFbeta-activation.

In ovo treatment with blocking antibodies raised against the R/S domain or the TSR-domains leads to an increase in apoptosis of E8 and E10 CG neurons as measured by TUNEL and Caspase 3 staining. Using the neuron-specific transcription factor Islet-1 as a marker for surviving neurons we counted neuron numbers in ciliary ganglia treated with anti-R/S and anti-TSR antibodies and found a loss of ~ 30% neurons at E10 compared to controls.

Taken together we show that F-spondin mediates the survival of CG neurons in ovo by a mechanism involving the Reelin/spondin and the TSR domains.

Supported by GIF G-739-97.1/2002

Kategorie: Vortrag

Vortrag 53

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:EINFLUSS DES LEUKÄMIE-ASSOZIIERTEN TRANSKRIPTIONSFAKTORS AF9/MLLT3 AUF DIE ENTWICKLUNG DES CEREBRALEN CORTEX DER MAUS

Autoren: Vogel T.(1),

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Abstract:

The cerebral cortex is subdivided into functional areas around a longitudinal and a radial axis. This subdivision is a conserved pattern amongst vertebrate species providing a clue for a conserved genetic program contributing to its formation. Much of the cortical cyto architecture is assembled prenatally involving the orderly migration of cells from the ventricular zone (VZ) through the subventricular/intermediate zone (SVZ) to the cortical plate. Neurons migrate mostly along radial glia towards the surface and as they arrive in the cortical plate, neurons assume progressively more superficial positions.

To understand the genetic basis of the formation of the cerebral cortex we attempted to isolate genes that are involved in this process. Using a microarray hybridisation assay,

we identified the leukaemia associated transcription factor Af9/Mllt3 as a gene that is expressed predominantly in the SVZ of the cerebral cortex, but also in the VZ and cortical plate as well as in the hippocampus, cerebellar cortex, septum and various thalamic structures.

Although expression of Af9 in the SVZ is overlapping with markers like Svet-1 and Cux-2, Af9 does not seem to determine a neuronal fate to upper cortical layers as proposed for these markers. Preliminary analyses of Af9 mutant mouse brains point to an involvement of Af9 in the specification of neurons of deep cortical layer VI. Af9 mutant brains display a reduction of cells in the VZ/SVZ specifying the boundary between the neocortical pallium and the subpallium, crucial for the correct migration of GABAergic interneurons to the neocortex.

Kategorie: Vortrag

Vortrag 54

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:REELER MUTANT MICE SHOW A WHISKER TO BARREL PATHWAY WITH TOPOGRAPHICALLY ORGANISED FUNCTIONAL MODULES

Autoren: Wagener R.(1),Haas C.(2),Zhao S.(1),Staiger J.(1),

Adressen:(1)Albert-Ludwigs-University|Department of Neuroanatomy|Freiburg|Germany; email:Robin.Wagener@gmx.net; (2)Albert-Ludwigs-University|Department of Neurosurgery|Freiburg|Germany

Abstract:

Perception of tactile information via the vibrissae is of high behavioural relevance for rodents. An anatomic substrate for tactile information processing is the whisker-to-barrel-pathway. It presents a somatotopic map of the sensory periphery in all processing stations, including the primary somatosensory (barrel) cortex. In the present study we asked whether this refined functional anatomy can also be found in a mouse model with a highly disorganised cortex, i.e. the Reeler mutant. The exploration of a novel environment led to the expression of the inducible transcription factor c-Fos at all stages of the whisker-to-barrel-pathway. Moreover, double staining of c-Fos and cytochrome oxidase showed a precise topographic organisation of the modules in the trigeminal brainstem nuclei and the thalamic ventrobasal complex. Also the barrel field seemed to be topographically ordered. Location, shape and borders were similar to the ones of wildtype animals. However, individual barrels within the posteromedial barrel subfield showed abnormalities in accordance with the inversion and smearing of the laminar organisation of the Reeler cortex. Barrels were spread much over the cortical thickness. Altogether, the alignment of the columns was less regular in shape. C-Fos upregulation was only found in columns of the barrel cortex, which corresponded to the stimulated vibrissae. From these results we draw the conclusion that an intact thalamic input into a disordered cerebral cortex allows the development of barrel-related columns and their organisation into a somatotopic map. Furthermore, a locally determined transcriptional response as a consequence of environmental stimuli processing is possible within these maps.

Kategorie: Vortrag

Vortrag 55

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:NUCLEAR AND UNCLEAR FUNCTIONS OF NEURONAL FLASH

Autoren: Nawrotzki R.(1),Liu X.(1),Böckers T.(2),Kirsch J.(1),

Adressen:(1)Heidelberg|Anatomie und Zellbiologie|Heidelberg|BW;
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Zellbiologie|Ulm|BW

Abstract:

During brain development, a surplus of neurons is generated that is subsequently eliminated by apoptosis. However, the proteins involved in neuronal cell death are poorly characterised. We have recently identified the caspase 8-associated protein FLASH as an interactor of gephyrin, a key player in the clustering of inhibitory neurotransmitter receptors. FLASH also binds to the death-inducing signalling complex (DISC) and to proteins implicated in TNF-alpha-mediated cell survival, indicating that FLASH may influence the decision over the fate of a cell. To determine the properties of neuronal FLASH, we analyzed the expression patterns of full-length FLASH and of a short variant we recently identified. By in-situ hybridization, we show that both variants are expressed in the adult brain, the full-length version being mostly neuronal and the short form being mostly glial. This pattern is currently verified by immunoblotting using specific antibodies. In cultured hippocampal neurons, FLASH antibodies revealed high concentrations of this protein in nuclear Cajal bodies (in tumour cells, FLASH has been implicated in the functions of nuclear bodies) and at synapses. We are now in the process of setting up assays using shRNA interference to address the role of FLASH in the survival of neurons.

In the light of recent findings that neuronal FLASH is highly regulated by the activation of synaptic glutamate receptors, we expect that FLASH is a key component in the programs that regulate the survival or death of maturing and mature neurons.

Kategorie: Vortrag

Vortrag 56

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE SURVIVAL OF MOTONEURON (SMN) PROTEIN MODULATES ACTIN DYNAMICS BY AFFECTING THE ROCK-PATHWAY

Autoren: van Bergeijk J.(1),Grothe C.(2),Claus P.(2),

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Abstract:

Spinal muscular atrophy is a neurodegenerative disease accompanied by a loss of motoneurons. Either mutations or deletions in the survival of motoneuron (SMN) gene are responsible for this defect. SMN is an assembly protein for RNA-protein complexes in the nucleus and is also found in axons of neurons. However, it is unclear which dysfunctions of SMN are important for disease progression.

We analyzed the effects of SMN on neuronal differentiation associated with outgrowth of neurites in PC12 cells as a model system for neurogenesis. Suppression of endogenous SMN protein levels by siRNA decreased significantly growth of neurites, whereas cells overexpressing SMN displayed increased lengths of neurites. Neurite outgrowth is associated with changes of the actin cytoskeleton. Remarkably, the knock-down of SMN led to a significant change of the G-/F-actin ratio indicating a role of SMN in actin dynamics. Rho-Kinase (ROCK) affects actin polymerization by phosphorylation of LIM-Kinase, which in turn regulates Cofilin. In our SMN knock-down model for spinal muscular atrophy we could show that the phosphorylation of these signaling molecules has been changed. The data suggest that actin-regulating proteins downstream of ROCK are involved in SMN-dependent neuritogenesis defects. Importantly, analyses of this pathway could help to elucidate new molecular targets for a therapy of spinal muscular atrophy.

Kategorie: Vortrag

Poster 1

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:EXPRESSION OF PROTOCADHERIN-1 (PCDH1) DURING MOUSE DEVELOPMENT

Autoren: Redies C.(1),Heyder J.(1),Staes K.(2),van Roy F.(2),

Adressen:(1)Friedrich-Schiller-Universität Jena|Institut für Anatomie
|Jena|Deutschland; email:redies@mti.uni-jena.de; (2)VIB-Ghent
University|Department for Molecular Biomedical Research|Ghent|Belgium

Abstract:

Members of the cadherin superfamily of cell adhesion molecules regulate morphogenesis and signal transduction during vertebrate development. Protocadherin-1 (Pcdh1) or axial protocadherin (AXPC) belongs to a recently identified subfamily of cadherins, termed delta-protocadherins (Vanhalst et al., 2005). In early *Xenopus* and zebrafish development, Pcdh1 is expressed in the somites, pronephros, heart, otic vesicle and brain and plays a role in prenotochordal cell sorting in the gastrulating embryo (Kuroda et al., 2002). Little is known about the expression of delta-protocadherins in other vertebrate species and at later stages of development. We therefore mapped the expression of Pcdh1 during mouse development from E10 to the adult stage by in situ hybridization. Results show that a number of additional tissues and organs express Pcdh1, for example, the lung, liver, kidney, uterus, intestines, the epithelial components of several glands, hair follicles, and sensory and visceral ganglia. In embryonic development, blood vessels throughout the entire embryo show prominent signal. Parts of the placenta are also positive. In the brain, Pcdh1 is expressed in a subset of grey matter structures, such as cortical layers and regions, and brain nuclei. In many of these tissues and organs, expression persists until the adult stage. Our results suggest that Pcdh1 expression is tightly regulated in several developing organs and tissues, also in the adult, shedding light on the possible role of Pcdh1 in development, tumorigenesis and other pathological processes in these tissues.

Kategorie: Poster

Poster 2

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:RESCUE OF DKK1 EXPRESSION PATTERN IN VITRO BY CHEMICAL HIF ACTIVATION AT ONSET OF GASTRULATION

Autoren: Pnitzner J.(1),Katschinski D.(2),Viebahn C.(1),

Adressen:(1)Georg-August-Universität Göttingen|Zentrum Anatomie, Abteilung Anatomie und Embryologie|Göttingen|Deutschland; email:jplitzn@gwdg.de;
(2)Georg-August-Universität Göttingen|Zentrum Physiologie, Abteilung Herz- und Kreislaufphysiologie|Göttingen|Deutschland

Abstract:

Embryonic development appears to be seriously impaired without the transcription factor hypoxia inducible factor 1(HIF-1) which activates specific signalling pathways to mediate cellular adaptation under conditions of hypoxia. This is suggested by the HIF-1a knock-out-mouse which is embryonic lethal and points to a reduced oxygen supply via the endometrium, especially prior to implantation. Ideally, culture conditions for experimental analysis of mammalian embryonic development should mimic physiological relative hypoxia throughout isolation and cultivation of embryos. Intrigued by an unexplained unphysiological gene expression pattern within otherwise normal morphological development after short-term in vitro culture of early gastrulation stages in the rabbit, we set up suspension cultures under normoxic conditions and treated them with dimethylxaloylglycine (DMOG), a prolyl 4-hydroxylase inhibitor which raises HIF activity by chemical stabilization. After culture the embryonic discs were analysed by in-situ hybridisation for the head inducing signalling molecule Dickkopf1, which after in vivo development shows an anterior expression domain running alongside the anterior marginal crescent. This physiological expression domain was regularly reduced to a small central domain in control embryos (under normoxic conditions, i.e. without DMOG), whereas in embryos cultured with DMOG the physiological, clasp-like Dkk1 expression domain was rescued in the majority of cases. Further investigations should answer the question whether standard normoxic culture conditions lead either to a disturbance of cell migration or a higher apoptotic activity, both of which could be reasons for the failing formation of the proper Dkk1 expression domain in the anterior pole of the early gastrulating embryo.

Kategorie: Poster

Poster 3

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:EXPOSITION TO HIGH DOSE 17 α -ETHINYLESTRADIOL DURING DEVELOPMENT DIFFERENTIALLY INFLUENCES GH, IGF-I, IGF-II AND ESTROGEN RECEPTOR- α ; GENE EXPRESSION IN FISH PITUITARY.

Autoren: Shved N.(1),Berishvili G.(1),Baroiller J.(2),Reinecke M.(1),Eppler E.(1),

Adressen:(1)University of Zürich|Division of Neuroendocrinology, Institute of Anatomy|Zürich|Switzerland; (2)Campus International de Baillarguet|UPR20 Aquaculture|Montpellier|France; email:eppler@anatom.unizh.ch

Abstract:

Growth and sexual development are closely interlinked in fish, however, no reports exist on potential effects of estrogen on the growth hormone (GH)/insulin-like growth factor (IGF)-axis in developing fish. We investigated whether estrogen treatment during early development affects GH, IGF-I, IGF-II and estrogen receptor-alpha (ER-alpha) in the pituitary. Tilapia were fed from 10 to 40 days post fertilization (DPF) with 17-alpha-ethinylestradiol (EE2). At 50, 75, 90 and 165 DPF, length, weight and sex ratio were determined, and pituitary GH, IGF-I, IGF-II and ER-alpha mRNA quantified by real-time PCR. Developmental exposure to EE2 had persistent effects on sex ratio and growth. GH mRNA was decreased in male pituitary at 165 DPF and in female pituitary at 75 and 90 DPF. In males, both IGF-I and IGF-II mRNA were raised at 75 DPF, downregulated at 90 DPF and reached normal levels at 165 DPF. In females, no change of IGF-I mRNA occurred, whereas IGF-II gene expression was upregulated at 75 DPF and reduced at 90 DPF. ER-alpha mRNA was significantly induced at 165 DPF in male, and at 75 and 90 DPF in female pituitary which is inverse to the GH gene expression pattern. The data are supported by in situ hybridisation. Our results show that developmental estrogen treatment impairs GH and IGF expression in fish pituitary - most likely via the estrogen receptor pathway -, and that the effects persist. Thus, long-lasting effects of estrogens on fish growth and reproduction may be exerted indirectly via inhibition of pituitary GH and IGF.

Kategorie: Poster

Poster 4

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:CELLS OF OCCIPITAL SOMITES USE AN EXTENSION-MECHANISM TO FORM A MUSCLE THAT STRETCHES BETWEEN HEAD AND SHOULDER IN THE AVIAN EMBRYO

Autoren: Theis S.(1),Scaal M.(1),Christ B.(1),Patel K.(2),Huang R.(3),

Adressen:(1)University of Freiburg|Institute of Anatomy and Cell Biology|Freiburg|Germany; (2)University of Reading|Institute of Zoology|Reading|UK; (3)University of Freiburg|Institute of Anatomy and Cell Biology|Freiburg|Germany

Abstract:

The development of skeletal muscles requires different mechanisms, even though most of them are derivatives of somites. The muscles in the deep back region and ventrolateral body wall are derived from the myotome, while limb and tongue muscles are formed by cells migrating from the dermomyotome into the limb bud and tongue anlage, respectively. The development of shoulder, pelvic girdle and cloacal muscles is more complex than that of the trunk and limb muscles. Their precursor cells migrate first into the limb similar to the precursors of intrinsic limb muscles and then translocate from the limb to their final position. In this study we report a yet unknown mechanism for muscle development. Based on observations from chick-quail-lineage tracing experiments, we found that the cucullaris muscle, which stretches across the head and shoulder and is considered to be a homologue of the mammalian trapezius and sternocleidomastoid muscles, is derived only from the first two somites. This led us to ask how somite cells translocate over an extended distance over 15 segments in the chick and 8 segments in mammals, to form a muscle. In situ hybridisation against the muscle regulatory factor MyoD and immunohistochemistry the muscle differentiation marker Mf20 reveals that the muscle precursor cells differentiate after they have arrived at their final position. Quail-to-chick transplantations and Myf5 in situ hybridisation argue for a possible involvement on branchial arches III and IV and indicate a contribution of two distinct myoblast populations, which passively become extended during the neck elongation.

Kategorie: Poster

Poster 5

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:TWO MECHANISMS INVOLVED IN SCLEROTOME FORMATION

Autoren: Wang B.(1),Proels F.(1),Christ B.(1),Scaal M.(1),Huang R.(1),

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Abstract:

Two mechanisms involved in sclerotome formation

Baigang Wang, Felicitas Proels, Bodo Christ, Martin Scaal, Ruijin Huang

Institute of Anatomy and Cell Biology, University of Freiburg,
Albertstr.17, 79104 Freiburg, Germany

Sclerotome is formed by epitheliomesenchymal transition (EMT) in ventral epithelial part of the somite. Up to date, it has been generally accepted that sclerotome formation is initiated by signals emanating from the notochord. In this process, Shh induces Pax-1 and Pax-9 expression in the ventral somite cells, which leads to disintegration of epithelial cell organisation and to chondrogenic differentiation. Simultaneously, BMP-inhibitors from the notochord, Noggin, Chordin and Follistatin, modulate the influence of BMP-signals from the lateral mesoderm and ensure the size of the sclerotome. However, we observed that Pax-1 and Pax-9 expression are located only in medial part of the sclerotome. Furthermore, we found that lateral part of the sclerotome, adjacent to the intermediate and lateral plate mesoderm, forms without signals from the notochord. Thus sclerotome can be subdivided into two parts. The medial part is induced by notochord signals and expresses Pax-1 and Pax-9, whereas the lateral part does not depend on the notochord signals and their cells do not express these Pax-genes. To determine the inductive signals for formation of the lateral sclerotome, we performed barrier implantations between the epithelial somite and the intermediate mesoderm. Without the lateral signals, ventrolateral part of the epithelial somite did not undergo EMT to form the lateral sclerotome. Ongoing work is to determine whether BMPs are involved in the initiation of the lateral sclerotome.

Kategorie: Poster

Poster 6

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:CYSTATHIONINE- γ -LYASE (CSE) IS SPECIFICALLY EXPRESSED IN THE DEVELOPING AVIAN KIDNEY

Autoren: Krück S.(1),Mittapalli V.(1),Christ B.(1),Pröls F.(1),Huang R.(1),Scaal M.(1),

Adressen:(1)Freiburg|Anatomie und Zellbiologie|Freiburg|Deutschland;
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Abstract:

Cystathionine-Gamma-Lyase (CSE) is specifically expressed in the developing avian kidney

Stefanie Krück, Venugopal Rao Mittapalli, Bodo Christ, Felicitas Pröls, Ruijin Huang, Martin Scaal

Institute of Anatomy and Cell Biology, University of Freiburg, Albertstr.17, 79104 Freiburg, Germany

Cystathionine gamma-lyase (CSE) is a key enzyme in the transsulfuration pathway for the biosynthesis of cysteine from methionine in mammals, and catalyzes the hydrolysis of cystathionine into cysteine. CSE has been described to be an inhibitor of cell proliferation acting on ERK –dependent cell cycle regulation. We isolated the chicken homologue of CSE and analyzed its expression pattern in the chicken embryo. We found distinct expression of CSE in the developing meso- and metanephric kidney, and an additional expression domain in the notochord. Our results indicate an important role of CSE during kidney formation and provide a new tool to study renal development in the avian model system.

Kategorie: Poster

Poster 7

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:HOXD13 EXPRESSION IN HUMAN ANOECTUM DURING EMBRYOGENESIS AND IN ADULTS.

Autoren: Illig R.(1),Schwarzer C.(2),Blumer M.(1),Fritsch H.(1),

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Abstract:

HOX genes and their homeodomain proteins play a significant role during embryonic development in particular in defining the body plan and in development of gut and urogenital tract. Deregulated Hox genes cause embryonic malformations and an oncogenic potential was found.

We examined the role of HOXD13 expression for the first time in the anorectal region of human embryos/fetuses as well as in adults. In-situ hybridisation and immunohistochemistry were performed on paraffin embedded sections of 22 human embryos/fetuses (5th to the 14th week p.c.) and on 8 samples of adult rectal tissue. Analysis in light microscopy showed an age-dependent and regional HOXD13 expression commencing in the rectal epithelium at the end of 6th week p.c. and in the surrounding mesenchyme in 7th week p.c.. During 8th and 10th week p.c. an increasing expression in the clearly structured and labelled rectal epithelium and surrounding mesenchyme (smooth muscular tissue) above the linea pectinata was observed. Analysis of HOXD13 expression in adult tissue showed a reduced expression in accordance with the histological layers from mucosa to adventitia. The chronological and increasing expression of HOXD13 during the 6th week p.c. in the rectal epithelium, thereafter (7th to 8th week p.c.) spreading towards the surrounding mesenchyme is synchronized with regulated proliferation and specific differentiation of epithelia and smooth muscular tissue in human hindgut, thus an involvement of HOXD13 in these processes is suggested. These data are inline with investigations obtained from laboratory animals where an epithelio-mesenchymal cross talk was observed.

Kategorie: Poster

Poster 8

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO CHONDROCYTES IN HIGH DENSITY CO-CULTURE: EVIDENCE FOR INTENSIVE INTERACTIONS BETWEEN MSC AND PRIMARY CHONDROCYTES

Autoren: Csaki C.(1),Matis U.(2),Mobasheri A.(3),Putz R.(1),Ye H.(1),Shakibaei M.(1),

Adressen:(1)LMU Munich|Institute for Anatomie|Munich|germany; (2)LMU Munich|Clinic of Veterinary Surgery|Munich|germany; (3)University of Nottingham|Division of Veterinary Medicine|Nottingham|UK

Abstract:

The use of mesenchymal stem cells (MSCs) for in vitro tissue engineering of cartilage is a promising concept for regenerative medicine. Previously, we have shown that high density co-culture of MSCs with isolated primary chondrocytes (PCH) leads to chondrogenesis. Thus far there have been no published studies on the role of physical interactions between MSC and PCH in vitro.

MSCs were cultured in a ratio of 1:1 with isolated PCH. To evaluate the cellular interactions, MSCs were membrane labelled with a red fluorescent dye and PCH were tagged with a green fluorescent dye. Live monolayer co-cultures were monitored in vitro over a period of seven days.

In high density co-cultures after 7 days cartilage formed. After 1 day in monolayer co-culture, both cell types exhibited extensive and directed pseudopodia. From day 3 PCH proliferated strongly in the vicinity of MSCs; numerous cell processes were observed and the extent of cell contacts increases thereafter. A number of PCH contained small red vesicles and others appeared to fluoresce in yellow suggesting that MSCs and PCH had fused. PCH that had no apparent connections with MSCs contained red vesicles suggesting either a prior interaction or the specific uptake of soluble factors produced by the MSCs.

We have demonstrated active and extensive interactions between MSCs and PCH which lead us to suggest that cell-cell communication might provide important signals for the differentiation of MSCs into PCH. This opens up new and exciting possibilities for the future use of MSCs and PCH co-cultures in cartilage tissue engineering.

Kategorie: Poster

Poster 9

Rubrik: 5.Entwicklungsbiologie

Abstract Nr.:5

Titel:EXPRESSION OF ADIPONECTIN AND ITS RECEPTORS IN RABBIT BLASTOCYSTS

Autoren: Fischer S.(1),Schmidt T.(1),Ramin N.(1),Navarrete Santos A.(1),Thieme R.(1),Fischer B.(1),

Adressen:(1)Martin-Luther-Universität Halle-Wittenberg|Institut für Anatomie und Zellbiologie|Halle|Deutschland; email:suenje.fischer@medizin.uni-halle.de

Abstract:

Recently we reported that adiponectin, a 26 kDa adipokine, is expressed in preimplantation rabbit blastocysts. We also found mRNA for the adiponectin receptors adipoR1 and adipoR2 and for the adiponectin paralogs CTRP2 and CTRP7 in rabbit and mice blastocysts. Adipokines have various functions in fatty acid and carbohydrate metabolism. Recent studies point to a role for adipokines, especially for leptin, in reproduction. Leptin is thought to be involved in embryo metabolism and the cross-talk between the embryo and maternal tissue prior to implantation. Hitherto little is known about a potential role for adiponectin in reproduction. We therefore studied adiponectin expression during the preimplantation period in the rabbit. We performed immunohistochemistry and real time PCR in blastocysts and endometrium. The blastocysts were separated into embryoblast and trophoblast. Adiponectin was detected in smooth muscle cells of rabbit myometrium and arterial vessels, in epithelial cells and within the lumen of uterine glands. On sections of day 8 p.c., the signal was specifically strong close to the implantation side and in primary anchoring villi. Whole mount immunohistochemistry showed a staining in the trophoblast and embryoblast. Real time PCR for adipoR2 revealed a high expression in trophoblast in early stages of gastrulation (stage 0/I, II), whereas expression was higher in the embryonic disc immediately prior to implantation (stage III). In summary, we report the spatial distribution of adiponectin and adipoR2 in rabbit preimplantation blastocysts and endometrium. Our findings point to a role of adiponectin in blastocyst differentiation and implantation.

Kategorie: Poster

Poster 10

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:TLR 3 AND TLR 4 EXPRESSION IN THE HUMAN ENDOMETRIUM AND THEIR IMPLICATIONS IN ENDOMETRIAL DISEASES

Autoren: Allhorn S.(1), Böing C.(2), Koch A.(1), Otterbach F.(3), Kimmig R.(1), Winterhager E.(1), Gashaw I.(1),

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Abstract:

Toll-like receptors (TLRs) serve an essential role in the innate immune system by initiating and directing immune response to pathogens. Although TLR3 and TLR4 are shown to be expressed in the human endometrium, the consequences of TLR-induced cytokine production on the endometrial function and pathogenesis remain unclear.

In this study, TLR3 and TLR4 expression was investigated in the menstrual cycle (n=55) and in post-menopausal endometrium (n=34) considering peritoneal endometriosis (n=20), hyperplasia (n=10) and endometrial adenocarcinoma specimens (n=16).

TLR3 and TLR4 were mostly localised to the glandular and luminal epithelium and their mRNA and protein expression pattern indicated no cyclical regulation in the endometrium. Expression of both receptors was elevated in post-menopausal tissues. In endometriosis, TLR3 and TLR4 mRNA expression decreased significantly 4- and 30-fold, respectively, in eutopic endometrium of the proliferative phase compared to healthy endometrium. Interestingly, endometriotic lesions showed a significant increase of TLR3 und TLR4 mRNA expression, compared to the corresponding eutopic tissues, indicating a local activation of the toll-like receptor pathway.

Like in endometriotic tissues, hyperplasia and endometrial adenocarcinoma revealed significantly reduced receptor levels. Within the different tumor stages, however, we could demonstrate a regain of TLR3 and TLR4 expression in G1, followed by a significant decrease in G2 and G3- carcinoma compared to postmenopausal controls. Our data suggest an involvement of TLR3 and TLR4 in endometrial diseases as we could demonstrate an altered expression in endometriosis and endometrial cancer. Further studies have to pinpoint the potential role of TLR3 and TLR4 in these endometrial diseases.

Kategorie: Poster

Poster 11

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:GASTRULATION DEPENDS ON INSULIN IN THE RABBIT BLASTOCYST

Autoren: Thieme R.(1),Ramin N.(1),Viebahn C.(2),Plitzner J.(2),Fischer B.(1),Navarrete Santos A.(1),

Adressen:(1)Martin-Luther-Universität Halle-Wittenberg|Institut für Anatomie und Zellbiologie|Halle|Deutschland; email:rene.thieme@gmx.de; (2)Georg-August-Universität|Institut für Anatomie und Embryologie|Göttingen|Deutschland

Abstract:

Gastrulation depends on insulin in the rabbit blastocyst

René Thieme, Nicole Ramin, Christoph Viebahn¹, Juliane Plitzner¹, Bernd Fischer and Anne Navarrete Santos

Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, D-06097 Halle (Saale), ¹ Department of Anatomy and Embryology, Georg August University Faculty of Medicine, D-37075 Göttingen, Germany

During day 6 of preimplantation development the embryonic disk of the rabbit blastocyst develops from a two cell layer (epiblast and hypoblast) to three germ layers. Early gastrulation proceeds in rabbit blastocysts in vitro as normal as in utero. Using the rabbit blastocyst in vitro model, we investigated the effects of insulin and IGF1 on early gastrulation events, employing morphological analysis and expression of the mesodermal marker gene Brachyury by real time PCR and in situ hybridisation. Blastocysts cultured in serumfree medium without growth factors did not develop, were arrested at an early gastrulation stage or died. Insulin supplementation, on the other hand, led to physiological upregulation of Brachyury and formation of the primitive streak, while morphogenesis and Brachyury expression were not influenced by supplementation of IGF1. The insulin effect was highly specific and restricted to the initiation of mesodermal differentiation because further enlargement of the primitive streak was delayed by insulin treatment. Our data show that insulin but not IGF1 specifically mediates initial pattern formation during gastrulation in the rabbit. Supported by Deutsche Forschungsgemeinschaft (DFG FI306/13-1)

Kategorie: Poster

Poster 12

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:THE PRO-ANGIOGENIC FACTOR CYR61 IS REGULATED BY EGF AND ESTROGEN IN ENDOMETRIAL CARCINOMA CELLS

Autoren: Riemke P.(1),Winterhager E.(1),Gashaw I.(1),

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Abstract:

CYR61 is a growth factor-inducible immediate-early gene, which displays a fundamental role in angiogenesis and tumorigenesis. In the endometrium, CYR61 reveals enforced expression during the proliferative phase correlated to enhanced levels of EGF and estrogen. In this study, we investigated EGF and 17 β -estradiol mediated induction of CYR61 mRNA and its connected pathways in RL95-2 human endometrial carcinoma cells expressing both estrogen receptors and the EGF receptor 1.

Application of estrogen for 0.5, 1 and 2h, respectively did not affect CYR61 mRNA levels in RL95-2 cells. Downstream genes of estrogen FOS and MYC were not upregulated as well. Treatment with EGF, however, led to an immediate 3-fold induction of CYR61 transcript levels. Activation of the EGFR accompanied with upregulated expression of CYR61 protein was shown by immunocytochemistry. Inhibition of EGFR via tyrphostin resulted in significant downregulation of CYR61 mRNA expression 0.5h after treatment. Activated EGFR operated through the MAP-kinase I and ERK1/ERK2 phosphorylation cascade on upregulation of CYR61 transcript levels, as evidenced by using the appropriate inhibitors.

Unexpectedly when EGF and estrogen were applicated simultaneously, CYR61 was further 35-fold upregulated after 2h. Addition of antiestrogens reduced the synergistic effect to control levels pointing to an involvement of the estrogen receptor.

In conclusion, EGF, but not estrogen alone, regulates CYR61 expression with activation of the EGFR/MAPK1/ERK pathway. However, application of estrogen and EGF led to a synergistic effect on CYR61 upregulation, pointing to an activation of estrogen signalling pathways through EGF signal transduction in endometrial carcinoma cells, which needs further evaluation.

Kategorie: Poster

Poster 13

Rubrik: 6.Reproduktionsbiologie

Abstract Nr.:6

Titel:MORPHOLOGICAL DEMONSTRATION OF STRUCTURAL MANIFESTATION OF PERSISTANT YELLOW BODY OVARIES OF COWS BY ITS INTERACTION WITH DENATURATED MAN'S PLACENTA

Autoren: Kapustin R.(1),Khokhlov A.(1),

Adressen:(1)Belgorod State Agricultural Academy|Department of Animal Morphology|Maiskii Belgorodskoi oblasti|Russia; email:romankapustin@mail.ru

Abstract:

The aim of the work is mastering of historical changes in sexual and immunocompetent organs of cows with persistent yellow body ovaries on the background of chlamydeous genital forms upon correction endocrine immune connections by a bionormalizator from man's placenta (PDS). The investigations have been carried out with keeping of rules with using the works of experimental animals. PDS calls inductance of follicle genesis in a grey matter substance of ovaries follicles on the different stages of growing and development of epithelium, endometria has less obesity, its cells are thickened with oval nucleuses, ending sections of uterine glands are characterized by small activity, vaginal tunic is covered with polylamellar epithelium which twice slimmer than in the control. Functional layer is presented by two – three rows of cells having solidier cytoplasm. Phenomena of desquamation are developed the method of cows treatment with persistent yellow body of ovaries in chlamydeous of genital form (patent RU 2294752 C1).

Kategorie: Poster

Poster 14

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:RELAXIN, RELAXIN-LIKE FACTOR AND RELAXIN-LIKE RECEPTORS LGR7 AND LGR8 AT THE OCULAR SURFACE

Autoren: Hampel U.(1),Klonisch T.(2),Paulsen F.(1),

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Abstract:

To analyse human relaxin 2 (RLN2), relaxin-like factor INSL3 and their cognate relaxin-like receptors LGR7 and LGR8, at the ocular surface, the lacrimal apparatus, and human corneal (HCE) and conjunctival (HCjE) epithelial cell lines. Human RLN2, INSL3, LGR7 and LGR8 were monitored in human tissue samples by RT-PCR. Specific antisera were employed to determine the tissue distribution of RLN2, LGR7 and LGR8. Western blot analysis was performed to detect RLN2 in tears. Transcripts of RLN2 and INSL3 were observed only in some lacrimal and Meibomian glands, conjunctivae, corneae and nasolacrimal ducts. In contrast immunohistochemistry revealed RLN2 protein in all investigated tissues. Transcripts and protein for LGR7 were detected in Meibomian gland but were absent in lacrimal gland, cornea and conjunctiva. INSL3 receptor LGR8 was expressed in all tissues analysed. Cell lines revealed specific transcripts for all, RLN2, INSL3, LGR7 and LGR8. RLN2 was present in tears. mRNA expression of RLN2 and INSL3 is only a few samples but detection of the respective proteins in these tissues suggests the transport of RLN2 and INSL3 via blood. This is supported by the detection of RLN2 in tears. Presence of LGR7 in nasolacrimal ducts and Meibomian glands as well as LGR8 in all tissues of the ocular surface and lacrimal apparatus suggest a role for RLN2 and INSL3 in ocular surface homeostasis. Both, HCE and HCjE may serve as models to elucidate possible roles of the relaxin-like ligand-receptor system at the ocular surface.

Kategorie: Poster

Poster 15

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:MORPHOLOGISCHE GEFAESSMESSUNGEN DES OPTISCHEN NERVS BEI
ENTKERNTEN RATTENAUGEN

Autoren: Bolintineanu S.(1),Vaida M.(1),Sargan I.(1),Haivas C.(1),Grigorita L.(1),

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email:bolint@upcnet.ro

Abstract:

The study aims at providing data on the vascular morphometry and micrometry of the optic nerve, to determine the effect of enucleation of one eye at birth on the microvascular development in the contralateral optic nerve. For this purpose, two groups of rats were used: five were unilaterally enucleated on the day of birth and studied on postnatal day 60; other five, were used as controls group. We analyzed parameters and compared the results statistically. The average diameter of microvessels up to 7.5 microns was found to be $4.9 \pm 0.3 \mu\text{m}$; in controls and $5.8 \pm 0.4 \mu\text{m}$; in experimental group. The density of microvessels represented by the number of sectioned capillaries per tissue area was $142 \pm 20/\text{mm}^2$ in the control group and $173 \pm 31/\text{mm}^2$ in the experimental group. Total length of capillaries per unit of volume (Lv) averaged 1065 ± 110 in control group and $2255 \pm 205 \text{ mm}/\text{mm}^3$ in experimental group. The internal capillary surface area available for metabolic exchange expressed per volume unit (Sv) was 15.7 ± 2.3 in control group and $38.6 \pm 3.1 \text{ mm}^2/\text{mm}^3$ in experimental group. These results suggest that an increase in the optic nerve metabolism, resulting from monocular vision, determined a rearrangements of the capillary system to the enucleated rats.

Kategorie: Poster

Poster 16

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel: EFFECTS OF NERVE GROWTH FACTOR (NGF), PRO-NGF, AND TUMOR NECROSIS FACTOR ALPHA ON CORNEAL AND CONJUNCTIVAL EPITHELIAL CELLS WITH REGARD TO PTERYGIUM PATHOLOGY

Autoren: Pfütze D.(1), Müller G.(2), Hauptmann S.(2), Paulsen FP.(1),

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Abstract:

To get deeper insights into the pathogenesis of pterygia which are invasive and highly vascularized growths at the ocular surface we examined nerve growth factor (NGF), its precursor (pro-NGF), as well as tumor necrosis factor alpha (TNF-alpha), a cytokine found commonly in inflammation processes with regard to cell proliferation and motility effects on corneal (HCE) and conjunctival (HCjE) epithelial cell lines as well as pterygia. HCE and HCjE were cultured in 96-well-plates, stimulated with several doses of NGF, pro-NGF and TNF-alpha, and incubated with bromodesoxyuridin bases. Absorption was measured with an ELISA-reader. Migration-assays were used to analyze differences in cell motility after stimulation of cultured cells. Moreover, NGF, pro-NGF, and TNF-alpha were localized by immunofluorescence (IF) and immunohistochemically (IHC). Proliferation of HCE and HCjE was significantly inhibited after stimulation with NGF and pro-NGF whereas TNF-alpha increases cell proliferation. All three factors had no significant effect on cell motility. IHC of human samples from limbus, cornea, conjunctiva, and primary pterygia revealed presence of pro-NGF and TNF-alpha especially in epithelial cells. Using IF, all three factors could be visualized in pterygia and HCE whereas in HCjE only NGF was detectable. Based on their inhibiting effects on cell proliferation, increased secretion of NGF and pro-NGF in pterygium may function in blocking growth of the pterygium itself. Further studies are necessary to clarify this aspect. However, hypothetically topical application of NGF and pro-NGF may support inhibition of pterygial recurrency after surgical excision.

Kategorie: Poster

Poster 17

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE PHOSPHORYLATION OF ENOS AT THR495 IN MERKEL CELLS OF THE RAT PALATAL MUCOSA

Autoren: Korkmaz H.(1),Arnhold S.(1),Bloch W.(2),Addicks K.(1),

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Abstract:

The Merkel cell is a special cell type in the basal layer of the palatal mucosa and enfolds the unmyelinated ending of the slowly adapting mechanoreceptive afferent fiber. Although the modulator role of different signal molecules were described in Merkel cells, the existence and regulation of the eNOS activity in Merkel cells is unclear. In endothelial cells, the phosphorylation (p) of human eNOS at Ser1177 results in the increase of eNOS activity, whereas the phosphorylation of human eNOS at Ser114 and Thr495 decreases the eNOS activity. To investigate the existence and regulation of the eNOS phosphorylation in Merkel cells of the palatal mucosa, rat hard and soft palate were perfusion- and post-fixed, decalcified, frozen-sectioned at 30 µm and immunoreacted with antisera against total (t)-eNOS and p-eNOS at Ser1177, Thr495 and at Ser116. In the basal layer of the palatal mucosa, Merkel cells were positive for t-eNOS and p-eNOS at Thr495. The detection of p-eNOS at Thr495 in Merkel cells is compatible with a decrease in the eNOS activity in Merkel cells. It is possible that the phosphorylation of eNOS at Thr495 in Merkel cells may be one of the key points in the control of NO and/or O₂- production in the absence of adequate BH₄ levels under inflammatory conditions. The different localization of p-eNOS residues in blood vessels, keratinocytes and Merkel cells under basal conditions indicates that the activity of eNOS is modulated by phosphorylation of the enzyme in a cell specific manner.

Kategorie: Poster

Poster 18

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:THE CHARAKTERISIERUNG OF EMMPRIN IN CELLS OF THE RAT TOOTH GERM

Autoren: Schwab W.(1),Harada H.(2),Kasper M.(1),Barth K.(1),

Adressen:(1)TU Dresden|Institut für Anatomie|Dresden|Deutschland;
email:wolfgang.schwab@tu-dresden.de; (2)Iwate Medical University|Department of Oral Anatomy II School of Dentistry|Iwate|Japan

Abstract:

In a recent immunocytochemical study we detected the differentiation-dependent co-expression of MMPs with EMMPRIN in the rat tooth germ, especially in ameloblasts and in odontoblasts.

The aim of the present in vitro study was to investigate the presence of EMMPRIN and the colocalization of EMMPRIN with caveolin in cells of the enamel organ.

Dental epithelial cells (HAT-7 cells) originating from epithelial loop of the rat incisor enamel organ were cultured according to the method of Kawano et al. (2004).

In the present study we show that two populations of EMMPRIN are detectable in the plasma membrane of HAT-7 cells. A discrete population of EMMPRIN was associated with lipid rafts, as determined by co-fractionation with the raft marker caveolin-1 after preparation of lipid rafts in the presence of Triton X-100. These fractions did not contain Golgi apparatus and endoplasmic reticulum as shown by detection of organelle specific protein markers beta-Cop and PDI, respectively. Double label immunofluorescence microscopy of caveolin-1 and EMMPRIN in HAT-7 cells confirmed the biochemical data.

Caveolin 1 constitutes not only the structural protein of caveolar raft membranes, but also concentrates many signalling molecules in caveolae and regulates their activity. Experimental datas from sucrose density gradient centrifugation of Triton X-100-insoluble membranes provide evidence of an association of a discrete portion of EMMPRIN with lipid rafts. Therefore, we assume a regulatory role of caveolin-1 in the EMMPRIN-induced expression of MMPs in the enamel organ.

Kategorie: Poster

Poster 19

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:TWO TYPES OF PERIVASCULAR CELLS IN THE CHORIOALLANTOIC MEMBRANE CAPILLARY PLEXUS

Autoren: Kurz H.(1),Fehr J.(2),Nitschke R.(3),Burkhardt H.(2),

Adressen:(1)Paracelsus Private Medical University|Anatomie - Tissue Dynamics Lab|Salzburg|Austria; email:haymo.kurz@pmu.ac.at; (2)Universität Freiburg|Computer Science - Image Processing|Freiburg|Germany; (3)Universität Freiburg|Biology - Life Imaging Center|Freiburg|Germany

Abstract:

In the chicken chorioallantoic membrane (CAM), we had previously described the expression of desmin (DES) in pericytes of the capillary plexus, and of alpha-smooth muscle actin (SMA) in vascular smooth muscle cells of the non-capillary microvessels (Kurz et al., Histochem Cell Biol 2000). Using refined confocal imaging, we now show that SMA can also be detected in perivascular capillary cells. The very delicate, circumferential SMA expression pattern, however, is clearly distinct from the coarser, and axially oriented DES pattern. Most probably, DES and SMA are not expressed by the same cells. Our conclusion that two types of perivascular cells may be present in the same microvascular bed is supported by the slightly different chromatin textures of their nuclei. In this context, we propose a new method for the identification of cells, even when only their DNA is stained, using voxel-based gray value invariants.

Kategorie: Poster

Poster 20

Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:ESTROGEN-DEPENDENT REGULATION OF CLAUDIN-5 IN ENDOTHELIAL CELL LINES/ ÖSTROGEN-ABHÄNGIGE REGULATION DES CLAUDIN-5 IN ENOTHELIALEN ZELLINIEN

Autoren: Burek M.(1),Drenckhahn D.(1),Förster C.(1),

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Abstract:

Claudin-5 is an integral membrane protein component of tight junction and plays a critical role in permeability of the blood-brain-barrier (BBB). The hormone 17 beta-estradiol (E2) and related estrogenic hormones play an important role in several physiological processes, including development of the female and male reproductive tracks as well as bone, vascular and neuronal function.

In the present study we have observed an increase in claudin-5 expression in mice endothelial cell lines, such as cEND (an in vitro model of cerebral BBB), cerebEND (an in vitro model of cerebellar BBB) and MyEND (myocardial endothelial cell line) after treatment with E2. A high estrogen receptor beta (ER-beta) expression has been demonstrated in cEND and MyEND cell lines and a weak ER-beta expression was detected in cerebEND cells.

To explain the effects of E2 on claudin-5 expression we have cloned the murine claudin-5 promoter region. Three fragments of claudin-5 promoter, -1030 bp/+101 bp, -601/+101 bp, and -367/+101 bp have been cloned and their functionality was evaluated in vitro using luciferase as the reporter gene. All the constructs showed high basal promoter activity in human adenocarcinoma cell line, MCF-7 cells. Moreover, E2 treatment induced the claudin-5 promoter activity (-367/+101 bp promoter fragment) up to 2-fold in comparison to untreated MCF-7 cells expressing claudin-5 promoter constructs. Further experiments are needed to explain whether E2-mediated transactivation require direct binding of the estrogen receptor to the claudin-5 promoter.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:ORM-PAI-1 INTERACTION PLAYS A ROLE IN CAPILLARY MORPHOGENESIS AND ANGIOGENESIS

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Abstract:

Orosomucoid (ORM) is an acute phase protein and increased in acute infection, inflammation and cancer. The biological function of ORM is largely unknown while some studies suggest that it may function as an immunomodulator. We recently showed that ORM is produced by endothelial cells and increased in urine samples of patients with bladder cancer. ORM forms a complex with plasminogen activator inhibitor-1 (PAI-1) at the surface of endothelial cells. However, it was not clarified yet whether ORM plays a role in capillary morphogenesis. To this aim we performed ORM-gene overexpression and ORM-gene silencing via siRNA technique in HDMECs. Supernatants of HDMECs-ORM and HDMECs-ORM-siRNA were used individually or in combination with VEGF in endothelial tube formation assay. Furthermore, recombinant ORM was also applied in combination with VEGF and anti-PAI-1 in endothelial tube formation assay. The present study revealed that application of ORM with VEGF simultaneously increased number and the network of VEGF-induced endothelial tube formation while ORM alone induced almost any endothelial tubes. The combined application of ORM, VEGF and anti-PAI-1 induced the highest number and the network of endothelial tubes. Taken together, our results let assume that in the presence of ORM there is a complex of ORM and PAI-1 which stabilizes PAI-1 resulting in inhibition of endothelial migration and angiogenesis. The additional application of anti-PAI-1 blocks the ORM-PAI-1 complex and increases the amount of free ORM which enhances angiogenesis. Thus, the interaction between ORM and PAI-1 and anti-PAI-1 system seems to be essentially involved in the VEGF-mediated capillary formation.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:CEACAM1-EXPRESSION PATTERN IN ENDOTHELIAL CELLS

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Abstract:

INTRODUCTION: Endothelial cells cover the luminal surface of all blood and lymphatic vessels and are crucial for the tube formation. Although blood vessel endothelial cells (BECs) and lymph endothelial cells (LECs) exhibit migration, proliferation, capillary tube formation, dependent on the vascular system they possess distinctive properties. In order to learn more about the primary events of endothelial determination we characterized an immortalized human dermal microendothelial cell line (CDC HMEC-1). **METHODS:** The endothelial cells were grown either to confluency for 4 weeks or were grown in logphase. Afterwards the cells were harvested and a FACScan analysis was performed to quantify to amount of expressed surface proteins. **RESULTS:** Analysing CDC HMEC-1 cells demonstated that the cells show different expression pattern and amount of total protein dependent on their growing conditions. Confluent CDC HMEC-1 cells showed in average much higher expression level of proteins than continuously growing cells. While the cells expressed common endothelial markers like CD31 and CD34, surprisingly they expressed both, lymph endothelial proteins such as podoplanin and VEGFR3, but also blood vessel endothelial proteins such as Tie-2. CEACAM1 was found to be expressed in confluent but only barely in growing cells. We therefore conclude that endothelial cells comprise an alterable system in which they change their surface molecules expression pattern dependent on the growing conditions and may therefore determine their lineage progression and signalling properties.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:RAT AORTA ENDOTHELIUM CHANGES AFTER INTRAVENOUS OR INTRAPERITONEAL LIPOFUNDIN ADMINISTRATION STUDIED EN FACE

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Abstract:

Recently we demonstrated that the intravenous administration of the lipid solution Lipofundin-S 20% (Brawn-Melsungen) in rats can induce light microscopic changes in the aortic endothelium. Here we investigate the effect of different ways of Lipofundin administration.

Male Wistar rats (250-350g) were used for the experiments. The Lipofundin was introduced by two ways – intravenous (10 days) or intraperitoneal (30 days). On the en face preparations the endothelial cells' borders were visualized with silver impregnation, the cell nuclei – with haematoxylin staining. For detection of possible lipid accumulations, an Oil red O staining was provided.

After intravenous Lipofundin administration significant changes in the endothelium were observed. There was an increased number of the inflammatory mononuclear cells. Another finding was the higher number of the binucleated endothelial cells. Rarely, signs of initial injury of the endothelial cells and small denuded areas were detected. The most interesting findings were the presence of local extracellular lipid accumulations and seldom presence of lipid-laden macrophages. After intraperitoneal Lipofundin administration a weaker cellular reaction was observed. The most distinctive finding was the increased number of mononuclear cells adherent to the endothelium. These inflammatory cells were scattered throughout the surface or rarely located in groups.

In conclusion, the direct exposure of the aortic endothelium in the cases of intravenous lipid administration is more crucial for the endothelium alteration. We suggest that after intraperitoneal administration the Lipofundin solution is subjected to first-pass effect of the liver and following a biotransformation or partial elimination, the effect on the aortic endothelium is weaker.

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:LOSS OF GLUCOCORTICOID RECEPTOR IN DEXAMETHASONE-TREATED MYOCARDIAL ENDOTHELIAL CELL LINE MYEND CAUSES A DECERASED NO PRODUCTION

Autoren: Blecharz K.(1),Drenckhahn D.(1),Förster C.(1),

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Abstract:

Nitric oxide (NO) is potent vasodilator synthesized by the endothelial NO synthase (eNOS). Under pathological conditions, like glucocorticoid-caused hypertension, NO production is reduced and subsequently, the vascular homeostasis- impaired. Our previous study [Förster C. J. Waschke et al. (2006) "Glucocorticoid effects on mouse microvascular endothelial barrier permeability are brain specific." J Physiol 573(Pt 2): 413-25.], could already show the down-regulation of the glucocorticoid receptor by glucocorticoids in the myocardial endothelial MyEND cell line but not in the cerebral endothelial cell line cEND. This fact is consistent with data shown for glucocorticoid-induced hypertension as side effects of glucocorticoid therapy. Unexpectedly, we measured a decreased NO production in dexamethasone distribution, but no changes in protein- and expression-levels of eNOS could be identified. Here, we attempt to demonstrate the negative influence of glucocorticoid treatment on the enzyme activity of eNOS, caused by the loss of the glucocorticoid receptor protein, required for the expression of GTP cyclohydrolase I (GTPCH I), the rate-limiting enzyme catalyzing the tetrahydrobiopterin synthesis. Tetrahydrobiopterin is reported as an essential cofactor for eNOS, playing a regulatory key role in the NO-production. Additionally, dexamethasone-treated and transfected with the glucocorticoid receptor MyEND cells showed a significant increase of GTPCH I mRNA levels compared with untransfected and untreated cells in accordance to GTPCH I expression and NO-production in glucocorticoid-treated cEND cells. These data proved our hypothesis, that glucocorticoid-dependent hypertension is caused by peripheral glucocorticoid receptor-degradation impairing the expression of GTPCH I.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel: INVOLVEMENT OF GROWTH-DIFFERENTIATION FACTOR-15 (GDF-15) IN IMMIGRATION OF MACROPHAGE SUBPOPULATIONS INTO THE INTERSTITIUM OF THE MYOCARDIUM

Autoren: Sackmann F.(1), Bonaterra G.(1), Vorwald S.(1), Metz J.(1), Strelau J.(1), Kinscherf R.(2),

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Abstract:

Growth-Differentiation Factor-15 (GDF-15) is a novel member of the TGF-beta superfamily. We found that GDF-15 is induced in macrophages of the human arteriosclerotic vessel wall. Other groups showed that patients with cardiovascular diseases have increased plasma GDF-15 levels. The aim of the study was to investigate the influence of GDF-15 on immigration of macrophage-subpopulations in the interstitium of the myocardium of ApoE deficient (ApoE^{-/-}) or -competent (ApoE^{+/+}) mice.

After crossing of ApoE^{-/-} with GDF-15 knockout (GDF-15^{-/-}) mice - developed in our group - we immunohistomorphometrically analyzed the myocardium by computer-assisted morphometry using CD68, MOMA-2, CD11b or BM-8 antibodies.

In the interstitium of the myocardium, the density of CD68-ir macrophages was about 5-15-fold higher than that of MOMA-2-ir macrophages and 10-50-fold increased in comparison with CD11b-ir macrophages. BM-8-ir macrophages were only rarely found. In myocardium of ApoE^{-/-}/GDF-15^{+/+} mice we didn't observe an increase of CD68-ir macrophages. However, a significant increase (6- or 8-fold) of MOMA-2-ir and CD11b-ir macrophages was found in comparison with ApoE^{+/+}/GDF-15^{+/+} mice.

In the myocardium of ApoE^{-/-}/GDF-15^{-/-} mice the density of CD68-, MOMA-2- or CD11b-ir macrophages was about 15-50% lower than in ApoE^{-/-}/GDF-15^{+/+} mice.

We conclude that 1) different macrophage-subpopulations exist in the myocardium; 2) ApoE deficiency leads to a diverse increase/recruitment of macrophage-subpopulations and 3) GDF-15 variably promotes an immigration of different macrophage-subpopulations. Future studies should show, whether GDF-15 also influences the development and progression of arteriosclerotic lesions and the recruitment of different macrophage-subpopulations in the arteriosclerotic vessel wall.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:INFLAMMATION-, APOPTOSIS- OR ADHESION-RELEVANT PROTEINS IN LYMPHOCYTES, MONOCYTES OR MACROPHAGES OF ARTERIOSCLEROSIS PATIENTS AND HEALTHY SUBJECTS – EFFECT OF AN ACETYLSALICYLIC ACID/STATIN THERAPY

Autoren: Bonaterra G.(1),Blessing E.(2),Hildebrandt W.(3),Traut U.(1),Metz J.(1),Kinscherf R.(4),

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Abstract:

Inflammatory proteins are used for diagnosis of arteriosclerosis and heart attack. We investigated, whether the percentage of mononuclear cells [MNC] (T- [CD3+], B- [CD19+], monocytes [CD14+] or macrophages [CD68+]), expressing inflammation- (CD40, COX-2, TNF-alpha), apoptosis- (PARP) or adhesion- (CD38) relevant proteins is different in arteriosclerosis patients and healthy subjects and, whether therapeutic intervention (acetylsalicylic acid [ASS]/statin) is effective.

The study included healthy subjects (group 1; n = 16), and patients with clinically relevant arteriosclerosis (group 2; n = 15). Group 2 consisted of patients without (group 2A; LDL>100mg%) and with ASS/statin therapy (Gruppe 2B; normolipidemic). MNC were analyzed by double immunofluorescence labeling via FACS.

Concentrations of blood lipids, C-reactive protein (CRP), oxidized Low-Density Lipoprotein (oxLDL) and number of leukocytes were determined.

CRP- and blood lipid levels - besides HDL - were similar in group 1 and 2. Group 2 revealed 4-fold in-creased oxLDL-levels and a significantly increased amount of MNC subpopulations expressing inflammation-, apoptosis- or adhesion-relevant proteins. CRP, total cholesterol-, LDL- and triglyceride concentrations were decreased in group 2B in comparison with group 2A or group 1. Therapeutic intervention with ASS/Statin did neither affect the increased oxLDL-levels nor the amount of MNC subpopulations express-ing inflammation-, apoptosis- or adhesion-relevant proteins. We show that an ASS/statin therapy leads to a decrease in CRP-, cholesterol-, LDL- and triglyceride concentrations. OxLDL levels, which are unchanged during therapy, are suggested to be the reason why the percentage of MNC subpopulations expressing inflammation-, apoptosis- or adhesion-relevant proteins remains increased even during ASS/statin therapy.

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:CALCIUM BINDING PROTEINS IN PANCREATIC ISLETS

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Abstract:

Stimulus coupled insulin secretion in the beta-cell is a result of a complex calcium-mediated transduction pathway that is not yet clearly understood. It has been suggested that calcium binding proteins are important mediators of calcium-mediated signal transduction pathways coupled to the rise in intracellular calcium. By using immunolabeling we show a specific localization of the calcium binding proteins calbindin, secretagogin, calmodulin and calreticulin in human and rat pancreatic islets. Double immunolabeling reveals the distribution of most of these proteins in beta-cells as well as in alpha-cells. In addition, the same proteins were localized in single beta-cells (INS1 rat insulinoma beta-cells). By using real-time RT-PCR the transcripts of all four calcium binding proteins were detected in INS1 cells and rat pancreatic islets.

In islets of the Goto-Kakizaki rat (GK rat), an animal model for type 2 diabetes, the localization of calcium binding proteins was altered. Real-time RT-PCR displayed significant increases in mRNA expression of calbindin, calmodulin, calreticulin and secretagogin. Our data show, that a diabetic metabolic status is associated with alterations in protein expression. Previously, our group reported that diabetic GK rats, as well as type 2 diabetic patients, showed decreased diurnal melatonin levels and an increased pancreatic melatonin-receptor status. Given the fact that many calcium binding proteins can interact with melatonin or are modulated by melatonin, it seems likely to assume a relationship between melatonin and calcium binding proteins, including their signal pathways. We want to investigate this functional relationship in further experiments using INS1 cells.

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel: MELATONIN AND ITS FEEDBACK ON THE EXPRESSION OF RAT PANCREATIC MT1- AND MT2-RECEPTORS

Autoren: Mühlbauer E.(1), Sucha H.(2), Bazwinsky I.(2), Peschke E.(2),

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Abstract:

We have previously published that the effects of melatonin are exerted on the pancreatic islet and beta-cell via Gi-coupled membrane receptors of the MT1 type. By using a highly sensitive real-time RT-PCR approach we were now able to detect transcripts of the MT2-receptor as well in rat islets and beta-cells, albeit with a much lower expression level compared to the MT1-receptor (86 fold in islets). Ligand binding to the melatonin receptors (MT1 and/or MT2) led to suppression of insulin secretion from beta-cells. The MT2-receptor may also have a specific function for the transmission of synchronizing signalling in the beta-cell.

In addition, we have pursued the question of a melatonin feedback on its own receptor expression. We have collected evidence for a transcriptional MT2- (but not MT1)-receptor upregulation (3.6 fold, $n = 4 - 8$, $p < 0.05$) in rat pancreas with chronic administration of melatonin in the drinking water. At the level of single beta-cells (INS1 rat insulinoma beta-cells) in contrast, the results concerning MT1- or MT2-receptor expression did not indicate a transcriptional melatonin influence in cell batches incubated with 1 nM, 10 nM, 100 nM, 1 μ M or 10 μ M of melatonin for 6 h. In summary, besides the melatonin MT1-receptor, also the MT2-receptor is expressed in the rat islet and beta-cell. Whereas the in vivo results indicate a positive feedback mechanism upregulating MT2-receptor expression by melatonin, in vitro on a single beta-cell (INS1 cell) level, this phenomenon was not observed.

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:CHARACTERIZATION OF MELATONIN RECEPTORS IN HUMAN PANCREATIC TISSUE OF TYPE 2-DIABETIC AND NON-DIABETIC PATIENTS

Autoren: Stumpf I.(1),Mühlbauer E.(2),Bazwinsky I.(1),Peschke E.(1),

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Abstract:

Recent results demonstrated the influence of the pineal hormone melatonin on insulin secretion as well as interactions between melatonin and glucose in type 2 diabetes. The aim of the present study was to determine the existence of melatonin membrane receptors and to examine the mRNA expression of nuclear orphan receptors in human pancreatic tissue, in an effort to explain differences between type 2 diabetic and metabolically healthy patients. Molecular and immunocytochemical investigations established the presence of the melatonin membrane receptors MT1 and MT2 in human pancreatic tissue and, notably, also in the islets of Langerhans. Results of a calculation model to determine mRNA expression ratios, as well as semiquantitative analysis of immunoreactions, showed elevated MT1 receptor expression in comparison to MT2 expression. mRNA transcript levels of melatonin receptors appeared to be significantly higher in type 2 diabetic patients than in a control group. An upregulation of receptor expression in type 2 diabetic patients was also observed in immunocytochemical investigations. In addition, transcripts of the nuclear orphan receptors ROR α , RZR α , ROR γ ; and RevErb α ; were detected in human pancreatic tissue and islets. In correlation with membrane melatonin receptors, data indicate increased mRNA expression levels of ROR α , RZR α , and ROR γ ; in type 2 diabetic patients. Thus, our data demonstrate the existence of the melatonin membrane receptors MT1 and MT2 as well as mRNA expression of nuclear orphan receptors in human pancreatic tissue, with upregulated expression levels in type 2 diabetic patients.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:ROLE OF IGF-I AND TGF- β ; ON CHONDROGENESIS IN VITRO

Autoren: Seifarth C.(1),Mobasheri A.(2),Csaki C.(3),Shakibaei M.(3),

Adressen:(1)LMU Munich|Institute for Anatomie|Munich|germany; (2)University of Nottingham|Division of Veterinary Medicine|Nottingham|UK; (3)LMU Munich|Institute for Anatomy|Munich|germany; email:mehdi.shakibaei@med.uni-muenchen.de

Abstract:

The main problem with autologous chondrocyte implantation (ACI) is that during in vitro expansion in monolayer culture chondrocytes rapidly dedifferentiate. The aim of this study was to determine whether the anabolic growth factors (IGF-I and TGF-beta) are capable of stabilizing the chondrogenic potential of dedifferentiated human chondrocytes and whether these cells treated with IGF-I and TGF- beta; in monolayer culture were still able to redifferentiate in high-density culture.

Human articular chondrocytes were cultured with interleukin-1beta (IL-1beta) to induce dedifferentiation, following the co-treatment with either IGF-I, TGF-beta; or both in combination at various concentrations (1, 10, 100 ng/ml). We investigated the effects of growth factors on monolayer and high-density cultures for evidence of chondrogenesis.

IL-1beta;-treated cells rapidly dedifferentiated; they lost their chondrocyte-like phenotype. Expression of collagen type II, beta1-integrin, extracellular regulated kinase (Erk) and the chondrogenic transcription factor Sox9 were down regulated. However, through co-treatment with IGF-I and TGF-beta; the cells redifferentiated increasing the expression of collagen type II, beta1-integrin, Erk and Sox9. In high-density cultures, we observed evidence for new cartilage formation after co-treatment with growth factors. We presume that IGF-I and TGF-beta; exert similar anabolic effects on chondrocytes in vitro and may stabilize the chondrogenic potential. In conclusion, the synergistic action of growth factors may find practical applications in the fields of tissue engineering and ACT.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:ANNEXIN-1 EXPRESSION IN CARTILAGE-FORMING SARCOMAS

Autoren: Dr. Gottschalk D.(1),

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Abstract:

We describe for the first time in the literature the expression of annexin1 in different cartilage-forming connective tissue tumors of bone, using standard and fluorescence immunohistochemical methodology. The results are discussed and possible diagnostic and treatment options are outlined.

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:HUMAN MESENCHYMAL STEM CELLS: PROLIFERATION AND DIFFERENTIATION IN A FIBRIN GLUE IN VITRO

Autoren: Baumgartner L.(1),Arnhold S.(2),Addicks K.(2),Bloch W.(3),

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Abstract:

Recently, tissue engineering has merged with stem cell technologies to develop new sources of transplantable material for injury or disease treatment.

The purpose of this study was to create a suitable matrix which provides a basis for the reimplantation of cells, minimizes necrotic areas and supports the capacity of cell-proliferation. Therefore multipotent stem cells isolated from adult human bone marrow (hMSC) were integrated into fibrin sealant (Tissucol, Baxter) and cultured for a period of 21 days in vitro. With the help of centrifugal force the creation of a new large-porous fibrin matrix was facilitated, which allows the hMSC to integrate and survive throughout the period of culture in vitro. Furthermore we compared the proliferation under normoxic (5%CO₂, 21%O₂) and hypoxic conditions (5%CO₂, 3%O₂).

Afterwards we analysed the cell growth of hMSC, particularly investigating morphology, proliferation and differentiation. Using RT-PCR a retention of multipotency as demonstrated by the expression of Oct-4 throughout the culture period is obvious. The proliferation capacity of the embedded hMSC was determined using the immunocytochemical proliferation marker anti Ki67 which could still be detected after 21 days in culture. Morphology and distribution of the cells inside the matrix was evaluated by light and electron microscopy. Surprisingly, cell behaviour did not vary depending on the culture conditions; in both conditions there is an unaltered cell proliferation.

It may be concluded that human mesenchymal stem cells especially in combination with the fibrin glue as potent scaffold material may have clinical applications in various fields of reconstructive surgery.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:CULTURE DEPENDANT EXPRESSION OF STEM CELL FACTORS BY MESENCHYMAL STEM CELLS (MSC)

Autoren: Schnell E.(1),Arnhold S.(1),Addicks K.(1),Nohroudi K.(1),

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Abstract:

Increasing interest in the application of stem cells for tissue engineering has led to more detailed attention to adult stem cells. In contrast to embryonic stem cells they are easily available as they can be taken directly from the patient. Mesenchymal stem cells (MSCs) are usually isolated from the bone marrow. They are expandable in vitro and multiple experiments have shown their pluripotency by differentiation into chondro-, osteo-, adipocytes, muscle cells and even neural phenotypes. However, under standard culture conditions containing 20% fetal calf serum (FCS) the differentiation potential of MSC rapidly declines with passage number in vitro, accompanied by an alleviated expression of stem cell transcription factors Oct-4 and Sox-2. In contrast, complete serum deprivation inhibits proliferation of MSC, thereby impairing their use for tissue engineering.

In order to elucidate the interactions of the MSCs with serum containing growth medium we have looked at MSC morphology, growth kinetics and stem cell factor expression under several conditions. Mesenchymal stem cells were isolated from the bone marrow of 3 month old wistar rats and grown with 5%, 10%, 20%, or without addition of FCS to the medium. We investigated MSC morphology, growth kinetics and expression of Oct-4 and Sox-2 stem cell factors during cell culture for up to passage 10. In conclusion, we show that an intermediate concentration of FCS serves for sufficient proliferation of MSC for the means of tissue engineering by conserving expression of stem cell transcription factors Oct-4 and Sox-2.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:QUANTIFICATION OF NUCLEAR PROTEIN IMPORT AND EXPORT PROCESSES IN LIVING CELLS BY INDUCED HETERODIMERIZATION

Autoren: Busch A.(1),Kiel T.(1),Drenckhahn D.(1),Hübner S.(1),

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Abstract:

A prerequisite of eucaryotic life is the continuous flow of molecules (cargos) between the nucleus and the cytoplasm. This flow occurs exclusively through the nuclear pore complex (NPC) involving transport receptors (importins and exportins). An important aspect is the correct subcellular delivery of cargos and in particular the kinetic with which this process takes place. Until today spatio-temporal measurements require chemical/mechanical permeabilization of the cytoplasmic membrane or microinjection using fluorescently labelled cargos. We have developed a new non-invasive in vivo system that allows us to quantify transport kinetics of cargos across the NPC after the addition of a membrane permeable heterodimerizer. The system is based on the ARGENT-TM-regulated heterodimerization Kit (ARIAD). The expression constructs used encoded dsRed-kanadap Δ NLS-FRB* (cytoplasm), NLS-GFP-3xFKBP (nucleus) and dsRed-NES-FRB* (cytoplasm and nucleus) fusion proteins. Coexpression of these fusions enabled us to investigate the importin α -dependent import and the exportin (Crm1)-dependent export components of transport processes once the heterodimerizer has been added to cultured cells. We found changes in the ratios of nuclear versus cytoplasmic fluorescence (Fn/c) for dsRed-kanadap Δ NLS-FRB* (in the presence of NLS-GFP-3xFKBP) from < 1 to > 8 , whereas the Fn/c for NLS-GFP-3xFKBP (in the presence of dsRed-NES-FRB*) changed from > 4 to < 1 after addition of the heterodimerizer. In the case of coexpression of NLS-GFP-3xFKBP and dsRed-NES-FRB* nuclear export of NLS-GFP-3xFKBP reached its maximum after 40 min. Future studies will implement this technique in order to investigate nuclear signalling in human cells suffering from nuclear envelopathies.

Kategorie: Poster

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Rubrik: 4.Zellbiologie

Abstract Nr.:4

Titel:DEER ANTLER REGENERATION: CELLS, CONCEPTS, AND CONTROVERSIES.

Autoren: Szuwart T.(1),Kierdorf U.(2),Kierdorf H.(2),

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Abstract:

The periodic replacement of antlers is an exceptional regenerative process in mammals, which in general are unable to regenerate complete body appendages. Antler regeneration has traditionally been viewed as an epimorphic process closely resembling limb regeneration in urodele amphibians, and the terminology of the latter process has also been applied to antler regeneration. More recent studies, however, showed that, unlike urodele limb regeneration, antler regeneration does not involve cell dedifferentiation and the formation of a blastema from these dedifferentiated cells. Rather, these studies suggest that antler regeneration is a stem-cell-based process that depends on the periodic activation of, presumably neural-crest-derived, periosteal stem cells of the distal pedicle. The evidence for this hypothesis is reviewed and as a result, a new concept of antler regeneration as a process of stem-cell-based epimorphic regeneration is proposed that does not involve cell dedifferentiation or transdifferentiation. Antler regeneration illustrates that extensive appendage regeneration in a postnatal mammal can be achieved by a developmental process that differs in several fundamental aspects from limb regeneration in urodeles.

Kategorie: Poster

Poster 36

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:TREFOIL FACTOR FAMILY PEPTIDE 3 PROMOTES RE-EPITHELIALIZATION OF CORNEAL WOUNDS

Autoren: Paulsen F.(1),Jansen A.(2),Woon C.(3),

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Abstract:

Purpose. The trefoil factor family peptide 3 (TFF3, also known as intestinal trefoil factor (ITF)) has been implicated in epithelial cell restitution. We have recently reported on the expression of TFF3 in both healthy and pathological human corneas. The expression of TFF3 seems to be induced in diseased corneas. The present study examined the biological role of Tff3 in maintaining corneal integrity and investigated the effects of Tff3 on corneal epithelial wound healing.

Methods. In two different models of corneal injury, alkali- and laser-induced corneal wounding, the wound healing process was evaluated in vivo and in a combined in vivo/in vitro system in mice with a wild-type (Tff3+/+) and Tff3-deficient (Tff3-/-) genetic background. We extended the study to assess the effects of topically applied recombinant human TFF3 (rTFF3) peptide on the rate of corneal wound healing.

Results. We found that Tff3 peptide is not normally expressed in intact corneal epithelium but its expression is induced following epithelial injury. Re-epithelialization of corneal wounds is impaired in Tff3-/- mice in comparison to Tff3+/+ mice. In addition, exogenous application of rTFF3 to the alkali-induced corneal wounds significantly accelerated healing in the in vivo and combined in vivo/in vitro systems for both Tff3+/+ and Tff3-/- mice.

Conclusions. These findings confirm a pivotal role for Tff3 in corneal epithelial restitution, opening new prospects to develop treatments that could enhance corneal wound healing following trauma, surgery or disease.

Kategorie: Poster

Poster 37

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:EFFECTS OF MAST CELL ACTIVATION AND THE NITRIC OXIDE PATHWAY ON CILIA-DRIVEN PARTICLE TRANSPORT SPEED

Autoren: König P.(1),

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Abstract:

Asthma is associated with IgE-mediated activation of mast cells, inflammation and increased production of nitric oxide (NO) in the airway epithelium. NO has been reported to increase ciliary beat frequency whereas application of allergen is associated with reduced mucociliary clearance. In this study, the effects of NO as well as mast cell activation on changes in cilia-driven particle transport speed were examined in the model of the acutely explanted mouse trachea. Exogenous application of NO by the NO donor DETA NONOate at concentrations as high as 100 μ M did not change cilia-driven particle transport speed, and blockade of the NO receptor soluble guanylyl cyclase by 100 μ M ODQ did neither affect muscarine- nor ATP-driven increases in particle transport speed. Application of the mast cell degranulating agent compound 48/80 increased particle transport speed at a concentration of 10 μ g/ml. However, specific activation of mast cells by anti-IgE antibodies (10 μ g/ml) effectively reduced particle transport speed. This indicates that the compound 48/80 effect is not attributable to mast cell activation.

This study shows that activation of mast cells can directly reduce cilia-driven particle transport speed, whereas, at least in mice, the NO-pathway does not seem to play a significant role and is therefore unlikely to counteract the effects of mast cell activation on mucociliary clearance.

Kategorie: Poster

Poster 38

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel: IMMUNE RESPONSE OF THE LUNG OF RAT AFTER ORAL
ADMINISTRATION OF MALATHION. AN ULTRASTRUCTURAL STUDY.

Autoren: Abd El-Aziz M.

Adressen: Alexandria, Egypt

Abstract:

Poster 39

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:EXPOSITION TO 17ALPHA-ETHINYLESTRADIOL DURING ONTOGENY LEADS TO PERSISTING IMPAIRMENT OF GROWTH AND THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM IN IMMUNE ORGANS OF A HIGHLY DEVELOPED BONY FISH, THE NILE TILAPIA.

Autoren: Häusermann E.(1),Shved N.(1),Baroiller J.(2),Eppler E.(1),

Adressen:(1)University of Zürich|Division of Neuroendocrinology, Institute of Anatomy|Zürich|Switzerland; (2)Campus International de Baillarguet|UPR20 Aquaculture|Montpellier|France; email:eppler@anatom.unizh.ch

Abstract:

The enormous enlargement of world-wide aquaculture for food production has led to increasing infectious diseases which enhanced the interest in the fish immune system. Preliminary evidence suggests that estrogen receptors (ERs) and the IGFs are produced in fish lymphoid tissues (spleen, head kidney) but their expression sites and potential interactions are unknown. Also few data exist on the melanomacrophage centres, leukocyte accumulations within the spleen considered as equivalents to mammalian lymphnodes. In an experimental study, tilapia were fed with 17alpha-ethinylestradiol (EE2)-enriched food from 10 to 40 days post fertilization (DPF) to induce functional feminization, an approach commonly used in aquaculture. Fish were sampled at 75 and 165 DPF. EE2-treated spleens were significantly smaller in size and weight. Morphometric evaluations revealed that number and size of the melanomacrophage centres were pronouncedly reduced. At 75 DPF, both in spleen and head kidney the expression of IGF-I mRNA was diminished while IGF-II mRNA in spleen was reduced at 75 and 165 DPF but increased in head kidney at 165 DPF. ER-alpha mRNA expression in spleen showed a trend to increase but was unchanged in head kidney. Thus, exposure to EE2 during early ontogeny differentially affected the IGFs in fish immune organs. It led to lasting impairment of spleen which can be attributed to interaction of EE2 with both IGFs. As places of antigen presentation and hematopoiesis, an impairment of spleen and melanomacrophage centres under estrogen might interfere with the antigen presentation capacity of the fish immune system and, thus, alter susceptibility to infection.

Kategorie: Poster

Poster 40

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:MORPHOLOGY OF THE MURINE INTESTINAL MUCOSA IN TWO-PHOTON MICROSCOPY: INTRAVITAL HIGH-RESOLUTION IMAGING BASED ON TISSUE AUTOFLUORESCENCE

Autoren: Klinger A.(1), von Smolinski D.(1), Orzekowsky R.(2), Koop N.(2), Hüttmann G.(2), Gebert A.(1),

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Abstract:

Two-photon laser scanning microscopy (TPLSM) presents a novel technique that enables three-dimensional imaging of intestinal mucosa in living animals. This approach is directed toward the intravital imaging of endogenous fluorophores, thus marker-free identification of different cell types with well defined cellular borders and subcellular resolution, in the most physiologically relevant setting. We developed an intravital experimental setup in anaesthetised mice that minimizes movement artefacts yet maintains tissue viability. At 730 nm excitation, fluorescence is dominated by NAD(P)H emission of the mitochondria and the remaining cytoplasm. In contrast, the signal source for lysosomes dominates at 850 nm excitation. This marker-free imaging created from optical serial sections could clearly identify enterocytes, goblet cells and enteroendocrine cells and the underlying lamina propria with its connective tissue, blood vessels and lymphoid cells, such as lymphocytes, macrophages and dendritic cells. Organized mucosal lymphoid tissue with follicle-associated epithelium (FAE), containing specialised membranous (M) cells could also be demonstrated. Fluorescence lifetime imaging (FLIM), which depends on the fluorescence decay differences between tissues to generate additional image contrast was also applied to distinguish between different cell types. This novel experimental setup can be efficiently used to identify the specific in-vivo histology of the mucosa and also evaluate various aspects of intravital cell function, including cell vitality and apoptosis, particle transport, endocytosis and blood flow.

Kategorie: Poster

Poster 41

Rubrik: 7.Immunbiologie

Abstract Nr.:7

Titel:SULFORAPHAN – EIN NEUES MEDIKAMENT IN DER RHEUMA THERAPIE?

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Abstract:

OBJECTIVE: Rheumatoid arthritis (RA) is a chronic inflammatory disease in which the synovial environment is characterized by intense immunological activity. We investigated whether sulforaphane (SF) could inhibit the expression of interleukin 6 (IL-6), and IL-1 as well as cell proliferation induced by tumor necrosis factor-alpha; (TNF alpha;) in human immortalised fibroblast-like synoviocytes (FLS). METHODS: FLS were cultured with or without SF. Cytokine and VEGF secretion levels were detected using ELISA. Cell proliferation of FLS induced by TNFalpha; was determined by Cyquant. NFkB and AP-1 activity was measured by Dual-Luciferase-Assay. RESULTS: ELISA and promoter-studies analysis revealed that the activity of NFkB and AP-1 and the levels of IL-6, and IL-1 secretion by FLS were reduced by SF in a dose-dependent manner. Cyquant assay showed that SF could inhibit proliferation of FLS induced by TNF-alpha;. CONCLUSION: Our results demonstrate that SF inhibits "pro-inflammatory"- transcription factors and -cytokines in FLS. In addition, it reduces TNF-alpha; induced FLS cell proliferation. These findings indicate that treatment of RA-patients with SF might be considered as a new therapeutic strategy to combat joint destruction and inflammation in RA.

Kategorie: Poster

Poster 42

Rubrik: 1.Methoden/Unterricht

Abstract Nr.:1

Titel:„TEACH THE TEACHER“ – A TEACHING PROJECT FOR LECTURERS AT THE INSTITUTE OF ANATOMY AND CELL BIOLOGY AT ULM UNIVERSITY

Autoren: Boeckers A.(1),Fassnacht U.(1),Öchsner W.(2),Boeckers T.(1),

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Abstract:

In the preclinical medical curriculum anatomy plays a prominent and very important role as reflected by a high number of teaching lessons. In contrast, teaching itself and the ability to teach depends widely on personal experiences or autodidactical principles. Meanwhile, there are several initiatives to improve teaching competences by didactic courses (f.e. Baden-Württemberg Certificate). These initiatives will be finally successful when young colleagues get the chance to further qualify in teaching by a formalized, implemented and directly applied educational program within their institutes.

Therefore during summer term 2007 six participants completed the first step of the structured curriculum „teach the teacher“ visiting 20 teaching units at our institute. We wanted to convey student centred didactical skills to less experienced colleagues. The project design is organized in three steps focussing on lecture presentation, small group teaching and practical training.

We offered 4 group meetings each lasting 4 teaching units: 1. needs-assessment, 2. definition of teaching objectives 3. preparation and 4. presentation of a lecture session within the histology lecture course. Participants were supervised by colleague experts. Their didactical performance was coached by the project leaders. We gave them check lists to ease the realization of the projects, 2UE supervision before and during the project phase as well as structured coaching (2UE) after the presentation. Students (n=10) were trained to evaluate the didactic elements of every lecture. We analyzed self assessment and evaluation results of the lecture series. Preliminary data already show that our young colleagues highly valued this internal educational program.

Kategorie: Poster

Poster 43

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel: APPROPRIATIONS OF DISTRIBUTION AND GROWTH ROMAN'S SHEEP OF MOSCOW'S REGION OF RUSSIA WITH THE CALCULATION THE IR TOPOGRAPHY

Autoren: Slesarenko N.(1),Tikhonova E.(1),Kapustin R.(2),

Adressen:(1)Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin|Department of Animal Anatomy and Histology|Moscow|Russia; (2)Belgorod State Agricultural Academy|Department of Animal Morphology|Maiskii Belgorodskoi oblasti|Russia;
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Abstract:

The aim of the work is to reveal the appropriations of growth and development of skeletal musculation`s sheep Roman's kind in ontogenesis. The investigations have been carried out with keeping of rules using the works of experimental animals. It is determined that the axis skeletal`s muscles constitute from 54,50 to 59,10% from the total mass` musculation but the peripheric muscles – 39,9-45,12%. The basic mass` musculation of the peripheric skelet are concentrated in the region`s girdles and stilopodium (74,88-79,68% - on the scapula and on the arm, 80,08-82,56 - on the pelvic girdle and on the femur), in the region of zeigopodium considerably less (17,68-26,88 on the forearm and 14,56-22,08% on the crus), in the region of autopodium only the tendons` muscles pass through. The functional groups of muscles outrun in the growth in the postnatal period, taking effect on the proximal articulations especially the adductors and the extensors of the hip joints and the adductors of the humeral articulations; minimum intensity increase`s mass is observed to the extensors` articulations pectoral fingers and especially of the pelvic limbs. The muscles of the distal limb`s articulations have shorter period of the intensive increase`s mass than the muscles of the proximal articulations.

Kategorie: Poster

Poster 44

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:THE CHARACTERISTIC OF BASIS MORPHOFUNCTIONAL INDICES OF NEW - BORN BABIES OF BELGOROD REGION OF RUSSIA IN A PERIOD FROM 1973 TO 2004 YEARS

Autoren: Martirosov E.(1),Krikun E.(1),Boldyr V.(1),Kapustin R.(2),

Adressen:(1)Belgorod State University|Department of Human Anatomy and Histology|Belgorod|Russia; (2)Belgorod State Agricultural Academy|Department of Animal Morphology|Maiskii Belgorodskoi oblasti|Russia;
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Abstract:

With the aim of exposure of trustworthiness temporary dynamics of morphofunctional indices of new – born babies they applied the analysis. For removal of casual component of the dynamics row an the exposure of the basic tendency of temporary changes, received empirical rows were smoothed out with the parabola of the 5th order and at the same time they were subjected to Kildishev - Abolentsev`s classification that allowed to mark out the stable row`s period of dynamics. On the whole for the majority of body`s seizes of new – born babies nave been characteristic temporary dynamics of decrease their level beginning from the middle of 80th years. The circle`s abdomen demonstrates a reverse picture its monotonous increase that is testified to decrease of the development of osteomusclar somatic body`s component by new – born babies at increase of the fatty component to the end of the XX century. According to the results of temporary changes of morphofunctional indices can be marked out three basic intervals with borders: 1973-1984 years, 1985-1991 years, 1992-2004 years. The regions with high and lower levels of the indices of physical development of new – born babies. The basic growth – weight characteristics of new- born babies from regions centre exceeded by a size than from district`s centres and villages that is more trustworthy the characteristics were shown by boys. Possibly the new – born girl`s organism is more adapted to changeable conditions of the environment.

Kategorie: Poster

Poster 45

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:DERMAGLYPHICAL CHARACTERISTICS OF POPULATION OF BELGOROD REGION OF RUSSIA LIVING IN DISTRICTS WITH DIFFERENT LEVELS OF ECOLOGICAL POLLUTION

Autoren: Krikun E.(1),Zinchenko I.(1),Nikityuk D.(1),Krikun Y.(1),

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Abstract:

We carried out the comparative investigation finger – shaped dermaglyphical patterns by population living in ecological clear (ECD) and dirty districts (EDD) of Belgorod region of Russia. Finger – prints were received with the method by T.D. Gladkova after that we carried out qualitative and quantitative rating. In all 571 men were investigated (from them: 132 male and 131 female – from ECD and 152 and 156 female – from EDD). The analysis of valuable facts allowed to determine some appropriations by population living on the territories with different ecological situation. Thus for inhabitants of ecological clear districts the greatest frequency of meeting the one deltoid pattern (the type stitch) with prevalence on the left hand by male and on the right hand by female has been characteristic. For inhabitants of ecological dirty districts the greatest senses two – deltoid patterns (the type curl and the central pocket) with equal distribution as by male since by female have been characteristic. The changeability of finger – shaped dermaglyphical patterns can be explained peculiar interaction of organism with environment at that by population living in ecological dirty districts the greatest frequency of meeting complex two – deltoid patterns are observed. This valuable fact confirms the hypothesis by B.A. Nikityuk about influence of unfavorable ecological factors to the rates of growing processes in embryogenesis in general and the influence of this factor to the crest – formation specifically.

Kategorie: Poster

Poster 46

Rubrik: 1.Methoden/Unterricht

Abstract Nr.:1

Titel:IN VIVO OBSERVATION OF SINGLE CELL MIGRATION USING LABELLING WITH IRON MICRO SPHERES AND MRT ALLOWS ONLINE OBSERVATION OF CELL BASED THERAPIES

Autoren: Nohroudi K.(1),Himmelreich U.(2),Hoehn M.(2),Berhorn T.(1),Addicks K.(1),Arnhold S.(1),

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Abstract:

Cell based therapy, mostly involving embryonic and adult stem cells, is one of the major research strategies to overcome the insufficiency of conventional therapies especially for malignant brain tumors like glioblastoma multiforme. However, several concerns like immunologic tolerance, distribution of the injected cells as well as their long term integration in the host tissues hamper the interpretation of experimental results and the predictability of clinical trials. Mesenchymal stem cells isolated from the bone marrow of the host eliminate immunologic risks and have never seen to form tumors after injection which clearly favours them over embryonic stem cells. Nevertheless, the follow up of injected MSC in vivo is still a major lack in the development of therapeutical approaches. Magnetic resonance tomography (MRT) is a promising technique to fill this gap, because of its non-invasiveness and good compliance, but the use of standard contrast agents like Sinerem requires high labelling loads, which strongly impair cell viability, still has a detection limit of 1000 cells on a spot and loss of specific signal over time anyway. We here present a new labelling technique for MSC introducing polystyrene coated iron micro spheres which are taken up by phagocytosis. The results point to the importance of careful titration of iron labelling concerning cell viability and migratory potential, but with an effective concentration conserving MSC viability and migration by providing sufficient contrast for in vivo observation by MRT in a rat glioma model with a detection limit of as few as 10 cells on a spot.

Kategorie: Poster

Poster 47

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:THE RELATIVE DEPOSITION INDEX: A NOVEL QUANTITATIVE METHOD TO ANALYZE THE DISTRIBUTIONS OF NANOPARTICLES WITHIN TISSUES AND CELLS-THEORY AND PRACTICE

Autoren: Mühlfeld C.(1),Mayhew T.(2),Geiser M.(1),Kapp N.(1),Gehr P.(1),Rothen-Rutishauser B.(1),

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Abstract:

Background: Translocation of nanoparticles (NP) from the pulmonary airways into other pulmonary compartments or the systemic circulation is controversially discussed in the literature. In a previous study it was shown that titanium dioxide NP were “distributed in four lung compartments (air-filled spaces, epithelium/endothelium, connective tissue, capillary lumen) in correlation with compartment size”. It was concluded that particles can move freely between these tissue compartments. To analyze whether the distribution of titanium dioxide NP in the lungs is really random or shows a preferential targeting we developed and applied a new method for comparing NP distributions within pulmonary tissue compartments.

Methods: Rat lungs exposed to an aerosol containing titanium dioxide NP were prepared for light and electron microscopy at 1h and at 24h after exposure. Numbers of titanium dioxide NP associated with each compartment were counted using energy-filtered transmission electron microscopy. Compartment size was estimated by unbiased stereology from systematically sampled light micrographs. Numbers of particles were related to compartment size using the relative deposition index and chi-squared analysis.

Results: Nanoparticle distribution within the four compartments was not random at 1h or at 24h after exposure. At 1h the connective tissue was the preferential target of the particles. At 24h the NP were preferentially located in the capillary lumen.

Conclusions: We conclude that titanium dioxide NP cannot move freely between pulmonary tissue compartments. The present study suggests a rapid transport from the airways to the connective tissue and a subsequent translocation to the systemic circulation.

Kategorie: Poster

Poster 48

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ANATOMICAL FEATURES OF THE OPENING OF NASOLACRIMAL DUCT AND HASNER'S VALVE FOR INTRANASAL SURGERY: A CADAVERIC STUDY

Autoren: Yurttas Saylam C.(1),Aktan Ikiz A.(2),Orhan M.(2),

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Abstract:

BACKGROUND: The location and size of the opening of nasolacrimal duct and lacrimal fold (Hasner's valve) are variable.

OBJECTIVE: The aim of this study was to demonstrate anatomical features of the opening of nasolacrimal duct and the lacrimal fold (Hasner's valve) and to discuss the importance of these structures for the surgical approaches to minimize the injury risk.

METHODS: Twenty sagittal head sections from formalin fixed cadavers were examined. The sections showed no evidence of pathology or trauma.

RESULTS: The opening type of the nasolacrimal duct was vertical sulcus in 14 of 20 (70%), oblique sulcus in two of 20 (10%), oblique fissure in two of 20 (10%) and sagittal fissure in one of 20 specimens(5%). Hasner's valve was present in 16 of 20 specimens (80%). Five different types of this valve were observed. Some measurements were evaluated morphometrically.

CONCLUSION: We believe that a detailed anatomic knowledge of the opening of nasolacrimal duct will be of help during surgical approaches to this area.

Key Words

Opening of nasolacrimal duct, Hasner's valve, intranasal surgery, lacrimal fold

Kategorie: Poster

Poster 49

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:DYNAMIK DES LIPPENSPALTENEREIGNISSES IN MENSCHLICHEN EMBRYONEN

Autoren: Puisoru M.(1),Forna N.(2),Fatu A.(2),Fatu R.(2),Fatu C.(1),

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Abstract:

Complex modifications occur in the cephalic part of the embryo under the influence of neural crests and the developing neural tube. Thus, in the 3rd week of gestation the primordium of the primitive mouth (stomodeum) appears. After the facial prominences develop, during the 5th to 8th weeks of gestation, their coalescence results in the definitive normal aspect of the initial part of digestive tube (oral cavity). We have studied a number of 35 human embryos of 4 to 8 gestational weeks, 24 normal embryos and 9 specimens with labial cleft.

After prelevation, the embryos have been fixed, photographed and paraffin-embedded. The slides have been usually stained and examined in optic microscopy. Our examination was focused on the aspect of orbicularis oris muscular fibers and subjacent mesenchymal tissue. In embryos with cheiloschizis (cleft lip) defects of orbicularis oris integrity and decreased mesenchymal density have been noted. In conclusion, the occurrence of cheiloschizis is a multifactorial process influenced by both endogenic and environmental conditions.

Kategorie: Poster

Poster 50

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:DENTAL PULP DERIVED MESENCHYMAL STEM CELLS FOR BONE REGENERATION IN A RAT CRITICAL-SIZE CALVARIAL DEFECT MODEL

Autoren: Arnhold S.(1),Nohroudi K.(1),Zöller J.(2),Scheer M.(2),Addicks K.(1),

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Abstract:

Recent studies suggest that the human dental pulp contains stem cells, which have similar properties as bone marrow derived mesenchymal stem cells. The objective of the present study was to assess the effect in the bone regenerating capability of human dental pulp derived stem cells (PDSC). The dental pulp of exfoliated deciduous teeth and of wisdom teeth was extracted and after crushing cells were isolated and cultivated on plastic culture dishes. Pluripotency was assessed by investigating the differentiation potential into the adipogenic, osteogenic and chondrogenic lineage. For evaluating the bone regenerating capacity an animal model was established by creating 5 mm critical-size circular defects in the rat calvaria using a trepan drill without damaging the dura. The defect created is the minimum bone defect size that requires treatment to heal rather than undergoing spontaneous bone regeneration. Pulpa derived mesenchymal stem cells were implanted into the calvarial defects with and without scaffold materials. As scaffold materials Tutobon®, BioOs® and Cerasorb® were used. Generally, the lesions were covered with the fibrin sealant (Tissucol®, Baxter). Specimens were harvested 4 weeks post-implantation and evaluated radiographically as well as histologically after decalcification in HNO₃. Radiodensitometric analysis revealed that Tutobon® seeded with PDSCs had noticeably the best bone tissue regeneration compared with lesions seeded with stem cells only or in conjunction with the other scaffold materials. This could also be confirmed by histological dyes using HE and Goldner for assessing the osteoid formation.

Kategorie: Poster

Poster 51

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:MORPHOLOGISCHE VERÄNDERLICHKEIT DER MINDERWERTIGEN SCHILDDRÜSE-ARTERIE

Autoren: Niculescu M.(1),Jianu A.(1),Niculescu V.(1),Bolintineanu S.(1),Ciobanu I.(1),Stana L.(1),Rusu M.(2),

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Abstract:

The vascularization of the thyroid is made by two superior thyroid arteries, which irrigate the superior and the anterolateral part of thyroid lobe and two inferior thyroid arteries irrigating the inferior and the inferomedial part. In 10% of the cases it's found middle thyroid artery "ima" with variability related to its origin: from aorta , brachiocephalic trunk or subclavicular artery. To point out the inferior thyroid artery we use the method of macroscopic dissection and the injection of vessels with colored plastic materials. During the dissection we followed the origin, the line, the size and the connections of inferior thyroid artery. Results: we didn't find the missing of inferior thyroid artery, neither unilateral or bilateral, although is written in anatomy literature; exceptional origin, in 0,5% of the cases, when inferior thyroid artery comes from the convexity of aortic arch together with vertebral artery, through a common trunk; a sinuous line with two arches, a lateral one with inferior concavity and another medial one superior concavity, in 75% of the cases. Inferior thyroid artery divides in terminal branches in different ways, more types in women comparing with men and is related with the opening of superior orifice on the chest; regarding the exceptional connections, we mention the passing of the inferior thyroid artery through a ring of the vertebral vein at the level of its inflexion towards the carotid tubercle; in less than 1% of the cases, inferior thyroid artery or a branch of it passes through a curl of a recurrent nerve.

Kategorie: Poster

Poster 52

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:VARIANTE EINES PARASTERNALEN MUSKELVERLAUFES - M. STERNALIS ODER M. SUPRACOSTALIS ANTERIOR?

Autoren: Reinehr M.(1),Doyscher R.(1),Herrmann M.(1),

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Abstract:

Muscles overlying the general body fascia in the thoracic region can often be found on the anterior trunk. About 5 per cent of all Europeans show an unilateral or bilateral developed muscle running parallel to the margin of the sternum which is termed as M. sternalis. This muscle shall be a remnant of a cutaneous muscle (M. panniculus carnosus).

During the dissection of a 92-years old woman we observed a bilateral muscular loop, running with tendinous passages to the sternocleidomastoid muscle across the sternoclavicular joint to end up at the thorax. This loop's fibres took course underneath the major and the minor pectoral muscles. On the right side the muscle showed a clear muscular body while being particularly tendinous on the left. The caudal part of the loop showed an innervation by a branch of the third intercostal nerve, whereas a bundle of vessels and nerves leaving the subclavius muscle entered the cranial part.

This muscular loop we describe is to be termed most likely as a M. supracostalis due to its course underneath the pectoral muscles. The additional examination of the nerval supply helps us to separate it from a M. sternalis.

Kategorie: Poster

Poster 53

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ERWORBENE ANOMALIEN DER GALLENWEGE IM VERLAUF DER
CHRONISCHEN CHOLEZYSTOLITHIASIS

Autoren: Sargan I.(1),Bolintineanu S.(1),Vaida M.(1),Haivas C.(1),Motoc A.(1),

Adressen:(1)Uni der Medizin Victor Babes|Anatomy|Timisoara|Rumanien;
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Babes|anatomy|Timisoara|Rumanien

Abstract:

Acquired abnormalities of the biliary tract from chronic gallstone disease are rare. Laparoscopic cholecystectomy has almost replaced open cholecystectomy as the therapeutic modality in the treatment of symptomatic gallstones. The aim of this study was to examine the frequency with these abnormalities occur and to asses the probability of encountering such an abnormality at laparoscopic cholecystectomy. Methods: laparoscopic cholecystectomy was performed in 10757 patients, in Surgical Clinic II Timisoara, during 1994-2005 .

Results:biliary tract abnormalities from chronic gallstone disease werw encountered in 87. 29 had a cholecystocholedocal and 35 had a stone impacted at the cystic duct-bile duct junction. 11 had cholecystoduodenal fistulas, three had cholecystocolic fistulas and five had an absent cystic duct with a normal bile duct. In 4 cases with absent cystic duct, the bile duct was mistaken for cystic duct.

Conclusions: This study demonstrates a similar incidence of acquired abnormalities of the biliary tract from chronic gallstone disease to that already reported. Patients with this condition are at high risk for for bile duct injury during Chronic gallstone disease.

Kategorie: Poster

Poster 54

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ECOLOGY-ANALYTICAL MONITORING OF MORPHOFUNCTOINAL RECOMBINATIONS OF MICRO-ARCHITECTONICS WITH A COMPACT SUBSTANCE OF A BONE

Autoren: Durtkarinov E.(1),Slesarenko N.(1),Kapustin R.(2),

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Abstract:

With methods of light and electronic microscopy have been studied micromorphological features of components of osseous tissue from diaphysis of thigh – bone of three species domestication of animals (a mink, a fox, a sheep) distinguished of morphofunctoinal type of limb. The investigations have been carried out with keeping of rules with using the works of experimental animals. Growing processes along the perimeter of diaphysical tube proceed irregularly. The structural making of compact substance has been completed before in those sections of diaphysis which test the main power – support loading: by foot – walking (a mink) and by finger – walking (a fox) in the medial, by phalange – walking (a sheep) in the caudal. The medial sector of diaphysis by foot – walking and by finger – walking is characterized by significant increase of quantity osteonal structures while by phalange – walking to this sign are marked out lateral and caudal sectors. Inside surrounding plates by foot – walking in a compact substance prevail over outside while by phalange – walking in the system of surrounding osseous plates outside ones dominate.

Kategorie: Poster

Poster 55

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:MORPHOLOGISCHE ASPEKTE UND KONSTITUTION DES BRACHIALPLEXUS

Autoren: Haivas C.(1),Bolintineanu S.(1),Vaida M.(2),Sargan I.(1),

Adressen:(1)Victor Babes|Anatomy|Timisoara|Rumanien; email:bolint@upcnet.ro;
(2)Victor Babes|Anatomy|Timisoara|Rumanien

Abstract:

We have made a study on 31 brachial plexus, which were provided from 16 human body from the Anatomy's laboratory of the University of Medicine and Pharmacy "Victor Babeș", from Timișoara.

These bodies were preserved in formaldehyde 10 % concentration.

We have studied 11 male body (21 brachial plexus) and 5 female body (10 brachial plexus)

The purpose of this study was to find out: the provenance and the number of the roots which form the brachial plexus, the diameter of roots, the number, the length and the diameter of the primary trunks, the length, the constitution and the diameter of the secondary trunks.

We made a comparative study between the left and the right side and between the male and female bodies.

Kategorie: Poster

Poster 56

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:VARIOUS CIRCULAR ARC RADIUSSES OF THE DISTAL VOLAR RADIUS AND ITS IMPLICATIONS ON VOLAR PLATE OSTEOSYNTHESIS.

Autoren: Clement H.(1),Tanzer K.(2),Pichler W.(2),Tesch N.(3),

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(2)MUG|Universitätsklinik für Unfallchirurgie|Graz|Österreich; (3)MUG|Institut für Anatomie|Graz|Österreich

Abstract:

Objectives: Purpose of this anatomical study was to explore the different circular arc radiusses of the distal volar radius and the implications on volar plate osteosynthesis.

Methods: The profile of the volar distal radius of one hundred cadaver specimens has been measured by using a common profile gauge. Profiles were copied to paper and analyzed by using calibrated curve templates.

Results: The measurements demonstrate clearly differing volar circular arc radiusses between the radial and ulnar side in a total of 55 percent.

Conclusion: This characteristic may lead to a false rotation position of the distal fracture fragment, following volar plate osteosynthesis. In Addition may suboptimal plate fitting due to discrepancy between plate angle and volar radius angle lead to an incorrect plate position.

Kategorie: Poster

Poster 57

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:MORPHOMETRIC ANALYSIS OF THE LISTER TUBERCLE AND ITS CONSEQUENCES ON VOLAR PLATE FIXATION OF DISTAL RADIUS FRACTURES.

Autoren: Pichler W.(1),Clement H.(2),Tanzer K.(2),Tesch N.(3),

Adressen:(1)Medizinische Universität Graz|Universitätsklinik für Unfallchirurgie|Graz|Österreich; email:Wolfgang.Pichler@klinikum-graz.at; (2)MUG|Universitätsklinik für Unfallchirurgie|Graz|Österreich; (3)MUG|Institut für Anatomie|Graz|Österreich

Abstract:

Objective of this study was to explore the various shapes of the Lister tubercle in order to find out under which circumstances the extensor pollicis longus tendon is menaced when doing volar plating of radius fractures. The length and the height of the Lister tubercle have been measured in one hundred cadaver specimens by using a sliding calliper. The deepness of the groove of the extensor pollicis longus has been quantified with a profile gauge. The measurements resulted in a distinct variant in size (height: $\bar{3.6}$ mm, ± 0.79 mm, range: 2 - 6 mm; length: $\bar{18.3}$ mm, ± 3.9 mm, range: 6 – 26 mm). In 63 percent a considerable groove to the tubercle deeper than 2mm has been observed. Deep grooves next to the tubercle or extremely prominent Lister tubercles may impede intra-operative fluoroscopy and screw length measurements. This may lead to prominent screw tips penetrating the third extensor compartment. On this account we recommend using shorter screws in the middle plate holes.

Kategorie: Poster

Poster 58

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:POSTNATAL CHANGES IN THE STYLOID PROCESS, VAGINA PROCESSUS STYLOIDEI AND STYLOMASTOID FORAMEN IN RELATION TO THE FUNCTION OF MUSCLES ORIGINATING FROM THE STYLOID PROCESS

Autoren: Krmpotic Nemanic J.(1),Vinter I.(2),Ehrenfreund T.(2),Marusic A.(2),

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Abstract:

We investigated age-related changes in the styloid process in 88 skulls from 6 months to 85 years of age. The osseous styloid process was not well developed in children. Its length increased significantly with age (from 2.3 mm in 11-20 age group to 16.3 mm in 61-85 group). The distance between the styloid process and stylo mastoid foramen decreased with age, from 0.7 in young adults to 0 mm in old age). The process was missing in 5% of the adult specimens. Changes in length and shape of the styloid process may be related to altered function of the three muscles originating from the styloid process – m. stylopharyngeus, m. stylohyoideus and m. styloglossus. They have a common function of lifting the aerodigestive elements upwards and backwards, after the descent of the larynx and final morphological differentiation of the vocal system during puberty. Relationship between altered muscle function and the morphology of the styloid process are important for understanding the clinical syndromes related to the styloid process, such as the Eagle's syndrome.

Kategorie: Poster

Poster 59

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:AN UNKOWN HAND MUSCLE AT THE HYPOTHENAR

Autoren: Claassen H.(1),Schmitt O.(2),Widmer S.(3),Wree A.(3),

Adressen:(1)Martin-Luther-Universität Halle--Wittenberg|Institute of Anatomy and Cell Biology|Halle (Saale)|Sachsen-Anhalt; email:horst.claassen@medizin.uni-halle.de; (2)Universität Rostock|Institut für Anatomie|Rostock|Mecklenburg-Vorpommern; (3)Rostock|Institut für Anatomie|Rostock|Mecklenburg-Vorpommern

Abstract:

Variations of hypothenar muscles are not seldom. In the dissection course an unknown hand muscle containing much connective tissue was detected at the right wrist joint of a 70-years-old woman. Interestingly, this muscle seems to take its origin in the forearm fascia proximally to the thenar eminence while it inserts into the lateral side of the hypothenar.

Wrist joint and forearm were dissected step by step. Dissection steps were documented by photographs and drawings. A biopsy for histological procedures was taken from the anatomical structure looking were similar to a muscle.

Histology: Skeletal muscle tissue together with a muscle spindle were found in sections stained with toluidin blue. Macroscopy: The unknown muscle takes its origin at the radial side in the palmar carpal ligament and undercrosses the flexor carpi radialis. Thereafter, it possesses a second origin at the flexor retinaculum. The muscle overcrosses the flexor digitorum superficialis and takes an oblique course in direction to the hypothenar eminence. After passing the ulnar rim of the flexor digitorum superficialis, muscle fibers are running parallel to the tendon of the flexor carpi ulnaris. Finally, it undercrosses the palmaris brevis and inserts into the radial rim of the abductor digiti minimi.

From the viewpoint of action, this muscle might support the flexor and opponens digiti minimi. The contraction might have been followed by a flexion of the fifth finger together with the hypothenar and the wrist joint. Further study will show if a similar muscle is present in the hand of claw apes.

Kategorie: Poster

Poster 60

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:CLINICALLY SIGNIFICANT VARIATIONS OF THE HYPOTHENAR MUSCLES

Autoren: Georgiev G.P.(1),JeleV L.(1),Surchev L.(1),

Adressen:(1)Medical University Sofia|Department of Anatomy, Histology and Embryology|BG-1431 Sofia|Bulgaria; email:surchev@medfac.acad.bg

Abstract:

Commonly unusual muscular structures can be observed in the human hypothenar region during anatomical dissections. Some of them represent only anatomical interests, but others have a definite clinical significance. Here, we present three cases of variant hypothenar muscles, which have a close relation to the course of the ulnar nerve and artery at the wrist.

In the first case, on the right side, the flexor digiti minimi brevis muscle was absent and a short muscular bundle between the pisiform bone and the flexor retinaculum was found. In the second case, on the left side, the flexor digiti minimi brevis had an aberrant lateral origin from the flexor retinaculum. Additionally, deep to abductor digiti minimi and flexor digiti minimi brevis an unknown variant muscle was described. It consisted of two well-defined muscular bellies - the lateral one arose from the lateral part of the flexor retinaculum and the medial one from the hamulus of the hamate bone. In the third case, a variant flexor digiti minimi brevis composed of three portions was observed in a left hand. The medial portion arose from the hamate bone; the intermediate originated from the fibers forming anteriorly the canal of Guyon; the lateral portion started from the medial part of the flexor retinaculum. In the literature there have been many reports of anomalous muscles inducing compression syndromes of the ulnar nerve and artery, so the variant structures described here may have such a compression role and must be borne in mind in the orthopedic practice.

Kategorie: Poster

Poster 61

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ANATOMISCHE UND RADIOLOGISCHE AUSWERTUNG DES FEMURALEN NEIGUNGSWINKELS, KLINISCHE BEDEUTUNG

Autoren: Vaida M.(1),Bolintineanu S.(1),Haivas C.(1),Sargan I.(1),Motoc A.(1),

Adressen:(1)Victor Babes|Anatomy|Timisoara|Rumanien;
email:monicaadrianavaida@yahoo.com

Abstract:

The femoral inclination angle represents one of the stringent problems facing surgeons due to the high incidence of femoral fractures in general, and in the elderly in special, to the diversity of existing anatomical shapes, the severity of vital and functional prognosis, the difficulties regarding treatment and consolidation and the severity of late sequelae. The anatomical study of the femoral angles was conducted on fifty bone pieces that had been photographed in order to assess the value of the inclination angle. The radiological study of the inclination angles was carried out on fifty scanned X-rays, subsequently assessing the value of each inclination angle. The study has proven that, technically, it is possible to describe with accuracy the variability of the geometrical characteristics of the upper femoral extremity and, specifically, to assess the inclination angle. The variability of the values obtained led to the conclusion that this might represent a reminiscence of a hip in pronounced flexion, as is the case in quadrupeds. Practically, this considerable variability of the femoral inclination angle should be taken into consideration in designing and producing better and better anatomical hip prostheses, especially when wanting to preserve the femoral neck.

Kategorie: Poster

Poster 62

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ANATOMOKLINISCHE STUDIEN DER MORPHOLOGIE DES TALUS

Autoren: Haivas C.(1),Bolintineanu S.(1),Vaida M.(1),Sargan I.(1),

Adressen:(1)Uni der Medizin Victor Babes|Anatomy|Timisoara|Rumanien;
email:bolint@upcnet.ro

Abstract:

The most frequently foot deformation encountered is the foot flat; the opposite situation is the vaulted foot. Many times we can meet halux valgus. These are the effects of an improper footwear but they can also be innated. We try to discover which of the two sex is more predisposed to develop one of these disease. With this purpose we measured the talus angles right on it, we made a selection depending on their origin; we represented them on a millimetric paper and we measured it. We followed radiological aspects and foot print. Knowing these parameters is usefull in ortopedic practice, in practicing interventions of osteosintesis when are used Steinman nails, screws and other osteosinthesis materials which have complementary sizes and are able to face each situation.

Kategorie: Poster

Poster 63

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:PRIMÄRE TUMEREN DES HIPOTALAMUS UND DIE BEDEUTUNG DES MRT
IN DER DIAGNOSTIK

Autoren: Bolintineanu S.(1),Vaida M.(1),Sargan I z.(1),Haivas C.(1),Motoc A.(1),Matu
C.(1),Niculescu M.(1),

Adressen:(1)Victor Babes|Anatomy|Timisoara|Rumanien; email:bolint@upcnet.ro

Abstract:

There were evaluated through a statistically retrospective method 52 cases of hypothalamic pathology detected by MRI. The patients were examined clinically, biologically, radiological, by CT scan and finally by MRI for detailed evaluation of topography and extension of lesions , the precise diagnosis being surgical confirmed by anatomic-pathological investigation. The MRI exams were done by a GE unit of 0,5 T using different types of sections: axial pre and postcontrast after i.v. bolus injection of 0,1 mmol/kg Gadolinium and sagittal and coronal pre and postcontrast images.We found 29 case of tumoral pathology, from witch 12 are primary tumours. Gliomas: 5 cases (42%);from witch diffuse infiltrative forms: 3 cases; and intratumoural necrosis forms: 2 cases; MRI appearance:T1: low/isosignal,T2: highsignal,intensely enhances and segmental ectasy with mass effect on Sylvius aqueduct, 4th ventricle and on cisternal spaces.

Craniopharyngiomas: 4 cases (33%); MRI appearance: lowsignal T1, highsignal T2 ,with intense enhancement,cystic lesion: lowsignal T1, highsignal T2 ,with peripheral intense enhancement,with calcifications.

Germinomas: 2 cases (17%); MRI appearance:unique hypothalamic forms: 2 cases,the second most frequent site after the pineal region,lowsignal T1, highsignal T2 with intense enhancement.

Lymphomas: 1 case (8%); MRI appearance: a multicentric primitive form ,infiltration, heterogeneous mass with lowsignal T1, highsignal T2 with contrast enhancement.

In conclusion,the MRI is the method of choice in hypothalamus pathology diagnosis due to: the very good natural contrast, the absence of bone artefacts, the 3D acquisition possibilities.

Kategorie: Poster

Poster 64

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:GLYCOCONJUGATE EXPRESSION AS PROGNOSTIC INDICATOR IN ADENOID CYSTIC CARCINOMA OF THE SALIVARY GLAND

Autoren: Teegen J.(1),Fehlauer F.(2),Altevogt P.(3),Löning T.(4),Schumacher U.(1),Thies A.(1),

Adressen:(1)Universitätsklinikum Hamburg-Eppendorf|Institut für Anatomie II: Experimentelle Morphologie|Hamburg|Deutschland; (2)Universitätsklinikum Hamburg-Eppendorf|Institut für Strahlentherapie|Hamburg|Deutschland; (3)DKFZ Heidelberg|Tumorimmunologie|Heidelberg|Deutschland; (4)Albertinen Krankenhaus|Albertinen Pathologie Hamburg|Hamburg|Deutschland

Abstract:

Adenoid cystic carcinoma (ACC) of the salivary glands shows persistent slow growth, multiple recurrences and distant metastasis. So far no reliable prognostic marker for development of systemic metastases exists. Aberrant glycosylation and up-regulation of cell adhesion molecules (CAMs) play a prominent role in metastatic spread. This study aimed to identify prognostic markers, which give insight into the mechanisms underlying ACC growth and spread. Expression status of CEACAM1 and L1 and binding of six lectins were analysed in 35 primary ACCs with different histologic subtypes (tubular, cribriforme, solid, mixed). Expression or binding status of these glycoconjugates was correlated with (i) recurrence free survival, (ii) metastasis free survival, and (iii) overall survival, respectively. Kaplan-Meier analyses revealed a significant positive association between death of ACC and intense L1 expression ($P=0.02$) as well as broad MAA binding ($>80\%$ of tumour cells; $P=0.05$) independent of the histologic subtype. 12/16 cribriforme ACCs contained pseudocysts, whose secretions showed intense CEACAM1 expression and intense HPA and GNA binding, whereas normal salivary gland secretions were devoid of these glycoconjugates. Presence of pseudocysts as such had no prognostic value, however, CEACAM1 expression or HPA and GNA binding sites in the secretions of the pseudocysts were significantly associated with lower rates of recurrence (CEACAM1: $P=0.02$; GNA: $P=0.05$), prolonged metastasis free survival (CEACAM1: $P=0.04$; GNA: $P=0.04$) and prolonged overall survival (CEACAM1: $P=0.04$; GNA: $P=0.04$). In conclusion, determination of L1 expression in primary ACCs improves risk estimations. As upregulation of L1 expression predicts fatal prognosis, L1 might be functionally involved in metastatic spread of ACC.

Kategorie: Poster

Poster 65

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:ULZERIERENDES MAMMA-KARZINOM.PATOLOGISCH-ANATOMISCHE
UND TERAPEUTISCHE BETRACHTUNG

Autoren: Sargan I.(1),Vaida M.(1),Bolintineanu S.(1),Haivas C.(1),

Adressen:(1)Uni der Medizin Victor Babes|Anatomy|Timisoara|Rumanien;
email:bolint@upcnet.ro

Abstract:

The ulcerous type from the advanced forms of breast cancer is a sad reality in the third millennium. Material and method: one have been studied the clinical and anatomopathological features on 32 patients, hospitalized and treated at Surgical Department, since of January 1995 to the 31 Decembre 2005. Results: The histopathological results certified the highest occurred of intraductal type associated with invasive carcinoma (65%). Because they were advanced forms, surgical treatments are: simple mastectomies with axillar lymphnodes removal in 21 cases or without axillar lymphnodes removal in 11 cases.Conclusions: ulcerative form of breast cancer is already a systemic disease. Intraductal type associated with invasive carcinoma exulcerated frequently.

Kategorie: Poster

Poster 66

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:QUANTIFICATION OF TUMOUR CELL LOAD IN LUNG TISSUE SECTIONS OF A HUMAN MELANOMA XENOGRAFT SCID MOUSE MODEL USING REAL-TIME PCR

Autoren: Stübke K.(1),Augustin C.(2),Püschel K.(2),Schumacher U.(3),Thies A.(3),

Adressen:(1)Universitätsklinikum Hamburg Eppendorf|Institut für Anatomie II: Experimentelle Morphologie|Hamburg|Deutschland; (2)Universitätsklinikum Hamburg-Eppendorf|Institut für Rechtsmedizin|Hamburg|Deutschland; (3)Universitätsklinikum Hamburg-Eppendorf|Institut für Anatomie II: Experimentelle Morphologie|Hamburg|Deutschland

Abstract:

Human melanoma cell/scid mouse xenograft models have been developed to analyse the molecular mechanisms underlying metastatic spread and to evaluate the anti-metastatic potential of new therapeutic approaches. Currently, the number of lung metastases is assessed microscopically in H&E stained serial lung sections. The aim of the present study was to establish a new time-saving DNA-quantification approach. The number of melanoma cells was microscopically assessed (by two independent observers) in 100 H&E stained lung tissue sections from a MV3 human melanoma xenograft scid mouse model and correlated with PCR quantification results. For PCR quantification, human DNA was extracted from the tissue sections and metastatic load was quantified by Real-Time PCR using forensically established primers specific for the XY-chromosomal amelogenin locus. For each lung section, analyses were performed in quadruplicate and in two independent experiments. A correlation between microscopically assessed tumour cell load and DNA amount measured by PCR was found for overall metastatic load ranging from 1 tumour cell to > 1200 tumour cells ($P=0.0062$; $r=0.33$). However, when analysing the discriminative power between low metastatic load (< 300 tumour cells) and high metastatic load (>300 tumour cells), the PCR quantification approach lost its accuracy. For tumour load > 300 cells, no correlation between the microscopic count data and PCR quantification was evident ($P= 0.29$; $r=0.1$). In conclusion, the PCR approach using primers specific for the amelogenin locus is not sufficient to replace microscopic quantification of tumour cell load.

Kategorie: Poster

Poster 67

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:DER WERT DER BIOPSIEAUSSCHABUNG FÜR DIE FRÜHE DIAGNOSE DES ENDOMETRIALEN KREBSSES

Autoren: Fatu C.(1),Puisoru M.(1),Fatu I.(2),Varvara G.(3),

Adressen:(1)University of Medicine and Pharmacy Gr. T. Popa|Anatomy|Iasi|Romania; (2)University of Medicine and Pharmacy Gr. T. Popa|Gynecology|Iasi|Romania; (3)Technical University Gh. Asachi|Department of Automatic Control & Industrial Informatics|Iasi|Romania

Abstract:

The endometrium is a specialised mesenchymal tissue with a remarkable lability, a special receptivity to ovarian hormones and an impressive capability to remake.

The endometrium is a high fidelity marker of the hormonal variations and the bioptic curettage gives the proof of its pathological modifications.

The uterine curettage is a mean of diagnosis, prognosis and therapy with great benefits for the patient.

A number of 1976 histopathological results in women aged between 15 and 73 years have been studied during 15 years period (1979-2006), in the Hospital of Obstetrics and Gynaecology Iasi. Results: the endometrial lesions were grouped in endometrial functional transformations (1150 cases), endometrial dysplasia (600 cases) and endometrial neoplasia (226 cases).

In conclusions, the endometrial bioptic curettage is an efficient, easier and not expensive method, available not only for early diagnosis of endometrial cancer, but also for its therapy and prognosis.

Kategorie: Poster

Poster 68

Rubrik: 2.Klinische Anatomie/Makroskopie

Abstract Nr.:2

Titel:MAGNETIC RESONANCE IMAGING OF DISTANT METASTASES IN A CLINICALLY RELEVANT HUMAN MELANOMA CELL XENOGRAFT SCID MOUSE MODEL

Autoren: Thies A.(1),Peldschus K.(2),Adam G.(3),Ittrich H.(3),Schumacher U.(1),

Adressen:(1)Universitätsklinikum Hamburg-Eppendorf|Institut für Anatomie II: Experimentelle Morphologie|Hamburg|Deutschland; email:thies@uke.uni-hamburg.de; (2)Universitätsklinikum Hamburg-Eppendorf|Abteilung für Diagnostische und Interventionelle Radiologie|Hamburg|Deutschland; (3)Universitätsklinikum Hamburg-Eppendorf|Klinik für Diagnostische und Interventionelle Radiologie|Hamburg|Deutschland

Abstract:

Human melanoma cell xenograft scid mouse models have been developed, however, these models mainly characterise lung metastases, while in the clinic multifaceted organ metastasis occur. The aim of this study was to characterise the metastatic behaviour of human melanoma FEMX-I cells in vivo after surgical excision of the primary melanoma nodule, thus more exactly paralleling the clinical situation. Development of distant metastases was monitored by magnetic resonance (MR) imaging and evaluated comparing MR data with microscopically assessed histopathologic findings. After excision of subcutaneous grown FEMX-I melanoma nodules, metastases were given 80 days to develop. Mice were anaesthetised and whole body MR imaging was performed to assess metastatic burden. MR analyses of eight tumour bearing mice revealed hyperintense suspicious lesions on T2 weighted images in the liver (n=4), adrenal glands (n=4), lymph node stations (n=3), brain (n=1), or in the abdominal (n=4) or mediastinal (n=1) stroma. MR lesions suspicious of metastases had a diameter larger or equal 500 µm. Histologic evaluation of H&E stained serial sections of lymph node regions and inner organs proved the high specificity (100%) and high sensitivity of MR image analysis of melanoma metastasis with a detection limit at about 500 µm (excluding lung metastasis, which could not be visualised due to respiration artefacts). In conclusion this study underlines (i) the importance of clinically relevant model systems as under clinical conditions FEMX-I cells showed high metastatic potency and (ii) demonstrates that MR imaging is a reliable method to monitor melanoma metastasis in mouse models.

Kategorie: Poster

Poster 69

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:INFLUENCE OF ABI-1 ON THE SYNAPTIC LOCALIZATION OF EPS8, WAVE, SOS1 AND C ABL IN HIPPOCAMPAL NEURONS

Autoren: Johannsen S.(1),Pröpper C.(1),Böckers T.(1),

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Abstract:

Fully functional neurons require a complex dendritic tree and mature synapses. In synaptogenesis, and during the maturation of synapses rearrangement processes of the actin cytoskeleton are necessary. Abl-interactor-1 (Abi-1) and its interaction partners are involved in the formation and reorganisation of the actin cytoskeleton in different model systems. Downregulation of Abi-1 protein expression in hippocampal neurons, using RNAi, led to a reduction of the density of synapses and an increase of filopodium-like spines. Thus, Abi-1 is crucially involved in the formation and morphological maturation of synapses.

The influence of Abi-1 on the synaptic localization of its interactors EGFR substrate 8 (Eps8), WASP family Verprolin-homologous protein (WAVE), son of sevenless1 (SOS1) and abl tyrosine kinase (c-Abl) was analyzed. Therefore, Abi-1 protein in primary rat hippocampal neurons was downregulated by RNAi or increased by overexpression and the colocalization of its interactors with the presynaptic protein Bassoon was determined by immunocytochemistry. Loss of Abi-1 did not prevent the localization of its interactors at synapses. Abi-1-RNAi increased the synaptic localization of Eps8 and c-Abl, Abi-1 overexpression led to an opposite effect. The localization of WAVE and SOS1 at synapses was not altered. Taken together, Abi-1 is not responsible for the synaptic localization of its interactors Eps8, SOS1, WAVE and c-Abl, but seems to regulate the amount of Eps8 and c-Abl protein at postsynaptic sites.

Kategorie: Poster

Poster 70

Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL LIKE CHANNEL 4 (TRPC4) IN THE DEVELOPING AND ADULT MOUSE CNS

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Abstract:

The TRPC- family, consisting of seven members (TRPC 1-7), represents a group of non-selective receptor and store operated cation channels. TRPC4 has been implicated to contribute to neuronal calcium signaling. Until now, little is known about the expression pattern of TRPC4 in the developing and adult brain. Here, we describe the widespread distribution of TRPC4 during development and adulthood. Expression of TRPC4 mRNA started as early as embryonic day 14.5 in the developing septal area and cerebellum. At E16.5 TRPC4 mRNA was additionally detectable in neurons of the cortical plate and of the hippocampal formation. Within the neonatal cortex, TRPC4 mRNA was mainly observed in layer II and III, but was also expressed by neurons located in the septum and developing cerebellum. Furthermore, with the exception of the hippocampus, TRPC4 expression declined during development, suggesting that TRPC4 may serve important functions during that phase. An involvement of TRPCs in axonal guidance during brain development has, e.g., been postulated for TRPC1 and 3. Within the adult CNS, high densities of TRPC4 expressing cells were observed in the olfactory bulb and hippocampus, whereas the cortex and septum displayed lower densities of cells positive for TRPC4 mRNA. This distribution pattern was confirmed by RT-PCR, Western blotting and immunohistochemistry. Concerning the hippocampus, TRPC4 may play an important role in neuronal signaling. The involvement of TRPCs in neuronal activity will be analyzed in future studies.

This study was supported by SFB 636/A5.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:MODULATION OF THE INTERNAL RIBOSOME ENTRY SITE-MEDIATED TRANSLATION OF ANTIAPOPTOTIC PROTEIN XIAP BY THE HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEINS C1-C2 FOLLOWING CEREBRAL ISCHEMIA

Autoren: Spahn A.(1),Blondeau N.(2),Rami A.(1),

Adressen:(1)Johann-Wofgang von Goethe Universität|Institut für Zelluläre und Molekulare Anatomie, Anatomie III, Uniklinik|Frankfurt/Main|BRD; email:rami@em.uni-frankfurt.de; (2)Université de Nice Sophia Antipolis|Institut de Pharmacologie Moléculaire et Cellulaire, UMR 6097, CNRS|Valbonne|France

Abstract:

XIAP is the prototype member of the family of inhibitor of apoptosis proteins. XIAP is critically involved in several cellular functions including caspase inhibition, signal transduction, and protein ubiquitination. Therefore the understanding of molecular mechanism that regulates XIAP upon ischemia is of particular interest. Although cap-dependent translation initiation is thought to be the prevalent mode of ribosome binding to mRNAs in eukaryotes, a growing list of cellular mRNAs exhibit an inherent ability to bypass the requirement for the cap structure. The translation of XIAP mRNA seems to be controlled by a potent internal ribosome entry site (IRES) element. To know if such regulatory mechanisms could exist in the CNS, we used the in vivo model of focal cerebral ischemia and the in vitro model of staurosporine-induced apoptosis in hippocampal HT22 cells. Both cerebral ischemia and staurosporine activate caspase-3 and trigger cell death. We found that both injuries slightly enhance XIAP protein levels. In addition to the XIAP expression, hnRNP C1-C2 protein which may regulate delayed neuronal death through its interaction with XIAP-translation was also up-regulated in the penumbra after ischemia and in HT22 cells after staurosporine-treatment. Here, we demonstrated that increased cellular levels of hnRNPC1-C2 may modulate XIAP expression, probably by interacting with the XIAP-IRES. We suggest that IRES-mediated translation by hnRNP C1-C2 could be selectively regulated to induce a protective role in the early stages of apoptosis as a part of a desperate attempt by the cell to save itself from destruction.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:IN VITRO AND IN VIVO ASSESSMENT OF POLYSIA AND POLYSIA BASED HYDROGEL IN TERMS OF SURVIVAL OF NEURAL AND GLIAL CELLS, IMMUNOLOGICAL REACTION AND NERVE REGENERATION

Autoren: Haile Y.(1),Haastert K.(1),Berski S.(2),Rode B.(3),Stummeyer K.(4),Gerardy-Schahn R.(5),Scheper T.(3),Dräger G.(2),Grothe C.(1),

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Abstract:

Polysialic acid (polySia) is a homopolymer of alpha 2, 8-linked sialic acid residues and a posttranslational modification of neural cell adhesion molecule. It displays a diverse array of physiological functions during the development of the nervous system. It is biocompatible, biodegradable by endoneuraminidase enzyme (endoN) and immunologically inert which make it potential candidate in tissue reconstruction therapies. We established a coating protocol and demonstrated polySia substrate did not entail negative impact on culturing of primary neurons and glial cells in vitro (Haile et al. Biomaterials, 2007). Only modified polySia based hydrogel supported adhesion and viability of cultured neurons and glial cells. In vivo study was performed to assess the impact or contribution of polySia in nerve regeneration. Thirteen mm silicone tube was filled either with hydrogel, matrigel, polySia combined with matrigel and Schwann cells in the presence or absence of endoN and bridged a 10 mm gap of the rat sciatic nerve. Three weeks post-operation, macroscopic analysis of the explanted tubes revealed that all animals transplanted in the presence of soluble polySia showed tissue cable regeneration whereas none of the animals transplanted with polySia hydrogel exhibit tissue cable growth. The preliminary results of immunostaining assay indicate that in vivo introduction of polySia or polySia hydrogel did not induce increased immunological reaction. The number of recruited or activated macrophages was similar to the controls. These results prove the immunologic inertness of polySia and demonstrate its biocompatibility and potential to be used as scaffold material in tissue engineering.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:GROWTH/DIFFERENTIATION FACTOR 15 (GDF15) IN FOREBRAIN DEVELOPMENT: EXPRESSION PATTERN OF LACZ & WNT3A IN GDF15 $-/-$ / LACZ KNOCK IN & WILDTYPE MICE

Autoren: Paech T.(1),Peterziel H.(2),Strelau J.(2),Unsicker K.(2),

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Abstract:

GDF15 is a distant member of the TGF β superfamily which has been shown to promote survival of embryonic dopaminergic midbrain neurons both in vitro and in vivo. Moreover, it is upregulated after cortical lesioning. These findings point to an important role of GDF15 during lesion and repair processes in the brain, underlining the importance to analyze the GDF15 expression pattern in the dynamically developing brain. The cortical hem comprises the rostro-caudal extension of the medial telencephalic wall, being located between the developing hippocampus and the cerebral choroid plexus. This region is defined by the expression of different Wnts & Bmps and constitutes one source of Cajal-Retzius (CR) cells, which are important for correct cortical and hippocampal layering. Using in situ hybridization for LacZ we found a clear signal in the cortical hem of embryonic GDF15 $-/-$ / LacZ knock in mice from E12.5 to E14.5. The signal covers the rostro-caudal extension of the cortical hem marker Wnt3a, however, it becomes progressively fainter towards the caudal direction. In the dorso-ventral axis both signals are only partially co-expressed with a more dorsal expression of LacZ. We could not observe a difference in Wnt3a expression in the hem region of GDF15 $-/-$ / LacZ knock in mice and wildtype mice, respectively, implicating that GDF15 does not (at least directly) regulate Wnt3a expression. Further studies of older embryos (E15.5 – E18.5) will be done to analyze the different expression of LacZ and Wnt3a in more detail.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:POLYSIALIC ACID AFFECTS NEURITE OUTGROWTH, MATURATION AND MYELINATION AFTER SCIATIC NERVE INJURY

Autoren: Bronzlik P.(1),Hellriegel H.(1),Eckhardt M.(2),Timmer M.(1),Grothe C.(1),Jungnickel J.(1),

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Abstract:

Polysialic acid (PolySia) is the most striking post-translational modification of the neural cell adhesion molecule (NCAM) and is involved in neurite outgrowth, migration and synaptic plasticity. After birth, the amount of PolySia progressively declines and is restricted to small, non-myelinated axons. After lesion of peripheral nerves, PolySia is up-regulated not only in axons but also in Schwann cells indicating an important function on neurite outgrowth and myelination. To understand the specific role(s) of PolySia after peripheral nerve lesion, we generated mice overexpressing PolySia exclusively in neurons and Schwann cells, respectively. One week after sciatic nerve crush, transgenic mice overexpressing PolySia in neurons, showed a dramatic increase in number and size of regenerating myelinated axons compared with wild-type littermates. In contrast, lesioned nerves of transgenic mice overexpressing PolySia in myelinating Schwann cells, displayed a significant reduction of myelin thickness and a decreased fiber number. These data argue for distinct roles of PolySia after lesion depending on the expression level in different cell types. PolySia is necessary for the axon outgrowth and maturation of regenerating fibers but inhibits the myelination process after peripheral nerve injury. Our results demonstrate for the first time a complex function of PolySia during peripheral nerve regeneration and could have an impact on the development of new therapeutic strategies.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:CHARACTERISATION AND DIFFERENTIATION OF CEREBELLAR PROGENITOR CELLS IN MONOLAYER IN VITRO CULTURES

Autoren: Saier T.(1),Kruttwig K.(2),Klinz F.(3),Nohroudi K.(1),Addicks K.(4),Arnhold S.(3),

Adressen:(1)Köln|Institut I für Anatomie|Köln|D; email:mail@tsaier.de; (2)Köln|Max-Planck Institut for neurological research|Köln|D; (3)Köln|Insitut I für Anatomie|Köln|D; (4)Köln|Instiut I für Anatomie|Köln|D

Abstract:

One of the major aims in neuroanatomy and experimental neurology is to achieve a broader understanding of mechanisms involved in proliferation, differentiation, and apoptosis of central nervous system derived neural progenitor cells. For this purpose especially the cerebellum, which contains up to 50% of the CNS neurons has evolved to become a rather interesting compartment. We were able to isolate and cultivate neural progenitor cells in a chemically defined serum deficient medium from early postnatal rat cerebellum in an in vitro monolayer culture technique. These nestin positive cells inherit progenitor cell characteristics, which could be detected by their single cell cloning capacity as well as their pluripotency, and the expression of molecular neural progenitor markers. Using immunohistochemical assays and FACS – analysis, we further investigated the differentiation capacity and apoptosis of these cells under the influence of the epidermal growth factor (EGF), the EGF – receptor inhibitor AG1478, the neurotrophins (BDNF, NT-3), as well as the cytokine interleukin – 6, and under hypoxic conditions. With the advantages of an adherent monolayer in vitro culture system, compared to so called floating neurospheres, we provide a simple but efficient model for neural stem cell research e.g. to investigate the influence of potential therapeutic substances in neurodegenerative diseases and possibly for cell replacement therapies. Furthermore, our results support the hypothesis that endogenous progenitor cells may specifically be recruited, stimulated and directed under the influence of substances such as EGF and neurotrophins.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:IMMORTALIZED MESENCEPHALIC PROGENITOR CELLS - CELLULAR AND MOLECULAR EVALUATION IN VITRO

Autoren: Nobre A.(1),Cesnulevicius K.(1),Wesemann M.(2),Tkachuk S.(3),Claus P.(1),Grothe C.(1),

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Abstract:

In Parkinson's diseases (PD) progressive cell loss of dopaminergic neurons in the substantia nigra leads to severe motor dysfunction. Exogenous cell replacement represents a putative alternative clinical therapy for late stages of PD. It is suggested that stem cells could be a promising cell source for transplantation. Neuronal progenitor cells (NPC) can be isolated from different parts of the brain, then expanded and differentiated in vitro into neurons, astrocytes and oligodendrocytes. The use of NPC for cell banking and transplantation may not be optimal, since their mitotic competence is limited which means that NPC can be stimulated to grow until they approach their natural senescence limit in culture. An alternative approach is to immortalize by arresting cells at specific stages of development and preventing their terminal differentiation. The SV40 largeT antigen is commonly used to immortalize mammalian cells, however interferes with multiple cell cycle components, including p53 and pRb, and usually produces undifferentiated phenotypes. NPC were immortalized with SV40 largeT antigen and four clones with different proliferation rates, were analyzed in this study. Morphological and molecular characterization of the clones was performed by using immunocytochemistry and semiquantitative RT-PCR analysis, respectively. All clones expressed several transcription factors involved in dopaminergic neurons production; however immunocytochemistry revealed only nestin and SV40 positive cells, whereas they were negative for TH, GFAP and α -tubulin. Since differentiation of the immortalized cells could be achieved by silencing of SV40 largeT antigen, we currently established two different silencing strategies to knock down SV40 largeT antigen expression.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:CLOSTRIDIUM BOTULINUM C3 PROTEINS: RHO-DEPENDENT AND - INDEPENDENT EFFECTS ON MORPHOLOGY AND FUNCTION OF NEURONS AND GLIA.

Autoren: Höltje M.(1),Hofmann F.(2),Just I.(2),Ahnert-Hilger G.(3),

Adressen:(1)Charité-Universitätsmedizin Berlin|Centrum für Anatomie|AG Funktionelle Zellbiologie|Deutschland; email:markus.hoeltje@charite.de;
(2)Medizinische Hochschule Hannover|Institut für Toxikologie|Hannover|Deutschland;
(3)Charité-Universitätsmedizin Berlin|Centrum für Anatomie|AG Funktionelle Zellbiologie|Deutschland

Abstract:

Small GTPases of the Rho family exert multiple impacts on the formation and development of neuronal axons and dendrites, effects often studied by the application of the enzymatically active Rho-inactivating Clostridium botulinum C3 transferase (C3bot). Recently, we found that enzymatically deficient C3bot exerted axonotrophic activity. We show here that a small peptide of 29 amino acids derived from the C-terminal region of C3bot promoted axonal as well as dendritic growth and branching of hippocampal neurons at submicromolar concentrations. Additionally, the number of synaptic contacts between hippocampal neurons was increased by C3bot aa154-182 as revealed by quantification of synaptophysin-immunoreactive spots contacting dendrites of eGFP-transfected neurons. Furthermore, full length enzymatically deficient C3bot as well as the peptide in addition exert axonotrophic activity in alpha-motoneurons of the spinal cord. Conversely, GABAergic Purkinje cells of the cerebellum are preferentially sensitive towards wild type C3bot. We also investigated the effects of C3bot on the release properties of astrocytes preincubated with [3H]glutamate. We found, that Rho-inhibition by C3bot results in a significantly enhanced exocytotic glutamate release. To a lesser extent, glutamine converted from glutamate by astroglial glutamine synthetase was also found to be released at higher levels after C3bot incubation.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:POSSIBLE CONNECTION OF PROSAP/SHANK FAMILY PROTEINS TO THE WNT SIGNALING PATHWAY IN MAMMALS AND DROSOPHILA.

Autoren: Grabrucker A.(1),Thomas U.(2),Schmeisser M.(1),Gundelfinger E.(2),Boeckers T.(1),

Adressen:(1)Universität Ulm|Institut für Anatomie und Zellbiologie|Ulm|Deutschland; email:andreas.grabrucker@uni-ulm.de; (2)IfN Magdeburg|Abteilung Neurochemie und Molekularbiologie|Magdeburg|Deutschland

Abstract:

Mammalian ProSAP/Shank proteins serve as scaffolding proteins within postsynaptic densities (PSDs) of excitatory synapses.

A single ProSAP homologue, DProSAP, has been identified in *Drosophila*.

Overexpression and RNAi-mediated knockdown of DProSAP cause a planar cell polarity (PCP) phenotype. Using a genetic modifier approach, 16 mutants were identified to either suppress or enhance the wing vein (PCP) phenotypes, among them mutants in *Dishevelled*, a component of Wnt signalling cascades. Since it is known that mutations affecting the Wnt pathway also cause a PCP phenotype, we reasoned that the similarity of phenotypes in flies might reflect a conserved functional interplay between ProSAP proteins and members of the Wnt pathway also in vertebrates.

Immunocytochemistry showed that the major components of the Wnt/beta-catenin pathway colocalize with ProSAP family members within PSDs of rat hippocampal neurons. Western Blot analysis using subcellular protein fractions substantiated this finding, showing the enrichment of the Wnt/beta-catenin pathway proteins within the PSD fraction. Transfected full size *Dishevelled* colocalizes with LAPSER1, a ProSAP binding partner, in HeLa cells and hippocampal neurons. CoIP experiments indicate that Dvl-3 and LAPSER1 act in a complex together with ProSAP2. LAPSER1 that directly interacts with ProSAP2 and beta-catenin can be found within the nucleus after NMDA stimulation together with beta-catenin.

These findings suggest that the ProSAP/DProSAP pathway is functionally interconnected with the Wnt/beta-catenin signalling cascade. A fully conserved Wnt/beta-catenin pathway possibly exists at PSDs of hippocampal neurons. A Crosstalk between this pathway and the activation of synapses upon stimulation might be present.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:EXPRESSION OF ADAMS IN THE EMBRYONIC CHICKEN BRAIN

Autoren: Lin J.(1),Luo J.(1),Redies C.(1),

Adressen:(1)Friedrich-Schiller-Universität Jena|Institut für Anatomie
|Jena|Deutschland; email:juntang.lin@mti.uni-jena.de

Abstract:

ADAMs (a disintegrin and metalloproteases) are transmembrane proteins that possess a disintegrin and a metalloprotease domain. ADAMs are involved in cell-cell and cell-matrix adhesion, in proteolysis and in signaling transduction; they play a role in morphogenesis and tissue formation during embryonic development (White, 2003; Blobel, 2005). However, the expression patterns of ADAMs during brain development have not been studied in detail. In the present study, full-length cDNAs or ORFs (open reading frames) encoding ADAM9, ADAM10, ADAM12, ADAM17 and ADAM23 of chicken were cloned with RT-PCR or RACE (rapid amplification cDNA ends) and identified by sequencing. The expression patterns of these ADAMs were investigated by in situ hybridization during chicken brain development. Moreover, the expression of ADAM10 and ADAM17 were analyzed by semi-quantitative RT-PCR. Our results show that all five ADAMs are expressed in the developing brain. The expression of ADAM17 is widespread whereas transcription of ADAM10 is temporally and spatially restricted to specific brain regions and cell types. Moreover, ADAM10 is expressed in embryonic (but not mature) blood vessels, suggesting a role in angiogenesis. ADAM9, ADAM12 and ADAM23 are expressed also in a regionally restricted fashion in diverse structures of the embryonic chicken brain. Altogether, the wide and differential expression of ADAM mRNAs suggests that ADAMs have versatile and multiple roles during embryonic brain development.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE ENERGY HOMEOSTASIS IS GENDER- AND AGE-DEPENDENTLY ALTERED IN A TRANSGENIC RAT MODEL OF HUNTINGTON'S DISEASE

Autoren: Gerding T.(1),Bode F.(1),Stephan M.(1),Dettmer S.(2),Lotz J.(2),von Hoersten S.(3),Nave H.(1),

Adressen:(1)Hannover Medical School|Functional and Applied Anatomy|Hannover|Germany; (2)Hannover Medical School|Diagnostic Radiology|Hannover|Germany; (3)University of Erlangen|Franz-Penzoldt-Zentrum, Department of Experimental Therapy|Erlangen|Germany; email:nave.heike@mh-hannover.de

Abstract:

The Huntington's disease (HD) is a lethal neurodegenerative disease often accompanied by a significant weight loss. Leptin is released by the adipose tissue and has profound anorexigenic effects. In the present study, a rat model transgenic of HD (tgHD) was evaluated in terms of the fat distribution (quantitative MR imaging) and the hepatic leptin/leptin receptor (Ob-R) expression (immunohistochemistry and semiquantitative image analysis). Eight animal groups were investigated: male/female 4 and 14 months old tgHD rats and their corresponding controls. Results showed that the body weight of male tgHD rats was significantly decreased compared to the healthy controls. Interestingly, the body weight of female tgHD rats was similar with the control rats. The hepatic leptin expression in male adult tgHD rats was increased and the Ob-R expression significantly decreased. These results may be one possible cause for the dramatic weight loss in adult tgHD rats. In female adult rats both the leptin and Ob-R expression were significantly decreased. No difference of the hepatic leptin system could be observed in young rats. Quantitative MR imaging revealed a significant decrease of total fat mass by 39% in tgHD rats with a notably fat loss in the intraabdominal compartment.

In conclusion the results of the present study show that notably the intraabdominal fat compartment is diminished and the hepatic leptin expression is gender-specific altered in tgHD rats. By interacting with other components of the energy homeostasis (e.g. NPY or ghrelin) leptin may play a role in the weight loss of subjects with HD.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE BRADYKININ-DEPENDENT PHOSPHORYLATION OF ERK1/2 IN NERVE FIBERS OF THE DENTIN-PULP COMPLEX

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Adressen:(1)Heinrich-Heine-University|Department of Operative and Preventive Dentistry and Endodontics|Düsseldorf|Germany; email:yueksel.korkmaz@uni-duesseldorf.de ; (2)University of Cologne|Department I of Anatomy|Cologne|Germany; (3)German Sports University|Department of Molecular and Cellular Sports Medicine|Cologne|Germany

Abstract:

Extracellular signal-regulated protein kinase (ERK1/2) is a mitogen-activated protein kinase (MAPK) that transduces extracellular stimulation into intracellular post-translational and transcriptional responses. In addition to the regulation of gene expression, cell proliferation and cell differentiation, the ERK1/2 have been implicated in the inflammation-dependent sensitization of nociceptors. The exact transduction mechanism of nociception in dentin-pulp-complex is still not clear and the inflammation-dependent modulation of the ERK1/2 in nerve fibers of the dentin-pulp complex is unknown. To test whether peripheral inflammatory stimuli induce activation of ERK in nerve fibers of the dentin-pulp complex, rat (n=4) molars without apical roots were treated for 1, 3 and 10 minutes with bradykinin (BK) (10^{-7} M) using organ bath experiments. After BK-treatment, the molars were immersion-fixed, decalcified, frozen-sectioned and analysed by quantitative immunolabelling. The numbers of p-ERK1/2 immunoreactive nerve fibers are increased within 3 minutes. After 10 minute BK-treatment there is a clear decrease in the number of ERK1/2 positive nerve fibers. These data indicate that basal activation of ERK1/2 localized in nerve fibers of the dentin-pulp complex and modulated by BK in a time-dependent manner. The role of ERK1/2 in pain signalling in the dentin-pulp complex nerve fibers is going to be clarified by our further experiments using double labelling for a nerve fiber typ localization of ERK1/2 in response to BK.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:A CADHERIN-BASED CODE FOR THE DIVISIONS OF THE MOUSE STRIATUM

Autoren: Hertel N.(1),K. K.(1),Nuernberger M.(1),Redies C.(1),

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Abstract:

The grey matter of the mammalian striatum is organized into patches ("striosomes") that are surrounded by a histologically uniform matrix. The two striatal compartments have different connections with various brain regions. The molecules, which regulate the morphogenesis and functional connectivity of the striatum, are largely unknown. Cadherins, a large family of adhesion molecules, are identified as important regulators for the development of the central nervous system. In the present study, the expression of twelve classic cadherins and delta-protocadherins was mapped in consecutive series of the postnatal and adult mouse striatum by in situ hybridization. Our results show that the patch and matrix compartments of the striatum express the cadherins differentially. Within the striatum, the cadherins are expressed in multiple, diverse and partially overlapping gradients. These gradients possibly relate to the different cortico-striatal connectivities of the matrix regions. The expression patterns persist in the adult striatum, indicating a role of cadherins also in the mature brain. Together, our results provide evidence that cadherins represent a molecular code for the compartments of the mouse striatum and possibly also for the functional differentiation and connectivity of this brain region.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:IDENTIFICATION OF THE MELATONIN RECEPTOR SUBTYPE INVOLVED IN THE PHOTOPERIODIC RESPONSE IN MAMMALS

Autoren: Yasuo S.(1),Korf H.(1),

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Abstract:

Reproduction is under photoperiodic control in most mammals. Photoperiod is transmitted by melatonin secreted from the pineal gland. Melatonin acts within the mediobasal hypothalamus, where the melatonin signal is converted to the expression levels of key genes for the photoperiodic gonadal response, type 2 and type 3 deiodinase (Dio2 and Dio3, respectively). In mammals, melatonin elicits its biological effects through two melatonin receptor subtypes, Mel1a and Mel1b. Here we investigated the functional importance of each receptor subtype for the photoperiodic response, using transgenic mice lacking the Mel1a-, the Mel1b-, or both receptor types. Expression of Dio2 and Dio3 under long- and short- day conditions was examined to determine the photoperiodic responsiveness. In wild type mice, Dio2 mRNA was observed in 1) in the ependymal cell layer lining the ventrobasal parts of the third ventricle (EC), and 2) in an area adjacent to the tuberoinfundibular sulcus. Dio3 mRNA was observed only in the EC. Dio3 mRNA levels were significantly higher in short day- than in long day- conditions. In both animal groups, no significant difference was detected in Dio2 mRNA levels. In Mel1a deficient mice, the photoperiodic changes in Dio3 expression were completely abolished and Dio2 expression was constitutively activated. Similar results were obtained in mice lacking both receptor types. In contrast, deletion of the Mel1b had no significant effects on the Dio2 expression. These data suggest that photoperiodic melatonin signals are mainly transmitted via the Mel1a receptor subtype.

*Supported by JSPS Postdoctoral Fellowships for Research Abroad

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE ROLE OF MPER1 IN THE REGULATION OF MELATONIN SYNTHESIS

Autoren: Christ E.(1),von Gall C.(1),Korf H.(2),

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email:E.Christ@med.uni-frankfurt.de; (2)J. W. Goethe Universität|Dr. Senckenbergische Anatomie, Institut für Anatomie II|Frankfurt am Main|Deutschland

Abstract:

The hormone melatonin is an important endocrine signal for darkness in all mammalian species. The rhythmic synthesis of this hormone in the pineal gland is driven by a master clock residing in the suprachiasmatic nucleus (SCN). Nocturnally low SCN activity gives rise to the release of norepinephrine from sympathetic nerve endings reaching the pineal gland. In mice, the activation of α -adrenergic receptors on the pinealocyte membrane leads to the elevation of intracellular cAMP and subsequently to the transcriptional activation of the penultimate enzyme for melatonin synthesis, the arylalkylamine N-acetyltransferase (AANAT). The transcriptional repressor mPER1 has been shown to be involved in the regulation of rhythmic gene expression in a variety of tissues and is strongly increased in the pineal gland during night time. However, the role of mPER1 in the regulation of melatonin synthesis is still unknown. Therefore, we compared circadian rhythms in the expression of Aaanat mRNA, AANAT enzyme activity and serum melatonin concentration in mice with a targeted deletion of the mPer1 gene (mPER1^{-/-}) and wild type (WT) mice. We found that Aaanat expression and AANAT enzyme activity during late night and early morning to be significantly elevated in mPER1^{-/-} mice compared to WT. This suggests an important role of mPER1 in the down regulation of Aaanat transcription.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:ENRICHED CULTURES OF ADULT CANINE SCHWANN CELLS SUITABLE FOR CELL TRANSPLANTATION IN PERIPHERAL NERVE REPAIR AND GENETIC MODIFICATION

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Abstract:

Schwann cells (SC) are crucially involved in recovery of peripheral nerves after injury and therefore adult SC are most interesting tools for autologous cell transplantation within nerve interponates for peripheral nerve repair after traumatic nerve lesion. Dogs get often injured in car accidents followed by complete function loss of one extremity. To provide adult SC for peripheral nerve repair in dogs, we established a system to culture adult canine SC in high purity. Evaluation of culture conditions for tissue predegeneration in vitro and different enrichment techniques resulted in an easy to use protocol. The well established use of melanocyte growth medium supplemented with forskolin, FGF-2 and pituitary extract as a selective, serum-free, culture medium for adult SC was combined with secondary enrichment, by immunopanning technique (Dynalbeads) to remove contaminating fibroblasts. Cultures were evaluated by immunocytochemical methods with regard to SC purity. Percentage of adult canine SC increased from 29.6 ± 6.6 % (mean \pm SD) after tissue dissociation up to 63.5 ± 11.7 % after one enrichment step and up to 78.8 ± 6.0 % following a second enrichment step. Enriched SC cultures are potential tools for transplantation of tissue engineered nerve grafts in peripheral nerve repair. Feasibility of genetic modification of adult canine SC is under current investigation. This approach would enable ex vivo gene therapy in peripheral nerve reconstruction in dogs.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:DISTRIBUTION PATTERN OF THE CGRP1 RECEPTOR SUBUNITS, RAMP1 AND CL RECEPTOR, IN THE NON-INFECTED AND SCHISTOSOMA MANSONI-INFECTED ILEUM OF WILD-TYPE AND C-KIT-DEFICIENT MICE

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Abstract:

In mouse, the acute phase of intestinal schistosomiasis is characterised by massive recruitment of mucosal mast cells (MMCs), in close association with extrinsic CGRP-expressing afferent nerve fibres. To unravel the distribution of CGRP receptors, we studied the protein and mRNA expression of the CGRP1 receptor subunits, RAMP1 and CL receptor (CLR), in non-inflamed and 8-week infected ileum of wild-type and c-Kit-deficient mice. RT-PCR revealed the mRNA expression of both subunits, while immunofluorescence showed the presence of both subunits in intrinsic and extrinsic enteric nerve fibres in the ileum of all murine types studied. In infected wild-type and Kit^{Wsh/Wsh} mice, a prominent network of RAMP1⁺/CLR⁺-ir (CGRP1 receptor-ir) nerve fibres surrounding the crypts of Lieberkühn was detected. Recruited MMCs did not express CGRP1 receptor subunits. In conclusion, expression of the CGRP1 receptor subunits in the ileal mucosa is increased in infected animals, but the distribution of these subunits and the degree of sprouting of CGRP-ir nerve fibres in the inflamed ileum are not altered by the presence or absence of MMCs. Although this study suggests a substantial role of CGRP1 receptor-mediated neuronal pathways in pathophysiological circumstances in the murine ileum, the present data support the hypothesis of Mousli and coworkers (1993) that CGRP-induced mediator release by MMCs is not a receptor-induced response, but involves a peptidergic pathway mediated by Gi-proteins (Ferry et al. 2002) and are inline with our previous in vitro data showing that exogenously applied CGRP leads to MMC secretion (De Jonge et al. 2004).

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Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE ULTRASTRUCTURE OF PERIPHERAL NERVE DEGENERATION AND REGENERATION: ARE SCHWANN CELL BASAL LAMINAE REALLY SCAFFOLD AND GUIDING TUBES FOR AXONAL REGENERATION?

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Abstract:

We have investigated the time course of ultrastructure of the distal part of the facial nerve in rats during degeneration after axotomy without nerve repair and during regeneration after immediate or delayed hypoglossal-facial crossed nerve suture (HFA). In 98 adult female Wistar rats we studied 4 ultrathin cross sections of each operated nerve through its the buccal and marginal mandibular branches 12mm and 18mm distal to the site of HFA or permanent axotomy. In addition to the well described phenomena of Wallerian anterograde degeneration such as macrophage invasion, demyelination and Schwann cell proliferation forego axonal sprouting (detailed monograph: G. Lundborg: Nerve injury and repair. 2nd ed., Elsevier 2004) we have observed quite drastic remodelling of the bands of Büngner and of the basal lamina tubes ensheathing them. During axonal outgrowth in the course of regeneration the bands of Büngner frequently split, but are at first still surrounded by one common basal lamina tube. Later this tube becomes fragmented and each fragment forms a new basal lamina tube around the now fully separated bands of Büngner and the regenerating axons attached to them. There is considerable plasticity of Schwann cell basal laminae during regeneration. These new findings are contrary to the general classical view (cf. Lundborg 2004) of Schwann cell basal laminae as a rather static scaffold of tubes guiding axons to same target as the original axon of that band of Büngner had innervated before the lesion.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:MILD PRE- AND POST-OPERATIVE TREADMILL TRAINING OF RATS IMPROVES MUSCLE REINNERVATION AFTER DE-LAYED REPAIR OF THE SCIATIC NERVE BY GRAFTING 11 MONTHS AFTER AXOTOMY

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Abstract:

Before and after surgery of the sciatic nerve rats were trained by treadmill-running and tested, whether training improves motor reinnervation and walking. In 26 adult Lewis rats we transected the right sciatic nerve. After 9 months of denervation 16 rats were trained (T) by walking 2x50 m/day at 15 cm/s and 10 weeks later (11 months post-axotomy) the right sciatic nerve was reconstructed with a 10 mm long congenic allograft. After 13 weeks of regeneration with identical training the evoked compound muscle-action potential (CMAP) of the soleus muscle was recorded. Then the soleus branch of the tibial nerve and the fibular communal nerve were cut and retrogradely labelled by application of Fast-Blue and Dil crystals, respectively. In 10 untrained rats (UT) the same recording and labeling was performed. Walking tracks were analysed before and after nerve transplant and the Sciatic Functional Index (SFI) was calculated. In normal rats the SFI was around 0, after sciatic nerve transection this value dropped to -46 (T) and -55 (UT) and was further reduced to -64 (T) and -59 (UT) after transplant. Dil plus Fast-Blue labelled 549±83 motoneurons in normal rats (mean±SD), 319±100 regenerated motoneurons in T, but only 216±71 in UT. The amplitude of the CMAP was 16.5 mV in normal rats, 4.0 mV in UT, but 8.1 mV in T. The latency of muscle contraction was 2.1 ms in normal rats, 3.2 ms in UT, but 2.3 ms in T. Mild training of 500 m walking per week before and after sciatic nerve transplant raised the number of regenerated motoneurons by 48%, doubled CMAP and almost normalized the latency of soleus muscle contraction.

Kategorie: Poster

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Rubrik: 3.Neuroanatomie/Neurobiologie

Abstract Nr.:3

Titel:THE SPINAL MUSCULAR ATROPHY PROTEIN AND THE ACTIN CYTOSKELETON

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Abstract:

Childhood spinal muscular atrophy is an autosomal, recessive disorder leading to a specific degeneration of motoneurons in the spinal cord and brain stem. The disease is caused by loss or mutation of the survival of motoneuron 1 (SMN1) gene and consequential reduction of the SMN protein level. The SMN protein mediates the assembly of small nuclear ribonucleoproteins (snRNPs) involved in splicing and participates in different processes within the nucleus. Furthermore, recent findings point to a new and additional function of the SMN protein in neurites. We have shown previously that SMN protein levels influence directly neurite outgrowth. ShRNA mediated knock-down of SMN led to shorter neuronal extensions and disturbed the dynamics of the actin cytoskeleton.

We have now demonstrated for the first time that the SMN protein interacts with actin in a common complex. The SMN protein co-localizes with monomeric G-Actin but not with filamentous (F) actin in growth cones of differentiated PC12 cells and at the leading edge of primary human fibroblasts. An interaction of both proteins could be confirmed by co-immunoprecipitation and pull-down experiments. Recent data of our group show that reduction of the endogenous SMN protein level had severe effects on the balance of the G- / F-actin ratio and caused hyperstabilization of filamentous actin. Our findings support the hypothesis of an important interaction of the SMN protein with the actin cytoskeleton.

Kategorie: Poster