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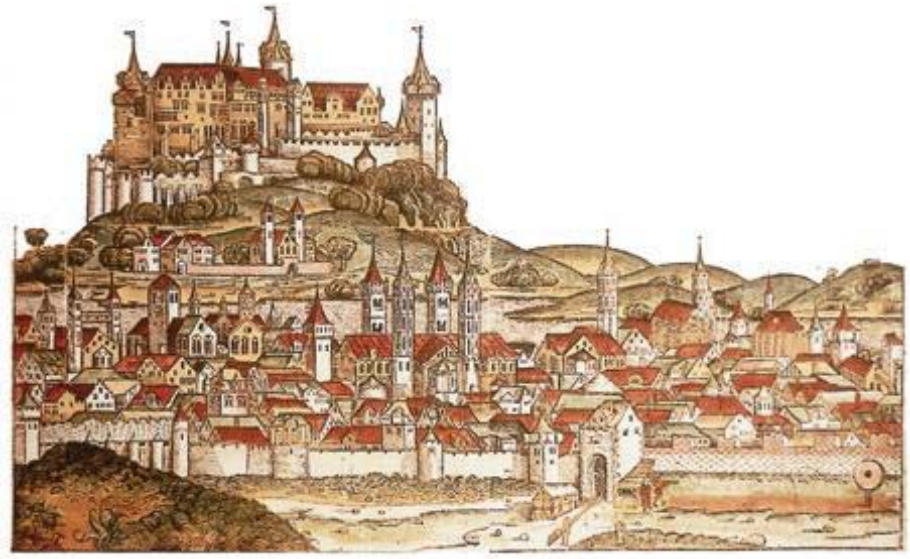
Zusammenfassung aller Vortrags- und Posterabstracts der 23. Arbeitstagung der Anatomischen Gesellschaft in Würzburg vom 27.09. bis 29.09.2006



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Vortrag 1

Characterization of cadherin-11 binding properties in the context of synaptic plasticity

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There is increasing evidence that the Ca^{2+}-dependent cell adhesion molecules, cadherins, are involved in modulation of synaptic structure, function and induction of long term potentiation (LTP). Interestingly, inhibition of N-cadherin function leads to suppression of LTP whereas loss of cadherin-11 enhances LTP formation. This study was performed to identify differences in the binding properties of cadherin-11 and N-cadherin focusing on the Ca^{2+}-dependency of their adhesive activities, because Ca^{2+}-influx (accompanied by a transient drop of Ca^{2+} in the synaptic cleft) is a key event of LTP induction. Cadherin-11-Fc fusion protein single-molecule AFM measurements revealed a Ca^{2+}-dependent binding activity of cadherin-11 with half maximal adhesion (K_D) of 0.2 mM Ca^{2+} and low negative cooperativity (Hill coefficient, n_H = 0.6). These data were confirmed by laser tweezer experiments which allow quantification of cadherin-11 binding between cadherin-11-coated microbeads and cadherin-11 expressed on cultured MDA-MB-231 cells (K_D = 0.1, n_H = 0.9). In addition, cadherin binding might also be modified by disconnection of cadherins from the actin filament cytoskeleton after induced intracellular increase of [Ca^{2+}]. Application of the Ca^{2+}-ionophore A23187 caused significant reduction of bead binding to 29% in laser tweezer studies. In conclusion, the observed biophysical properties of cadherin-11 differ profoundly from those of N-cadherin (K_D = 0.7 mM Ca^{2+}; n_H = 2). Whereas N-cadherin will be significantly reduced at Ca^{2+}-concentrations between 0.3 and 0.8 mM (supposed concentrations in the synaptic cleft during induction of LTP), cadherin-11 binding will remain unchanged due to its low Ca^{2+}-affinity. This suggests a stabilizing role for cadherin-11 during synaptic activity whereas N-cadherin–mediated adhesion becomes modulated by synaptic activity and would allow activity-dependent shape changes underlying synaptic plasticity.

Vortrag 2

Getrennte Funktionen von Neuroligin 1 in Synaptogenese und Synapsenreifung

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Synapsen des zentralen Nervensystems bestehen aus zwei Kompartimenten: den in Axonen lokalisierten präsynaptischen Spezialisierungen, die Neurotransmitter freisetzen, und den in Dendriten gelegenen postsynaptischen Spezialisierungen, die Neurotransmitterrezeptoren beinhalten. Wie diese beiden Spezialisierungen generiert werden und wie sie funktionell reifen, ist Gegenstand unserer Forschungsarbeit.

In Zellkultur auswachsende Axone sind in relativ frühen Differenzierungsstadien in der Lage, präsynaptische Kompartimente zu bilden, während Dendriten die Fähigkeit zur Synaptogenese später entwickeln. Als Folge daraus entstehen Synapsen nur bei Kontakt von Axonen mit Dendriten eines gewissen Reifegrades. Wir zeigen hier anhand von Primärkulturen aus dem Hippocampus, dass das dendritische Transmembranprotein Neuroligin in der Lage ist, die Bildung präsynaptischer Spezialisierungen in jungen Axonen zu induzieren. Wir zeigen weiterhin, dass diese neu gebildeten Orte der Neurotransmitterfreisetzung einen unerwartet fortgeschrittenen Differenzierungszustand aufweisen. Ferner finden wir, dass die extrazelluläre Domäne von Neuroligin die Neubildung präsynaptischer Spezialisierungen bewirkt, während die intrazelluläre Domäne deren funktionellen Zustand mitbestimmt. Unsere Studie legt ein Modell für die molekularen Mechanismen einer doppelten Funktion von Neuroligin 1 nahe.

Vortrag 3

Transforming growth factor-beta 2 (TGF-beta2) is required for the development of functional synapses.

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Synaptogenesis is a complex process that is regulated by different factors. Recently, studies in drosophila mutants showed that different components of the transforming growth factor-beta (TGF-beta)-signalling pathway play a crucial role in the development of synapses. TGF-betas constitute a superfamily of cytokines that are involved in different aspects of development, including cell proliferation, differentiation, survival and apoptosis. Although their role in vertebrate synaptogenesis is discussed, it has not been analysed yet. In the present work, the influence of TGF-beta2 in synaptogenesis is explored in embryonic TGF-beta2 -/- mice at E18.5. As these mice die perinatally due to hypoxia, the peripheral and central respiratory system was investigated. This included whole-mount analysis of the diaphragm with staining for the phrenic nerve and acetylcholine receptor-clusters (AChR-clusters). Further, functional analysis was performed in acute slices of the pre-Boetzinger-complex in the ventral medulla which contains the respiratory rhythm-generating network using patch-clamp recordings. The results showed no obvious alteration of the branching of the phrenic nerve, but a pronounced fibre disorganisation of the diaphragm muscle. Additionally, there was a significant decrease in the number of AChR-clusters in the knockout. Moreover, inhibitory synaptic transmission in the acute slices was fairly diminished in TGF-beta2-/- compared to control mice. Together, these results suggest that the loss of inhibitory synaptic transmission might cause the lethal phenotype of TGF-beta2-/- mice and they provide first insight into the putative role of TGF-beta2 in synaptogenesis.
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Vortrag 4

FLASH is a component of synapses and mitotic centrosomes

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FLASH (Flice-associated huge protein) is a 219 kDa protein that interacts with pro-caspase 8 and other components of the death-inducing signalling complex involved in Fas-induced apoptosis. In addition, FLASH has been implicated in TNFα-mediated cell survival, indicating that, depending on specific signals, FLASH can profoundly influence the life of a cell. We have previously shown that FLASH is also a component of synapses in postmitotic neurons, at which it is present as a 21 kDa isoform. Whether FLASH is involved in neuronal cell death remains to be shown. Recently, a screen using short interfering RNAs has identified FLASH as an essential polypeptide in HeLa cells. Upon down-regulation of FLASH, HeLa cells survive until the beginning of mitosis but then die of apoptosis. Here, we examined FLASH's role in the cell cycle in more detail.

We report that FLASH has a dual distribution in HeLa cells. In interphase, it was detected in speckled compartments of the nucleus, but during mitosis FLASH was dramatically redistributed and colocalised precisely with γ-tubulin, suggesting that it is a component of mitotic centrosomes. Overexpression of FLASH deletion constructs in COS-7 cells showed that centrosomal targeting was mediated by an N-terminal region including a coiled coil motif. Finally, FLASH was found to interact with tubulin, as shown by copolymerization assays and by pharmacological interference with the stability of tubulin.

Our data suggest that the FLASH may be an essential microtubule-associated component of the mitotic centrosome. Current in-vitro mutagenesis and loss-of-function studies are designed to examine i) whether the N-terminal coiled-coil is indeed a critical part of FLASH, ii) which coiled-coil protein interacts with FLASH, and iii) to which molecular event(s) FLASH contributes during the cell cycle.

Vortrag 5

Polysialylierung des neuralen Zelladhäsionsmoleküls (NCAM) ist an der korrekten synaptischen Verschaltung beteiligt

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Die posttranslationale Polysialylierung, die ausschliesslich am neuronalen Zelladhäsionsmolekül (NCAM) stattfindet, ist entwicklungsabhängig reguliert und weist ein perinatales Maximum auf. Dieser Verlauf korreliert zeitlich mit der Zielfindung axonaler Projektionen und der Ausbildung synaptischer Kontakte. Die Synthese der Polysialinsäuren (PSA) erfolgt über zwei Polysialyltransferasen ST8Sia2 und ST8Sia4. Während ST8Sia2 hauptsächlich während der Entwicklung exprimiert wird, ist ST8Sia4 vor allem im adulten Gehirn nachweisbar. Nach Behandlung hippocampaler Schnittkulturen mit 8 mM des biochemisch hergestellten Sialinsäurevorläufers N-Propanoylmannosamin konnten wir die Synthese von PSA hemmen. Diese Hemmung der NCAM-Polysialysierung führt zu aberranten Moosfaseraxonen ins Stratum pyramidale von CA3. In neuen Untersuchungen können wir zeigen, daß Moosfaseraxone nach Inhibition der PSA-Synthese an CA1 Pyramidenzellen funktionelle Synapsen bilden. Elektrophysiologische Untersuchungen an diesen Moosfaser-CA1-Pyramidenzell-Synapsen belegen, dass der präsynaptische Bouton seine charakteristischen elektrophysiologischen Eigenschaften an der falschen Zielzelle (CA1 statt CA3) beibehält. *In vivo* führt die Hemmung der PSA-Synthese zu Resultaten, die mit jenen aus *in vitro* Untersuchungen vergleichbar sind. Typischerweise gleicht *in vivo* das Muster aberranter Mossfaseraxone jenem nach Inaktivierung des ST8Sia2-Gens.
Unsere Befunde belegen, dass die Polysialysierung von NCAM bei der korrekten synaptischen Verschaltung eine wichtige Rolle spielt und dass die physiologischen Eigenschaften der Moosfasersynapsen trotz aberranter Termination beibehalten werden.

Vortrag 6

The spinal muscular atrophy gene product regulates neurite outgrowth

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Spinal muscular atrophy (SMA) is a neurodegenerative disease accompanied by a massive loss of motoneurons. Mutations or deletions of the survival of motoneuron (*SMN*) gene are responsible for this defect. However, it is unclear which dysfunctions of SMN are important for disease progression. SMN has been well characterized as an assembly protein for small nuclear ribonucleoprotein particles (snRNPs) involved in splicing. To fulfill this function, SMN is associated with proteins in the so called “SMN-complex”, e.g. the Gemin-proteins. Is this SMN-Gemin complex responsible for the SMA phenotype? In our study, we analyzed the effects of different proteins of the complex with regard to neurite outgrowth in PC12 cells by a combination of strategies involving knock-down by siRNAs, over-expression and rescue experiments.

First, we analyzed expression of SMN and some of its interaction partners during neuronal differentiation. SMN expression was not co-regulated with expression of partner proteins of the SMN-complex, indicating different functions in this developmental context. Second, we evaluated the effects of SMN knock-down. Suppression of endogenous SMN protein levels decreased significantly growth of neurites. Interestingly, down-regulation of the interacting protein gemin2 had the opposite effect. Neurite outgrowth is tightly associated with actin dynamics. The SMN knock-down led to a significant change of the G-/F-actin ratio indicating and defining a new role of SMN in actin metabolism. Selective overexpression of the SMN C-terminal domain promoted neurite outgrowth similar to full-length protein and could rescue the SMN knock-down effects. Therefore, our data suggest a functional role of SMN in microfilament dynamics in neurites.

Vortrag 7

Ciliary neurotrophic factor (CNTF) stimulates neural stem cell renewal in neurosphere cultures.

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A remarkable property of the adult nervous system is the continuous generation of new neurons in the subventricular zone of the forebrain and the subgranular zone of the dentate gyrus. It is unknown to date which molecular mechanisms regulate self-renewal of multipotent neural stem cells, their transition into more restricted and more rapidly proliferating progenitor cells and the differentiation into mature neurons. We have previously shown that members of the IL-6 family of cytokines via signaling by the JAK/STAT-pathway play a crucial role in regulating neurogenesis in the adult nervous system. We now extended these studies to an in vitro system, namely cultures of neural stem cells growing as neurospheres, to elucidate this effect of IL-6 cytokines in more detail. When neural stem cells derived from the adult subventricular zone are cultured in the presence of CNTF or LIF, proliferation is reduced as compared to control cultures. However, when neurospheres grown in the presence of CNTF/LIF are redissociated and cultured in the absence of these growth factors, they produce a higher number of secondary spheres. This indicates that in the presence of CNTF/LIF a higher number of stem cells, capable of forming new neurospheres were generated. Immunostaining with antibodies for neural stem cells showed that their number increased in the presence of CNTF. In addition, quantitative PCR analysis of genes expressed in stem cells or their progeny showed that CNTF-treatment increased expression of stem cell markers and reduced expression of differentiation markers. Finally, using neurosphere cultures derived from mouse mutants with impaired JAK/STAT3-signaling corroborated these findings.
In summary our results suggest, that CNTF and related cytokines regulate the size of the stem cell pool at the level of the cell cycle, probably by favoring symmetrical over asymmetrical cells divisions.
(Supported by Deutsche Forschungsgemeinschaft, DFG)

Vortrag 8

PRG-1/Ras-GRF2 Proteininteraktion kontrolliert axonales Wachstum über die Deaktivierung von N-Ras

PRG-1/Ras-GRF2 interaction controls axonal elongation via N-Ras deactivation

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Plasticity-related gene-1 (PRG-1) was the first member of the plasticity-related gene family (PRG-1-5) to be identified and belongs to the LPP family. The latter is characterized by its six transmembrane domains, ectoenzymatic activity on the external surface of the plasma membrane, as well as intracellularly located N- and C-terminals. PRG-1 is expressed in the brain from postnatal stages to adulthood and *in vivo* data shows an up-regulation after brain lesion. Cell culture experiments show that PRG-1-overexpressing cells attenuate LPA-induced neurite retraction. To elucidate the associated pathway, a yeast two-hybrid screening was performed, in which one of the putative interaction partners was Ras-specific exchange factor 2 (Ras-GRF2). Ras-GRF2 belongs to a family of calcium/calmodulin-regulated guanine nucleotide exchange factors that activate Ras proteins. In particular, Ras-GRF2 can activate Ras-GTPases via their Cdc25-like catalytic domains. The signals involved in Ras-GRF2 activation have not been fully characterized, but its key role in mediating neuronal functions, such as neurite growth, is clear.
Using co-immunoprecipitation assays, our experiments confirmed the interaction between PRG-1 and Ras-GRF2 in mammalian cells. Ras-activation assays demonstrated clear inhibition of the N-Ras protein, an interesting finding since the latter is known to induce neuronal outgrowth. We consequently performed morphology analyses on primary neurons, overexpressing and knocking down PRG-1, using an siRNA technique. Initial results show that PRG-1 overexpression leads to a decrease in axon length. Further studies will be conducted to determine whether the molecular trigger of PRG-1/Ras-GRF2 interaction is extracellularly or intracellularly located. Data already at hand indicates an LPA dependency. Our discussion of the findings focuses on the underlying molecular signaling pathways.

Vortrag 9

The ins and outs of SGLT1: Constitutive cycling of the sodium / D-glucose cotransporter and its regulation by Rab-GTPases

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The sodium/D-glucose cotransporter SGLT1 plays a central role in the reabsorption of D-glucose in small intesine and kidney. We studied post-translational regulation and trafficking of endogenous SGLT1 in Caco-2 cells, a model for human enterocytes. The investigation of fluorescently labeled SGLT1 in Caco-2 cells revealed that SGLT1 is endo-/exocytosed under basal conditions, thus undergoes constitutive cycling. Inhibition of endo-/exocytosis with tannic acid (0.5 mM) significantly reduced (50% inhibition after 30 min) sodium-dependent D-glucose uptake into Caco-2 cells. To elucidate the molecular mechanisms of SGLT1 regulation we screened a Caco-2 cell cDNA library for SGLT1 interacting proteins using the yeast two hybrid system and identified the small GTPase Rab5a. SGLT1-Rab5a association was further confirmed by co-immunoprecipitation from Caco-2 cell lysate and by an *in vitro* binding assay. Rab5a regulates clathrin-coated pit mediated endocytosis from the plasma membrane to early endosomes. SGLT1 exocytosis from early endosomes to the plasma membrane can occur via a direct route (Rab4-dependent), or via recycling endosomes (Rab11-dependent). Transfection of Caco-2 cells with the dominant negative mutant Rab11(S25N) did not alter the distribution of endogenous SGLT1, whereas transfection with dominant negative Rab4(S27N) lead to an accumulation of SGLT1 in Rab4-positive endosomes. Our data suggest a constitutive cycling of SGLT1 between the plasma membrane and early endosomes, whereby the endocytosis step involves clathrin coated vesicles and a regulation by Rab5a. Exocytosis from early endosomes to the plasma membrane follows a direct route and is regulated by Rab4. Furthermore, cycling of SGLT1 between the plasma membrane and intracellular sites is essential for the sodium dependent uptake of D-glucose into Caco-2 cells. A more detailed knowledge of SGLT1 trafficking and regulation in epithelial cells may provide clues for novel therapies for trafficking diseases such as the glucose galactose malabsorption syndrome.

Vortrag 10

Proteome analysis in podocytes: Downregulation of specific annexins by high glucose

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Diabetic nephropathy is one of the most common complications of diabetes mellitus and the leading cause of end-stage renal disease. A reduction in the number of podocytes and podocyte density has been documented in the kidney of these patients. The main symptom is an elevation of glucose concentration resulting in cellular changes. To better understand the molecular changes leading to loss of podocytes induced by diabetes, the purpose of the present study was to investigate the protein profile in podocytes under long term high glucose exposure (30 mM for 2 weeks) as compared to that of podocytes under control conditions (10 mM) by proteomics. The proteins were separated by two-dimensional gel electrophoresis and identified by peptide mass fingerprinting using MALDI-TOF/TOF mass spectrometry and Mascot software. Among the 1547 spots that were detected on the gel, about 200 spots were selected for identification. 78 of the 149 proteins identified were differentially expressed: 13 proteins were significantly upregulated (2-4.5 fold) and 65 proteins were significantly downregulated (2-10 fold) under high glucose exposure. Among the differentially expressed proteins, annexin III and VI were significantly downregulated under high glucose conditions. Members of the annexin family are known to be importantly involved in membrane organization. Expression of annexin II, III, IV, V, VI and VII in podocytes was verified by RT-PCR and immunostaining. Real time RT-PCR confirmed the downregulation of annexin III and VI (50 and 76%, respectively) in podocytes cultivated in high glucose medium for 2 weeks. Our data show that high glucose concentrations strongly modify the protein expression pattern of podocytes. Downregulation of annexins may alter membrane organization of podocytes in diabetes.

Vortrag 11

Die Rolle von Megalin auf den luminalen Transport des proximalen Tubulus

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Apikal lokalisierte Ionentransporter und –kanäle des proximalen Tubulus werden durch zelluläres Trafficking in ihrer Aktivität gesteuert. Hierbei beteilgte, interagierende Proteine sind noch unzureichend bekannt. Das 600 kDa Membranprotein Megalin ist als „Scavenger-Rezeptor“ des proximalen Tubulus der Niere mit 4 heterogenen, extrazellulären LDL-Bindungsdomänen für die Resorption vieler strukturell verschiedener Proteine und Peptide aus dem Ultrafiltrat verantwortlich. Interaktionen von Megalin und den Transportern wurden postuliert.

Wir untersuchen die Hypothese, dass Megalin bei Translokationsvorgängen der Bürstensaum-Membran (BBM)-Proteine Na^+/H^+-Austauscher 3 (NHE3), Na^+, PO^{--}-Kotransporter IIa (NaPi-IIa) und Aquaporin1 (AQP1) eine Rolle spielt. Wir gehen weiterhin der Frage nach, welche Bedeutung diese Interaktion bei pathophysiologischer Belastung (Proteinurie) spielen kann. Hierzu wurde in Kontrollmäusen und in Mäuse mit konditionellem, nierenspezifischen Knockout für Megalin eine Proteinurie induziert. Die Nieren wurden für die histochemische Auswertung perfusionsfixiert oder für die biochemische Analyse entnommen. Immunhistochemie, Immunelektronenmikroskopie, In situ Hybridisierung, Real time-PCR und Ko-Immunpräzipitation, Pull down assays and Western blots wurden durchgeführt. Bindungsanalysen ergaben eine spezifische Wechselwirkung von NHE3, NaPi-IIa und AQP1 mit Megalin. Für NHE3 und AQP-1 konnte die Bindung an alle 4 Bindungsdomänen von Megalin, für NaPi-IIa nur an die 4 Domäne gezeigt werden. In Megalin-defizienten Tieren zeigte sich eine verstärkte BBM-Lokalisation von NHE3, NaPi-IIa und AQP1. Diese war unter Proteinurie bei NaPi-IIa und AQP1 verringert, nicht jedoch bei NHE3. Wir schlussfolgern, dass Megalin spezifisch an der Translokation der proximal tubulären Transporter und Kanäle beteiligt ist und somit zur Regulation des Salz- und Wasserhaushaltes beiträgt. Eine Proteinüberladung, häufig bei vielen nephrologischen Erkrankungen, führt zu einer Megalin-abhängigen Steigerung von NHE-3. Dies könnte eine entscheidende pathogenetische Rolle für die Volumenretention bei proteinurischen Erkrankungen spielen.

Vortrag 12

Rho and Rac differentially regulate barrier functions dependent on the origin of endothelial cells

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It is generally believed that Rho A activation leads to endothelial barrier breakdown while Rac1 stabilizes endothelial barrier properties. This concept emerged primarily based on studies using macrovascular endothelium. We provide evidence that roles of Rho-GTPases in endothelial barrier regulation fundamentally differ dependent on the origin of endothelial cells. Activation of Rho A induced stress fiber formation in three different cell lines. This was paralleled by barrier breakdown in macrovascular pulmonary artery endothelial cells (PAEC) and microvascular mesenteric endothelial cells (MesEnd) but not in microvascular myocardial endothelial cells (MyEnd). Moreover, activation of Rac1 and Cdc42 increased permeability in PAECs but not in MesEnd cells and even strengthened barrier properties in MyEnd cells. Inactivation of Rho GTPases impaired barrier function in all cell lines studied. These data indicate that a general antagonistic function of Rho A and Rac1 is not given.

Vortrag 13

T-Helferzellen fördern axonale Regeneration nach Cortex- und Rückenmarksverletzung durch Interleukin-4-abhängige Neurotrophinrezeptor-Hochregulation

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Die Rolle von T-Helferzellen in der axonalen Regeneration nach mechanischer Hirn- und Rückenmarksschädigung ist bisher unverstanden. Wir konnten zeigen, dass T-Helferzellen vom Subtyp 2 (Th2) axonale Regeneration *in vitro* und *in vivo* fördern. Nach experimenteller Kompressionsverletzung des Rückenmarks *in vivo* führte die Injektion von Th2-Zellen (im Gegensatz zu Th1-Zellen) in die Läsionsstelle zu hochsignifikanter Steigerung der regenerierenden Nervenfasern distal der Läsionsstelle sowie zu einer signifikanten Steigerung der Nervenfasern, die die Mittellinie überschreiten. Ebenso steigerte die intrazerebrale Injektion von Th2-Zellen nach entorhinaler Cortex-Läsion (ECL) die Expression des Proliferationsmarkers GAP-43 in der Molekularschicht des deafferentierten Gyrus dentatus sowie die Expression der Neurotrophinrezeptoren (NTR) TrkA, B, C und p75NTR. *In vitro* zeigte sich in Auswachs- und Reinnervationsassays, dass aktivierte Th2-Zellen axonale Regeneration fördern, während naive, unstimulierte T-Zellen oder CD28-aktivierte regulatorische T-Zellen keinen Effekt zeigen. Ferner reduzierte weder die Anreicherung noch die Depletion der IL-10-produzierenden Th2-Zellen die axonale Wachstumsstimulation. Hingegen blockiert die Inhibition von Interleukin-4 sowohl die NTR-Hochregulation als auch die Stimulation des Axonwachstums.

Diese Daten zeigen, dass aktivierte Th2-Zellen *in vitro* und *in vivo* durch IL-4-Sekretion die Neurotrophinrezeptoren hochregulieren und nach traumatischer Hirn- und Rückenmarksläsion die axonale Regeneration fördern.

Vortrag 14

Reduction of secondary neuronal damage by the immunosuppressant mycophenolate mofetil as shown in an in vivo model of controlled cortical impact injury

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Lesions of the CNS induce specific morphological and functional changes of glial cells such as proliferation, cell shape transformation and secretion of proinflammatory cytokines and NO. It has been suggested that suppression of glial cell activation in the aftermath of the neuronal injury inhibits the development of secondary damage and contributes to improved neuronal survival. In previous studies with organotypic hippocampal slice cultures and glial single cell cultures we have shown that mycophenolate mofetil (MMF) reduced the number of degenerating neurons after excitotoxic injury, inhibited the proliferation of glial cells and suppressed the activation state of microglial cells. To prove that MMF exerts equally potent neuroprotective effects in vivo, we investigated rats subjected to controlled cortical impact (CCI) injury, an established in vivo model for neurotrauma. 8-12-week-old Wistar rats (350g-450g) received a unilateral CCI injury (4.5-5.5 m/sec velocity, 2mm depth, 400 ms dwell time) and vital parameters were monitored for up to 2h after the injury. Injured animals were treated with vehicle or MMF up to 14 days. Animals were sacrificed 4h, 1, 3 or 14 days after the trauma. The lesion volume was calculated by quantative morphometry on serial sections stained with hematoxylin/eosin. In vehicle treated animals CCI caused a moderate cortical contusion, swelling (1 day) and massive neuronal cell loss underneath the injury. The lesion volume showed an average of 31.41 mm^3 and the lesion edges were poorly defined. Immunohistochemistry showed microgliosis, astrogliosis, massive invasion of blood borne monocytes and a high number of proliferating glial cells. In contrast, MMF treatment led to a significantly reduced lesion volume of 19.41 mm^3. The lesion was sharply demarcated against neighboring NeuN immunoreactive neurons. Immunohistochemically a strong suppression of glial activation and proliferation was visible and the number of proliferating glial cells was attenuated. Our findings show that the immunosuppressant MMF protects in vivo neurons from secondary neuronal damage, inhibits the proliferation of astrocytes and reduces the activation state of glial cells resulting in a smaller lesion volume. MMF may be used as a neuroprotective agent in treatment of acute traumatic CNS pathologies. *This study was supported by Dr. August Scheidel-Stiftunng.*

Vortrag 15

Dose-dependent and time-dependent effects of Interleukin-1 receptor antagonist on neuronal damage in organotypic hippocampal slice cultures (OHSC)

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Interleukin (IL)-1 is an important mediator of neuronal demise and glial activation after acute central nervous system (CNS) damage such as spinal cord injury or stroke. IL-1 receptor antagonist (IL-1ra) has been shown to improve neuronal survival in different lesion models. Here we investigated the effect of IL-1ra on neurons and microglial cells in organotypic hippocampal slice cultures (OHSC): OHSC derived from rats were excitotoxically lesioned after 6 days in vitro (div) by application of N-methyl-d-aspartate (NMDA; 50µM) and treated with IL-1ra (40, 100, or 500 ng/ml) for 4 h or up to 9 div. Degenerating neurons in OHSC were labelled with propidium iodide, microglial cells stained with fluorescent isolectin B_4, and the preparations were analyzed by confocal laser scanning microscopy. Application of IL-1ra alone in unlesioned OHSC did not induce significant changes in the number of microglial cells or degenerating neurons. Treatment of NMDA-lesioned cultures with IL-1ra significantly attenuated neuronal damage and reduced the number of microglial cells. The maximal effect was observed, at an IL-1ra concentration of 100ng/mL. We than determined the time frame of neuroprotective effects of IL-1ra after delayed application and treated the OHSC with IL-1ra (100 ng/mL) at different time points (4, 8, 16, 24, 36 or 48 h) after induction of neuronal injury. Application of IL-1ra strongly suppressed neuronal damage if it was initiated within the first 16 h after the onset of excitotoxic injury ($p<0.05$), and less potent but nevertheless significant neuroprotection was observed if treatment with IL-1ra was delayed up to 24 h. IL-1ra also suppressed the increase in the number of microglial cells if its application was delayed up to 24 h ($p<0.05$), and a significant but less pronounced decrease in the number of microglial cells was found up to 36 h after the initiation of excitotoxicity. Our findings indicate that a) even short term application of IL-1ra reduces neuronal cell death and induces a dose-dependent decrease in the number of microglial cells after excitotoxic damage and b.) IL-1ra exhibits significant neuroprotective effects when given during the first 16h after the lesion. These data support the use of IL-1ra as a neuroprotective treatment after acute CNS pathologies. This study was supported by Dr. August Scheidel Stiftung.

Vortrag 16

Chemical inhibitor of caspase-independent cell death with therapeutic potential for ischemic brain injury

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Previous studies on mechanisms of apoptotic cell death have demonstrated that besides cytochrome c, mitochondria contain a number of other pro-apoptotic molecules that are released during apoptosis (Smac/DIABLO, AIF and HtrA2). HtrA2 is localised in mitochondria and is released to the cytoplasm of fibroblasts in response to apoptotic stimuli. HtrA2 is able to induce both caspase-dependent cell death by interacting with IAPs and caspase-independent cell death that relies on its protease activity. In this report, we have investigated whether (1) HtrA2 is translocated from the mitochondria to the cytosol in brains subjected to focal ischemia (2) this translocation correlates with XIAP degradation and induction of neuronal apoptosis and (3) pharmacological inhibition of HtrA2 may protect neurons from ischemic damage. Ischemia was performed in rats by occluding the middle cerebral artery. Rats were pre-treated with ucf-101, a specific inhibitor of the serine protease HtrA2. We found that the level of HtrA2 protein is up-regulated after ischemia. This up-regulation was followed by the release of HtrA2 from mitochondria to the cytoplasm and degradation of XIAP. Furthermore, we show that the proteolytic activity of HtrA2 is necessary and essential for ischemia-induced cell death. Treatment with ucf-101, a specific inhibitor of HtrA2, counteracts IAPs degradation in ischemic cells and increases their survival. These results point to the IAPs as potential pharmacological targets in cerebral ischemia.

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Vortrag 17

Identification of proinflammatory cytokine receptors on rodent primary afferent neurons: evidence for their function as direct immunosensors.
(Identifizierung proinflammatorischer Zytokin-Rezeptoren auf primärafferenten Neuronen des Nagers belegt ihre Funktion als direkte Immun-Sensoren)
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The proinflammatory cytokines TNF□ and Interleukin-1 beta (IL-1□ are known as important mediators of inflammatory pain by activating peripheral nociceptors. However, the exact cellular sites of their biosynthesis in nociceptive neurons are still controversially discussed. Thus, we reexamined the cellular expression pattern of TNF□ and IL-1ß and their signal transducing receptors at the mRNA level in the dorsal root ganglion (DRG) in a lipopolysaccharide (LPS)- inflammation model using RT-PCR combined with laser microdissection and semi-quantitative *in situ* hybridization. After LPS treatment we found both IL-1ß and TNF□ exclusively expressed in a non-neuronal subpopulation of DRG, but not in neurons. The signal-transducing cytokine receptors, however, were expressed constitutively in non-neuronal cells as well as in neurons. While TNF receptor type 1 transcripts were present in virtually all primary afferent neurons, IL-1 receptor type 1 mRNA was found predominantly in a subset of DRG neurons co-expressing markers of nociceptive neurons such as substance P, CGRP and TRPV1. Our results explain how TNF□ and particularly IL-1ß cause hyperalgesia along primary sensory neurons and why cytokine effects on primary afferents are likely to be related not only to pain as currently conceptualized but also involve sensory modalities beyond pain perception. Cytokine receptor bearing primary afferents including presumed nociceptive neurons may be relevant in sickness behaviour and fatigue syndromes as seen under bacterial infections.

Vortrag 18

Synapsen exprimieren Aromatase

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Unsere vorausgegangenen Untersuchungen haben gezeigt, dass hippocampale Neurone Östradiol synthetisieren und dass dieses lokal gebildete Östradiol einen entscheidenenden Einfluss auf die synaptische Plastizität im Hippocamus hat. In diesem Rahmen wird unter anderem die Hypothese diskutiert, dass Östradiol auch als Neurotransmitter fungiert (Balthazart & Ball, 2006, Trends Neurosci). Östrogene als Neurotransmitter machen die Präsenz der östrogensynthetisierenden Aromatase in Boutons wahrscheinlich und ihre Expression würde die Hypothese der Neurotransmitterfunktion von Östrogen substantiieren.
Aus diesem Grund haben wir mit verschiedenen Aromatase-Antikörper die Verteilung des Proteins im Hippocampus der Ratte mittels immunhistochemischer Kolokalisationsexperimente, mit Immunogold-Markierung und mit Westernblots an Synaptosomen- und Post-Synaptischen-Dichte (PSD)-Präparationen untersucht. Die immunhistochemischen Untersuchungen zeigten, dass die Aromatase nicht nur cytoplasmatisch in den Perikaryen lokalisiert ist, sondern sich auch in den Dendriten und den Synapsen befindet (Kolokalisation mit Synapsin). Doppelinkubationen mit den spezifischen prä- bzw. postsynaptischen Markern Synaptophysin und Spinophilin, sowie die Ergebnisse der Immunogold-Markierung zeigen sowohl prä- als auch postsynaptisches Vorkommen der Aromatase. Diese Befunde wurden durch die Westernblots an synaptosomalen Proteinen bzw. PSDs bestätigt.

Vortrag 19

Synaptogenesis: promoted by cholesterol or estradiol?

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Cholesterol of glial origin has been demonstrated to promote CNS synaptogenesis *(Science 294, 1354, 2001*). As neuron-derived estradiol also regulates synapse density, we questioned whether cholesterol promotes synapse formation directly or indirectly by providing elevated substrate levels for neuronal estrogen synthesis. In this study, we provide evidence that cholesterol-induced synaptogenesis results from its metabolization to estradiol. Estradiol release from the cultures into the medium was stimulated by cholesterol. Cholesterol-promoted synaptogenesis, as demonstrated by spine synapse counting and by quantitative evaluation of pre- and postsynaptic protein expression, is abolished when cholesterol and letrozole, a potent aromatase inhibitor, are simultaneously applied to hippocampal cultures. Most importantly, downregulation of synapse formation after knock-down of StAR is only rescued by estradiol but not by cholesterol.
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Vortrag 20

The influence of estrogen on cytokine expression by astrocytes

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Astrocytes represent important players during inflammatory processes in the CNS. They respond to pathological stimuli, interact with other types of immune cells, and synthesize and secrete a set of cytokines. Recent studies have revealed that 17ß-estradiol can interfere and regulate immunological/inflammatory processes in the brain and acts as a neuroprotective factor. Since we have shown, that astrocytes express estrogen receptors and are functionally regulated by estrogen, we put forward the concept, that estrogen may control brain inflammatory processes through the control of astrocytes properties. Primary astrocytes cultures from the midbrain and cortex were exposed to lipopolysaccharides (100ng LPS, 4h) in the absence of estrogen or after pre-treatment with estrogen (10^{-8} M, 24 h). In unstimulated astrocytes, only marginal cytokine expression (TNF-α, IL-1α, IL-6, IL-18) was found. LPS stimulation yielded a significant induction of all respective cytokines, although in a region- and time-dependent way. Estrogen application prevented the induction of TNF-α, IL-1α and IL-6 in midbrain astrocytes by about 30% and IL-18 about 80% but to a lower degree (15%) in cortical astrocytes.
Taken together, our data clearly show that estrogen is capable to antagonize LPS-induced expression of cytokines in astrocytes. This interference of estrogen with inflammatory responses in the brain may represent an important cellular mechanism to perform a neuroprotective role.

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Vortrag 21

Differential effects of Trichostatin A (TSA) on gene expression of T47D and MCF7 breast cancer cells

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Growth and differentiation of breast cancer cells are influenced by estradiol and progesterone. Both steroids act via multi-protein-complexes composed of the specific hormone receptor and different cofactors. One group of cofactors are class I histone deacetylases (HDACs), which were recently established as drugable targets in cancer therapy. Here we study the effects of the HDAC inhibitor TSA on the estradiol (E) and medroxyprogesterone acetate (MPA)-regulated expression of estrogen receptor (ER) alpha, Na,K ATPase and clusterin. Expression of these genes was studied in T47D and MCF7 breast cancer cells. Cells were cultured with a) vehicle, b) E or c) E+MPA. In addition, cells of these three groups were treated with different doses of TSA. Analyses were performed by real-time RT-PCR and Western Blot.
Treatment of T47D cells with 0.2 µM/L and treatment of MCF7 cells with 0.2 as well as 0.5 µM/L TSA did not influence the expression of the ER alpha under the tested hormonal conditions.
Na,K ATPase mRNA was significantly upregulated by MPA in T47D but not in MCF7 cells. TSA did not exert an additional effect on Na,K ATPase mRNA expression in both cell lines. In T47D cells clusterin mRNA expression was suppressed by MPA. This decrease was abolished by TSA. In MCF7 cells clusterin mRNA expression was not influenced by steroids. However, TSA treatment significantly elevated clusterin mRNA expression in all treatment groups.
We conclude that steroid and TSA treatment exert highly specific effects on gene expression of different breast cancer cell lines. Consequently, tumor type and hormonal status of premenopausal women has to be taken into consideration when HDAC inhibitors are administered during breast cancer therapy.

Vortrag 22

Nitric oxide prevents ascorbic acid-induced cytotoxicity in MA-10 tumor cells

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Recently, ascorbic acid was shown to exert prooxidative, cytotoxic effects in various cancer cell types (Chen et al., PNAS 2005, 102:13604-13609). However, the molecular mechanisms involved as well as potential effects induced by nitric oxide (NO), known to regulate cellular growth, have not yet been elucidated.
In order to address these aspects, we examined the effects of ascorbic acid and NO on MA-10-Leydig tumor cells by proliferation assays, immunological approaches and a newly developed hydrogen peroxide detection assay, allowing - in combination with a modified MTT assay - to discriminate between anti- and prooxidative effects of ascorbic acid.
Ascorbic acid was found to lead to a loss of cell viability that could be reversed by catalase, indicating an involvement of hydrogen peroxide. In agreement, administration of exogenous H_2O_2 also resulted in a dose-dependent decrease of cell number. Ascorbic acid-induced production of H_2O_2 was shown to take place extracellularly and to be independent of the presence of serum proteins. Concentrations of ascorbic acid > 1 mM were shown to be antioxidative, whereas ascorbic acid concentrations < 1 mM were found to be prooxidative as indicated by generation of detectable levels of H_2O_2. Surprisingly, ascorbic acid-induced cytotoxicity was prevented by NO. This NO effect was cGMP-independent, since the NO donor sodium nitroprusside failed to augment cGMP accumulation and, in accordance, MA-10 tumor cells were found to lack the cGMP-generating NO receptor soluble guanylate cyclase, and especially the cGMP-dependent protein kinase I, mediating most cGMP effects on proliferation, whereas NO synthases are expressed. Under differentiation, forced by serum deprivation, expression of NO synthases increased and NO was more efficient in preventing cytotoxicity of ascorbic acid.
These results indicate that cytotoxic, prooxidative effects of ascorbic acid on tumor cells are extracellularly and serum-independently mediated by H_2O_2 and can be prevented by NO in a cGMP-independent manner.

Vortrag 23

Identification of Candidate bHLH Transcription Factor Gene Involved in a Novel Myopathy

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The genome encodes basic helix-loop-helix transcription (bHLH) factors conserved in evolution that are required for skeletal muscle development. We have analyzed gene expression profiles in a patient who suffered from a severe lethal myopathy with an unfused myoblast phenotype. Using cDNA microarray we could previously show that many genes have significantly altered expression levels. Among them, the expression of a bHLH transcription factor gene, *atonal homolog 8* (*ATOH8*), was significantly reduced (ratio<-4). Using quantitative real-time RT-PCR the down-regulation of the expression of this patient's *ATOH8* was confirmed. The patient's *ATOH8* was further submitted for sequencing. The results show that there is a point mutation near the bHLH domain coding area, which changes the predicted protein sequence. The bioinformatical analysis shows that bHLH domains of ATOH8 are highly conserved among different species. As there is no report describing the expression pattern of *ATOH8* during embryogenesis, we cloned a fragment of *cAtoh8* and performed *in situ* hybridization in chicken embryos of different stages with the probe. The results show that in the chick, *cAtoh8* is specifically expressed in the somites (dermomyotome/myotome) and muscle cells. Silencing of *cAtoh8* with RNA interference constructs significantly inhibits *MyoD* expression, and upregulates *Pax3* expression in the chick embryos. Our preliminary results suggest that beside some known bHLH transcription factors, like MyoD, Myf5 and Myogenin, ATOH8 that had not been reported in the context of myogenesis before, represents another bHLH transcription factor involved in muscle development.

Vortrag 24

New roles for neuropeptide and classical transmitter co-transmission in the human skin (Co-Transmission von Neuropeptiden mit klassischen Neurotransmittern in der humanen Haut)

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The skin is a complex organ that is regulated by cutaneous nerve endings that secrete a complex mixture of classical neurotransmitters and neuropeptides. Determining the chemoanatomy of peptide/classical amine transmission is a necessary step in understanding the chemical basis for neuronal control of cutaneous function. We employed antibodies against the vesicular acetylcholine (VAChT) and monoamine (VMAT2) transporters, markers for catecholamine biosynthesis (TH, AADC, DBH), and several neuropeptides in these studies of human skin.

Sympathetic nerves supplying human sweat glands and AV anastomoses (AVA) co-express cholinergic (VAChT) and full noradrenergic (TH, AADC, DBH, VMAT2) traits (Weihe et al., J. Comp. Neurol., 2005). We now show that the neuropeptides VIP and CGRP co-exist in these cholinergic/noradrenergic sudomotor and vasomotor nerves. The vasomotor supply to AVA also codes for SP. SP is fully co-expressed with neither cholinergic nor adrenergic markers, and is therefore mostly sensory. NPY is present in vascular innervation but is absent from sudomotor and pilomotor innervation, which is reflected by absence from some TH-positive stellate ganglion neurons in the Rhesus macaque. VAChT/VIP co-positive nerves were also seen around non-AVA arterial vessels. The majority of CGRP-, SP- and PACAP-positive innervation lacking cholinergic and adrenergic phenotypes is most likely sensory.

Our results suggest that the sympathetic control of skin functions including vascular, fluid and electrolyte, thermoregulatory, and sensation, is likely to result from a balance of neuropeptide and cholinergic-adrenergic co-transmission. Pre- and postjunctional interaction of multifunctional neuropeptides with classical transmitters of sympathetic and sensory nerves may have pathophysiological relevance for diverse skin disorders like hyper-/anhidrosis, neurodermatitis, diabetic neuropathy, pain and photoaging.

Vortrag 25

Regulation der kationischen Aminosäure Transporter, hCAT1- und 2, bei der Psoriasis

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Psoriasis ist eine entzündliche Hauterkrankung, von der etwa 1-3% der europäischen Population betroffen ist. Sie ist durch ein abnormales Proliferationverhalten der Keratinozyten und einen reduzierten Harnstoffgehalt im Stratum corneum gekennzeichnet. Ein Faktor der beide Ereignisse verbindet, ist L-Arginin, das über zwei Wege metabolisiert werden kann. (1) Durch die Stickstoff-monoxidsynthase (NOS) in L-Citrullin und Stickstoffmonoxid (NO) und (2) durch die Arginase, die L-Arginin in L-Ornithin und Harnstoff umwandelt. Die aus dermatologischer Sicht interessanten Substanzen sind hierbei NO und Harnstoff. NO nimmt auf die Zellproliferation und die Unterhaltung von Entzündungsprozessen Einfluss; Harnstoff erhöht als Moistruizer die Wasserbindungskapazität in der Haut. Eine limitierende Komponente beider Stoffwechselwege ist der Transport von L-Arginin, der in Säugerzellen zu ca. 80 % durch den y^+-Transporter gewährleistet wird. Der y^+-Transporter wird durch die Gene hCAT1, 2a, 2b, 3 und 4 codiert und durch die Proteine hCAT1, 2, 3 und 4 repräsentiert. In der vorliegenden Studie haben wir die Verteilung der kationischen Aminosäuretransporterproteine 1 (hCAT1) und 2 (hCAT2) in gesunder und psoria-tischer Haut immunhistologisch analysiert. Zusätzlich sind wir der Hypothese nachgegangen, ob L-Arginin und L-Ornithin die Expression von hCAT1 und 2 beeinflussen. Dazu wurden native Keratinozyten in Zellkultur mittels Real-time-PCR and Western blot untersucht. Die immunhisto-chemischen Vergleiche von gesunder und psoriatischer Haut zeigten einen signifikanten Abfall der hCAT1-Protein-expression im Stratum granulosum von psoriatischer Haut; das Verteilungsmuster von hCAT2 ist hingegen kaum verändert. Die Zellkulturexperimente zeigen, dass sowohl L-Arginin als auch L-Ornithin die Expression ihres eigenen Transporters über feed-back-Mechanismen beeinflussen. Die Ergebnisse rücken L-Arginin als mögliches Therapeutikum zur Behandlung der Psoriasis-Symptome in den Mittelpunkt.

Vortrag 26

Role of peroxisomes in ossification and bone metabolism

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Patients with peroxisomal diseases display pathological defects in the skeleton, such as skull deformities and calcific stipplings in the epiphyseal areas of long bones with growth defects, indicating that peroxisomes might play an important role both in desmal and chondral ossification processes. However, only sparse information is available on metabolic pathways of peroxisomes in different cell types of the skeleton. In the present study, we characterized the peroxisomal compartment in different cell types of the skeleton of C57Bl/6J mice of different postnatal age (P0.5-3 months) by means of 1) double- and triple-immunofluorescence preparations of paraffin sections of decalcified bones and joints using several antibodies against marker enzymes of matrix and membrane proteins of peroxisomes, 2) isolation and culture of primary osteoblasts from the calvaria of newborn mice, 3) immunofluorescence with the same antibodies as used for in situ stainings on primary osteoblasts of different time in culture and distinct differentiation (as shown by specific bone markers). Immunostainings for the localization of Pex14p, a peroxisome biogenesis protein, revealed the presence of peroxisomes in all cell types of the skeleton, however, with highest abundance in hypertrophic cartilage cells (chondrocytes of reserve zone < proliferative chondrocytes < hypertrophic chondrocytes > osteoblasts > osteocytes). In addition, all peroxisomal metabolic enzymes, such as catalase, SKL-containing matrix enzymes and the two ABC transporters-PMP70 and ALDP, were selectively enriched in hypertrophic cartilage cells. Furthermore, we noted a proliferation of peroxisomes during differentiation of osteoblasts with a concomitant increase of peroxisomal marker enzyme expression. In addition, osteoblast differentiation was markedly retarded in primary cultures of osteoblast from peroxisome-deficient mice, showing lower expression of secreted bone matrix proteins. Taken together, our data suggest an important role of peroxisomes for ossification processes.

Vortrag 27

Adenoviral transduction of alginate derived chondrocytes is more efficient than using primary monolayer chondrocytes

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Gene transfer into cultured chondrocytes using adenoviral vectors has potential applications in treating cartilage disorders. The present study was undertaken to compare and optimize two chondrocytes culture conditions for adenoviral transduction efficacy using primary human articular chondrocytes that were either cultivated a) directly in a monolayer condition or b) outgrowth from alginate-stored chondrocytes cultures and to evaluate longterm transgene expression in threedimensional chondrocyte culture.
Either primary or alginate derived chondrocytes were transduced with an enhanced green fluorescent protein (EGFP) gene bearing adenoviral vector (1000 and 3000 virus particles/cell). Immunohistochemistry and flow cytometric analysis were employed to determine the expression of cartilage extracellular matrix proteins and the $\alpha v\beta 5$ integrin receptor which is a precondition for adenoviral cell entry. To study longterm transgene expression, EGFP expressing chondrocytes were cultivated in alginate culture 24 h post transduction. Primary chondrocytes exhibited only moderate transduction rates (mean 22,2% and 46,9% EGFP positive cells at 1000 and 3000 virus particles/cell 72 hours post transduction), whereas alginate-derived chondrocytes revealed significantly higher transduction efficacies (95,7% and 99%) ($p < 0{,}001$). Both, primary and alginate-derived chondrocytes expressed $\alpha v\beta 5$ integrin, type II collagen and cartilage proteoglycan. The mean fluorescence intensity of $\alpha v\beta 5$ integrin was significantly higher ($p < 0{,}001$) in the primary chondrocytes, compared to the alginate cultured ones, implying that transduction efficacy may be independent on the $\alpha v\beta 5$ integrin expression levels in chondrocytes. Significant elevated EGFP expression was evident during 4 weeks in alginate culture. Our results indicate that adenoviral transduction of alginate-derived chondrocytes is more efficient than that for primary chondrocytes and may be a suitable tool to achieve sufficient transduced and differentiated chondrocytes for experimental applications and cartilage repair.

Vortrag 28

The role of Hif-3 alpha 4 in Rheumatoid Arthritis (RA)

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Joints from patients suffering from rheumatoid arthritis (RA) show increased Hif-activation resulting in new blood vessel formation, synovitis and pannus formation leading to articular cartilage and bone destruction. Hence Hif is involved in the persistence of inflammation and progression of neovascularisation during RA. Using K4M synoviocytes overexpressing Hif-3 alpha 4, a novel splice variant of the Hif-3 gene and a dominant-negative regulator of Hif, we were able to inhibit the Hif-activaton via hypoxia as well as cytokines. Inhibition of Hif-activation was measured utilising a hypoxia respose element-luziferase assay, VEGF-ELISA and real time RT-PCR. Inhibition of Hif-activation by pharmacological induction of Hif-3 alpha 4 - expression could potentially be a new strategy for the treatment of RA.

Vortrag 29

Different Density of the $Gaba_{b1}$ Subunit in Somato-Dendritic Compartments Of Cck- and Pv-Containing Hippocampal Interneurons

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$GABA_B$ receptors, composed of $GABA_{B1}$ and $GABA_{B2}$ subunits, mediate metabotropic effects at pre- and postsynaptic sites resulting in a reduction of transmitter release and slow inhibitory postsynaptic potentials (IPSP). In the hippocampus high levels of the $GABA_{B1}$, but not the $GABA_{B2}$, subunit, have been observed in somata of many interneurons. However, the identity of these cells and the subcellular localization of functional receptors remained unknown. In the present study we have investigated the cellular and ultrastructural localization and distribution of the $GABA_{B1}$ subunit by immunofluorescent and high-resolution pre-embedding immunogold labeling techniques in two interneuron subpopulations, cholecystokinin (CCK)- and parvalbumin (PV)-containing basket cells. At the light microscopic level, strong immunoreactivity for the protein was found in somata of CCK+ interneurons, whereas the PV+ interneurons were weakly stained. At the electron microscopic level, the $GABA_{B1}$ protein was found on the extrasynaptic plasma membrane of dendritic shafts and axon terminals of both types of interneurons. Quantitative analysis revealed a significantly higher density of the protein in dendrites than in boutons. In addition, the density of the subunit was higher in dendritic shafts of CCK+ cells in comparison to PV+ neurons. These data show that the $GABA_B$ receptors are present at different densities on the somato-dendritic surface of the two subpopulations of basket cells, indicating differential regulation of activity of these perisomatic inhibitory cells by dendritic inhibitory interneurons.

Vortrag 30

Compartment-Dependent Colocalization of Kir3.2-Containing K^+ Channels and $Gaba_b$ Receptors in Hippocampal Pyramidal Cells

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The G-protein-coupled inwardly rectifying K^+ channels (Kir3 channels), coupled to metabotropic $GABA_B$ receptors ($GABA_B$Rs), are essential for the control of neuronal excitation. To determine the distribution of Kir3 channels and their spatial relationship to the $GABA_B$Rs on hippocampal pyramidal cells, we used high-resolution immunocytochemical approaches. Immunoreactivity for the most abundant Kir3.2 subunit was mainly localized postsynaptically to the extrasynaptic plasma membrane of dendritic shafts and spines of principal cells. Quantitative analysis of immunogold particles for the protein revealed an enrichment of the subunit around glutamatergic synapses in dendritic spines, similar to that of the $GABA_{B1}$ subunit. Consistent with this observation, a high-degree of coclustering of Kir3.2 and $GABA_{B1}$ was found around excitatory synapses by the highly sensitive SDS-digested freeze-fracture replica immunolabeling method. In contrast, in dendritic shafts receptors and channels were found to be mainly segregated. These results suggest that Kir3.2-containing K^+ channels in dendritic spines preferentially mediate the effect of GABA, whereas channels in dendritic shafts are likely to be activated by other neurotransmitters as well. Thus, Kir3 channels, localized to different subcellular compartments of hippocampal pyramidal cells, appear to be differentially involved in synaptic integration in principal cell dendrites.

Vortrag 31

Partial loss of the cisternal organelle in the axon initial segment of βIV-spectrin-deficient mice

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The cisternal organelle (CO) is a putative calcium store found exclusively in the axon initial segment (AIS) of telencephalic neurons. It consists of stacks of smooth endoplasmic reticulum and interdigitated electron-dense material. At present, little is known about its cell biology and the mechanisms involved in its selective targeting to the AIS. To test whether □IV-spectrin, a membrane cytoskeleton adaptor protein highly enriched in the AIS could be involved in the targeting mechanism, we analyzed the hippocampus of quivering mice lacking a functional □IV-spectrin gene. The AIS of hippocampal principal neurons was identified using immunostaining for phosphorylated I□B□pl□B□ and the CO was immunolabeled with antibodies against the actin-associated molecule synaptopodin. Confocal microscopy revealed that the prevalence of synaptopodin-positive puncta in the AIS of hippocampal neurons was markedly reduced in the mutants as compared to littermate controls, suggesting a partial loss of the CO. Based on this observation, we performed electron microscopy of pl□B□-immunostained sections of quivering mouse hippocampus. In this material, the prevalence of the CO was significantly reduced as compared to controls (1.9% ± 0.7 vs. 5.9% ± 1.3). Our study suggests that (1) □□V-spectrin contributes to the AIS-specific localization of the CO, and that (2) quivering mice may help to unravel the biological relevance of the CO in the AIS.

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Vortrag 32

Oxidative stress is an important mediator of neuronal death in peroxisomal biogenesis disorders

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Zellweger syndrome (ZS) is the most severe form of a peroxisomal biogenesis disorder, characterized by focal neuronal death and major migration defects in the medial neocortex of the patients. In addition, children with ZS suffer from neonatal seizures and general hypotonia, they develop liver cirrhosis, endocrine pathologies and die during the first year of life. In recent years, we have developed several animal knock-out mouse models (e.g. Pex11β-deficient mice), exhibiting the same phenotype as the ZS patients, to study the molecular pathogenesis of organ defects in this devastating disease.
The purpose of the present study was to investigate the molecular pathogenesis of neuronal death occurring in ZS. Therefore, we established primary neuronal cultures from the medial cortex from E19 Pex11β (+/+), (+/-) and (-/-) mice. All experiments were performed after 6 days of primary neuronal culture. The number of peroxisomes was reduced in neurons from PEX11β-KO mice in comparison to heterozygous and control animals. In contrast, peroxisomal catalase immunoreactivity of individual peroxisomes was similar in cortical cultures from all three genotypes. Determination of cell death revealed 11% and 45% apoptotic neurons in cultures from (+/+) and (-/-) animals, respectively. Interestingly, we also found 32% apoptotic neurons in cultures from (+/-) animals. Subsequently, intracellular ROS levels were measured using the oxidant-sensitive dye dihydroethidine for examination whether reactive oxygen species (ROS) contribute to neuronal death in cultures from (+/-) and (-/-) PEX11β-mice. The mean cellular ROS levels in cultures from (+/-) and (-/-) mice were about 2- and 3-fold higher than in cultures from wild type mice, respectively. Furthermore, we found differences in the protein levels of the mitochondrial antioxidant enzyme SOD-2 in distinct genotypes. Treatment with vitamin E reduced cellular ROS levels and neuronal cell death in (+/-) and (-/-) cultures to wild type values. In conclusion, our data suggest that oxidative stress plays an important role in the development of neuronal dysfunction in peroxisomal biogenesis disorders (e.g. ZS).

Vortrag 33

Morphological reorganization and implications in functional properties by C3bot proteins and Rho GTPases: Studies on neurons and neuroglial cells

Morphologische Reorganisation und Einfluss auf funktionelle Eigenschaften durch C3bot Proteine und Rho GTPasen: Untersuchungen an Neuronen und Neuroglia

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C3 proteins of various bacterial origin serve as biochemical tools to investigate Rho functions in a great variety of cells. C3bot derived from *Clostridium botulinum* in addition exhibits Rho-independent effects. In this respect, we were able to show that enzyme deficient C3bot as well as C3bot derived peptide preparations such as the amino acids 154-182 have similar neurotrophic effects on axons. C3 proteins, especially C3bot in its Rho-inactivating form might be used as therapeutic tools to foster neuronal regeneration. Consequently, we also investigated the effects of C3 proteins on the function of other CNS cells like astrocytes and microglia. Upon inhibition of Rho with C3bot astrocytes exhibited an increased glutamate uptake capacity. Based on proteinbiochemical, pharmacological, as well as immunofluorescence methods we could show an increased NF□B-mediated expression of GLT-1 glutamate transporter at the plasmamembrane. Conversely, expression of GLAST, the glutamate transporter of undifferentiated astrocytes, was reduced. Additionally, the calcium-dependent release of glutamate from storage pools was amplified after incubation with C3bot. When looking at cultivated microglia cells Rho inhibition by nanomolar doses of C3bot leads to morphological activation similar to the one observed by the application of lipopolysaccharide (LPS). The observed morphological changes were accompanied by a proinflammatory response characterized by the release of various factors like the cytokines NO, TNF□ or chemokines like KC.

Vortrag 34

Region- and cell specific expression of Na^+-HCO_3^- cotransporter (SLC4A4) variants pNBC1 and kNBC1 in mouse brain and regulation in health and disease.

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Maintenance of pH homeostasis is a prerequisite for proper brain function. However, study of the pH in the CNS reveals considerable differences to other tissues, mainly because neuronal activity is accompanied by rapid pH shifts. These activity-dependent pH changes occur in neurons, as well as in glia cells, and affect both the extracellular and intracellular compartment. Alteration of pHi may affect ion channel conductance and synaptic transmission, whereas a decrease in the pHo accompanies many pathological states, such as ischemia, hypoxia, and CNS injury, and prevents long-term potentiation. In the present study we have investigated expression and distribution of the electrogenic Na+/HCO3- cotransporter variants pNBC1 and kNBC1 in adult mouse brain by PCR, immunohistochemistry, double immunofluorescence and electron microscopy. Moreover, effects of acid-base disturbances were studied in vitro using mouse primary hippocampal cultures. The results show expression of both NBC1 variants in mouse cerebellum, cerebral cortex, olfactory bulb and hippocampus. kNBC1 was expressed in cerebellar Purkinje cells, in non-pyramidal cell bodies and synaptic compartments in cerebral cortex, in periglomerular and mitral cells of the olfactory bulb, and in granular cells of the dentate gyrus. PNBC1 immunoreactivity was observed in Bergmann glia and in perivascular astroglial processes and lamellae, as well as in apical pyramidal cell dendrites in hippocampus. Induced acidosis and alkalosis in vitro differentially regulated NBC1 variants. The results suggest involvement of NBC1 in pH regulation of neural cells, implicate region- and cell specific distribution of NBC1 variants in mouse brain and demonstrate differential regulation mechanisms of these transporters during acid base disturbances.
Funded by grants from the Deutsche Forschungsgemeinschaft.

Vortrag 35

ActivinA and TGFß-1 Apoptosis-Signaling in Oligodendroglial Cells

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ActivinA (ActA) and transforming growth factor beta-1 (TGF-ß1) are cytokines which modulate numerous cell activities like cell growth, differentiation, proliferation and apoptosis. ActA- and TGF-ß1-induced cell death is mediated through different signalling pathways, including Smad proteins, MAP kinase cascades as well as NfkB and PKB/Akt, depending on cell type, differentiation and expose to other growth factors.
In this study we analysed the signalling pathways of ActA- and TGF-ß1-induced apoptosis in an oligodendroglial cell model, Oli-neu. Most surprinsingly, both cytokines lead to cell death independently of each other through activation of different pathways. In Oli-neu cells TGF-ß1-induced caspase activation is followed by downregulation of anti-apoptotic Bcl-2 family members. ActA on the other hand activates another pathway of the intracellular apoptosis network involving Caspase3-independent nuclear condensation resulting in activation of the apoptosis inducing factor (AIF).
Thus, this study shows for the first time that two members of the TGF-ß superfamily activate two independent pathways leading to apoptosis in the same cellular system, a neural oligodendroglial cell line.

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Vortrag 36

TGF-β2 and GDNF in the Development of the Murine Nervous System: Evidence from Double Mutant Mice

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Neuronal survival and death is a central issue both in development and in regeneration. A number of neurotrophic growth factors have been identified that promote neuronal survival and differentiation. Therefore they are good candidates to be responsible for many developmental and neurodegenerative diseases such as Amyotrophic Lateral Sclerosis and Parkinson's Disease. The transforming growth factors-beta (TGF-β) constitute a family of multifunctional cytokines. Their functions include control of cell proliferation, differentiation and regulation of cell survival and death. Glial cell line-derived neurotrophic factor (GDNF) itself is distantly related to TGF-β. It maintains survival of various neuronal populations such as midbrain dopaminergic neurons and motoneurons. Many recent advancement have revealed that growth factors acting in synergy can regulate neuronal survival much more potently than individual factors alone. Several evidences suggest that GDNF may require cofactors for acting as neurotrophic factor. Current work aims at elucidation of TGF-β2 and GDNF synergism *in vivo* via generation of TGF-β2/GDNF double mutant mice. For that purpose, heterozygous animals (*Tgfβ2*$^{+/-}$*Gdnf* $^{+/-}$) were crossed to receive mice lacking both TGF-β2 and GDNF. The analysis focused on midbrain dopaminergic neurons, hindbrain serotonergic neurons, lumbar spinal motoneurons, enteric neurons, dorsal root ganglionic neurons, superior cervical sympathetic ganglionic neurons and cranial ganglionic neurons. Our results suggest crucial roles for TGF-β and GDNF in the development of the central and peripheral neurons.
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Vortrag 37

TGF-β/GDNF cooperativity in superior cervical ganglion development

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Neuronal survival, proliferation, differentiation and death are fundamental topics in neurobiology research.
Among different families of growth factors, members of the TGF-β superfamily have been identified as key mediators of neuronal survival and differentiation. Thereby they may act in cooperation with either members of other families of growth factors or neurotrophins, or with members of the same superfamily. Such synergistic mode of action may imply interaction at the receptor level, convergence of the respective signaling pathways or colaboration at the transcriptional level.
The superior cervical ganglion (SCG) has been established as a model for investigating the molecular mechanisms underlying development and survival of peripheral neurons, though its genesis is an intricate process. It begins with migration of neural crest derived neural precursors to their final destination where gangliogenesis occurs and proceeds with proliferation, target innervation, and growth factor-dependent survival.
The aim of the present study was to investigate a potential synergistic effect of TGF-β and GDNF in the development of SCG neurons in vitro and in vivo. To that end, mouse primary SCG cells were cultured, treated with different factors and subsequently processsed for survival assays. In addition, the role of TGF-β and GDNF was examined in vivo by analyzing TGF-β/GDNF double mutant mice. The results demonstrate a crucial role for both TGF-β and GDNF in the development of SCG neurons. These results provide profound insights and contribute to a better understanding of the molecular machinery dictating PNS development.
Funded by DFG Forschungszentrum CMPB.

Vortrag 38

Transforming growth factor beta and Bone morphogenetic protein synergize in mediating programmed retinal cell death

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Transforming growth factor beta (TGF-ß) and Bone morphogenetic protein (BMP) are extracellular molecules known to mediate programmed cell death (PCD) in the developing retina. In the present study we investigated the expression profiles and activity levels of ligands and receptors of the TGF-ß and BMP4 family during the physiological PCD periods of the developing chick and mouse retina and possible interactions of both pro-apoptotic molecules in mediating apoptosis in chick and murine retinal wholemount cultures. Immunocytochemical double labeling studies with the established ganglion cell marker Islet revealed overlapping expression patterns for TGF-ß and BMP4 ligands and receptors on the surface of retinal ganglion cells. The bi-phasic peak of activity and expression levels of TGF-ß and BMP4 ligands and receptors – revealed by Western Blots and MLEC assays - coincided with the two main periods of retinal chick and murine PCD. In organotypic retinal cultures, we were able to increase apoptosis over basal levels by application of recombinant TGF-ß or BMP4. Double factor treatment induced an additional increase of apoptosis suggesting a cooperation of both pro-apoptotic pathways. A significant increase in the number of apoptotic cells in the ganglion cell layer was observed in a TUNEL-staining of retinal wholemounts treated with recombinant TGF-ß or BMP4, suggesting a concerted action of both factors in triggering ganglion cell death. Blockage experiments revealed that both pathways do not interact at the ligand, receptor or Smad protein level but converge at the transcriptional level of the TGF-ß immediate-early response gene TIEG and the transcriptional co-activator Gcn5.

Vortrag 39

F-spondin expression in the chicken ciliary ganglion and its effects on survival and intracellular signalling of ciliary ganglion neurons.

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F-spondin is a secreted extracellular membrane (ECM)-associated protein of 807 amino acids. It is expressed at the floor plate during embryonic development (floor plate: F-spondin), and acts as a guidance molecule with an important role in patterning the axonal trajectory in the spinal cord. In situ hybridisation for F-spondin mRNA in E8 chicken embryos revealed a strong signal in the ciliary ganglion (CG) and in cells surrounding the axonal path of CG neurons. Costaining for the neuronal marker NeuN proved that F-spondin is exclusively expressed on non-neuronal cells. Full-length F-spondin efficiently promoted survival and neurite outgrowth; an effect which is dependent on the TGF-beta activating property of the 6th thrombospondin type 1 repeat (TSR6) at the carboxyterminus of the protein. Treatment of CG neurons with F-spondin induced rapid phosphorylation of the intracellular adaptor molecule disabled-1, and protein kinase B/Akt. This effect was mediated by the full-length protein and a construct lacking the TGF-beta activation domain TSR6. The reelin/spondin domain has been shown to interact with amyloid-beta precursor protein (APP). Real-time PCR experiments showed that APP mRNA is highly expressed in CG neurons, indicating that APP might be an F-spondin interacting protein/receptor in these cells. Taken together we show that F-spondin medates intracellular signalling in CG neurons via its N-terminal reelin/spondin domain, whereas the TSR6-mediated activation of latent TGF-beta is additionally required for the survival promoting effect.

Vortrag 40

Both, over-expression and absence of polysialic acid affects peripheral nerve regeneration

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Polysialic acid (PSA) is the most striking post-translational modification of the neural cell adhesion molecule (NCAM) and is involved in neurite outgrowth, migration and synaptic plasticity. To examine the expression and function of endogenous PSA during development and regeneration of the peripheral nervous system, we evaluated sciatic nerves from PSA mouse mutants by using immuncytochemical, Western-Blot and morphometric analysis. After birth, the amount of PSA progressively declines and is absent in myelinated fibers of adult wild-type mice. Also in sciatic nerves from transgenic mice, which display an enhanced level of PSA in neurons, PSA is expressed on small, nonmyelinated axons. With regard to the number and size of myelinated axons, we found no differences in intact sciatic nerves between wild-type and transgenic mice. One week after crush injury, PSA over-expressing mice revealed significantly more regenerating myelinated fibers with a decreased axon caliber suggesting that PSA is essential for axonal outgrowth during early regeneration but not during development of the peripheral nerve. In a second series of experiments sciatic nerves from adult wild-type and PSA knock-out mice were analyzed. After crush lesion, Western-Blot revealed a strong up-regulation of PSA in wild-type mice in contrast to knock-out mice. Quantification of regenerating axons showed significantly increased axonal size of myelinated fibers with no alterations in number and myelin thickness in the absence of PSA. The results indicate, first that the normally occurring down-regulation of PSA is not a prerequisite for myelination during development. Second, the up-regulation of PSA is important for axonal outgrowth, but inhibits the size of myelinated fibers during peripheral nerve regeneration.

Vortrag 41

Modulation von kultivierten neuralen Stamm- und Vorläuferzellen aus dem enterischen Nervensystem von Säugern

In vitro modulation of neural precursors and stem cells isolated from mammalian enteric nervous system

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The enteric nervous system (ENS) is the largest and also by far the most complex division of the autonomic nervous system, with neuron numbers comparable to that of the spinal cord and a broad range of neurotransmitters. The isolation of enteric human neuronal precursor cells as well as their transplantation after *in vitro* expansion may be a new approach for cell-based therapies.
In this study, we investigated new regulatory elements of enteric neurones and the biological potential of ENS derived neural cells *in vitro*. Therefore, we expanded ENS precursors isolated from murine and human gut tissues and evaluated in detail their differentiation capacity *in vitro*. The proliferated neural progenitors could be differentiated into neuronal and glial cells and were able to integrate into chick embryo and murine intestinal tissue after transplantation.
Several molecules influencing cell proliferation and differentiation of enteric precursor cells have been described. The repulsive guidance molecule (RGM) is a recently identified protein implicated in axonal guidance, neuronal apoptosis and neural tube closure in the CNS. In ENS a specific biological function of RGM is still unknown. Therefore, we investigated in detail the expression pattern and biological effects of RGM subtypes on enteric neurons *in vitro*. Thus, strong expression of subtypes RGMa and RGMb as well as the receptor Neogenin was detected in enteric neuronal and glial cells of the fetal and adult gut *in vivo* and in ENS derived precursor cells *in vitro* analyzed by immunohistochemistry, *in situ* hybridisation, RT-PCR, and Western blot analysis.
In this study, we could demonstrate that RGM strongly influenced the differentiation of proliferated neuronal precursors shown by outgrowth and collapse assays. The knowledge about the microenvironmental mechanisms and factors modulating differentiation processes of stem cells are of particular interest for further investigations in the field of Regenerative Medicine.

Vortrag 42

CCL11/Eotaxin abhängige Rekrutierung von Entzündungszellen – ein möglicher therapeutischer Ansatz für allergische Erkrankungen

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In allergischen Erkrankungen werden auf den Schlüsselreiz des Allergen-Kontaktes hin eine Vielzahl von Zytokinen (z.B. Il-5, Il-13) und Chemokinen (z.B. CCL11/Eotaxin) freigesetzt, was zu einer Proliferation, Differenzierung und Rekrutierung von eosinophilen Granulozyten führt. Zum genaueren Verständnis dieser Mechanismen wurde die Rekrutierung von Eosinophilen durch chemotaktische Reize des CC-Chemokinrezeptor 3 (CCR3)-Agonisten CCL11/Eotaxin in Abhängigkeit von der Expression der Eotaxin-spaltenden Dipeptidylpeptidase 4 (DP4) untersucht. Zum anderen wurde durch eine intravenöse Applikation von Eotaxin versucht, eine Verminderung der inflammatorischen Reaktion in einem OVA-induzierten Ratten-Asthmamodell zu erreichen.
Die i.v. Applikation von humanem CCL11/Eotaxin führte zu einer Zeit- und Dosis-abhängigen Erhöhung der Anzahl von Eosinophilen im Blut in Abhängigkeit von der DP4-Expression des Ratten-Substammes. Im Rattenmodell für Asthma bronchiale führte die intravenöse Gabe von CCL11/Eotaxin zu einer Erhöhung der Anzahl eosinophiler Granulozyten im Blut. Allerdings konnten diese Eosinophilen nicht mehr aus den Gefäßen in die Lungen auswandern, was in einer Internalisierung des CCR3 begründet war. Daraus ergibt sich ein möglicher therapeutischer Ansatz für allergische Erkrankungen im CCR3 als Zielrezeptor des CCL11/Eotaxin.

Vortrag 43

Impaired receptor-mediated activation of natural killer (NK) cells by leptin as a novel mechanism of NK cell dysfunction in diet-induced obesity

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Leptin, the product of the *ob* gene is primarily secreted from adipocytes, acting as a hormonal feedback signal to hypothalamic nuclei and herewith regulating energy homeostasis. However, leptin receptors (Ob-R) are not only expressed in the brain but also in peripheral tissues and cells, suggesting a role for leptin beyond the hypothalamus. In contrast to its effect on T- and B-lymphocytes, only few data exist on the modulatory functions of leptin on NK cells (e.g. proliferation, activation or migration). To determine the *in vivo* effect of leptin on NK cells leptin was injected in lean and obese i.v. cannulated rats. Blood was collected 4h after the application. Blood NK cell numbers in blood and spleen were analysed by FACS and immunohistologically. The activity was measured *in vitro* with by the chromium release assay. In order to determine whether NK cells express Ob-R an RT-PCR was performed with NK cells from lean and obese rats. The i.v. leptin application resulted in higher NK cell numbers in the blood in lean and obese rats and in increased NK cell activity only in lean animals compared to vehicle-treated lean and leptin-treated obese littermates. Furthermore results clearly showed the expression of Ob-R mRNA and protein on NK cells.

In conclusion, the results of the present study show that leptin seems to play a role as a modulator of NK cell numbers and activity *in vivo* and *in vitro*.
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Deutscher Titel:
Leptin mobilisiert und aktiviert Rezeptor-vermittelt Natürliche Killerzellen in normalgewichtigen Lewis-Ratten

Vortrag 44

Increased surfactant protein (SP) A expression in asthmatic *Brown Norway (BN)* rats*

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Introduction: Pulmonary surfactant stabilizes alveolar airways and prevents alveolar collapse during exhalation. Surfactant proteins (SP) are necessary for preservation and stabilisation of active surfactant components. In addition, the surfactant–associated hydrophilic proteins SP-A and SP-D are members of the host defence lectins (collectins), which are important components of the innate immune system. Using the established asthma model of Brown Norway (BN) rats we tested the hypothesis that an acute asthma attack influences the expression of both proteins. Methods: Animals were sensitized twice with 1 mg Ovalbumin (OVA), 200 mg $Al(OH)_3$ s.c. and $5x10^9$ heat-killed Bortedella pertussis bacilli (Chiron Behring, Marburg, Germany) i.p.. Challenge was carried out intratracheally with 0.5% OVA in 0.9% NaCl on day 13. We compared sensitized NaCl- (n = 6) and OVA-challenged (n = 5) BN rats. 24h after challenge, bronchoalveolar lavage (BAL) was obtained from the left lung. The superior lobe of the right lung was filled with OCT and frozen on dry ice (Immunohistochemistry), the inferior lobe was frozen in liquid nitrogen (qRt-PCR, Western Blot). Results: After asthma induction 1) the total amount of SP-A was increased in lung tissue and in the BALF, 2) a higher surface fraction of SP-A labelled alveolar epithelial cells type II (AEII) was found, 3) the level of SP-A-m-RNA in lung tissue was elevated, 4) a higher number of macrophages labelled with SP-A per mm^2 was counted. Preliminary data showed also an elevation of SP-D in the BAL and a tendency to a higher surface fraction of SP-D labelled AEII. The number of SP-D positive alveolar macrophages did not differ. Conclusions: There is an increased gene expression and secretion of SP-A after sensitization and asthma induction.The secreted SP-A may operate as an opsonin and promote phagocytosis by alveolar macrophages.Thus, the increased levels of SP with its immunomodulatory functions after allergic challenge may be a part of the response to the acute airway inflammation.

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Vortrag 45

Embryoblast and trophoblast specific signalling of insulin and IGF-1 in rabbit blastocysts

Embryoblast- und Trophoblast-spezifische Signalwege von Insulin und IGF-1 in Kaninchenblastozysten

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The insulin growth factor (IGF) family is one of the most important endocrine and paracrine systems regulating foetal and placental growth. Although preimplantation embryos do not synthesize insulin they have access to maternal insulin via oviduct and uterine fluid. The preimplantation embryo expresses receptors for insulin and IGF (IR, IGF-1R). Signalling and function of the IGF system during embryo pre- and perimplantation development is still unclear. We have investigated the localisation of IR and IGF-1R, the activation of their downstream signalling pathways and target genes c-fos, early growth response gene (EGR) 1 and phosphoenolpyruvate carboxykinase (PEPCK) in 6 day old rabbit blastocysts. Target genes were quantified in whole blastocysts and in the separated embryoblast (Em) and trophoblast (Tr) by real time PCR. At the blastocyst stage the IR and IGF-1 receptor are expressed in a specific expression pattern in Em and Tr cells. Whereas the IR was expressed in both compartments, the IGF-1R was mainly localized in the Em. In rabbit blastocysts insulin acts via mitogenic-activated protein kinase (MAPK) while IGF-1 activated both the MAPK and phosphatidylinositol 3-kinase (PI3K) signalling pathway. The induction of the c-fos and EGR-1 genes was different in the Em and Tr. In separated Em and Tr, insulin induced c-fos and EGR-1 in the trophoblast but not in the Em. In contrast, IGF-1 increased c-fos and EGR-1 in the Em, but not in the Tr. The cell-line specific signalling of insulin and IGF-1 correlates with a specific expression pattern of the IR isoforms A and B and the IGF-1R in Em and Tr. In whole blastocysts the PEPCK expression was decreased by insulin and not affected by IGF-1. Both, the receptor specific expression pattern and signalling indicate that insulin and IGF-1 exert cell-line specific effects during blastocyst development. Analysis of the impact of insulin and IGF-1 on pre- and postimplantation development is of high interest for embryologists and clinicians. *Supported by the Deutsche Forschungsgemeinschaft (DFG FI306/13-1) and the Wilhelm-Roux-Programme of MLU.*

Vortrag 46

RS1 regulates the exocytotic pathway of the Na^+-D-glucose cotransporter SGLT1

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The intracellulary localized regulatory protein RS1 (*RSC1A1*) downregulates the human Na^+- D-glucose cotransporter hSGLT1 and other plasma membrane transporters via transcriptional and posttransriptional mechanisms. In this study, we investigated the posttranslational regulation of hSGLT1 and the human organic cation transporter hOCT2 by human RS1 using the *Xenopus laevis* oocyte expression system. Injection of purified hRS protein in transporter expressing oocytes lead to a downregulation of SGLT1 mediated methyl-α-D-glucopyranoside (AMG) uptake or hOCT2 mediated tetraethylammonium (TEA) uptake within 30 min. Inhibition of AMG uptake by injected hRS1 protein was abolished by coexpression of a dominant-negative dynamin I mutant and increased after stimulation of protein kinase C. Inhibition remained unaltered when endocytosis was blocked by chlorpromazine, imipramine or filipin but was prevented when exocytosis was inhibited by botolinum toxin B or when the release of vesicles from the TGN and endosomes was inhibited by brefeldin A. Inhibition of hSGLT1 or hOCT2 by hRS1 protein were decreased at enhanced intracellular AMG concentration. Coexpression with deletion mutants of hRS1 identified three active peptide sequences. After injection of the respective oligopeptides hSGLT1 or hOCT2 mediated transport was inhibited by about 30-40%. The data suggest that hRS1 protein exhibits glucose dependent short term inhibition of hSGLT1 and hOCT2 by inhibiting the release of vesicles from the TGN whereby distinct short amino acid sequences of hRS1 are involved in the exocytotic regulation of hSGLT1 and hOCT2.

Vortrag 47

Cyclic expression of CYR61 in endometrium and its regulation in vitro

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CYR61 (cysteine-rich protein 61, CCN1) is a member of a family of growth factor-inducible immediate-early genes, which have been shown to be crucial for angiogenesis and involved in endometriosis. In this study, we investigated CYR61 expression pattern in human endometrium during cycling and its possible regulation mechanisms in endometrial cell lines. CYR61 mRNA and protein expression were investigated in endometrial tissues from 54 cycling women obtained at different stages of menstrual cycle and dated histologically to early, mid and late proliferative, early, mid and late secretory as well as menstrual phase. To investigate further the oestrogen-, cytokine- and oxygen-dependent regulation of CYR61 expression we used two endometrial epithelial cell lines HES and RL95-2, which express both estrogen receptors (ESR1 and ESR2) and the EGF receptor 1 (HER1). CYR61 mRNA levels were significant higher in proliferative compared to the secretory phase. In the secretory phase, the levels constitutively increased from early to late secretory stage. Maximal CYR61 mRNA expression, however, was detected in menstrual tissues corresponding to a 5-fold upregulation compared to mean expression in endometria from proliferative and secretory phase. CYR61 protein has been localized mainly to glandular and luminal epithelia and was also present in endothelial cells and some macrophages of endometrium. Estrogen and EGF are agents active in proliferative phase of the cycle and induced synergistically an early gene response of CYR61. However, only the HES cells did respond to treatment with 17-β-estradiol increasing 7-fold CYR61 mRNA levels. Both cell lines responded to EGF treatment with a significant upregulation of CYR61 transcripts after 30min. Most of the pro-inflammatory cytokines (TNFα, PGE2, IL1α, IL1β, PGF2α and IL8) as well as hypoxic conditions elevated CYR61 transcripts in endometrial cell lines. Taken together, we presented defined cyclic changes in CYR61 expression correlated to the transforming processes in the endometria during cycling and regulative mechanisms for CYR61 in human endometrial cell lines, which could represent the regulatory mechanisms for increased CYR61 expression during the proliferative phase and menstruation.

Vortrag 48

Azetylcholin im Urothel: Synthese- und Freisetzungswege

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Azetylcholin (ACh) ist nicht nur ein Überträgerstoff des Nervensystems, sondern auch in vielen nicht-neuronalen Zellen, insbesondere Epithelien, enthalten. Entsprechend wird ACh auch als ein vom Urothel gebildeter Mediator angesehen, der die darunter liegenden afferenten Nervenfasern sowie den M. detrusor steuern soll. Wir untersuchten den ACh Gehalt des Urothels und das Vorkommen der molekularen Komponenten der Synthese und Freisetzung von ACh mittels ACh-Assay, RT-PCR und Immunhistochemie. Sowohl im abgeschabten Urothel der Maus als auch in dem des Menschen ist ACh nachweisbar. Mittels RT-PCR konnte im Urothel statt des klassischen ACh-Syntheseenzyms Cholinazetyltransferase (ChAT) das alternative Syntheseenzym Carnitinazetyltransferase (CarAT) nachgewiesen werden. Der vesikuläre ACh-Transporter (VAChT) wird nicht vom Urothel exprimiert, in der Immunhistochemie zeigt sich aber ein VAChT-positiver Nervenplexus unmittelbar unterhalb der Basalzellschicht. Das Urothel exprimiert hingegen das Mediatophor und die organischen Kationentransporter (OCT) 1 und 3. OCT1- und OCT3-Immunreaktivität waren insbesondere in der Membran der Basalzellen zu finden. Der M2/M3-Rezeptor Antagonist Trospiumchlorid inhibiert die humanen Transporter OCT1 (IC_{50} = 7,1 µM) und OCT2 (IC_{50} = 0,8 µM), kaum jedoch OCT3 (IC_{50} = 871 µM). Zusammenfassend kann festgestellt werden, dass das Urothel ACh enthält. Seine Herkunft differiert jedoch sowohl von den im Nervensystem als auch von anderen Epithelien bekannten Mechanismen, indem das klassische Syntheseenzym ChAT nicht nachweisbar ist. Als ACh-Quellen des Urothels kommen eine bisher unbekannte Spleißvariante der ChAT, die CarAT sowie eine Aufnahme des unter dem Epithel freigesetzten neuronalen ACh in Frage. Die Freisetzungs-/Transportmechanismen des urothelialen ACh differieren ebenfalls vom neuronalen System (dort ist VAChT verantwortlich) und sind daher differenziell pharmakologisch adressierbar, unter anderem durch den muskarinischen Rezeptorantagonisten Trospiumchlorid.

Vortrag 49

Tracheale Zilienfunktion und Zelldifferenzierung bei muskarinischen Rezeptor Knockout Mäusen

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Muskarinische Rezeptoren (MR) spielen eine wichtige Rolle beim mukoziliären Transport des Atemwegsepithels, und sollen in dessen Differenzierung involviert sein. Welche Funktionen die einzelnen MR bei diesen Prozessen spielen, ist nicht bekannt. Um diese Frage zu beantworten, wurden MR-defiziente (-/-) Mäuse und ihr jeweiliger Wildtyp (Wt) morphologisch und funktionell untersucht. Veränderungen der Morphologie des Trachealepithels wurden auf licht- und elektronenmikroskopischer Ebene bestimmt und die relative Anzahl der Zelltypen des Trachealepithels gezählt. Außerdem wurde die ziliäre Partikeltransportgeschwindigkeit (PTG) und die Zilienschlagfrequenz (ZSF) nach Stimulation mit Muskarin (M) und ATP untersucht. Es ergaben sich keine Unterschiede in der Anzahl und Morphologie der zilientragenden, nicht-zilientragenden und der Basalzellen bei M1R-/-, M2R-/- und M3R-/- Mäusen. In Wt Mäusen induzierte sowohl die Zugabe von M als auch die Zugabe von ATP einen Anstieg der PTG, parallel zu einem Anstieg der ZSF. In M3R-/- Tieren steigerte M weder die PTG noch die ZSF. Auch die initiale PTG und die PTG nach ATP Zugabe war in M3R-/- Mäusen im Vergleich zu Wt stark erniedrigt. Wie in M3R-/- Mäusen, zeigte sich auch in M1R-/- Mäusen eine erniedrigte initiale PTG und verringerte Antwort auf M und ATP, obwohl die ZSF vergleichbar mit der des Wt war. In M2R-/- Tieren war die initiale PTG mit den Wt vergleichbar, die Reaktion auf M und ATP war jedoch erhöht. Gegenüber den M3R-/- Mäusen, zeigten M2/3R-/- Mäuse einen Anstieg der PTG auf M und ihr Anstieg nach ATP war vergleichbar mit dem Anstieg des Wt, was darauf hindeutet, dass der M2R für die verminderte Reaktion auf ATP in M3R-/- Mäusen verantwortlich ist. Diese Ergebnisse zeigen eine essenzielle Rolle der M1R, M2R und M3R bei der Regulation der PTG, jedoch nicht bei der Differenzierung des Trachealepithels.

Vortrag 50

Tamm-Horsfall protein is involved in the modulation of ion transport in the thick ascending limb

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Tamm-Horsfall protein (THP) is synthesized exclusively in the thick ascending limb of Henle
 INCLUDEPICTURE "http://www.marathonmultimedia.com/graphics/alphabet/apos.jpg" * MERGEFORMATINET s loop (TAL) and abundantly released into the urine. Together with NKCC2, the major ion transporter of TAL, intracellular THP is integrated in lipid rafts for polar sorting. Human mutations link both products with defective urinary concentration. We have previously described reduced urine concentrating ability under water deprivation in THP-deficient mice (THP-/-); steady state expression of distal tubular ion transporters was increased in these mice suggesting defective sodium reabsorption in TAL. We therefore hypothesize that THP interferes with NKCC2 trafficking, possibly via the assembly of lipid rafts. To detect compensatory adaptation of distal tubule we applied short term thiazide (HCT) treatment (50mg/kg) under osmotic diuresis in THP-/- and control strain. 10 urine fractions were collected in 15 min intervals for urinanalysis. Apical trafficking of NKCC2 was studied by histochemistry and Western blot in AVP (30 min. dDAVP; 1ng/g b.w.) or vehicle treated THP-/- and controls. Lipid raft assays were performed with these materials. There were no major differences in urine parameters between strains during osmotic diuresis alone. Addition of HCT induced a significantly higher sodium excretion in THP-/- compared to the control strain (+53% and +57% in the final two fractions). dDAVP induced a +25% increase in NKCC2 in membrane preparations from control mice, whereas THP-/- mice showed no difference, suggesting diminished apical trafficking of NKCC2 in the THP-/- strain. dDAVP administration further resulted in a rapid integration of NKCC2 into lipid rafts in controls, but less so in THP-/-. We suggest that THP interferes with the assembly and apical trafficking of lipid rafts containing NKCC2. This could explain the compromised concentrating ability in THP deficient mice or in patients with mutated THP.

Vortrag 51

Regulation of adhesion by dimerisation and oligomerisation of vascular endothelial cadherin (VE-cadherin)

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VE-cadherin is a cytoskeleton-anchored membrane protein which mediates Ca^{2+}-dependent cell-cell adhesion of endothelial cells by homophilic trans-interaction with cadherins of neighbouring cells. As cadherin trans-interaction is a low-affinity reaction (KD~10^{-4} M), adhesive strength might be altered not only by Ca^{2+}-dependent changes in cadherin-cytoskeletal linkage but also by dimerization or clustering of cadherins. In order to analyse the role of dimerization and oligomerization on cadherin-mediated cytoskeletal-independent adhesion, CHO cells were transfected with constructs encoding VE-cadherin fusion proteins, in which the cytoplasmic domain of VE-cadherin was replaced by a FK506 binding domain which allowed dimerization or oligomerization by a membrane-permeable FK506 crosslinker. We applied laser tweezer technique and single molecule fluorescent microscopy to characterise VE-cadherin-mediated adhesion between VE-cadherin expressing cells and VE-cadherin-coated microspheres or soluble VE-cadherin dimers respectively. The experiments showed that formation of cadherin cis-dimers is necessary for VE-cadherin trans-interaction with soluble and bead-bound VE-cadherin dimers. Dimerization increased binding of VE-cadherin-coated microspheres to transfected cells 75 % above control levels. However, oligomerization of VE-cadherin-molecules did not further increase microsphere binding activity. If oligomerization was induced before bead addition, no increase of adhesion compared to untreated cells was observed. These results imply that, strong binding occures only if VE-cadherins form dimers and are mobile in the plane of the lipid bilayer thereby allowing the dimers to accomodate to VE-cadherin of the opposing cell surface (beads).

Vortrag 52

Regulation of ectodermal Wnt-6 expression by the neural tube is transduced by dermomyotomal Wnt-11: A mechanism of dermomyotomal lip sustainment

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Ectodermal Wnt-6 plays an important role during development of the somites and the lateral plate mesoderm. In the course of development, *Wnt-6* expression shows a dynamic pattern. At the level of the segmental plate and the epithelial somites, *Wnt-6* is expressed in the entire ectoderm overlying the neural tube, the paraxial mesoderm and the lateral plate mesoderm. With somite maturation, expression becomes restricted to the lateral ectoderm covering the ventrolateral lip of the dermomyotome and the lateral plate mesoderm. To study the regulation of *Wnt-6* expression, we have interfered with neighboring signaling pathways. We show that Wnt-1 and Wnt-3a signaling from the neural tube inhibit *Wnt-6* expression in the medial surface ectoderm via dermomyotomal Wnt-11. We demonstrate that Wnt-11 is an epithelialization factor acting on the medial dermomyotome, and present a model suggesting Wnt-11 and Wnt-6 as factors maintaining the epithelial nature of the dorsomedial and ventrolateral lips of the dermomyotome, respectively, during dermomyotomal growth.

Vortrag 53

Desmoglein 1 undergoes multivalent Ca^{2+}-dependent low affinity trans-interaction

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Desmoglein 1 is a desmosomal member of the cadherin family expressed in stratified epithelia. Desmoglein 1 is the target adhesion molecule of severe blistering skin diseases such as pemphigus or bullous impetigo. However, despite this enormous pathological relevance, the molecular binding properties of desmoglein 1 are largely unknown. Using single molecule atomic force microscopy we provide evidence that desmoglein 1 undergoes Ca^{2+}-dependent (K_D = 0.8 mM Ca^{2+}) homophilic trans-interaction. While the single unit unbinding force is comparable to other cadherins (~ 40 pN at retrace velocity of 300 nm/s), significant differences with respect to the affinity and stoichiometry were observed. Desmoglein 1 molecules displayed Ca^{2+}-dependent self-aggregation of the extracellular domains which was different from other cadherins. Thus, besides the biophysical characterization of Desmoglein 1, an important outcome of the study is that Desmoglein 1 substantially differs from other members of the cadherin family in terms of molecular binding properties.

Vortrag 54

Molecular proof for the transport mechanism of the organic cation transporter OCT1

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Polyspecific transporters for organic cations from the SLC22 family participate in uptake, excretion and tissue distribution in organs as kidney, liver, or brain. They translocate a great variety of organic cations, e.g. monoamine neurotransmitters or choline. However, the molecular basis of polyspecific substrate recognition and the transport mechanism is not understood. It was discussed, if translocation of substrates occurs by a conformational change of the binding pocket from extracellular to intracellular or if a channel-like pathway, including some selectivity filter exists. Recently, based on extensive mutagenesis studies, we presented a structural model of the rat organic cation transporter 1 (rOCT1). This model shows a large substrate binding pocket with different interaction sites for diverse substrates that is flanked by several transmebrane helices. We identified two amino acids in position 447 and 448 within the 10^{th} transmembrane helix that are localized within the binding pocket and are involved in corticosterone binding.
Employing electrical measurements in oocytes of *Xenopus laevis*, we now investigated corticosterone binding to rOCT1 and point mutations of rOCT1. Corticosterone binds with low affinity to rOCT1 (IC_{50} ~ 200 µM) and no difference between interaction from the intra- or extracellular side was observed. Exchange of lysine in position 447 for tyrosine significantly increased the affinity of corticosterone binding from both sides of the membrane. Furthermore, this mutation also brought about an asymmetry in binding affinities since the affinity increase was significantly larger on the intracellular side. In contrast, exchange of glutamine 448 to glutamate only affects corticosterone binding from intracellular. Analysis of the structural model of rOCT1 was consistent with an accesibility of leucine 447 from extracellular in the outward conformation of the substrate binding pocket and from intracellular in the inward conformation. The data provide molecular evidence for a classical transporter type functionality of an organic cation transporter including a conformational change of the binding pocket from extra- to intracellular.

Vortrag 55

Ethinylestradiol (EE2) differentially affects IGF-I in male and female bony fish

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Growth and sexual differentiation are closely interlinked in fish, but the underlying physiology is not understood yet. Studies in mammals suggest that estrogen(s) may interfere with the IGFs but few data exist in fish and nothing is known about potential effects on IGF-I during development. A population of tilapia was fed during 10-40 day post fertilization (DPF) with the optimal dosis of EE2 to induce feminization. The effects were studied in males and females at 75, 90 and 165 DPF using RIA for serum IGF-I and RT-PCR for IGF-I and estrogen receptor α (ERα) in liver, gonads, brain and gills. Estrogen treatment affected both sexual differentiation and growth, i.e. it led to a significant shift of sex ratio 86.5% females vs. 47.2% (control) and to significant impairment of growth (165 DPF: body weight -51.2%, body length –19.2%). At 75 DPF, serum IGF-I was decreased (7.35 ± 2.35 ng/ml, controls: 10.57 ± 3.37 ng/ml) and later recovered. In correspondence, IGF-I mRNA in liver was reduced at 75 DPF (females -46%, males -60%) and recovered. Liver ERα was transiently (75 DPF) induced by about the 30-fold. Thus, growth impairment by estrogen(s) in fish may be due to prolonged suppression of liver IGF-I synthesis and release. In testes, IGF-I mRNA expression was highly suppressed (75 DPF, -80%) and partially recovered but in ovary only slightly suppressed. ERα transiently (75 DPF) decreased in testes (-90%) and ovaries (-43%). IGF-I mRNA in male brain was shortly reduced but in female brain suppressed (75 DPF, -71%), increased (90 DPF, 32%) and lowered to the normal. At 90 DPF, ERα was transiently increased (100%) in female brain. In gills, there was a trend to increase IGF-I mRNA expression. Thus, during fish ontogeny IGF-I may be a target for the disturbing effects of estrogen(s) on growth and reproduction. The IGF-I response seems to be tissue-specific with a sex difference in gonads and brain. The findings provide initial insight into the molecular processes underlying the crosstalk between growth and sexual hormone systems in fish. Supported by the SNF (NRP 50, project 4050-66580).

Vortrag 56

Pxmp 2 is a mediator of the metabolism of reactive oxygen species

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Peroxisomes are subcellular organelles that are present in virtually all eukaryotic cells. They are involved in the metabolism of lipids and of reactive oxygen species (ROS). The peroxisomal membrane contains a number of integral and peripheral membrane proteins involved in the import of peroxisomal matrix proteins and the transport of metabolites across the membrane. Pxmp2 is the most abundant peroxisomal membrane protein in higher eukaryotes. However, little is known about the function of this protein.
We have investigated a potential function of Pxmp2 in ROS metabolism. 2'7' dichloro-fluorescin (DCF)-fluorescence is increased upon oxidation and DCF therefore serves as a reporter for visualization of intracellular oxidants. Using a single cell fluorescence assay, we measured DCF-fluorescence levels as an indicator for oxidative stress. Our experiments revealed that intracellular ROS-levels were significantly higher in Pxmp2-deficient cells compared with wildtype cells. These data were confirmed by Laser scanning microscopy using dihydroethidine as an alternative probe for fluorometric determination of ROS production in viable cells. In order to understand the adaptive reactions of cells to compensate for Pxmp2-defciency, we compared wildtype and Pxmp2 knockout cells with respect to expression levels of genes involved in peroxisome biogenesis, pathways of peroxisomal metabolism and cellular metabolism of ROS by real time RT-PCR. We could show, that expression levels of PEX genes as well as of genes encoding for metabolic enzymes were altered differentially in Pxmp2-deficient cells. Taken together, our data demonstrate a higher susceptibility of Pxmp2 knockout cells to oxidative stress suggesting a role of Pxmp2 for the cellular response to ROS.

POSTER 1

Virtueller mikroskopisch-anatomischer Kurs

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Es wird eine Sammlung von mehr als 100 virtuellen histologischen Schnitten aus verschiedenen anatomischen Instituten präsentiert und den Teilnehmern der Arbeitstagung zum selbständigen virtuellen Mikroskopieren zur Verfügung gestellt. Die Präsentation erfolgt nicht über das Internet sondern unter Verwendung einer externen USB 2.0-Festplatte als Datenspeicher. Diese Konfiguration erlaubt ein virtuelles Mikroskopieren in Echtzeit.

POSTER 2

Two-photon fluorescence excitation: differentiation of fluorescence signals for in-vivo imaging of tissue components

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Two-photon laser scanning microscopy (TPLSM) provides intrinsic three-dimensional resolution of optical sections less than 1µm in thickness in living tissue. The most commonly used femtosecond lasers provide a large wavelength tuning range, from about 700 nm to over 1000 nm. Within this range they excite native biological fluorophores, such as NADPH (autofluorescence) as well as highly organized structures, like collagen fibrils, and the most commonly used fluorophores. We have developed an assay concept that is able to distinguish between the tissue autofluorescence and fluorescent markers, by combining excitation profiles with fluorescence lifetime imaging and short-wavelengh second harmonic generation (SHG). Our data show that the common fluorescent dyes FITC, Cy2 and Alexa 488 which exhibit nearly identical fluorescence excitation and emission profiles in one-photon excitation, display different excitation characteristics in two-photon absorption. Whereas Alexa 488 and FITC display fluorescence excitation even in the low infrared range (710–750 nm), Cy2 reveals characteristic fluorescence excitation only above 800 nm. Our observations also show that the fluorescent light emitted by the cyanine dye Cy2 shows average lifetimes of 1200 ps whereas Alexa 488, tissue autofluorescence and FITC display average lifetimes of 1700 ps to 1900 ps. The ability to distinguish the different fluorescence signals provides the oppurtunity to discriminate endogenous fluorophores from applied fluorescent markers in order to differentiate subcellular, cellular and extracellular tissue components.

POSTER 3

Multiphotonen-Mikroskopie und Laser-Nanochirurgie der Kornea mittels Naher-Infrarot Nanojoule Femtosekunden Laserpulse

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Die Vorzüge der Multiphotonen-Mikroskopie sind die selektive *in-vivo* Darstellung von Gewebebestandteilen mit subzellulärer Auflösung bei hohem Kontrast ohne vorangehendes Anfärben und Schneiden, die hohe Eindringtiefe von NIR-Licht in Gewebe und die Nutzung endogener intrazellulärer Fluorophore. Das macht die Multiphotonen-Mikroskopie zu einem neuartigen Diagnosewerkzeug für die *in-vivo* Differenzierung von Gewebeschichten, für die dreidimensionale optische Darstellung von Epithelzellen, Keratozyten und Endothelzellen sowie von Kollagenlamellen. Basierend auf 80MHz Nanojoule (nJ) NIR fs gepulsten Ti: Sa Lasersystemen, wird die Fähigkeit dieser Methode zur Nanodissection anhand histologischer Resultate belegt. Innerhalb des behandelten Gewebes treten keinerlei schädliche Effekte auf und die Regeneration und Wundheilung erfolgt vollständig ohne Narbenbildung. Die Multiphotonen-Mikroskopie ist somit ein leistungsfähiges Instrument zur in-vivo 3-D Raumanalytik von Geweben und gleichzeitig auch ein leistungsfähiges Werkzeug für die Laser-Nanochirurgie.

POSTER 4

Two-photon laser scanning microscopy of the eye – A step towards in-vivo histology.

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Two-photon laser scanning microscopy (TPLSM) is a new high-resolution optical technique that allows ex-vivo and in-vivo investigations of biological tissues without the use of artificial stains or fixatives. The eye resembles an eligible organ for optical imaging due to its transparent tissue components. First results from ex-vivo TPLSM imaging of ocular tissues from different species are shown to demonstrate potentials and limitations of this new method.
TPLSM imaging, fluorescence-lifetime measurements (FLIM) and second harmonic generation (SHG) were applied to unfixed ex-vivo ocular tissues from pig, mouse and rabbit.
Ocular surface analysis showed corneal and conjunctival components in cellular and subcellular resolution. Whole-mount dissection enabled retinal structure analysis. Wavelength-adaptation lead to detailed differentiation of cellular and non-cellular components with tomographic reconstruction of image stacks. FLIM and SHG allowed further cellular characterisation.
TPLSM imaging is a promising technique to analyze morphology and function of ocular tissues in detail. This method has great potential in ocular basic research as well as in clinical investigations as for the first time non-invasive optical imaging provides details so far generated by tissue probing and histological preparation only.

Zwei-Photonenmikroskopie des Auges – Auf dem Weg zur in-vivo Histologie

POSTER 5

In-vivo two-photon laser scanning microscopy: a novel technique for long-time investigations of the intestinal mucosa in living mice

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Two-photon fluorescence excitation allows in-vivo microscopy to be performed in the murine intestine based on autofluorescence of the unstained tissue. While establishing this novel technique, the most important methodical aspects to be considered were the positioning of the mouse and its intestine, the inhibition of intestinal peristalsis and the development of an optical setting that allows high quality imaging over several hours. We constructed a special operating table that keeps the temperature of the animal and the exteriorized gut at a physiological level and permits permanent moistening of the intestinal mucosa during long-time examination. It also fixes the intestine in a position suitable for microscopy. Reduction of intestinal peristalsis proved to be one of the crucial aspects in our experimental setting. We found that classic antidiarrheals such as butylscopolamine or loperamide failed to reduce the persistalsis sufficiently while papaverine directly applied onto the mucosa inhibited intestinal motility adequately. Our data show that tissue-autofluorescence is inducable within a wavelength range between 720 nm and 910 nm. Most detailed imaging was obtained at 750 nm, exciting mainly native biological fluorophores such as NADPH, thereby reaching more than 100 µm in depth. Using these optimized laser settings we were able to perform in-vivo two-photon microscopy for up to 7 hours and defined areas of the intestine could be monitored for more than 3 hours without visible tissue damage. This experimental setting enables us to perform long-time investigations of the intestine of living mice and thus provides the opportunity to observe complex physiological and pathophysiological events on the intestinal mucosa in-vivo for the first time.

POSTER 6

"SonoBasics", a multimedia program of the ultrasonography of the pancreas in dogs and cats.

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The ultrasonography of the canine and feline pancreas causes difficulties even to experienced examiners. The purpose was to design a computer based program, which combines the advantages of books and multimedia tools and also reduces the necessary training time for ultrasonography in dogs and cats.
The program has been designed to meet high didactic and ergonomic requirements. The recommendations of the International Organisation for Standardisation (ISO) regarding software and design ergonomics have been considered.
First of all topography and patient preparations are shown. Scanning positions are described in detail with regard to the respective ultrasound images. Many photos of specimens and many collages including photos and graphics illustrate the anatomy and scanning positions. Characteristic ultrasonographies of all parts of the pancreas are presented. The last chapter of the program includes the colour flow imaging of the pancreatic vessels. The canine pancreas is described on 28 main pages, the feline pancreas on 19 main pages. Corresponding pages of dogs and cats are linked. The topic is illustrated by 110 images and 23 films. Every image and film can be blown up in high resolution and shown without and with clear labelling.
The non experienced ultrasound user should best work through the program step by step such as going through a book page by page. The more experienced examiner can move freely, using the navigation bar, text links, or the detailed table of contents. Nevertheless a clear labelling of every page preserves from being “lost in hyperspace”. Accordingly the CD has the advantages of a book, such as a consistent structure, a well linked index and a list of references. Additionally, films improve the understanding of and dealing with ultrasonography. The consistent and comprehensive structure of the multimedia presentation enables the veterinarian to learn ultrasonography of the pancreas in the dog and cat autodidactically.

POSTER 7

Necessity of the somatotype differentiation for the correct measurement of inner organs ultrasound parameters

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Complex investigation of 108 practically healthy urban girls 12 – 15 years old and 103 boys 13 – 16 years old was carried out. Selected girls and boys after prior psychophysiological and psychohygienic questionnaire examination underwent to detailed clinicolaboratory investigation included ultrasonography of inner organs, X-ray examination, spirography, cardiography, rheovasography, estimation of basic blood biochemical indices, pric-test with mixt-allergens etc. Anthropometrical investigation consists of evaluation of body length and body mass, longitudinal, circumference, transversal, anteroposterior body sizes and thickness of dermatofatty folds (aggregate amount – 52 sizes). For the somatotype evaluation the scheme of J.Carter and B.Heath (1990) was used. Statistical processing of data was faced out in package "STATISTICA 5.5" (license number AXXR910A374605FA) with the usage of parametric and nonparametric methods of findings estimation.

Under the construction of regression models of normative ultrasound sizes of different organs according to anthropometrical indices of the body the age and the sex of the adolescents were considered. However, received indices of determination in majority models were greatly lesser than 50%. Under disjunction of adolescents according to somatotypes (generally marked mesomorphs, ectomorphs and meso-ectomorphs) index of determination in models increase practically in all cases to 80-95% under inclusion to regression linear polynomial only 3-4 anthropometrical indices. Findings denoted to necessity of obligatory accounting not only age and sex but a somatotype in adolescents under the calculation of normative ultrasound sizes of inner organs.

POSTER 8

Wirkung einiger Umweltfaktoren auf die physikalische Entwicklung der Neugeborenen und der Kinder im ersten Lebensjahr

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Diese Untersuchung wurde durchgeführt, damit die Wirkung einiger klimatisch-meteorologischen Umweltfaktoren und solarer Aktivität auf die somatometrische Hauptkennzeichen der physikalischen Entwicklung der Neugeborenen und der Kinder im ersten Lebensjahr (Wuchs, Gewicht, Brustkorb- und Kopfumfang) zu erforschen. 4886 Neugeborenen und der Kinder im ersten Lebensjahr, die von 1980 bis 1996 geboren wurden, wurden untersucht. Die Materialverteilung je nach der Periode, die den Grad solarer Aktivität charakterisieren, wurde entsprechend dem Zählwert von Wolf-Zahl, für die eine bestimmte Periodizität (11-jährige Zyklus) kennzeichnend ist, durchgeführt. Für die Bearbeitung des Materials wurden „die Methode der Zeitalterschichtung“ und das Verfahren der statistischen Auswertung benutzt. Es wurde festgestellt, dass während der untersuchenden Zeit die jahresdurchschnittliche Lufttemperatur und die jahresdurchschnittliche relative Luftfeuchtigkeit weitgehend variierten. Der jahresdurchschnittliche Barometerdruck veränderte sich in dieser Zeit gleichmäßiger, nur im Jahre 1985 wurde seine intensive Senkung beobachtet, was auch mit der Senkung der jahresdurchschnittlichen Lufttemperatur und der jahresdurchschnittlichen relativen Luftfeuchtigkeit dazu noch mit der minimalen Sonnenaktivitätskennzeichen zusammengefallen ist. In dieser Zeit vergrößerte sich die Körperlänge bei den Jungen auf 0,74 cm und das Körpergewicht auf 172,0 g ($P>0,05$), bei den Mädchen bzw auf 0,82 und 143,0 ($P>0,05$) im Vergleich zu den Jahren mit der maximalen Sonnenaktivität und hohem klimatisch-meteorologischen Kennzeichen. Gleichartige Veränderungen wurden bei den Parametern des Brustkorb- und Kopfumfanges beobachtet, die vergrößerten sich und bis zum Jahre 1985 maximale Größen erreichten, danach bis zum Jahre 1991 wurde ihre allmähliche Senkung und bis zum Jahre 1996 Stabilisierung dieser Kennzeichen beobachtet. Die Arbeit wurde unter der finanziellen Unterstützung der Humanistisch-wissenschaftlichen Stiftung N 0506055608 a /14.

POSTER 9

Ultrasonographic examination data of the obturative function of myocardial sphincters of the right pulmonary vein.

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To study the function of myocardial sphincters of pulmonary veins we have performed the ultrasonic heart examination of 50 men aged from 19 to 27 years. All examined individuals had no history of heart diseases. The blood flow in the right superior pulmonary vein (RSPV) was recorded in the mode of impulse Doppler ultrasonography. Besides we measured the orifices diameters of this vein during atrial systole and diastole. During the investigation we have obtained the following data: the systolic blood component in RSPV was 0,51±0,01 m/sec, of the diastolic one – 0,54±0,02 m/sec and of the atrial one - 0,34±0,01 m/sec ($p \leq 0,001$). The orifice diameter of RSPV to be 12,01±0,26 mm during the systole; it being 14,11±0,29 mm during the diastole ($p \leq 0,001$).
The lumen of RSPV orifice is narrowed during the atrial systole but a complete closing doesn't occur and a retrograde blood flow normally occurs. The value of the retrograde flow in RSPV (atrial component) is lower during the atrial systole than the blood rate from this vein to the atria during the atrial diastole. So, the function of myocardial sphincters is to prevent the excessive amount of blood passing to the veins in atrial systole thus regulating the blood flow to the ventricles. The presence of the retrograde blood flow in pulmonary veins eliminates the possibility of repletion of ventricles.

POSTER 10

Anatomo-Clinical Considerations of Mitral Valve in Cardiac Tumors

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The study assesses the changes that occur at the level of the left atrioventricular valve in cases of intracardiac neoplasm. These are observed both macroscopically and microscopically, and compared to similar structures coming from human cadavers from the laboratory of anatomy of the "Victor Babes" University of Medicine and Pharmacy, Timişoara, Romania. The case that was examined was that of a patient operated in the 2nd Department of Cardiovascular Surgery of the Cardiology Institute in Timişoara. The patient underwent a surgical procedure for valvular prosthesis. The neoplasm developed in the left ventricle and it insinuated in the left atrium, altering in the functionality of the mitral valve. The bioptic examination confirmed the diagnosis of sarcoma. The slides were coloured at the Department of Morfopathology in HE stain and trichromic Masson, and were examined with 140, 280x objectives.

POSTER 11

Study of the Morphological Variability of the Jugular Veins

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Knowing the variants of the veins of the head and neck is extremely important both for the surgeon operating at this level, for the radiologist performing catheterization and for the clinician in general. The study of the trajectory and affluents of the external and internal jugular veins was carried out on 60 cadavers preserved in formalin, in the laboratories of the Department of Anatomy of the “Victor Babes” University of Medicine and Pharmacy, Timisoara, using the macroscopic dissection method. Of the total of 60 cadavers, 20 were men and 40 women. The following results were obtained: in 58 of the cases the external jugular originates in the bulk of the parotid gland from the confluence of the posterior auricular vein with the posterior branch of the retromandibular vein; the lingual vein unites with the facial vein forming a linguofacial trunk that drains into the external jugular vein, and than this one drain into the internal jugular vein above the anterior belly of the omohyoid muscle, an anomaly that was not found anywhere in literature (2 cases); the internal jugular vein receives the lingual and facial veins through a linguofacial venous trunk and independent the superior thyroid vein (30 cases); the internal jugular vein receives the lingual, facial and superior thyroid veins through a thyrolinguofacial trunk (20 cases); the lingual, facial and superior thyroid veins independently drain into the internal jugular vein (8 cases).

POSTER 12

Vascular Morphometry and Micrometry of the Orbital Region of the Human Optic Nerve

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The study aims at providing data on the vascular morphometry and micrometry of the orbital region of the human optic nerve. The disposition of the microcirculatory trunk in the orbital region has been studied on 40 samples taken post-mortem, as well as on 10 pieces from subjects having undergone enucleation. Histological specimens were prepared from the optic nerves using eosin-hematoxylin dye-staining, the Romhany-Bârzu staining and the Gomory trichromic staining. Functionally active capillaries make up 19.380 per cent (7 – 14 μ group), respectively 21.318 per cent (15 – 20 μ group) of the total vessels in the area, having an average diameter of 10.01 ± 0.0826085 μ, respectively 17.9272 ± 0.0651995 μ, for P = 0.05. There are 169 reserve capillaries (with a diameter between 2 – 6 micron), representing 32.752 of the total of vessels counted. The existence of reserve capillaries allows, at a certain point, for the supplying of the obstruction or of the vasospasm on the territory of the functionally active capillary.

POSTER 13

Klinische Bedeutung von Varianten der Armarterien: Oberflächlich verlaufende Armarterien

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Variationen der Armarterien kommen häufig vor. Oberflächlich verlaufende Armarterien werden eher selten entdeckt. Klinische Bedeutung gewinnen oberflächliche Armarterien, wenn sie durch die Ellenbeuge verlaufen. Hier können sie bei einer intravenösen Blutentnahme versehentlich punktiert werden. Es wurde der Arterienbauplan der Arme von 30 Körperspendern des Institutes für Anatomie (Universität Rostock) untersucht.
Bei 3 von 30 Armen (10%) wurden oberflächlich verlaufende Armarterien gefunden. Fall 1: Bei nach distal verschobener Medianusgabel teilt sich die A. brachialis in der Mitte des Oberarms in einen ober-flächlichen und tiefen Ast. Der oberfläche Ast spaltet sich in die A. radialis und die A. ulnaris auf, welche beide ebenfalls oberflächlich auf den Unterarmbeugemuskeln verlaufen. Aus dem tiefen Teil, der vom N. medianus überquert wird, gehen die Aa. interosseae communis, anterior und posterior hervor. Der Arcus palmaris superficialis wird von der A. ulnaris gebildet. Fall 2: Die A. brachialis teilt sich in der Ellenbeuge in die Aa. radialis, ulnaris und interossea communis, welche oberhalb des N. medianus liegen. Die oberflächlich verlaufende A. ulnaris zieht über den M. flexor digitorum superficialis hinweg und bildet den Arcus palmaris superficialis. Fall 3: Etwa in der Mitte des Oberarms geht aus der A. brachialis die A. radialis hervor. Die A. radialis überkreuzt in der Ellenbeuge die A. brachialis und den N. medianus. Die A. ulnaris, wohl als Fortführung der A. brachialis zu betrachten, bildet den Arcus palmaris superficialis.
Die Ausbildung von oberflächlichen Armarterien liegt im Bereich der Variabilität des Arterienbauplans der Säugetiere. Bei einigen Säugetieren gibt es eine oberflächlich und eine tief verlaufende A. brachialis. Bei den hier geschilderten Fällen sind die oberflächlichen Armarterien durch eine besondere Lage zum N. medianus gekennzeichnet. Oftmals ist einem Merkmalsträger ein an ungewöhnlicher Stelle pulsierendes Gefäß schon bekannt. Im Ultraschall läßt sich der genaue Verlauf derartiger Arterien untersuchen, so daß eine unbeabsichtigte Punktion im Rahmen der klinischen Diagnostik vermieden werden kann.

POSTER 14

Die Anatomie des Ramus perforans der Arteria fibularis und seine klinische Relevanz

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Bei Operationen am oberen Sprunggelenk kann es aufgrund des unterschiedlichen Verlaufs und Lage des Ramus perforans der Arteria fibularis zu Blutungen kommen, die zu massiven Hämatombildungen, Knochennekrosen und Wundheilungsstörungen führen können. Das Ziel unserer Untersuchung war es festzustellen, in welchen Abstand zum oberen Sprunggelenk der Ramus perforans zu erwarten ist und welchen Durchmesser er hat. Dazu wurden 45 nach Thiel konservierten Leichen (30 m/ 15 w), jeweils 23 rechte Extremitäten und 22 linke Extremitäten, präpariert und analysiert. Der Abstand von der hinteren Kante der Facies articularis inferior tibiae bis zum Ursprung des Ramus perforans aus der Arteria fibularis und dessen Durchmesser wurden mit dem Gleitzirkel gemessen. Zusätzlich wurde der Ramus perforans und seine möglichen Anastomosen auf 16 Röntgenbildern dargestellt.
Der größte Abstand vom Ursprung des Ramus perforans bis zur hinteren Kante der Facies articularis inferior tibiae betrug 150mm, der kleinste Abstand 1mm (Mittelwert: 46,3mm). Der mittlere Durchmesser der Rami perforantes betrug 0,86mm. An 5 Extremitäten wurden zwischen zwei und drei Rami perforantes beobachtet. Für den postoperativen Verlauf ist die Erhaltung dieses Gefäßes eine unabdingbareVorraussetzung, um eine gute Heilung zu gewährleisten.

POSTER 15

Gefäßanatomische Untersuchungen für zweiseitig gefäßgestielte Beckenkammtransplantate

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Bei Unterkieferrekonstruktion nach Tumorresektion mit gefäßgestieltem Beckenkammtransplantat wird gewöhn-lich die A. circumflexa ilium profunda verwendet. Bei großen oder segmentierten Tranplantaten kann dabei die Ernährung des Transplantates in stielfernen Arealen grenzwertig sein. Für eine optimale Einheilung wäre daher eine Blutversorgung über zwei gegenüberliegende Pedikel (Gefäßstiele) wertvoll. Ziel war es daher die anatomischen Grundlagen für die Verwendung eines zweiten, dorsalen Pedikels durch Lumbalgefäße zu untersuchen.
An 24 phenol-/formalinfixierte Beckenhälften wurden A./V. circumflexa ilium profunda (ACIP), A./V. iliolumbalis (AIL), A./V. lumbalis 3 (AL3) und 4 (AL4) im Verlauf präpariert. Mit einer elektronischen Schublehre wurden die planen Querschnitte am Abgang/Abfluß und die Längen bis zum Eintritt in die Muskulatur vermessen. Die Lage zu den Nn. cutaneus femoris lateralis, iliohypogastricus, ilioinguinalis und genitofemoralis und Mm. psoas, iliacus, quadratus lumborum wurde beschrieben.
Die AIL hatte lateral des M. psoas eine Länge von 56mm+/-19, Gesamtlänge 119mm+/-32. Der Querschnitt der Arterie lag hier bei 4,4mm+/-1,3, der Vene bei 3,4mm+/-1,3. Die Länge der ACIP lag bei 100mm+/-18, der Querschnitt bei 3,6mm+/-0,7, der Vene 4,7mm+/-1,3. Die AL4 teilte sich in 50% der Fälle dorsal oder lateral des M. psoas in 2 Äste auf. Die Gesamtlänge vom Abgang aus der Aorta war 145 mm+/-25, lateral des Psoasrandes bei 61mm+/-13. Der Querschnitt lag bei 4,3mm+/-0,9. In 41% der Fälle verliefen hier die zugehörigen Venen paarig. Ihr Querschnitt lag lateral des M. psoas bei 2,7mm+/-0,8. Die AL3 zeigte eine Gesamtlänge von 106mm+/-3,1, die Länge lateral der Mm. psoas und quadratus lumborum bei 51mm+/-13. Bei weiterer Präparation Richtung Abgang von der Aorta kreuzt diese den Truncus sympaticus. Der Verlauf bzw. die Länge der Venen entsprach den Arterien.
Durch den hohen Abgang und der lateral der Mm. psoas und quadratus lumborum nur kurzen verbleibenden Strecke bis zum Eintritt in die Muskulatur ist die AL3 nicht geeignet zur Verwendung als 2. Pedikel für gefäßgestielte Beckenkammtransplantate. Eine tiefere Präparation wäre wegen der Nähe zum Truncus sympaticus komplikationsträchtig. Die AL4 weist eine ausreichende Länge und einen Gefäßdurchmesser auf, der mit der ACIP vergleichbar ist. Die Präparation kann durch einen ähnlichen, nach dorsal erweiterten operative Zugang erfolgen, wie er auch für die Beckenkammentnahme mit ACIP nötig ist. Zwar bedeutet diese Methode einen größeren operativen Eingriff, doch könnte der damit gewonnene 2. Pedikel an großen oder segmentierten Beckenkammtransplantaten die Voraussetzungen für eine bessere Ernährung und Einheilung schaffen.

POSTER 16

Ultrasound-guided block of the greater occipital nerve: accuracy of a selective new technique confirmed by anatomical dissection

(Die Ultraschall-gestützte Blockade des N. occipitalis major: Beleg der Treffsicherheit einer neuen, selektiven Technik mittels anatomischer Präparation)

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Background and aims: Greater occipital nerve (GON) block is used to diagnose occipital neuralgia or pain arising from the innervated area of the GON. The present study describes a new ultrasound-guided approach to this nerve and determines its accuracy using anatomical dissection control. Materials: Twenty ultrasound-guided approaches to the GON were performed in 10 embalmed cadavers. After injection of 0.1 ml of dye the cadavers were dissected to evaluate needle position and colouring of the nerve. Results: All twenty needle tips were located at the exact target point directly at the GON. In nineteen of these cases the entire nerve was coloured and in one case the nerve was partly coloured. In contrast to the standard “blind” approach of GON block we targeted the nerve more central where it is usually not divided. Just after it runs around the caudal edge of the obliquus capitis inferior muscle, it can easily be found and visualized by ultrasound. The median (range) diameters of the nerve measured by ultrasound were: 4.0 (3.2 – 5.6) x 1.8 (1.2 – 2.6) mm. It was found 27.6 (18.9 – 32.6) mm lateral to the spinous process of the C2 vertebrae. Conclusions: The anatomical dissections confirmed that our new ultrasound-guided selective approach to the GON near its origin out of the C2 root is accurate. Thus, ultrasound could become an attractive alternative to the “blind” standard techniques of GON block in pain medicine and may lead to more accurate block of the nerve.

POSTER 17

The angiogenetic factor VEGF (vascular endothelial growth factor) used at coated suture in meniscal tears: an experimental study in sheep.

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BACKGROUND:One of the most frequent sports traumas are meniscal tears in the knee joint. The potential of healing is reduced for the central two-thirds.There is no vascularization. In some studies were shown that VEGF is an important factor for vascularization. We wanted to find out which influence locally applied VEGF coated suture have. METHODS:In 18 Sheeps we created an 15mm long tear in the avascular zone of the medial meniscus.The tear was repaired with an uncoated suture (group 1, n=6), a PDLLA coated suture (group 2, n=6) and a PDLLA/VEGF coated suture (group 3, n=6). After 6 weeks the menisci were investigated macroscopically, immunhistochemically with MMP-1,-2,-3,-9,-13,TIMP-1,-2,VEGF,VEGFR-1,-2 and factor VIII, an ELISA with TIMP-2 and a realtine RT-PCR for VEGF. RESULTS: In group 3 one meniscus partial healed. There is no activity of the invesigated MMPs and only a less of endothelialisation. In group 2 one meniscus partial healed and one completly. The activity of mediators is shown. In group 1 three menisci partial healed and there is a lot of enzym acticity.This group shows a good endothelialisation. In all groups the endogenous production of VEGF was not influenced.CONCLUSION: Single locally applied VEGF does not promote healing. It is a complex system to stimulate vascularization.

POSTER 18

Die Rolle von VEGF im Verlauf der experimentell induzierten Arthrose im Kaninchenmodell

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Ziel der vorliegenden Studie ist die Untersuchung der Rolle von VEGF im Verlauf einer experimentell induzierten Arthrose am Kaninchengelenk.12 Neuseelandkaninchen wurde das vordere Kreuzband reseziert, 12 weitere erhielten eine Scheinoperation. Nach 3, 6 und 12 Wochen wurden die makroskopischen und mikroskopischen (Mankin) Arthrosekennzeichen evaluiert. Die Kreuzbanddurchtrennung führte zu zeitabhängig ansteigenden Arthrosegraden sowie einer signifikanten Steigerung der VEGF-Expression. Der Arthrosegrad korreliert positiv mit der seit der Operation vergangenen Zeit ($r = 0,52$, $p < 0,05$). Es liegt jeweils eine positive Korrelation zwischen der VEGF-Expression und dem histologischen Arthrosegrad ($r = 0,767$, $p < 0,01$) sowie zwischen der VEGF-Expression und dem makroskopischen Arthrosegrad ($r = 0,518$, $p < 0,02$) vor. Da zusätzlich auch ein Zusammenhang mit den makroskopisch und histologisch nachweisbaren Gewebeveränderungen besteht, ist eine wichtige Rolle von VEGF in der Frühphase der Arthroseentwicklung anzunehmen.

POSTER 19

Morphologische Veränderungen des Kniegelenkknorpels in Abhängigkeit von Varus- und Valgusfehlstellungen – eine longitudinale In-vivo-Untesuchungung mittels MRT

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Studien zur Untersuchung Struktur-modifizierenden Knorpelmedikamente (DMOADs) erfordern Patienten-kollektive, in denen der Knorpelabbau (Matrixverlust) relativ schnell voranschreitet, damit Aussagen über die Wirksamkeit von Medikamenten (proof of concept studies) zeitnah erfolgen können. Bislang gibt es wenig quantitative Informationen über Risikofaktoren, die die Progression des Knorpelverlustes beeinflussen. Hier testen wir die Hypothese, dass Varus- und Valgusfehlstellungen des Kniegelenks zu einer höheren Progression im belasteten Kompartiment führen als dies bei neutraler Beinachse der Fall ist.

Untersucht wurden 81 Patient(inn)en (Alter 72 ± 9 J., BMI 29.9 ± 5.5, 73% Frauen) mit der klinischen Diagnose einer Arthrose. Die Stellung des Knielgelenkes wurde aus Ganz-Bein-Röntgenaufnahmen bestimmt: 34 Patient(inn) hatten keine Fehlstellung (-2° bis +2° Abweichung der biomechanischen Achse), 27 wiesen eine Varusdeformität und 20 eine Valgusdeformität auf. Magnetresonanztomographische Aufnahmen wurden mit einer coronaren FLASHwe Sequenz zum Studienbeginn und im zeitlichen Abstand von 2 Jahren angefertigt. Aus diesen wurde nach Segmentierung die Morphologie der Knorpel (Volumen, Dicke, etc.) dreidimensional bestimmt.

Der Knorpelvolumenverlust (per annum = p.a.) betrug ohne Fehlstellung 1.1% ($p<0.05$) im medialen femorotibialen Kompartiment (0.7% an der Tibia und 1.8% am Femur) und 0.9% (n.s.) lateral (0.1% an der Tibia, 1.5% am Femur). Bei Varusfehlstellung betrug der Volumenverlust 3.8% p.a. medial ($p < 0.01$) und 1.3% lateral (n.s.), bei Valgusfehlstellung 2.9% p.a. lateral ($p < 0.05$) und -0.1% medial (n.s.).

Die Ergebnisse zeigen, dass das Ausmass des Knorpelverlustes im Femorotibialgelenk massgeblich von Fehlstellungen des Kniegelenks beeinflusst wird. Die Patient(innen) weisen eine deutlich höhere Änderungen in dem durch die Fehlstellung belasteten Kompartiment (medial bei Varus- und lateral bei Valgusgonarthrose) auf als im kontralateralen Kompartiment oder als Patient(inn)en ohne Fehlstellungen. Bei der Testung von Struktur-modifizierenden Experimenten ist daher die Auswahl von Patienten mit Fehlstellungen des Knies empfehlenswert.

POSTER 20

Ein Beitrag zur Theorie der Mineralisation osteogener und chondrogener Matrix

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Die Mineralisation des Osteoids während der Knochenbildung sowie der Matrix des hypertrophen Knorpels erfolgt an Kollagen, Phosphoproteinen und/oder Proteoglycanen. Nach physikochemischen Überlegungen ist das Ionenprodukt aus Kalzium und Phosphat im Plasma für die spontane Bildung eines Kalziumphosphates an der Kollagenfibrille ausreichend. Um eine unphysiologische Kalzifizierung zu verhindern, wäre demnach ein Hemmstoff nötig. Die Arbeitsgruppe um Fleisch konnte schon 1961 anorganisches Pyrophosphat als einen solchen entdecken. Pyrophosphat (PP) als ubiquitärer Hemmstoff hätte auch den Vorteil, dass bei der notwendigen hydrolytischen Spaltung des PP am Ort der physiologischen Mineralisation anorganisches Phosphat (Pi) bereit stehen würde. Diese elegante Theorie konnte in vitro am dekalzifizierten Knochen oder Dentin mit Salzlösungen unterschiedlicher Ionenprodukte von Ca und PO_4 bestätigt werden. Die bei jedem Mineralisationsprozess in hoher Aktivität vorliegende Alkalische Phosphatase (ALP) soll auch eine Pyrophosphataseaktivität aufweisen, wodurch der Hemmstoff am Ort der Mineralisation hydrolysiert werden würde. Neuerdings sind andere membranständige Pyrophosphatasen beschrieben worden, durch die PP gespalten werden könnte. Allerdings ist diese Theorie noch nie experimentell bei der Osteogenese oder Knorpelkalzifizierung bestätigt worden. An Organoidkulturen von Osteoblasten und Chondroblasten wurde der Effekt von PP auf die Mineralisation und die ALP Aktivität untersucht. PP hemmte deutlich sowohl die durch ß-Glycerophosphat als auch die durch anorganisches Phosphat induzierte Mineralisation. Allerdings hatte PP alleine keine Mineralisationspotenz, wurde also in den Kulturen nicht hydrolysiert. Die Aktivität der ALP wurde durch PP in den Osteoblasten- wie auch in den Chondroblastenkulturen deutlich erhöht. In diesem histotypischen in vitro Modell konnte die Hemmung der Mineralisation durch PP experimentell bestätigt werden, nicht jedoch seine hydrolytische Spaltung.

POSTER 21

Die trabekuläre Architektur der Processus articulares superiores

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Ausgehend von der vielfach bestätigten Theorie, wonach sich die Spongiosatrabekel nach der haupt-sächlichen Beanspruchung ausrichten, soll die trabekuläre Architektur der Procc. art. sup. der Wirbel LW2 sowie S1 dazu benützt werden, auf die Belastungssituation der kleinen Wirbelgelenke in diesen Bereichen rückzuschließen. Dazu wurden die Gelenkfortsätze von 6 männlichen sowie 9 weiblichen Leichen aus dem Bestand des Präparierkurses mittels µ-CT untersucht. Dabei wurden die Struktur-parameter: Verhältnis Knochenvolumen zum Gesamtvolumen (BV/TV), Connectivity Densitiy (Conn-Dens), Structure-Model Index (SMI), Trabekelanzahl (Tb N), Trabekeldicke (Tb Th), Distanz zwischen den einzelnen Trabekeln (Tb Sp) und Degree of Anisotropy (DA) in 5 Abschnitten zu 20% jedes Gelenkfortsatzes statistisch miteinander verglichen.
Die Bone-Volume Fraction ist in allen Abschnitten bei S1 größer als bei LW2, was auf eine durchweg höhere Trabekelzahl zurückzuführen ist. Auch die einzelnen Trabekel der mittleren Bereiche sind von S1 signifikant dicker als in LW2. Dies gilt auch für die Trabekelanzahl. Tendenziell weist LW2 einen höheren Trabekelabstand in den mittleren Abschnitten auf. Eine Vorzugsrichtung der untersuchten Trabekel ließ sich mittels DA auf Höhe S1 nachweisen. Die anderen Parameter zeigten keine statis-tisch signifikanten Unterschiede. Vergleicht man die Parameter der Abschnitte innerhalb von LW2 oder S1in axialer Richtung, so findet sich ein (oftmals) signifikanter Unterschied zwischen den oberen sowie mittleren und unteren Abschnitten der Gelenkfortsätze. Die Analyse der Bilder zeigt bei den Fortsätzen LW2 eine Spitzbogenarchitektur der Trabekel in der Transversalebene.
Aus unseren Untersuchungen ergibt sich, dass die Procc. art. sup. des Os sacrum hauptsächlich auf Druck in der Sagittalebene beansprucht werden, die der 2. Lendenwirbel im Rahmen der Begren-zungsmechanismen der Rotation zusätzlich auf Biegung in der Transversalebene. Betrachtet man die Materialverteilung innerhalb der einzelnen Processus, so kann man auf eine durchweg höhere Intensität der Belastung im jeweils oberen Bereich schließen.

POSTER 22

Lamellar Resistance Structures of the Viscerocranium

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Abstract. Classical literatures describes two resistance arches: the maxillary arch and the mandibular arch, from which the masticatory forces direct themselves towards the functional resistance structures of the viscerocranium. Three pairs of vertical pillars ascend from the maxillary arch: fronto-nasal, zygomatic and pterygoid. Certain authors add a median arch to the previous two: the bony part of the nasal septum. From the mandibular arch, the force lines act towards the condyle and the coronoid process of the mandible. Recent trends indicate five vertical blades at the upper level of the viscerocranium: one median, two medial and two lateral, the latter including the pillars contained in the classical description. These vertical blades are joined by three horizontal laminae: upper, middle (interrupted by the median line), and lower (included in the concavity of the maxillary arch). These laminar structures induce the formation of some resistance cylinders and cones. There are three mandibular arches described in the lower part of the viscerocranium: upper (the classical mandibular arch), middle (directed obliquely) and lower.
Keywords: maxillary arch, vertical pillars, force lines, laminar structures, resistance cylinders and cones

POSTER 23

Resistance Belts And Nodes of the Neurocranium

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Abstract. Classical authors consider the functional resistance of the neurocranium as consisting of arcs at the arch level, rafters at the base and pillars at the joint the arcs and rafters, the latter also connecting the framework of the neurocranium to that of the viscerocranium. Recent trends replace the term pillar with that of resistance node, while arcs and rafters are considered to form a common structure called resistance belts. there are eight resistance belts, as follows: one transversal, three sagittal, two in the frontal plane and two in oblique position. Resistance nodes are placed at the intersection of the resistance belts.

Keywords: arches, rafters, pillars, resistance belts, resistance nodes

POSTER 24

Die Kiefergelenksköpfchen in Dysfunktion des Unterkiefers: eine experimentelle Studie

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Bei den Dysfunktionen des Unterkiefers kommt es zu morphologische Veränderungen am Kiefergelenk, besonders am Caput mandibulae. Die Erkrankungen von Biβ- und Kaustörungen wurde es in Klinik vielmals gesehen. Dafür ist es in der Stomatologie eine wichtige Problem. Bei diesem Experiment wurde die morphologischen Veränderungen des Caput mandibulae untersucht. Diese Studie wurde bei 7 Hunden durchgeführt. Die Zähne im lateralen und vorderen Teil des Unter- und Oberkiefer haben wir präparatorisch derart verändert. In jeden Monat wurden von den Kiefergelenken für Vergleichszwecken Röntgenaufnahmen im lateralen Strahlgang angefertigt. Nach 7 Monaten wurde der Zwischenraum des Kiefergelenks eine deutliche Verengung und am Caput mandibulae der präparieren Seite die Abflachung festgestellt. Diese Befunde erweisen die adaptive Gewebereaktionen am Caput mandibulae an Dysfunktion des Unterkiefers und die Ursache der funktionelle Erkrankungen im orofazialen Organ deutlich.

POSTER 25

Quantitative Densitometrie des Caput mandibulae im hochaufgelösten Schädel-CT

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Mit der allgemeinen Verbreitung der Computertomographie findet diese auch in der präoperativen Planung der Mund-Kiefer- und Gesichtschirurgie Anwendung. Unter dem Aspekt der Strahlenhygiene bei Verwendung eines hochauflösenden Niedrigdosis-Multidetektor CT wurde untersucht wie gut sich kortikale und trabekuläre Anteile des Caput mandibulae quantitativ analysieren lassen.
Untersucht wurden 27 formalinfixierte Schädel mit hochauflösendem Multislice Spiral-CT (GE LS16, Schichtdicke 0,625mm, SFOV 250mm, Matrix 512*512, Pixelgröße 0,488mm) im Niedrigdosis-Modus. Zur exakten Quantifizierung der Knochendichten in mg/ccm statt nur in Hounsfield-Units wurden alle Untersuchungen mit einem Kalibrierphantom (Mindways, Austin/Texas, USA) durchgeführt. In den axialen Schichtbildern wurden in den Kieferköpfchen jeweils auf Höhe des Unterrandes des Tuberculum articulare die Zone des Kieferköpchens segmentiert und mit der Software Geanie 2.1 (Bonalyse, Finnland) weiter quantitativ analysiert. Die trabekulären Schwellwerte wurden auf 100 bzw. 400 mg/ccm festgelegt.
Die Dichtewerte wurden wie folgt bestimmt: Gesamtdichte (kortikal + trabekulär) 338+/-133mg/ccm. Trabekuläre Dichte: 240+/-97mg/ccm. Kortikale Dichte anterior: 740+/-190mg/ccm. Kortikale Dichte posterior 440+/-130mg/ccm. Die intraindividuellen Korrelation zwischen beiden Seiten betrug für die Gesamtdichte r = 0,72, für den trabekulären Anteil r = 0,79 und r = 0,82 bzw. r = 0,75 war für die anterioriore bzw. posteriore Kortikalis (alle signifikant mit $p<0,05$).
Die Untersuchung zeigt, daß im hochauflösenden Multidetektor-CT im Niedrigdosis-Modus bei Verwendung eines Kalibrierphantoms und einer geeigneten Auswerte-Software die quantitative Analyse der trabekulären und kortikalen Anteile gut möglich ist. Bedingt durch die dünne kortikale Dicke im Bereich der maximal möglichen Pixelauflösung erscheinen die kortikalen Werte durch den „partial volume effect“ gegenüber bekannten kortikalen Dichten (1000 – 1200mg/ccm) deutlich reduziert. So kann hier nur von einer apparenten Dichte gesprochen werden. Entsprechend sind quantitative Aussagen zur Kortikalisdicke nicht möglich.

POSTER 26

Modeling of individual parameters of a dental arch at adolescents of different regions of Ukraine.

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Following the basic contemporary references by definition of the individual sizes of a dental arch we have rechecked parameters received according to indexes Ponn, Linder and Heart, Korhaus with parameters of a normal occlusion of adolescents of Ukraine. We determine these methods do not allow for précised determination of the Ukrainian adolescents dental arch.

For definition, we used derivation of specific features of a dental arch method mathematical modeling, which considers not only the sizes of a separate teeth, but also anatomic parameters of a head. We determine, that dental arch represented as not standard geometrical function, and as a complex of metric parameters of key points of a dental arch. Our method considerably raises diagnostic value, quality of treatment, early diagnoses, and prophylaxis of dental arch abnormalities. By studying odonto- and kephalometric parameters of adolescents from different regions of Ukraine, all as whom have a normal occlusion; we found significant gender differences in linear and angle sizes of a teeth and head. We also noted a characteristic correlation between odonto- and kephalometric parameters and linear parameters of a dental arch.

On the bases of this data we constructed mathematic models of individual normal dental arch forms. We subsequently developed the graphical program for the design of normal dental arch based on adolescent’s odonto- and kephalometric parameters.

POSTER 27

New Approaches to Studying of Stress-Strengths Conditions of the Human Teeth

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The aim of the given research was to create three-dimensional computer model of the second upper molar of the person on a basis of morphometric dates. Research is carried out on 24 natural preparations of a teeth and 15 X-ray films of dent's areas of persons of IX age group - the second period of mature age (the man of 36-60 years, the woman of 36-55 years). On the basis of the received data the finite-element model of a tooth consisting of 15378 triangular and tetraidle parabolic elements in the size of 0,8 mm with tolerance of 0,04 mm is constructed. Algorithms of research of biological objects are described by a method of final elements. At calculations the data about is stress - strengths condition of 3-D model in different conditions of static compression (modeling of various chewing pressure) are received.
Key words: teeth, computer three-dimensional models, a method of final elements.

POSTER 28

Postnatal, structural and morphological changes of the palate

Postnatale, strukturelle und morphologische Veränderunges des Gaumens

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We studied postnatal changes of the hard palate on our collection of skulls, ranging in age from birth to 100 years of age, as well as of the palatine mucosa, plicae palatinae transversae and ruge palatinae from the sucking period to adult age. We measured the anterior and the posterior width as well as the length and shape of the hard palate. Before the first dentition, the osseous palate was concave, smooth and without alveolar processes. From the end of the first to the end of the fourth year of age, balloon-like osseous formations appeared bilaterally in front of the osseous palate behind the milk teeth, containing the elements of the permanent teeth. With permanent dentition, the alveolar processes became massive. With age, the concavity of the palate diminished, to become flat with the loss of the teeth. The palatal surface displayed ridges and spines from the first year of age, which disappeared sometimes completely in old age. Plicae palatinae transversae, which are said to disappear after the sucking period, were found in adult female specimens as a constant formation but were missing in 6% adult male specimens.

POSTER 29

Die variantenreiche Anatomie des Humerusschaftes – hat sie eine Relevanz für chirurgische Eingriffe?

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Chirurgische Eingriffe am Humerusschaft sind zur täglichen Routine geworden. Wenn auch die Verplattung des Humerusschaftes in letzter Zeit der Einbringung von Marknägeln Raum gegeben hat, gibt es noch immer Indikationen, die eine Verplattung erfordern. Unsere Untersuchung zielte darauf ab, ob die Struktur des Humerusschaftes eine Verplattung jederzeit ohne Schwierigkeiten zulässt, oder ob Varianten unerwartete Schwierigkeiten bieten könnten. Dazu untersuchten wir 13 isolierte Humeri sowie die Humeri von 14 Skeletten. An keinem der Humeri oder der Skelette waren makroskopisch pathologische Auffälligkeiten festzustellen. Abgesehen von den wohlbekannten Varianten fiel uns auf, dass an drei isolierten Humeri und an vier Skeletten jeweils ein deutlicher, in der Nomenklatur nicht erfasster, „Margo anterior“ festzustellen war, der bei den Skeletten zwei mal seitengleich und je einmal am rechten sowie am linken Humerus ausgeprägter war. Eine Abweichung der Schaftachse distal des Sulcus nervi radialis nach lateral um bis zu 9° gegenüber der Achse proximal des Sulcus konnte bei den isolierten Humeri drei mal, bei den Skeletten seitengleich zwei mal festgestellt werden. Bei diesen Humeri kam es auch zu einer Verkippung der beiden Abschnitte der Facies posterior humeri proximal und distal des Sulcus nervi radialis um bis zu 39 °. Gerade die letzte Variante aber auch ein ausgeprägter „Margo anterior“ sowie die geknickte Schaftachse können beim Aufbringen von Platten große Schwierigkeiten bereiten, so dass die Kenntnis solcher Abweichungen von der Norm bei chirurgischen Eingriffen von großer Bedeutung sein kann. Ein Seitenvergleich mit der gesunden Seite des Patienten kann jedenfalls hilfreich sein.

POSTER 30

Die Ruptur der Bizepssehne – eine experimentelle Studie im Vergleich zur klinischen Realität

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Die Ruptur der Bizepssehne ist ein bekanntes, wenn auch relativ seltenes Phänomen. In der reichlichen Literatur sind der Ort, die möglichen Ursachen und der histopathologische Hintergrund gut aufgearbeitet. Das Ziel unserer Studie war es, festzustellen, ob mit nach Thiel konservierten Präparaten die klinischen Ergebnisse nachzuvollziehen sind. Dazu wurden die Befunde von 14 Patienten sowie 14 Präparate zur vergleichenden Untersuchung herangezogen. Bei den Präparaten wurde der Radius mit intaktem M. biceps brachii entnommen und sowohl der Radius als auch die Bizepssehne an ihrem Übergang zum Muskel in den Autographen von SHIMADZU eingespannt und die Sehne in Spannung gebracht. Anschließend wurde die Sehne bis zum Riss belastet. Es stellte sich heraus, dass die Art und der Ort de Risse den Patientendaten entsprachen. Auch die histologischen Schnitte der Sehnen wiesen die in der Literatur beschriebenen Degenerationserscheinungen auf. Wir kommen damit zum Schluss, dass Experimente mit nach Thiel konservierten Präparaten ohne weiteres mit der klinischen Realität vergleichbar sind.

POSTER 31

Anatomische Grundlagen der lumbalen Plexusanästhesie

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Die regionale Anästhesie des Plexus lumbalis ist in den letzten Jahren zunehmend klinisch relevant geworden, was in zahlreichen Fallbeispielen beschrieben wird. Ziel dieser Studie ist es, die intramuskulären Verläufe der Nn. cutaneii femores laterales, femorales und obturatorii als Grundlage für ein besseres Verständnis von Komplikationen und Risken der Punktion darzustellen.
Die Studie beruht auf 95 Retrositen, aus denen 190 Mm. psoae entnommen werden konnten. Nerven und Wirbelkörperhöhen werden am Muskel markiert. Für die Auswertung werden Schublehre und Winkelmesser verwendet. Vermessen werden Breite und Dicke des Muskels. Der intramuskuläre Nervenverlauf wird individuell erfasst. Ein- und Austrittsstellen der Nerven in Bezug auf den Muskel werden aufgesucht.
Abgesehen vom Umstand, dass Muskeln weiblicher Leichen eine geringere Masse aufweisen als Muskeln männlicher Leichen. zeigt der Verlauf der Nerven auf der untersuchten Höhe der Oberkante des 5. Wirbelkörpers die größten Übereinstimmungen. In vielen Fällen können alle drei Nerven an dieser Stelle am Muskel aufgefunden werden. Die Punktion des M. psoas scheint daher auf dieser Höhe am sinnvollsten. Skoliose - an unseren Präparaten als altersbedingte Veränderung (n=14) fallweise beobachtet - verändert die Muskelmorphologie und kann Einfluss auf den Nervenverlauf haben. Dies muss bei der Anästhesie berücksichtigt werden.

POSTER 32

Der Arcus tendineus musculi levatoris ani

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Bei gynäkologischen Eingriffen ist die Kenntnis der Strukturen des kleinen Beckens unabdingbare Voraussetzung. Der Arcus tendineus m. levatoris ani stellt eine wichtige Landmarke dar, um die Abgrenzung des Beckenbodens von kranial beurteilen zu können. In den meisten Büchern ist der Arcus geradlinig vom R. superior ossis pubis beginnend und bis zur Spina ischiadica ziehend, dargestellt. Bei Präparierkursen fiel uns jedoch auf, dass der Arcus in seinem ventralen Bereich wesentlich weiter kranial zu finden war. Unsere Untersuchung an 24 nach Thiel konservierten weiblichen Leichen ergab, dass der Arcus tendineus m. levatoris ani jeweils nahe der Umrahmung des Canalis obturatorius beginnt und sich in sanftem Bogen der Spina ischiadica nähert. Eine eindeutige Identifizierung dieser Struktur ist wichtig, da bei einer Verwechslung eventuell mit dem Arcus tendineus fasciae pelvis der Beckenboden durchtrennt und die Fossa ischioanalis erreicht wird. Auch bei der rekonstruktiven Beckenbodenchirurgie (TVT,TVT-O, PROLIFT) ist die exakte Kenntnis des Verlaufs des Arcus von entscheidender Bedeutung für den Erfolg des operativen Eingriffs.

POSTER 33

Die Analmembran ist Ort eines biphasischen Verschlusses an epithelialen Grenzen

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In neueren Arbeiten konnte gezeigt werden, dass die Kloakenmebran bei sieben Wochen alten Embryonen reißt und jeweils eine Öffnung für das Anorektum und den Sinus urogenitalis entsteht, dabei aber das zwischen beiden Öffnungen gelegene Septum urorectale nicht beteiligt ist. Kurz nach dem Riss der Kloakenmembran wurde im Anorektum ein epithelialer Verschluss beschrieben, dessen Bedeutung nicht näher analysiert wurde. Ziel der vorliegenden Untersuchung war es zu klären, welche funktionelle Bedeutung dem epithelialen Verschluss im Anorektum zugrunde liegt.
In die Untersuchung wurden 27 menschliche Embryonen zwischen der 6. und 15. Schwangerschaftswoche (SSW) einbezogen, von denen konventionelle, meistens sagittale Paraffinschnitte (4µm) angefertigt wurden. Für eine erste immunhistochemischen Analyse wurden verschiedene epitheliale Marker verwendet.
Am Ende der siebten Embryonalwoche war das Anorektum bedingt durch engen Kontakt der epithelialen Wandauskleidung partiell verschlossen. Da die epithelialen Auskleidungen von Rektum und Analkanal durch Labelling mit Cytokeratin 18 breits unterschieden werden konnten, wurde der Verschluss auf Höhe der epithelialen Linea anorectalis lokalisiert. Bei acht Wochen alten Embryonen war das Lumen auf dieser Höhe offen. Weiter aboral an der Linea anocutanea konnte jedoch erneut ein Verschluss durch einen proliferierenden epithelialen Zellhaufen beobachtet werden. Bei neun Wochen alten Feten ist das Lumen des Rektums gänzlich durchlässig. In der weiteren Entwicklung bilden sich an den epithelialen Grenzen die Auskleidungen der Vavulae anales und der Hautfalten aus.
Unsere Ergebnisse zeigen, dass die Analmembran eine von der Kloakenmebran zeitlich und örtlich verschiedene Struktur ist. Sie befindet sich vorübergehend dort, wo an epithelialen Grenzen vermehrte Proliferation stattfindet.

POSTER 34

Messung der maximalen Zugfestigkeit am Aufhängeapparat des Klauenbeins beim Rind

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In der jüngsten Literatur wird eine alters- und nutzungsbedingte Lockerung der lamellären Aufhängung des Klauenbeins beschrieben, besonders bei Milchkühen mit einer höheren Zahl an Laktationen.
Es war das Ziel dieser Studie durch Zugversuche die lokale, maximale Belastbarkeit des Aufhängeapparates für das Klauenbein in Abhängigkeit von der Laktationszahl zu testen. Für diese Untersuchung wurden 40 Färsen und Kühe mit einem minimalen Alter von 18 Monaten ausgewählt. Entsprechend der Anzahl an Laktationen wurden die Schlachttiere in vier Gruppen eingeteilt: nullipar, primipar, multipar mit 2-4 Graviditäten und multipar mit mehr als 4 Graviditäten. Je ein Vorder- und Hinterfuß wurden zufällig ausgewählt. An 64 Messpunkten wurden die Zugfestigkeit des Aufhängeapparates in der Materialprüfmaschine getestet. Eine repräsentative Anzahl an Proben wurde histologisch ausgewertet.
Die Klauen der Jungtiere zeigten nur relativ wenige und geringgradige Veränderungen äußerlich an den Klauen. Mit zunehmendem Alter wurden diese häufiger und in der Ausprägung schwerer. Die Zugfestigkeit nahm von 2,6-3,9 MPa bei Färsen auf Werte zwischen 1,9-2,6 MPa bei multiparen Tieren ab. Mit zunehmendem Alter bzw. Laktationen verlagerte sich die Versagensgrenze vom lamellären Abschnitt des Coriums in die tiefere Faserschicht.
Die Ergebnisse legen nahe, dass der Aufhängeapparat nutzungsbedingt mit steigender Zahl an Trächtigkeiten an Zugfestigkeit verliert. Daraus resultiert eine geringgradige Verlagerung des Klauenbeins innerhalb der Klaue mit erhöhtem Druck auf die Sohlenlederhaut. Deswegen sind vor allem ältere Milchkühe besonders für die Bildung von Rusterholz'schen Sohlengeschwüren prädestiniert.

POSTER 35

Die Besonderheiten der Asynchronentwicklungsprozesse der Körpermuskulaturmasse bei den Ziegen der orenburgieschen Daunenrasse

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Das Ziel der Untersuchung besteht darin, die Besonderheiten der Asynchronentwicklungsprozesse der Körpermuskulaturmasse bei den Ziegen der orenburgieschen Daunenrasse zu bestimmen, die Prozesse ihrer Formierung unter der Technologiebedingungen und der Intensität der Tierhaltung zu erforschen. Es wurden die morphologischen Untersuchungsverfahren mit den weiteren biometrischen Bearbeitung der Ergebnisse ausgenutzt. Die Untersuchungen wurden laut den Regeln der Arbeit mit den Untersungstieren durchgeführt. Die Intensität der Entwicklungsprozesse des Muskelgewebes ist in den ersten 3 Monate nach der Geburt besonders bedeutend. Etwa 41% der Muskelmasse und 39% der Körpermasse der Ziege formieren sich in den letzten 2 Monaten der intrauterinen Periode und in den ersten 3 Monaten der postnatalen Periode, in dieser Zeit ernährt sich die Frucht völlig durch den Körper der Mutter und dann wird das Ziecklein mit der Muttermilch gefüttert. Besonders intensieves Wachstum der Muskulatur und des Körpers der Ziege wird in den ersten Stufen der intrauterinen Entwicklung beobachtet, dann senkt es standing, der erste Monat der postnatalen Entwicklung bildet die Ausnahme, weil es im Laufe dieses Monats bedeutend steigert. Während der intrauterinen und postnatalen Periode enwickelt sich die Körpermuskulatur intensiver, als das Ziegenorganism insgesamt. In der untersuchenden Periode steigt die Muskulaturmasse in 971,4 Mal und Körpermasse der Ziege in 829,1 Mal. Das Verhältnis der Muskelmasse zur Körpermasse des Tieres in den verschiedenen Perioden der Ontogenese ist sehr flexibel und schwankt zwischen 25,4 und 33,2%, was die Asynchronentwicklungsprozesse der Körpermuskulaturmasse und der anderen Systeme des Ziegenorganismus insgesamt wiederspiegelt.

POSTER 36

Estimative Research of Possible Antiinflamatory Effects of an no Donor on Rats with Induced Granuloma

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Einschätzende Forschung der gegen entzündlichen Wirkungen eines No-Spenders auf Ratten mit eingeleitetem Granuloma

The NO Donors Are Significant Cellular Mediators; Their Therapeutic Potential Is Very Disputated, Offering Unexplored Possibilities To Investigate Target Organs. This Experiment Evaluates A Possible Antiinflammatory Effect Of Synthetic Original NO Donor (NX) On Rats with induced granuloma. Material and method: we have used 5 lots od 11 wistar male: lot 1 – witness; lot 2 – received Levamisol 10 mg/kg body weight (day); lot 3 – received Prednison 5 mg/kg body weight/day; lot 4 – received Indometacin 1,42 mg/kg body weight/day; lot 5 – received NX 4,5 mg/kg body weight/day. All the substances were intraperitoneally administered, 2 weeks daily. In the 8th day we produced a dorsal granuloma weith a Sterile cotton of 62 mg subcutaneous. We prelevated granullar tissue from the euthanasized rats, proceeding it specifically for histological exam. Results and discussions: the rats from lot 2 presented a moderate inflammatory reaction, similar weith that of lot 3; lot 4 presented only discrete signs of tissular inflammation; lot 5, receiving NX-hod a moderate inflammatory reaction, compared with lot 4. Conclusion: NX derivation of NO cam be are alternative to the classic antiinflamatory therapy. Key words: NO, derivative, antiinflammatory, Prednison

POSTER 37

Dreidimensionale Rekonstruktion des Hirnstammatlas von Paxinos und Huang

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Die Fülle bildgebender Verfahren in der Neurologie und die enormen Fortschritte im Anwendungsbereich von CCT und MRT machen die Entwicklung neuer dreidimensionaler Referenzatlanten für klinische Bilder immer wichtiger. Gerade stereotaktische Biopsieverfahren sowie die Neurochirurgie selbst sind auf verlässliche Korrelationen zwischen Patientenbildern und Vergleichsatlanten angewiesen. Die hier vorgestellte Arbeit soll einen Teilaspekt dieses Problems lösen, indem sie die digitale Umwandlung eines Hirnstammatlanten in ein 3D-Format zeigt und dessen Projektion auf MRT-Datensätze von Patienten. Der „Atlas of the Human Brainstem“ von Paxinos und Huang ist ein neuroanatomischer Atlas, der insbesondere die Kerngebiete des Hirnstammes kartiert. Die 64 Templates der linken Hirnstammseite sind assoziiert zu einem Koordinatensystem relativ zum Obex in Millimeterschichten repräsentiert. Die Templates wurden hochauflösend digitalisiert und anschließend dreidimensional aufeinander ausgerichtet. Hierbei wurde anhand des Koordinatensystems eine landmarkenbasierte Registrierung vorgenommen, die mithilfe von Matlab-Routinen realisiert wurde (Translation, Rotation, Scaling). Anschließend wurde der dreidimensional ausgerichtete Datensatz in die Software 3D-Slicer importiert. Diese erlaubt es, eine Segmentierung der verschiedenen Strukturen vorzunehmen und diese dreidimensional darzustellen. Der digitale Hirnstammatlas soll dazu genutzt werden, die betroffenen Hirnstammkerngebiete bei lakunären Hirnstamminfarkten zu lokalisieren und zu elektrophysiologischen Messergebnissen in Bezug zu setzen. (gefördert durch das IZKF der FSU Jena, TP 1.5)

POSTER 38

PLI-Faserorientierungskarten des menschlichen Hirnstammes

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Insbesondere in der Detektion von Wallerscher Degeneration nach Schlaganfall erlangt die DTI-Bildgebung (Diffusion Tensor Imaging) eine immer größere Bedeutung. Doch bislang fehlen Methoden zu deren Validierung. In diesem Projekt werden 18 in Formalinlösung fixierte menschliche Hirnstämme seriell geschnitten, mithilfe einer Polarisationsoptik digitalisiert und mit einer selbst entwickelten Software analysiert. Die Methode der PLI-Bildgebung (Polarized Light Imaging) erlaubt es unter Ausnutzung der optischen Eigenschaften der im polarisierten Licht durchleuchteten Hirnschnitte, für jeden Punkt des Präparates die Faserorientierung zu berechnen und diese in so genannten Faserorientierungskarten darzustellen. In diesen Faserorientierungskarten werden einzelne Faserbündel detektierbar und können segmentiert werden. Die dreidimensionale Registrierung der Faserorientierungskarten und die anschließende Segmentierung der Faserbündel ermöglicht eine 3D-Darstellung der Faserbahnen in ihrer anatomischen Struktur. Aus der Zusammenfassung der 18 3D-Rekonstruktionen resultiert ein Modell eines probabilistischen Nervenfaseratlasses, deren erste Ergebnisse hier vorgestellt werden sollen. Dieser Atlas dient zum einen der Validierung von DTI-Datensätzen und zum anderen als Wissensbasis und Ergänzung zu anderen Atlanten, die bislang keine reliable Darstellung der Faserbündel garantieren. Desweiteren fungiert er als Normalisierungsdatensatz von Populationsdaten und findet weitere Anwendung in der OP-Vorbereitung, Stereotaxie, Neuronavigation oder auch minimal-invasiven Chirurgie. (gefördert durch DFG Ax 20/3-1)

POSTER 39

Histological variants of meningiomas and their topographical localisation

Histologische Variante des Meningiomas und ihre topographische Lokalisierung

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Meningiomas are one of the most common tumours of the central nervous system, accounting for 13-26% of all primary brain tumours. Meningiomas are generally benign tumours, usually attached to the dura, that arise from the meningothelial cell of the arahnoid. Various histological patterns can be observed in these lesions: syncytial, fibroblastic, transitional, psammomatos, secretory, microcystic etc. The objective of our study is to review the clinical and histological aspects of primary intracranial meningiomas and their localisation. The incidence of various histological subtypes of intracranial meningiomas was examined in 150 patients with surgically treated meningiomas in the Iaşi Department of Neurosurgery, between 2000 and 2003. Of the 150 intracranial meningiomas, the majority occurred in adult females. They were benign in nature (over 70%) and more often were located on the cerebral convexity (60%). We found some correlation between the age and sex of patients, the location of tumours and the histological subtypes.

POSTER 40

Anatomic and radiological correlations in the glioblastomas prognosis

Anatomische und radiologische Wechselbeziehungen bei Glioblostoms Prognose

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Glioblastomas derive from astrocytes and are the most malignant tumors of central nervous system. Glioblastoma has a great number of tumoral glial cell nuclei and a great nuclear pleomorphism; mitotically active cells are very often observed. Our study reveals the multiple aspects of the nucleus in the glioblastomas, the topographical situation of the glioblastomas and the importance of this fact regarding the prognosis of this type of tumors. Our study was realized on 115 tumors from the patients, which were operated in the "Sfânta Treime" hospital Iaşi. Glioblastomas with small cells are highly cellular, but rather monotonous. Tumor cells are poorly differentiated with brisk mitotic activity. There is also prominent microvascular proliferation. Giant cell glioblastomas presented a low tumor cells density with numerous multinucleated giant cells, with prominent nucleoli. The mitotic activity is lower compared with small cell glioblastomas. Also the mitotic activity in the endothelial tumor cells is lower. We observed that the giant cell glioblastomas are more often localized in the temporal and parietal lobes and the glioblastomas with small cells are localized more often in the frontal lobe. Both types are very rare in the occipital lobe. Probably, the nuclear morphology, the mitotic activity and their localization explain the different prognosis between these different histological variants of gliobastomas.

POSTER 41

Anti-proliferative effects of Curcumin on human neuroblastoma cells through increased apoptosis are mediated by inhibition of Nuclear Factor kappa B.

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Neuroblastoma, a malignant tumour of the sympathic nervous system, represents the third most common childhood malignancy, which is largely incurable in children over 1-year old using current treatment protocols. After dissemination to the bone the survival rate is <7%, indicating a need for novel therapeutic targets. One interesting approach is the polyphenol natural product Curcumin (diferuloylmethane). Curcumin is isolated from tumeric (Curcuma longa), giving the specific flavour and colour to the common spice curry. Curcumin was shown to exert strong anticancer effects against diverse human malignancies, through various molecular mechanism of action, depending on the tumour cell type. One of these mechanism is the inhibition of the signalling pathways of Nuclear Factor kappa B (NF-κB), which seems to play a crucial role in apoptosis. Therefore we tested the anti-proliferative effect of curcumin in a broad concentration range (0,00001-100 µM) on the growth of the human neuroblastoma cell lines Lan5, SKNSH and Kelly using XTT cell proliferation assays. To investigate the effect of curcumin on NF-κB activation, the protein levels of the NF-κB subunit p65 of curcumin treated cells were compared to untreated cells via quantitative western blotting. A possible induction of apoptosis through curcumin treatment was assed by detection of DNA fragmentation. Curcumin showed a significant dose dependent anti-proliferative effect on all three neuroblastoma cell lines starting at a concentration of 0,001 µM. The highest concentration of 100 µM significantly reduced the viable cell count of SKNSH to 48%, of Kelly to 22% and of Lan5 to 8%, respectively. This anti-proliferative effect was mediated through an increased induction of apoptosis. This apoptotic effect of curcumin was induced through an inhibition of NF-κB, underlying the anti-apoptotic potency of
NF-κB. Our results suggest, that curcumin might be a promising approach in the treatment of patients suffering from neuroblastoma.

POSTER 42

The maturation of the tree shrew striate cortex (V1) anticipates its visuotopic organization

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Histological serial sections, three-dimensional reconstructions, and morphometry served to study the postnatal maturation of V1 in tree shrews (*Tupaia belangeri*) in relation to the representation of the visual space or retinal topography, respectively.
On postnatal day 1 (P1), V1, discernable by a conspicuous granular layer IV, demarcated a crescent-like region on the superior surface of the occipital cortex including the poles and the upper part of the medial wall. In the central part of this region, at the transition of the superior to the medial surface, lamina IV was differentiated into its sublayers IVa and IVb indicating the formation of the binocular part. With age, V1 spread and maturated in a centro-peripheral gradient which is succeeded by the differentiation into its sublayers IVa and IVb. On P15 the binocular part has reached its medial boundary on the inferior side. The V1 appearing on this side presented a layer IV which did not show any sublayers which is distinctive for the monocular part. The expansion of V1 lasted until adolescence. The expansion of the monocular part ceased after the binocular part had reached its supero-lateral boundary.
In reference to the retinotopic map of V1 (Bosking et al., *J. Neurosci., 2000, 20:*2346-2359; Kaas, in S.O.E. Ebbesson “Comparative neurology of the telencephalon”, Plenum Press, New York, 1980, p. 483-502), regions emerged in a coherent temporo-spatial sequence delineating the retinal topography in a central to peripheral gradient beginning with the visual streak representation. Early bilateral enucleation in primates showed that the laminar pattern of the striate cortex develops independently from visual afferents (Dehay et al., *J. Comp Neurol. 1996,* 367:70-89). Thus, the observations give evidence that the course of maturation of V1 anticipates its visuotopic organization.

POSTER 43

N-cadherin and R-cadherin – two proteins expressed in the tectorial membrane during chicken cochlear development

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For the process of hearing, the interaction of tectorial membrane and hear cells is fundamental. During chicken cochlear development the tectorial membrane originate from homogene cells at the lateral wall of the cochlear duct and from supporting cells at the basilar papilla. However, the origination of the tectorial membrane has not been indicated by any protein.
In the present study, we performed light and electron microscopic immunohistochemistry to investigate the expression of cadherins during tectorial membrane development.
Our results demonstrate that in the tectorial membrane, N-cadherin (N-Cad) and R-cadherin (R-Cad) are expressed in both layers, in the amorphous upper layer and in the fibrous sublayer. Furthermore, N-Cad and R-Cad are found in a patched fashion in the amorphous upper layer, whereas it is encountered in a uniform manner in the fibrous layer.
Moreover, N-Cad is expressed in hair and supporting cells, where the predominant expression occurs in the supporting cells localized in the inferior part of the basilar papilla.
By electron microscopy, the expression of N-Cad is also found in the tip of the tallest stereocilia, which points to the fact that N-Cad is involved to the connection of stereocilia to the tectorial membrane.
In homogene cells R-Cad is expressed only. In electron microscopical pictures R-Cad can be seen in the cytoplasm and the apical surface of homogene cells.
In conclusion, N-Cad and R-Cad are present in the tectorial membrane. They are secreted from supporting cells and homogene cells respectively. Therefore N-Cad and R-Cad can be used as markers to define the origination of the tectorial membrane during chicken cochlear development.

POSTER 44

Developmental expression of AMPA receptors and SV2 in the chick cerebellum

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In the developing cerebellum, the appearance and localisation of glutamate receptors and their proper integration in the postsynaptic (and/or presynaptic) membrane is still an open issue. The present study focuses on the expression of the AMPA subunits GluR 2/3 in the developing cerebellum of the chick. Single as well as double labeling immunohistochemistry experiments show the temporal and spatial expression pattern of GluR2/3 in the developing cerebellar cortex, and its correlation with the expression pattern of the presynaptic marker SV2.

We found that Purkinje cells (PCs) express AMPA receptor subunits 2/3 in the soma and in the primary dendrites. Their location tends to follow patterns of developing climbing fibre innervations which express presynaptic SV2. In addition, the granular layer exhibit a similar expression pattern with GluR 2/3 positive granule cell dendrites/glomeruli in close apposition to SV2 immunopositive puncta, presumably mossy fibre terminals. Both proteins, GluR2/3 subunits postsynaptically in Purkinje cell and granule cell dendrites, and SV2 in presynaptically in climbing and mossy fibres are present from embryonic stages (E16) on until adulthood.

Our data revealed that the principal cerebellar neurons (Purkinje cells, PCs and granule cells, GCs) express the AMPA receptor subunits 2/3 postsynaptically and SV2 presynaptically in contacting terminals as soon as they are formed. With ongoing development, the cell body and an increasingly elaborate dendritic tree was outlined by immunoreaction products suggesting an early clustering of synaptic proteins during synapse formation and an ongoing subcellular redistribution during the period of synaptic investment especially of Purkinje cells.

POSTER 45

Expression of epigenetic factors in mouse brain development

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Polycomb group (PcG) genes are well described regulators of body segmentation and cell growth, being therefore important players during development. PcG proteins form large complexes (PRC) and are involved in various epigenetic phenomena, such as maintenance of Hox gene expression patterns, X chromosome inactivation and chromosomal imprinting. Methylation of histone H3 and subsequent ubiquitination of histone H2A are among the epigenetic functions of PcG proteins that lead to the specific inheritance of a transcriptional program. Recently, epigenetic chromatin modifications are discussed in the context of neurodevelopmental diseases such as schizophrenia.
Although expression of PcG genes in the brain has been noticed, the involvement of PcG genes in the processes of brain development is not understood. In this study we analysed the expression patterns of PRC1 complex members to reveal PcG proteins that might be relevant for mouse brain development. Using in situ hybridisation we show PRC1 activity in proliferative progenitor cells during neurogenesis, but also in maturated neuronal structures. PRC1 complex compositions vary in a spatial and temporal controlled manner during mouse brain development, providing cellular tools to act in different developmental contexts of cell proliferation, cell fate determination and differentiation.

POSTER 46

Funktionelle Analyse der Bedeutung von CALEB/NGC für die Cortexentwicklung von Mäusen durch die Technik der *in utero* Elektroporation

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Der klassische Ansatz, um die Funktion eines Proteins *in vivo* zu untersuchen, ist die Analyse einer *knockout* Maus. Dieser Ansatz unterliegt jedoch einigen Einschränkungen: 1) Das Einfügen von fremder DNA in ein Genom kann unerwartete Effekte auf die Regulation von anderen Genen haben, was zu Kompensationserscheinungen und dem Fehlen eines Phänotyps führt, oder zu einem Phänotyp, der keinen direkten Bezug zum untersuchten Gen hat. 2) Mit diesem Ansatz ist es nur bedingt möglich, ein Genprodukt nur in einem bestimmten Zeitfenster der Entwicklung funktionell zu blockieren. 3) Für verschiedene Fragestellungen ist es notwendig, ein Genprodukt nur in einer Subpopulation von Zellen und nicht in allen Zellen funktionell zu verändern. Um diese Einschränkungen zu umgehen haben wir die neue Technik der *in utero* Elektroporation angewendet, um die Funktion von CALEB/NGC für die Cortexentwicklung von Mäusen *in vivo* zu analysieren. Mit dieser Technik ist es möglich, die Expression von Genen durch RNA Interferenz zu unterdrücken, Genprodukte funktionell zu inaktivieren, oder die Expression von spezifischen Genen in einer Subpopulation von Zellen während einer bestimmten kritischen Phase der Entwicklung zu steigern. Wir geben eine Beschreibung dieses experimentellen Ansatzes, und präsentieren Daten zur funktionellen Analyse von CALEB/NGC für die Differenzierung von kortikalen Neuronen *in vivo* unter dem Fokus der Entwicklung der Dendritenbäume.

POSTER 47

Developmental deficits in central and peripheral mouse nervous system after knockout of SNARE Vti1a and Vti1b.

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In eukaryotic cells, molecules need to be transported to their correct intracellular destination without compromising the structural integrity of cellular compartments. To achieve this, transport vesicles bud from intracellular donor organelle and then target, dock and fuse with an acceptor organelle. SNARE (Soluble NSF attachment protein receptor) proteins have been implicated as central in most, if not all intracellular trafficking events studied so far. SNAREs Vti1a and Vti1b have 30% similarity in their amino acid sequences and have distinct but overlapping subcellular localization. Vti1a has been associated with recycling process after endocytosis and early endosomal fusion whereas Vti1b with late endosomal fusion and lysosomal degradation events.
Mice deficient of both endosomal SNARE proteins, Vti1a and Vti1b, die during intrauterine life just before birth, whereas single knockouts and triallelic mice survive and reach normal age without difficulty. The reason for this differential behaviour is completely unknown. These KO mice have various changes in central (CNS) as well as peripheral nervous system (PNS). In CNS they show wide ventricles, lack several fibre tracts and some axons do not reach to their targets suggesting a deficit in axonal guidance and neurite outgrowth. Additionally, the KO mice also show more neurons in inner cortical layers. This could be due to altered productivity in the ventricular zone during cortical layer development or defect in neuronal migration along radial glia cells. On the other hand in PNS, KO mice show various degrees of neurodegeneration in different types of ganglia. Our data provide the neuroanatomical basis for ongoing work on possible mechanism in which these SNARE's are involved during nervous system development.

POSTER 48

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POSTER 49

Induction and specification of sertonergic neurons of the ventral rhombencephalon

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Serotonin (5-HT) producing neurons of the formatio reticularis are involved by their projections in the modulation of behaviour as anxiety, sleep and mood. Dysfunction of the 5-HT system is associated with disorders as depression, schizophrenia and migraine. Their development depends critical on the floor plate signal Shh, on FGF8 from the isthmus-organizer and the pre-patterning signal FGF4 during early develop-ment. So far the transcription-factors Nkx2.2., Lmx1b, Gata2 and Pet1 have been described to act downstream of Shh, leading to the differentiation of progenitor cells and specification of the 5-HT phenotype. In order to complete this network, we performed a cDNA micro-array, comparing the gene expression pattern of ventral mesencephalon and ventral rhombencephalon of mice E11, a time point where 5-HT neurons are first generated. Subsequently, micro-array results were validated by RealTime PCR and in situ hybridization.

The results show differential expression of several genes in ventral hindbrain, compared to ventral midbrain. These results together with the observation of the selective agenesis of paramedian raphe 5-HT neurons in TGFβ2 knock-out mice at E18.5 indicate that TGFβ2 is an essential member of this network. Current in vitro experiments focus on the effects of TGFβ1-treatment, of functional blocking of endogenous TGFβ as well as the inhibition of TGFβ by receptor-blocking on differentiation of progenitor cells towards serotonergic fate.

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POSTER 50

The effects of TGF-β and persephin on differentiation of mouse mesencephalic progenitor cells towards dopaminergic neurons *in vitro*.

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Mesencephalic dopaminergic neurons play a paramount role in the control of voluntary movement. Degeneration of these cells results in Parkinson's disease. Recently, an increasing number of studies have highlighted the role of TGF-□ as a factor involved in the induction and maintenance of dopaminergic neurons. Furthermore, other members of the TGF-□ superfamily, viz., GDNF, artemin, neurturin and persephin (PSP) have been shown to play essential roles as target-derived factors promoting survival of mesencephalic dopaminergic neurons. In the present study, we examined the effects of TGF-□ and PSP on the differentiation of mouse E12 ventral mesencephalon progenitor cells towards dopaminergic neurons. Furthermore, differentiation signaling pathways for both TGF-□ and PSP were analyzed. Therefore, progenitor cells were isolated from mouse E12 ventral mesencephalon and cultured in suspension in the presence of FGF2. Neurospheres were formed and plated onto PORN/laminin coated coverslips. Cells were treated with TGF-□ and PSP in the presence or absence of ALK-, p38-MAPK- or PI3-kinase-inhibitor, fixed, and processed for immunocytochemistry. The results show that TGF-□ together with PSP significantly increased the number of TH immunoreactive cells compared to controls and to single TGF-□ treatment. Application of ALK 4,5,7-, p38- or PI3 kinase inhibitor significantly reduced the number of TH positive cells, compared to factor treatment. The data suggest cooperation of TGF-□ and PSP in induction of a dopaminergic phenotype *in vitro*, and indicate that these effects are receptor-mediated, involving the p38- and PI3-kinase pathway.
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POSTER 51

Identification and characterization of new candidate genes involved in the differentiation of ventral mesencephalic dopaminergic neurons

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Parkinson's disease, a neurodegenerative disorder of the central nervous system, is associated by a loss of dopaminergic neurons of the substantia nigra. The mesencephalic dopaminergic system regulates a diversity of brain functions, including movement control and behavior. Therefore, the molecules and mechanisms involved in the differentiation of progenitor cells into dopaminergic neurons are of major interest. In the present study we have screened for new candidate genes involved in the differentiation of dopaminergic neurons. A cDNA microarry analysis was performed, using tissue from ventral and dorsal mouse mesencephalon at embryonic day 12. The array results were validated by Real Time PCR and in situ hybridisation. Moreover, gain of function and loss of function experiments were performed for candidate genes. The microarray analysis revealed the upregulation and downregulation of several hundred genes of the ventral, compared to dorsal midbrain. Real Time PCR for selected genes confirmed the micrarray results. In addition, a gradient expression pattern of candidate genes was observed in mouse midbrain. Two candidate genes, namely F-Spondin, an extracellular matrix protein and Sim1, a transcription factor, were selected for gain of function and loss of function analysis using the MN9Dcell line.Overexpreesion of F-Spondin increased tyrosine hydroxylase (TH) expression, a marker for mature dopaminergic neurons, as assessed by RT-PCR and Western blot. However, the expression of Pitx3, an early dopaminergic marker, was not changed due to the overexpression of F-Spondin. These data indicate that F-Spondin might promote the differential potential of progenitor cells toward mature dopamingeric neurons but this effect is not mediated via Pitx3.
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POSTER 52

Survival promoting effect of BDNF, NT-4 and insulin during retinal programmed cell death periods

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The balance between cellular suicide and survival program is important not only for physiological apoptosis during embryonic development but also for the pathogenesis of a wide spectrum of diseases. A multitude of studies analyzed survival promoting effects of neurotrophins under pathological condition. However, the cell intrinsic survival promoting mechanisms of neurotrophin signalling under physiological conditions of naturally occurring, programmed cell death (PCD) has not been investigated so far, although it has been proposed that neurodegenerative disorders involve a pathological reactivation of developmental apoptotic programs. In the mouse postnatal retinal PCD phases comprise peaks at postnatal day (P)2 (ganglion cell death), P9 (amacrine cell death) and P15 (ganglion and photoreceptor cell death). We have evidence that insulin has a strong anti-apoptotic effect during early PCD periods of the developing retina. Besides, several studies indicate that BDNF as well as NT-4 support the survival of target deprived rat retinal ganglion cells. Against this background we investigated the anti-apoptotic capacity of the neurotrophic factors insulin, brain derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) in the context of developmental retinal PCD using a murine *in vivo*-like organotypic retinal wholemount culture system. Dose response experiments with recombinant insulin, BDNF and NT-4 revealed that (i) during all postnatal murine PCD peaks (P2, P9 and P15) the murine retina is responsive to these factors, (ii) all neurotrophins have an anti-apoptotic potential at physiological concentrations and (iii) insulin seems to be the most potent survival promoting factor, especially at early PCD peak P2.

POSTER 53

Expression profiles of neurotrophic factors and receptors during murine retinal programmed cell death periods

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Neurotrophic factors like brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) are a class of cell signalling molecules critical for the differentiation and survival of neuronal cells. Besides, insulin and (ciliary neurotrophic factor) CNTF have been reported to be potent survival promoting factors in retinal cell death decisions. Most investigators focussed on expression of neurotrophins in the adult retina, although it has been proposed that neurodegenerative disorders involve a pathological reactivation of developmental apoptotic programs. Against this background, we characterized the spatial and temporal expression of insulin, BDNF, NT-4 and CNTF and their receptors during physiological murine retinal cell death periods. Using semi-quantitative PCR, ELISA assays and Western Blot analyses, we set out to correlate temporal expression peaks of neurotrophic factors and receptors with maxima of murine retinal PCD at postnatal day (P)2, P9 and P15. As different cell types die within the PCD peaks of the mouse retina, we investigated the cellular localization of the neurotrophins and their receptors by immunocytochemical double labelling studies with cell specific markers in order to determine the possible target cells of the survival promoting effect of different neurotrophic factors. While insulin, BDNF, NT4 and CNTF expression displayed no correlation with the main PCD peaks, the expression of the TrKB und the insulin receptor peaks at P2, P9 and P15. Immunocytochemical stainings indicate a strong expression of all factors in the ganglion cell layer of the developing murine retina and additional staining of cell bodies in the inner nuclear layer homing amacrine cells.

POSTER 54

Expression and function of erythropoietin during retinal development

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The hematopoietic hormone erythropoietin (Epo) has also been found to protect neurons from apoptosis. To examine whether Epo plays a role in cell death decisions during neurogenesis, we made use of the developing mouse retina as an excellent model system for the central nervous system. The retina is easily accessible and less complex than other neuronal tissues. Complete retinas were explanted and homogenised for RNA-extraction or cultured as organotypic explants in DMEM for subsequent factor treatment. Epo and Epo receptor (EpoR) expression in the retina was determined by RT-PCR on postnatal days (P)0 to P20. We detected a continuous expression of Epo- and EpoR-mRNA during postnatal retinal development. Epo expression in the retina was compared with Epo-mRNA levels in the developing liver and kidneys. Treatment of P15 retinal whole-mount cultures with recombinant Epo resulted in a significant decrease of apoptosis. Moreover, transforming growth factor beta (TGF-ß)-induced apoptosis was completely blocked by Epo when both factors were applied simultaneously. These data indicate that Epo antagonises physiological neuronal cell death as well as TGF-ß mediated retinal apoptosis.
Our data indicate that a balance between pro-apoptotic TGF-ß and anti-apoptotic Epo in the developing mouse retina may contribute to proper development in the CNS.

POSTER 55

Evaluation of TGF-β target genes in primary cortical and hippocampal neurons

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Transforming growth factors beta (TGF-βs) are pleiotropic and multifunctional cytokines and mediate a wide range of biological activities in a context dependent manner.
It has been recently shown that TGF-βs play an important role in the developing nervous system in regulating proliferation, differentiation, survival and death of neural cells. Deregulation of the signalling pathways is also involved in several pathological conditions including neurodegenerative disorders.
Many of these TGF-β transmitted responses result from changes in the expression of key target genes.
In this study we aim to isolate and investigate downstream target genes that may mediate TGF-β activity in primary cortical and hippocampal cultures isolated from E18.5 mouse embryos. The expression levels of selected target genes are quantified by RT-PCR and real-time-PCR after treatment with exogenous TGF-β.
The identification of TGF-β regulated genes might provide us with new targets for the development of pharmacological drugs to treat different CNS pathologies.

POSTER 56

Compartmental proteome analysis of the adult rat brain

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The scope of our study is to differentially analyse the healthy brain structures caudate-putamen complex (CPU), substantia nigra, olfactory bulb and the cerebellum in comparison to 6-OHDA leasioned brains (Parkinsonian rat model). Before, performing comparative proteomics we optimized the 2D-PAGE with regard to the rather fatty brain tissue. Furthermore, the whole IEF and SDS electrophoresis were efficiently improved in terms of reproducibility. Here, we performed a transcardial perfusion followed by an exact dissection of brain structures. Directly, after dissection the storage and most steps of processing the probes have been performed under frozen conditions. Major steps which have been adapted to the belongings of brain tissue were: 1) Homogenisation step, followed by an additionally performed TCA precipitation which turns out to produce less artifacts in the 2D-gels, 2) first dimension (desalting and IEF) including an appropriative rehydration buffer and prolongation of the focusing time, 3) equilibration step of the second dimension. The 2D-gels have been compared visually. In the 11.5 cm gels of the left and the right CPU of the same control rat no differences with consideration to spot pattern and staining intensities have been found. Furthermore, we observed, many spots in different areas within the same control brain which are constant in terms of optical density and 2D-distribution. Overall, our optimized approach gives rise to a reliable differential proteome analysis of brain areas with great significance for differential studies of models of the idiopathic Parkinson syndrome.

POSTER 57

Biochemical and morphological characterization of peroxisomes during postnatal development of the mouse brain

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The importance of peroxisomes in cellular metabolism is emphasized by the devastating consequences of peroxisome deficiency in patients with peroxisomal biogenesis disorders (e.g. Zellweger syndrome). Zellweger syndrome patients exhibit neuronal migration defects and severe hypo- or demyelination in distinct brain regions, the molecular pathogenesis of which is completely unknown. Unfortunately, very little is known about the exact distribution and physiological role of peroxisomes in the brain. With the methods used in former publications, an increase in the number of peroxisomes in the rodent brain was observed in the first 3 weeks after birth, whereas hardly any peroxisomes were detected in the brain of adult rodents. In this study, enriched peroxisomal fractions were prepared from the cerebellum, hippocampus, and medial cortex of 2-, 15- and 49-day-old C57Bl/6J mice. The specific activity of the peroxisomal marker enzyme catalase was highest in brain regions from 2-day-old mice and significantly decreased in 15- and 49-day-old mice. Western blots of the enriched peroxisomal fractions were probed with antibodies against peroxisomal matrix (e.g. catalase, acyl-CoA oxidase 1, thiolase A) and membrane proteins (PMP70, Pex14p, Pex13p) as well as specific markers for distinct cell types of the CNS (MAP2, GFAP, CNP & MBP). Highest levels of peroxisomal matrix and membrane proteins were detected in all brain regions of P2-mice. Double-immunofluorescence preparations of paraffin sections of distinct brain regions of P2-/P15-/P38-mice, probed with all antibodies, corroborated the biochemical data. All morphological data obtained with P2-brain sections were confirmed in primary cultures of neurons and astrocytes from newborn mice. Our data clearly show age-dependent differences in distribution and protein levels of catalase, peroxisomal □-oxidation enzymes, and peroxisomal membrane proteins in distinct brain regions. These results indicate the presence of distinct peroxisomal populations with different enzymatic compositions during postnatal development of the brain, suggestive for differences in peroxisomal function both in distinct cell types as well as during development.

POSTER 58

Axonal spines in the early postnatal cerebellum of AnkyrinG-deficient mice

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AnkyrinG (AnkG) is a membrane adapter protein that is required for restriction of voltage-gated sodium channels at the axon initial segment (AIS). We have recently studied a mouse model with a cerebellum-specific knock-out for AnkG. Surprisingly, a fraction of Purkinje cell (PC) axons in AnkG -/- mice was characterized by cytoplasmic protrusions closely resembling dendritic spines. In the present study we have examined whether such axonal spines are already detectable in early stages of cerebellar development. To this end, coronal sections (50 µm) were cut from the cerebellum of AnkG -/- mice which were sacrificed at postnatal days P1, P5, P10, and P20. Sections from littermate controls were processed in a similar manner. The morphology of PC axons was visualized by immunofluorescent labeling against calbindin. Interestingly, spines were detectable in the cerebellum of AnkG -/- mice as early as P5 and were also present at P10 and P20. PC giving rise to spiny axons often tended to form clusters in circumscribed regions of the cerebellum, including lobules IX and X. At P5 the spine-like protrusions preferentially exhibited a filopodia-like shape, whereas in later developmental stages (P20) these protrusions exhibited a stubby or mushroom-like shape. In conclusion, axonal spines are already detectable in the early postnatal cerebellum of AnkG-deficient mice. This observation implicates that the AnkG-based membrane cytoskeleton of the AIS plays an important role for early axonal differentiation.
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POSTER 59

Cloning and characterization of LAPSER1, a member of the Fezzin family of PSD proteins

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The postsynaptic density (PSD) is an electrondense structure underneath the postsynaptic membrane of excitatory synapses in the central nervous system. PSD proteins are known to anchor and cluster membrane receptors, cell adhesion molecules, components of signal transduction cascades and cytoskeletal elements. Recently, we have identified a novel family of postsynaptic scaffolding proteins, the "*Fezzins"*, consisting of four molecules (ProSAPiP1, PSD-Zip70, LAPSER1 and N4BP3), comprising C-terminal FEZ1 domains with yet unknown function in the brain.
The aim of our study was to characterize the "*Fezzin"*- family member LAPSER1, a novel PSD-protein. LAPSER1 consists of about 690 aa (74kDa) encoding a central coiled coil domain, a C-terminal FEZ1 domain and C-terminal PDZ domain binding motif (ATEI), the latter interacting with the PDZ domain of ProSAP/Shank.
LAPSER1 is suposed to be a membrane-associated protein, expressed in several parts of the rat brain shown by in situ hybridisation and immunolabeling of rat brain sections. mRNA and the corresponding protein is especially abundant in the cerebellum from early developmental stages onwards. Furthermore, LAPSER1 one is also expressed in non-neuronal tissue, for example in heart, kidney and testis. Recent results indicate that LAPSER1 binds to the RAP-GAP domain protein SPAR via the FEZ1 domain and coiled coil domain. Therefore, we conclude that the *"Fezzins"*- family seems to consist of closely related PSD proteins localizing to the synapse by ProSAP/Shank proteins providing a physical link to the PSD protein SPAR.

POSTER 60

RhoSAP, a novel Rho-GAP protein of the PSD

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Glutamatergic synapses in the central nervous system are characterized by an electron dense network of cell adhesion proteins, cytoskeletal proteins, scaffolding and adaptor proteins, membrane bound receptors and channels, G-proteins and a wide range of different signalling modulators and effectors underneath the postsynaptic membrane. This so called postsynaptic density (PSD) ressembles a complex signaling machinery. We performed a Yeast-Two-Hybrid screen with the PDZ domain of the PSD scaffolding molecule ProSAP2/Shank3 as bait and identified a novel interacting protein without any known functions. This molecule was named after its Rho-GTPase domain: RhoSAP (Rho GTPase Synapse Associated Protein). Performing database analysis yield to the human analogue RICH-2 and displayed a close relation with Nadrin. Interestingly, RhoSAP also contains a N-terminal situated BAR domain known that could facilitate membrane curvature and a Pro-rich domain at its C-terminus that could possibly act as a SH3 binding motif. Due to the finding of three splice variants of the C-terminal region, a second screen was carried out with RhoSAP's Pro-rich C-Terminus as bait to discover interacting proteins. Surprisingly, Syndapin I, a molecule involved in vessicle endocytosis via direct interaction with Dynamin I was found. Furthermore, Syndapin I contains a C-terminal SH3 domain that suggests to be the binding motif for RhoSAP's Pro-rich Domain. Providing new insight into RhoSAP's function, this interaction as well as the interaction with ProSAP2's PDZ domain was verified by Pull Down Assays and Co-Immunprecipitations. Taken together, RhoSAP is a novel ProSAP2/Shank3 interacting PSD protein that displays several protein/protein interaction domains. Due to the N-terminal BAR domain, and the interaction with Syndapin I, RhoSAP might act within endocytotic processes of the postsynaptic membrane. Project supported by DFG, SFB 497/ B8

POSTER 61

Cloning and characterisation of a Spar homologous protein, SerSAP3 (Serine-rich Synapse Associated Protein 3)

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In a yeast two hybrid screen using the PSD protein ProSAP interacting protein 1 (ProSAPiP1) as bait, we isolated partial clones of a novel Rap-Gap domain containing protein. This protein, termed SerSAP3, is characterized by several serine rich stretches, a PDZ domain and a C-terminal coiled coil region. The SerSAP3 - ProSAPiP1 interaction is mediated by leucine zipper containing coiled-coil regions of SerSAP3 and ProSAPiP1 as revealed by blot-overlay assay and co-transfection experiments. SerSAP3 mRNA and protein is widely expressed in neurons of the brain and is found in dendrites, spines and PSDs. High protein levels are especially detected in the hippocampus and cerebellum, where SerSAP3 is predominantly localized in cerebellar Purkinje cells. SerSAP3 binds to actin and codes for two distinct regions that are responsible for the targeting to dendritic spines and PSDs, where it colocalizes with proteins of ProSAP / Shank family. SerSAP3 shows some structural similarity to the spine associated Rap-Gap, SPAR, which is able to alter size and complexity of dendritic spines. This effect is not seen after transfection of the full length SerSAP3 protein, an N-terminal SerSAP3 construct, however, leads to the formation of numerous longer and thinner spines paralleled by a significant reduction of the dendritic arbor. The cloning of SerSAP3 adds a new member to the growing list of proteins that are able to regulate small GTPases at spines and synapses. This new molecule might therefore well be important for the structural remodeling of spines and PSDs.

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POSTER 62

Characterization of a ProSAP1/Shank 2 “knock out” mouse

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ProSAP1, ProSAP2, and ProSAP3 constitute a family of proteins (also called Shank-family) that function as molecular scaffolds in the postsynaptic density (PSD). Family members are characterized by an identical pattern of several protein-protein interaction domains including a SH3, PDZ and a SAM domain. Meanwhile it has been shown that several splice variants exist where some of the above described domains are deleted. ProSAP1/Shank2 is expressed in the central nervous system and in several other tissues like kidney, thymus or pancreas. In brain it localizes to the PSD of excitatory synapses via the C-terminal SAM domain. Up to now several ProSAP/Shank interacting proteins have been identified that link glutamate receptor clusters to the actin based cytoskeleton. Moreover, proteins that are involved in several intracellular signalling pathways (i.e. dynamin, IRSp53) have been shown to be attached to ProSAP/Shank.
In search for more data concerning the functional role of the protein we generated a ProSAP1/Shank2 KO mouse by the genomic targeting of ProSAP1 exon 7 that codes for the first part of the ProSAP1 PDZ domain. ProSAP1 knock-out mice have been analyzed with a wide set of methods. We found that the survival rate of KO mice was only 50% compared to controls. The surviving mice, however, have the same life span expectancy as do WT mice. In addition KO mice are hyperactive and show significant learning disabilities. The overall morphology of brain structures as well as synapse ultrastructure was not altered, synapse number, however, was significantly lower compared to WT. Results show that ProSAP1/Shank2 KO mice show severe behavioural defects that might be explained by a dramatic reduction of synapse number.

POSTER 63

Synaptogenese in primären kultivierten Hippokampus-Neuronen

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Neuronale Informationsverarbeitung beruht u.a. auf der synaptischen Verschaltung zwischen Nervenzellen. Man unterscheidet hemmende und erregende Synapsen, wobei exzitatorische Synapsen u.a. durch ein dichtes Proteinnetzwerk unter der postsynaptischen Membran charakterisiert sind, die postsynaptische Dichte (PSD). Primäre Kulturen mit Hippocampus-Neuronen sind ein häufig genutztes Modell für die Untersuchung von synaptischen Strukturen. Da es bisher wenig Informationen zur Synaptogenese in diesen Kulturen gibt, haben wir die Entwicklung von Synapsen embryonaler Ratten-Hippokampusneuronen (Embryonaltag 20) nach 3, 7, 10, 14 und 21 Tagen in Kultur ultrastrukturell analysiert. Beginnend mit Tag 7 wurden reife exzitatorische Synapsen mit einer eindeutig definierten postsynaptischen Dichte an Dendriten und mit präsynaptisch lokalisierten synaptischen Vesikeln identifiziert. Mit zunehmender Kultivierungszeit wurde ein Anstieg der PSD-Anzahl in den Kulturen nachgewiesen. Es konnten jedoch keine signifikanten Unterschiede zwischen den Längen und Flächen der PSDs bei den verschiedenen Kultivierungs-Zeitpunkten festgestellt werden. Reife Synapsen an dendritischen Dornen konnten ab Tag 10 beobachtet werden, zweifache bzw. multiple Synapsen fanden sich vereinzelt nach 14 bzw. 21 Tagen. Sogenannte *dense core* Vesikel, verantwortlich für den Transport von Proteinen zur aktiven Zone der präsynaptischen Endigungen, wurden nach dem 7. Kultivierungstag im Bereich der Präsynapse bei allen Zeitpunkten nachgewiesen. Zusätzlich zu den elektronenmikroskopischen Untersuchungen wurde mittels Immunfluoreszenz eine zeitabhängige Zunahme der Expression des PSD-spezifischen Proteins ProSAP1/Shank2 und des präsynaptischen Proteins Bassoon festgestellt, was mit den ultrastrukturellen Ergebnissen zu korrelieren war. Zusammenfassend konnte eine regelhafte Ausbildung und Reifung exzitatorischer Synapsen gezeigt werden, die mit Tag 7 in Kultur beginnt.

POSTER 64

Influence of Abi-1 on the actin cytoskeleton in hippocampal neurons

Einfluss von Abi-1 auf das Aktin-Zytoskelett in hippocampalen Neuronen

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The rearrangement of the actin cytoskeleton plays an essential role for the outgrowth of dendrites and the development as well as the maturation of synapses. The regulation of these processes, however, is not known in detail. In different model systems it was shown, that Abl-interacting protein 1 (Abi-1) and its interaction partners WAVE2, Rac, Eps8, SOS1 and Abl tyrosine kinase are very important for the formation and reorganisation of the actin cytoskeleton.
To investigate, if Abi-1 has a similar role in neurons, down regulation of Abi-1 by a vector based RNAi method was established in primary hippocampal neurons from rat.
Transfection of neurons with Abi-1 RNAi led to a highly branched dendritic arbor. Overexpression of Abi-1 resulted in an opposite phenotyp with a simplification of the dendritic tree. Additionally, Abi-1-down regulation by RNAi in neurons decreased the density of synapses and reduced the number of mature synapses, while Abi-1 overexpression increased the number of synapses and led to more mature synapses.
These results point out the importance of Abi-1 in actin cytoskeleton rearrangement processes in neurons. Abi-1 protein concentration was shown to be essential for regulated outgrowth of dendrites as well as development and maturation of synapses.
This project is supported by the DFG and the Land Baden-Württemberg (1423/74).

POSTER 65

Synaptic Activation of Hippocampal Mossy Cells is Reduced in *Reeler* Mice

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Development alterations of cortical structures can cause increased propensity for epilepsy. In *Reeler* mutant mice migration defects during development result in altered lamination of the hippocampus and the dentate gyrus. To test whether these alterations can support epileptogenesis, we have investigated morphological and physiological properties as well as synaptic activation of mossy cells, neurons that provide excitatory feed-back within the dentate networks. Analysis of the morphology of intracellularly-filled mossy cells in acute slices of *reeler* mice revealed that the dendrites extend aberrantly into the molecular layer and receive synaptic input from putative perforant path axon terminals. Consistent with this observation, extracellular stimulation in the molecular layer revealed monosynaptic excitatory response in the neurons during the whole-cell patch-clamp recordings. Despite this aberrant excitatory input, mossy cells show a reduction in their synaptic activation compared to wild type mice. In *reeler* mossy cells strong $GABA_A$ receptor-mediated inhibition contained excitation. Blockade of inhibition resulted in a dramatic increase in the excitability of mossy cells in both wild-type and *reeler* slices, but no spontaneous seizure activity was observed. Thus our data show that developmental alteration do not result in an enhanced, but rather a lowered excitability of mossy cells and the dentate circuits of the *reeler* mouse.
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POSTER 66

Lack of Sortilin - a p75 co-receptor - rescues axotomized cholinergic but not GABAergic septo-hipocampal neurons

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Sortilin is a member of the recently discovered family of Vps10p-domain receptors, and is expressed in a variety of tissues, notably the brain and spinal cord. It has recently been shown to act as a co-receptor and molecular switch governing the p75NTR-mediated pro-apoptotic signal induced by proNGF. Pro-apoptotic signals mediated via proNGF and p75 are known to play a crucial role in the survival of cholinergic medial septum neurons during development and after lesioning. In the present study we investigated the survival of medial septum neurons in sortilin knockout mice during development and after fimbria-fornix transsection. To this end we immunostained serial sections of the medial septum for ChAT, p75 and parvalbumin at three months of age as well as in young adult mice three weeks after lesioning of the septo-hippocampal projection. We quantified the number of cholinergic and GABAergic medial septum neurons by computer-based stereology. In contrast to p75 mutants, in sortilin knockout mice we found no changes in the number of cholinergic or GABAergic neurons at the end of development as compared to wildtype littermates. However, three weeks after axotomy lack of sortilin leads to a significant, twofold increase in the number of surviving cholinergic medial septum neurons as compared to lesioned wildtype littermates. In contrast, the survival of GABAergic medial septum neurons was not improved in sortilin knockout mice. Our results indicate that p75/sortilin mediated pro-apoptotic effects play a role in the cell death of cholinergic septo-hippocampal neurons after axotomy. However, disturbance of this pathway by deletion of sortilin only partially rescues lesioned medial septum cholinergic neurons since cell numbers observed in sham operated mice are by far higher.

POSTER 67

Involvement of the abnormal-Cannabidiol-sensitive receptor in excitotoxic secondary neuronal damage, as assessed in organotypic hippocampal slice cultures

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In the aftermath of acute CNS pathologies several metabolic and immunological mechanisms contribute to the delayed loss of neurons, initially unaffected by the lesion. In this so called “secondary damage”, activated microglial cells accumulate at sites of neuronal injury and endanger neurons that have survived the primary damage. Cannabinoids have been shown to be involved in neuroprotection and this may reflect their actions on microglial cells. Activated microglial cells mainly express the CB2 and the abn-CBD receptor. Abn-CBD receptors are activated by abnormal cannabidiol (abn-CBD) and 2-arachidonoylglycerol (2-AG) and are antagonized by the phytocannabinoid cannabidiol (CBD) and the synthetic cannabinoid O-1918, respectively. In the present study we investigated the effects of agonists/antagonists of the abnormal-Cannabidiol-sensitive receptor (abn-CBD receptor) on microglial cells and neurons after excitotoxic lesion of organotypic hippocampal slice cultures (OHSC). OHSC derived from 8-day-old (p8) Wistar rats were lesioned by the application of N-methyl-D-aspartate (NMDA) after 6 days in vitro (div) and treated with abn-CBD (0.1-10µM) or 2-AG (0.001-1µM) up to 9 div. Propidium iodide (PI) and FITC-conjugated *Griffonia simplicifolia* isolectin B_4 (IB_4) were used to visualize the extent of neuronal damage and microglial cells, respectively. The OHSC were analyzed by confocal laser scanning microscopy. The dentate gyrus of control OHSC contained almost no damaged PI^+ neurons and few, ramified microglial cells. Both, abn-CBD and 2-AG had no effect on microglial cells and neuronal cell death in non-lesioned OHSC. NMDA-treated cultures displayed a massive increase in PI^+ neurons and large numbers of amoeboid microglial cells accumulating at the sites of neuronal injury. Treatment of lesioned OHSC with abn-CBD resulted in a concentration-dependent decrease in the number of microglial cells, compared to OHSC lesioned with NMDA only. Maximal reduction occurred at an abn-CBD concentration of 10µM. This effect was blocked by the abn-CBD receptor antagonists CBD and O-1918. Abn-CBD protected granule cells from excitotoxic injury in a concentration dependent manner with maximal reduction of degenerating neurons at 10µM abn-CBD, which was only reversed by the phytocannabinoid CBD but not by O-1918. In lesioned OHSC 2-AG also reduced the number of microglial cells with maximal reduction at 0.001µM, and as compared to abn-CBD this effect was antagonized by CBD and O-1918. Similar to abn-CBD, 2-AG significantly reduced the amount of degenerating neurons and surprisingly this was only antagonized by O-1918, but not by CBD. Our findings show that the number of microglial cells and the amount of neuronal cell death induced by excitotoxicity is reduced by both abn-CBD and 2-AG. Different binding sites may be responsible for the different effects of natural and synthetic antagonists of the abn-CBD receptor, as observed in our model system.

POSTER 68

Combination of laser microdissection with microarray analysis allows plaque-associated transcriptome analysis in APP23 transgenic mice

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Amyloid plaques are a characteristic feature of Alzheimer´s disease (AD). They are regularly accompanied by plaque-associated inflammatory reactions, which may have beneficial (removal of amyloid) as well as detrimental (aberrant axonal sprouting) effects on neighboring brain tissue. Although novel immunological treatment strategies aimed at the removal of amyloid from brain have recently been developed, the local plaque-associated cascade of inflammatory events has not been elucidated so far.
In the present study, aged APP23 transgenic mice were employed to study plaque-associated inflammatory changes. These mice develop amyloid plaques with age and show a strong plaque-associated glial reaction. In order to unravel the network of inflammatory compounds which might be involved in plaque-associated inflammation, we have combined the power of microarray analysis with the strength of laser microdissection for isolating defined regions in APP23 transgenic mice. Amyloid plaque-associated and non-plaque-associated tissue fragments were microdissected in APP23 transgenic animals as well as tissue from non-transgenic controls. Bioinformatic tools were employed to confine the data sets to target genes up- or downregulated in amyloid-associated tissue. A preliminary network analysis of the microarray data points at modifiers of interferon (IFN) family members as well as regulators of macrophage phagocytosis. In order to increase reliability and functional usefulness of the microarray data, gene expression changes of selected mediators were verified by quantitative RT-PCR. These studies will identify candidate molecules which could be involved in the local regulation of the amyloid-induced inflammatory response and may pinpoint putative targets for therapeutic intervention.

POSTER 69

Untersuchungen zur β Amyloid 1-42-induzierten Rezeptoraktivierung und Endozytose in Gliazellen

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Die genauen Ursachen des Nervenzelluntergangs beim Morbus Alzheimer sind weitestgehend unbekannt. Ebenso unklar sind die Mechanismen, über die das in die Erkrankung verwickelte β Amyloid Peptid (Aβ 42) Neurone und Gliazellen (Astrozyten, Mikroglia) in ihrer Aktivität beeinflusst. Es gibt Hinweise darauf, dass Aβ42 den G-Protein-gekoppelten und an der Vermittlung der Chemotaxis beteiligten *Formyl-Peptide-Receptor-like* 1-Rezeptor (FPRL1) aktiviert und zur Internalisierung benutzt, die nachfolgend ablaufenden Signalwege sind jedoch nicht näher charakterisiert. Anhand der Bestimmung des cAMP-Spiegels, durch Western-Blot-Analysen sowie Untersuchungen zur Endozytose des Aβ42 (Immunfluoreszenz, ELISA) von Aβ42-behandelten Gliazellen sollte die Rolle des FPRL1 Rezeptors für die Aktivität und Internalisierung des Aβ42 näher analysiert werden. Die Untersuchungen zeigten, dass der FPRL1 in Gegenwart von 1µM Aβ42 sowohl in Astrozyten als auch Mikroglia internalisiert wird und zu einem cAMP-Anstieg führt. Mit dem FPRL1-Antagonisten CDCA konnte dieser Effekt inhibiert werden. Die Quantifizierung der Internalisierung mit Hilfe einer ELISA-Technik verdeutlichte eine zeitabhängige Zunahme der Endozytose des Aβ42 in den Gliazellen. Eine Hemmung des FPRL1 Rezeptors führte zu einer Abnahme der Endozytose-Rate. Die Untersuchungen deuten auf eine wichtige Rolle des FPRL1 Rezeptors in der Vermittlung der Aktivität und Endozytose des Aβ 42 in Gliazellen hin. Weitere Kenntnisse über die Funktion und Regulation des FPRL1 Rezeptors können eine Grundlage für die Entwicklung von Therapiestrategien für die Alzheimer-Demenz bilden.

POSTER 70

Estrogen regulated dopamine uptake in astrocytes

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Dopamine is actively eliminated from the extracellular space by the dopamine transporter. The availability of dopamine is important for the function of nigrostriatal circuits under normal and pathological conditions. Estrogen is known to regulate the activity of dopaminergic neurons at the synaptic level and to improve dopaminergic function. Therefore, we have studied the role of estrogen on the regulation of dopamine transporter expression and uptake kinetics in cultured mesencephalic and striatal astrocytes. Cultures were treated with 17ß-estradiol(10^{-8} M, 24 h) and dopamine transporter mRNA levels were measured by PCR. The dopamine uptake into astrocytes was measured by HPLC. We showed a decrease of dopamine transporter expression after 17ß-estradiol treatment by 85 % in mesencephalic and 63 % in striatal cultures. Dopamine uptake into astrocytes was totally inhibited by the dopamine transporter antagonist *GBR 12935*. Consequently uptake into cultures pre-treated with 17ß-estradiol showed a decrease in both brain regions. Our data reveal that astrocytes play a crucial role in the regulation of dopamine elimination by an active and highly specific transport mechanism. The dramatic downregulation of astroglial dopamine transporter expression results in coherent diminished dopamine uptake and therefore in higher extracellular concentrations of dopamine. As a consequence, well-known beneficial effects of estrogen on the activity of the nigrostriatal dopaminergic system during degenerative disorders such as M. Parkinson might be the result of an impaired glial dopamine clearance rate.

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POSTER 71

Der Verlust von FGF-2 hat keinen Effekt auf die Integrität des dopaminergen Systems in MPTP-behandelten Mäusen

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Die Zugabe von exogenem Fibroblasten-Wachstumsfaktor 2 (FGF-2) fördert das Überleben dopaminerger Neurone in-vitro und schützt dopaminerge Neurone vor MPTP-induziertem Zelltod. Anhand von FGF-2–knockout Mäusen untersuchten wir, ob das Fehlen von endogenem FGF-2 Einfluss auf das dopaminerge System hat. FGF-2 defiziente Mäuse zeigen weder eine Veränderung der Tyrosin-hydroxylase (TH) positiven Fasern im Striatum oder der Amygdala noch eine Veränderung des Dopamingehalts im Striatum. Zudem fand sich auch keine signifikante Veränderung der Dichte dopaminerger Zellen in der Substantia nigra bei diesen Tieren. Da ein eventuell vorhandenes Defizit bei den FGF-2 defizienten Tieren möglicherweise erst unter pathologischen Gegebenheiten zum Tragen kommt, behandelten wir FGF-2 knockout Mäuse mit MPTP. Sowohl FGF-2 defiziente Mäuse als auch Kontrolltiere entwickelten eine Reduktion der striatalen, dopaminergen Faserdichte um 71% und des Dopamingehaltes um 80% nach MPTP-Behandlung. Bei beiden Tiergruppen tritt zudem eine starke Reduktion der TH-positiven Zellen in der Substantia nigra auf. Es bestand jedoch kein signifikanter Unterschied zwischen den beiden Gruppen bezüglich dieser Untersuchungsparameter. Diese Daten weisen darauf hin, dass endogenes FGF-2 keine essentielle Rolle für die Integrität des dopaminergen Systems in MPTP-behandelten Mäusen spielt.

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POSTER 72

Die Antidepressiva Amitriptylin und Citalopram haben unterschiedliche Einflüsse auf Neurodegeneration und Verhalten in einem Modell der Depression

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Die „olfaktorische Bulbektomie“ (OB) ist ein etabliertes Tiermodel der Depression und wurde meist an Ratten untersucht. Bulbektomie induziert Verhaltensänderungen, die durch Behandlung mit Antidepressiva abgeschwächt werden können. Zudem ist bekannt, dass Bulbektomie zu neurodegenerativen Veränderungen in einzelnen Gehirnbereichen führt. Wir untersuchten OB-induzierte Verhaltensänderungen und Neurodegeneration, und deren Reversibilität durch Antidepressiva in Mäusen. Unsere Ergebnisse zeigen, dass OB bei Mäusen, ähnlich wie bei Ratten, die lokomotorische Aktivität erhöht und Defizite im „passive avoidance“-Test bedingt. OB führt auch zu Neurodegeneration (visualisiert mit Hilfe des Neurodegenerationsmarkers Fluoro-jade B) im piriformen Kortex und im posterolateralen kortikalen Hirnkern der Amygdala (PLCo). Chronische Behandlung mit Amitriptylin reduziert Neurodegeneration in beiden Hirnarealen und vermindert die abnormale lokomotorische Aktivität, die durch OB induziert wurde. Chronische Behandlung mit Citalopram schwächt die durch OB induzierten Verhaltensdefizite ab, schützt jedoch nicht vor Neurodegeneration im piriformen Kortex oder im PLCo. Diese Daten zeigen, dass OB erfolgreich auch bei Mäusen eingesetzt werden kann und dass Amitryptilin nicht nur als Antidepressivum wirkt, sondern zudem auch über neuroprotektive Eigenschaften verfügt.

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POSTER 73

Spinocerebellar ataxias types 2 and 3: Isolation of the cerebellum by severe damage to the precerebellar brainstem nuclei

Die spinozerebellären Ataxien Typ 2 und 3: Isolation des Kleinhirnes durch schwere Schäden in den präzerebellären Hirnstammkernen

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The precerebellar nuclei act as a gate for the entire afferent input destined for the cerebellum. Despite their important physiological role in cerebellar circuits no pathoanatomical studies of these brainstem nuclei had yet been performed in spinocerebellar ataxia type 2 (SCA2) or type 3 (SCA3). To address this question, we carried out a detailed postmortem study of the precerebellar brainstem nuclei in six clinically diagnosed and genetically-confirmed SCA2 and seven clinically diagnosed and genetically-confirmed SCA3 patients. Unconventionally thick serial tissue sections through the brainstem of these patients were stained for lipofuscin granules and Nissl substance and used for systematic pathoanatomical studies. All of the SCA2 and SCA3 patients' precerebellar nuclei underwent considerable neurodegeneration. Widespread damage to these brainstem nuclei separates the three phylogenetically defined regions of the cerebellum and substantially contributes to the occurrence of a variety of SCA2 and SCA3 disease symptoms: gait, stance, limb and truncal ataxia, dysarthria, truncal and postural instability, impairments of the vestibulo-ocular reaction and optokinetic nystagmus, slowed and saccadic smooth pursuits, dysmetrical horizontal saccades, and gaze-evoked nystagmus.

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POSTER 74

Proteinkinase C (PKC) vermittelte Oberflächenexpression von Glutamattransportern in kultivierten cerebellären Körnerzellen der Maus *

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Die Glutamattransporter EAAC1 und GLT1v kommen hauptsächlich in zytoplasmatischen Vesikeln und weniger in der Zellmembran von Neuronen vor. Aufgrund dieser Lokalisation werden sie als Reservetransporter für die Wiederaufnahme synaptisch freigesetzten Glutamats betrachtet. Der Transfer dieser transporterhaltigen Vesikel zur Zellmembran wird möglicherweise durch PKC vermittelt. So haben Untersuchungen an verschiedenen Zelllinien gezeigt, daß EAAC1 nach Aktivierung von PKC durch Phorbol-myrastat-acetat (PMA) vermehrt durch Exocytose in die Zellmembran eingebaut wird. Bisher fehlen jedoch Ergebnisse über die Rezirkulierung von Glutamattransportern in glutamatergen Neuronen. Ziel der vorliegenden Untersuchung war es, einen möglichen PKC vermittelten Transfer von EAAC1 und GLT1v an glutamatergen cerebellären Körnerzellen (Maus) in Primärkultur mittels Immunfluoreszenzmikroskopie und quantitativenWesternblot-Analysen nachzuweisen. Die PKC-Aktivierung von Körnerzellen erfolgte mit PMA und die Inhibierung mit Staurosporin. Als Kontrolle diente die Inkubation mit dem für die beiden Substanzen verwendeten Lösungsmittel (DMSO). Für die Membrananalysen mittels Westernblot wurde eine Biotinilierungsmethode eingesetzt. Die immuncytochemischen Untersuchungen ergeben, daß nach Aktivierung der PKC EAAC1 und GLT1v in den Oberflächenmembranen der Neuritenvarikositäten von Körnerzellen lokalisiert sind, während sie unter Kontrollbedingungen oder bei PKC-Inhibierung vor allem vesikulär gebunden im Zytoplasma vorliegen. Die quantitativen Westernblot-Analysen zeigen, daß die Oberflächenexpression von EAAC1 - verglichen mit Kontrollen - nach PKC-Aktivierung um 31% zu- und nach Inhibition um 22% abnimmt. Dadurch ergibt sich ein Unterschied von 69% in der Oberflächenexpression zwischen Aktivierung und Inhibition der PKC. Die Unterschiede zwischen den verschiedenen Experimentergebnissen sind statistisch signifikant ($p<0,05$). Ähnliche Ergebnisse wurden für GLT1v erzielt. Die Untersuchungen lassen den Schluss zu, daß die Oberflächenexpression von EAAC1 und GLT1v in glutamatergen Körnerzellen des Kleinhirns durch PKC vermittelt wird. Unter physiologischen Bedingungen dürfte eine PKC-Aktivierung durch Glutamatbindung an metabotrope Glutamatrezeptoren erfolgen.

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POSTER 75

Mechanismen neuraler Differenzierung: Microcarrier als kontinuierlicher Pool für neurale Stammzellen zur Therapie neurodegenerativer Erkrankungen

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Die Transplantation eines konstanten Pools fetaler neuraler Stammzellen könnte als Behandlungsstrategie zur Therapie von neurodegenerativen Erkrankungen dienen. Da die therapeutischen Optionen für dieses Krankheitsbild immer noch sehr beschränkt sind, war es Ziel der vorliegenden Untersuchung, die Potenz von neuralen embryonalen Stammzellen aus dem Cortex embryonaler Ratten (E18) für einen therapeutischen Einsatz unter experimentellen Bedingungen zu prüfen.
Besonderes Interesse für einen therapeutischen Einsatz stellt die Isolation von neuralen Vorläuferzellen in Form von 3D Aggregaten, den sog. Neurosphären dar. Innerhalb von Neurosphären werden allerdings neben mitotisch aktiven Zellen viele apoptotische und nekrotische Zellen identifiziert. Ziel dieser Arbeit war es daher, eine geeignete Matrix zu finden, die als Trägermaterial für die zu reimplantierenden Zellen dient, nekrotische Areale und die Differenzierung der Zellen minimiert sowie die Proliferationskapazität der Zellen begünstigt. Microcarrier aus einer spezifisch aufgearbeiteten Kollagenmatrix wurden hierfür *in-vitro* mit den isolierten neuralen Stammzellen beladen und anschließend die von den Microcarriern abgewanderten und unterschiedlich differenzierten Zellen immunhistochemisch untersucht.
Es konnte mittels Immunfluoreszenz mit Antikörpern gegen MAP-2, GFAP, β3-Tubulin, O4 und Nestin gezeigt werden, dass die Zellen auf den Microcarriern im Vergleich zu Neurosphären weniger in die drei neuralen Zelltypen (Neurone, Astrozyten, Oligodendrozyten) differenzieren, sondern eher in einem undifferenzierten Zustand (Nestin positiv) verbleiben.
Diese Befunde zeigen, dass die Kultivierung der neuralen Stammzellen auf dieser Trägermatrix für einen therapeutischen Einsatz bei neurodegenerativen Erkrankungen besser geeignet ist.

POSTER 76

Efferent connections of the parabigeminal nucleus to the superior colliculus and the amygdala in the rat. A double labeling fluorescent retrograde tracing study

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A recently revealed important function of the amygdala (Am) is that it acts as the brain's „lighthouse", which constantly monitors the environment for stimuli which signal a threat to the organism. In patients with extensive lesions of the striate cortex unseen "fearful" and fear-conditioned faces elicit increased Am responses. Apparently, extrageniculostriate pathways are involved. Retinal impulses reach the Am via a multisynaptic pathway: superior colliculus (SC) – pulvinar – Am. The parabigeminal nucleus (Pbg) is a small structure along the lateral border of the mesencephalon. Pbg is interconnected with several subcortical visual centers. Especially, the reciprocal connections between the Pbg and SC are rather strong. We recently found (Uzunoff et al. 2006) that the Pbg projects also to the Am. This projection might be an element of a further disynaptic connection from the SC to the Am. In order to understand whether the neurons of the Pbg are able to innervate more than one target by means of divergent axon collaterals, we performed a double labeling retrograde tracing study. Fluoro-Gold was stereotaxically injected in the right Am and fluoro-emerald was placed in the SC on the same side. Here, we report that some neurons in Pbg emit branching axons that innervate simultaneously the SC and the Am, but that these connections arise also from separate neuronal populations. The present study demonstrates that Pbg projects bilaterally to the Am and SC. The ipsilateral projections arise from separate cell populations, whilst the contralaterally projecting Pbg neurons emit branching axons that simultaneously innervate Am and SC.

POSTER 77

Nitric oxide synthase-containing neurons in the amygdaloid nuclear complex of the rat

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The nitric oxide-producing neurons in the rat amygdala (Am) were studied using reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) histochemistry. Almost all nuclei of the Am contained NADPHd-positive neurons and fibers, but the somatodendritic morphology and the intensity of staining of different subpopulations varied. The strongly stained neurons displayed labeling of the perikarya and the dendritic trees with Golgi impregnation-like quality, whilst the dendrites of the lightly stained neurons were less successfully followed. Many strongly positive neurons were located in the external capsule and within the intraamygdaloid fiber bundles. Stained cells were present in the amygdalostriatal transition area, the lateral amygdaloid nucleus, the basolateral nucleus, the basomedial nucleus, and the central nucleus. The medial amygdaloid nucleus contained numerous moderately stained neurons and displayed the strongest diffuse neuropil staining in Am. From the cortical nuclei, the most appreciable number of stained neurons was seen in the anterior cortical nucleus. The intercalated amygdaloid nucleus lacked NADPHd-positive neurons but an appreciable plexus of fine, tortuous axons was present. In the intraamygdaloid part of the bed nucleus of the stria terminalis some lightly stained cells were seen but along the entire course of stria terminalis strongly stained neurons were observed. Some Am nuclei, and especially the central lateral nucleus and the intercalated nucleus, display considerable species differences when compared with the primate Am. The age-related changes of the nitrergic Am neurons, as well as their involvement in neurodegenerative diseases is discussed.

POSTER 78

Charakterisierung der Melatoninsynthese beim Menschen in autoptisch gewonnenen Pinealorganen

Characterization of human melatonin synthesis using autoptic pineal tissue

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The mammalian pineal gland rhythmically synthesizes the hormone melatonin that provides the body with a signal coding the duration of the night period. The ultimate step in melatonin synthesis is catalyzed by the hydroxyindole *O*-methyltransferase (HIOMT), but the enzyme that drives melatonin synthesis is the arylalkylamine *N*-acetyltransferase (AA-NAT). Regulation of melatonin synthesis shows remarkable differences among mammalian species. In rodents, but not in sheep and bovine, activation of melatonin synthesis depends on transcriptional activation of the *Aa-nat* gene. The mechanisms controlling melatonin formation in man are elusive. Therefore, pineal tissue, taken from regular autopsies (n=69; postmortem intervals ranging from 9.5 to 147 h) was analyzed simultaneously for 1) *Aa-nat* and *Hiomt* mRNA levels by PCR, 2) AA-NAT and HIOMT activities by radiometric assays with ^{14}C-acetyl-coenzyme A and S-Adenosyl-*L*-[^{14}C]-methionine, respectively, and 3) melatonin content employing an ELISA. Results were allocated to asserted time-of-death groups (day: 1000 h-1630 h; dusk: 1630 h–2200 h; night: 2200 h–0730 h; dawn 0730 h-1000 h). Degradation rates of *Gapdh*, *Aa-nat* and *Hiomt* mRNAs ran in parallel and therefore, data normalization could be established, irrespective of post-mortem delay in tissue sampling. *Aa-nat* and *Hiomt* mRNA levels and HIOMT activity showed no diurnal rhythm. In contrast, a significant rhythm was found for the correlation between time of death and both, AA-NAT activity and melatonin content, with elevated values during dusk and night. Presented data demonstrate that post-mortem brain tissue can be used to detect the remnant of pre-mortem adaptive changes in neuronal activity. In particular, our results give for the first time strong experimental support that post-transcriptional mechanisms are dominant for the generation of rhythmic melatonin synthesis in the human pineal gland.
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POSTER 79

Immunocytochemical and immunohistochemical characterization of the endocannabinoid system in the rat pineal gland

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The cannabinoid system consists of distinct receptor types and exogenous or endogenous ligands (endocannabinoids). The most abundant endocannabinoids are arachidonolyglycerol (2-AG), arachidonolyethanolamide (AEA) and palmitoylethanolamide (PEA) that are synthetized and degraded by specific enzymes. These substances are known to modulate many neuronal and neuroendocrine circuits. Recently, we have discovered in rats that the cannabinoid system also influences photoneuroendocrine functions: several phytocannabinoids like tetrahydrocannabinol attenuate the NE-driven biosynthesis of melatonin, the neuroendocrine hand of the circadian clock. Since such pharmacological data suggest the existence of an endocannabinoid system, we have investigated in the rat pineal gland the presence of cannabinoid receptor proteins and enzymes responsible for endocannabinoid synthesis and degradation by use of immunocytochemistry and immunoblotting. Virtually all pinealocytes displayed immunoreactions for the two cloned cannabinoid receptors 1 and 2 and two enzymes involved in the degradation of 2-AG and AEA, the fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL). Ongoing studies focus on enzymes involved in the biosynthesis of endocannabinoids like the NAPE-specific phospholipase D and address the question whether cannabinoid receptor proteins and enzymes controlling the biosynthesis and degradation of endocannabinoids undergo diurnal and circadian variations in the pineal gland.

POSTER 80

Differential gene expression in the pars tuberalis of wild-type and MT1-melatonin receptor-deficient mice

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The hypophyseal pars tuberalis (PT) is an important target tissue for melatonin and an excellent model to study the molecular mechanisms that decode the melatonin signal. Previous investigations have shown that melatonin is an essential signal to drive rhythmic clock gene expression in the PT. In mice with a targeted deletion of the MT1 melatonin receptor gene ($MT1^{-/-}$), rhythmic expression of the clock genes *mPer1*, *mCry1*, *Bmal1*, *Clock* is reduced. In the present study we have used cDNA microarray analysis to screen for additional genes in the PT which are under control of the MT1 receptor. To this end, we compared the gene expression in the PT of wild-type (WT) and $MT1^{-/-}$ mice of 45101 genes at two different timepoints, midsubjective day (CT06) and midsubjective night (CT18). CT06 is the timepoint of maximal mPER1 protein levels in the PT of WT and CT18 is the timepoint of high melatonin levels in the circulation. In the PT of WT mice, 1753 genes were increased at CT18, whereas 998 genes were decreased as compared to CT06. In the PT of $MT1^{-/-}$ mice, 2061 genes were increased at CT18, whereas 1302 genes were decreased as compared to CT06. This suggests that there is fluctuation of gene expression in the PT of $MT1^{-/-}$ mice which must be regulated independently from mPER1 protein and MT1 receptor signaling. At CT06, we found 1174 genes to be decreased in the PT of WT-mice, whereas 1570 genes were increased compared to $MT1^{-/-}$ mice. At CT18, we found 650 genes to be down regulated in the PT of WT-mice, while 979 were up regulated as compared to $MT1^{-/-}$ mice. In the end we found 24 genes of interest according to the assumed impact based on daytime, melatonin or mPER1. The differentially expressed genes encode for proteins involved in G-protein-coupled receptor signaling and transcriptional regulation.

POSTER 81

Impact of melatonin receptors on pCREB and clock gene protein levels in the murine retina

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In several mammalian species, the retina is capable to synthesize melatonin and contains an autonomous circadian clock depending on interlocking transcriptional/translational feed back loops involving several clock genes, such as *Per1, Cry2*. Our previous investigations have shown remarkable differences in retinae of melatonin-deficient (C57BL) and melatonin-proficient (C3H) mice with regard to the protein levels of PER1, CRY2 and phosphorylated (p) cyclic AMP response element binding protein (CREB). To elucidate the melatonin receptor type possibly responsible for these differences we have now performed immunocytochemical analyses for PER1, CRY2 and pCREB in retinae of melatonin-proficient wild type (WT) mice and mice with targeted deletions of the MT1 receptor (MelaaBB) or the MT1 and MT2 receptors (Melaabb) at four different timepoints. Immunoreactions for PER1, CRY2 and pCREB were localized to the nuclei of cells in the inner nuclear layer (INL) and ganglion cell layer (GC) of all strains. Surprisingly, in MelaaBB and Melaabb the day/night rhythm in pCREB, PER1 and CRY2 levels was not abolished, but the maxima and minima of PER1 were 180 degrees out of phase as compared to the WT. These data suggest that MT1 and MT2 melatonin receptors are not necessary to maintain rhythmic changes in clock gene protein levels in the murine retina. However, as shown for PER1, they appear to be involved in internal synchronization.

POSTER 82

Cloning and expression pattern of clock gene homologs in *Branchiostoma lanceolatum*

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Many rhythmic processes in physiology and behavior of vertebrates are under the control of an endogeneous pacemaker called the circadian clock. The core oscillator of the clock is composed of autoregulatory transcription/translation-based negative feedback loops that control the rhythmic expression of a limited number of clock genes (such as *Per*, *Cry*, and *Bmal* genes). In order to gain insight into the evolution of the circadian clock of vertebrates, we tried to identify clock genes in *Branchiostoma lanceolatum* (Amphioxus). The cephalochordate amphioxus is widely believed to be the living invertebrate most closely related to vertebrates. Using RT-PCR we cloned partial sequences of putative amphioxus clock genes that show significant homolgy to *Per* and *Cry* genes, respectively, from other vertebrate species. Using in-situ hybridization experiments with digoxigenin-labelled oligoprobes we investigated the expression pattern of the amphioxus *Per* homolog in cryostate sections. We identified a circumscribed cluster of cells in the anterior part of the neural tube rostrally of Hatchek's pit expressing the putative amphioxus *Per* gene. This is the first study to present data on the circadian system of amphioxus.

POSTER 83

VE-cadherin expression is regulated by glucocorticoids in blood brain-barrier endothelial cells

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The demonstration that glucocorticoid-mediated activation can control occludin expression in brain capillary endothelial cells has provided a new avenue of research in the field of blood brain-barrier permeability regulation. In order to identify more key genes involved in glucocorticoid-mediated regulation of BBB permeability, we used cDNA microarrays to study changes in gene expression in dexamethasone-treated cEND brain capillary endothelial cells: Primarily, we observed changes in expression of the VE-cadherin gene involved in cell adhesion. Vascular-endothelial-cadherin (VE-cadherin) is an endothelial cell-specific adhesion protein localized in cell-cell contacts. It is known as an important determinant of vascular architecture and endothelial cell survival. Quantitative real-time polymerase chain reaction showed an up-regulation of VE-cadherin expression exclusively in cEND cells, in accordance to previous observations made for the occludin gene. Subsequently, we verified divergent transcriptional activation of the VE-cadherin gene by dexamethasone. Furthermore, we measured the change in protein levels of VE-cadherin in the cEND cell line. Additionally, we demonstrated an transactivation of the VE-cadherin promoter in cENDs via dexamethasone.
To find cell- or tissue-specific ligands that could be used in therapeutic regime of barrier disorder diseases it is very necessary to study and understand the molecular mechanisms of the beneficial effects of glucocorticoid action on BBB-genes involved in BBB-permeability regulation.

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POSTER 84

Influence of glucocorticoids on the tight junction protein occludin

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The 65kDa protein occludin is an essential element of the blood-brain-barrier. This integral membrane protein is an important part of the tight junctions, which are responsible for the increased resistance and decreased permeability between capillary cells in the brain.
In former projects we could already show that treatment of cerebral endothelial cells with glucocorticoids results in an increased expression of occludin in cell-cell-contacts. Our study aims to identify the molecular mechanisms of this glucocorticoid mediated protein induction and the sequences responsible for the genomic regulation of occludin, the hormone responsive elements. We could find one distal and one proximal putative degenerate fullsite glucocorticoid responsive element (GRE) within the occludin promoter as well as several halfsites. In order to investigate the functionality of these putative binding sites we performed a promoter reporter assay using a 1863 bp promoter fragment with both elements and a 833 bp fragment containing only the proximal element in front of the luciferase gene. This revealed a higher transactivation level after transfection with the distal part of the promoter. To verify and concretize the results we proceeded with a chromatin immunoprecipitation assay and the side directed mutagenesis of the distal putative element.

POSTER 85

Voluntary exercise combined with Schwann cell transplantation promotes peripheral nerve regeneration after sciatic nerve transaction

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Transplantation of Schwann cells (SC) as part of nerve guidance channels sutured between cut peripheral nerve stumps are well known to help regeneration across long gaps. Recently, we demonstrated that grafting of genetically modified SC over-expressing isoforms of fibroblast growth factor-2 promote regeneration of high numbers of myelinated axons (MA) and positively influence functional recovery. It is also known that voluntary wheel running increases axonal regeneration after peripheral nerve crush injury. Here we investigated the combined effects of cell transplantation and voluntary running exercise post transplantation on nerve regeneration and motor recovery. Silicone tubes were used to bridge a 10 mm gap in adult rat sciatic nerves, and 3 days later individual animals were randomly exposed to cages with or without a running wheel.

1st set of experiments: tubes were filled with Matrigel alone or non-transfected SC resuspended in Matrigel. Analysis of the sciatic function index (SFI) demonstrated a significant improvement of motor performance in SC transplanted rats after 7 days of exercise. In correlation to this functional finding, exercise elevated mRNA levels of GAP-43 and Synapsin I in the lumbar spinal cord of SC transplanted rats, suggesting that exercise enhances implant-promoted axonal regeneration.

2nd set of experiments: rats received non-transfected SC, SC transfected with an empty vector and SC over-expressing FGF-2 isoforms. 4 weeks of periodical analysis of the SFI after transplantation demonstrated a significant improved motor performance in exercising animals. However, time pattern and grade of motor recovery due to exercise differed between the groups. Morphometrical analysis of numbers of MA in the regenerated nerve tissue revealed significant more regenerated MA after somatic gene transfer of high molecular weight as well as 18kDa-FGF-2 in exercising animals as compared to sedentary rats.

Exercise during rehabilitation after peripheral nerve reconstruction seems to increase beneficial effects of SC transplantation by increasing speed of axonal outgrowth and establishment of functional connections.

POSTER 86

Pharmacological inactivation of RhoA enhances peripheral nerve regeneration

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NOGO and myelin-associated glycoprotein (MAG) are expressed in the peripheral nervous system and may be involved in the limited functional recovery after peripheral nerve lesion. We demonstrate neuronal binding sites and inhibitory effects of NOGO and MAG on axonal growth by sensory neurons dissociated from adult rat dorsal root ganglia. Since the inhibitory effects of NOGO and MAG are mediated via activation of the small GTPase RhoA, we analyzed the effects of C3 transferase, an enzyme from Closteridium botulinum that blocks Rho function by ADP ribosylation of the effector domain, on peripheral axon regeneration. C3 (10 µg/ml) enhances axonal elongation and collateralization (branching) of adult DRG neurons in vitro and in the sciatic nerve lesion model in vivo. C3 lacking enzymatic activity was ineffective in both experimental paradigms. Corroborating an involvement of RhoA in peripheral axon growth, we observed that down-regulation of RhoA by silencing RNAs enhances neurite outgrowth and blocks LPA-induced growth cone collapse in pheochromocytoma (PC12) cell and adult DRG neuron cultures. Taken together, our data indicate a central role of RhoA in mediating the effects of myelin-derived inhibitors on peripheral axon regeneration. Therefore, pharmacological inactivation of RhoA represent novel therapeutic strategies to enhance peripheral nerve regeneration.

POSTER 87

NGF-mediated transcriptional regulation of *KLF10* and *KLF11*

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KLF10 (*Tieg1*) and *KLF11* (*Tieg2/3*) belong to a family of Krüppel-like transcription factors that are induced as immediate early response genes after TGF-ß treatment. Overexpression of *KLF10* is known to promote apoptosis in epithelial cells of lung, liver and pancreas, whereas *KLF11* is downregulated in human cancers, inhibits cell growth in vitro and in vivo and suppresses neoplastic transformation. The aim of this study was to investigate whether *KLF10*/*KLF11* expression is regulated via TGF-ß independent signalling pathways. PC12 cells lack TGF-ß receptor-type-II and therefore served as a suitable cell system. Following treatment with NGF and different inhibitors, PC12 cells were harvested, total mRNA or proteins were isolated and RT-PCRs and Luciferase assays were performed. The results show that in NGF treated PC12 cells *KLF10* mRNA was upregulated and *KLF11* mRNA was downregulated. Upregulation of *KLF10* could not be blocked by inhibiting known NGF/TrkA signalling pathways, including inhibition of PKA, PKB or PKC. However, NGF-induced KLF10 upregulation could be prevented following application of CaMKII inhibitor, suggesting a role of CaMKII in the NGF dependent transcriptional regulation of *KLF10* in PC12 cells. In contrast, downregulation of *KLF11* appears to be mediated through PI3-kinase as well as MEK. In summary, our data provide evidence that *KFL10* and *11* may be regulated in a TGF-ß independent manner and may be regulated by a non-canonical pathway of NGF and possibly also by other neurotrophins.
Supported by grants from the Deutsche Forschungsgemeinschaft.

POSTER 88

Assoziation von Phospho-eNOS Ser-1176 mit dem Golgi-System und den Nucleoli von Glioma-Zellen

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Die Aktivität der endothelialen NO Synthase (eNOS) wird durch posttranslationale Modifikationen, Protein-Protein-Interaktion und subzelluläre Lokalisation reguliert. So wird z.B. durch Phosphorylierung der eNOS am Ser-1176/1177/1179 (Ratte/Mensch/Rind) die Freisetzung von NO stimuliert. An Endothel-Zellen des Rinds wurde die Assoziation von Phospho-eNOS Ser-1179 mit dem Golgi-System beschrieben. Wir zeigen hier mittels konfokaler Fluoreszenzmikroskopie, daß Phospho-eNOS Ser-1176 mit dem Golgi-System von C6 Glioma-Zellen der Ratte assoziiert ist. Überraschenderweise beobachten wir im Nucleus der Glioma-Zellen mehrere Phospho-eNOS Ser-1176-positive Strukturen. Doppelfluoreszenz-Analysen mit dem Nucleolus-Marker-Protein Fibrillarin demonstrieren die selektive Assoziation von Phospho-eNOS Ser-1176 mit den Nucleoli von Glioma-Zellen. Es ist bekannt, daß in mitotischen Zellen die Nucleoli während der Prophase abgebaut und während der Telophase zurückgebildet werden. Doppelfluoreszenz-Untersuchungen an mitotischen C6 Glioma-Zellen zeigen, daß die Nucleoli und die Assoziation von Phospho-eNOS Ser-1176 während der Prophase verschwinden und in der Telophase zurückgebildet werden. Die Anwesenheit von Phospho-eNOS Ser-1176 in den Nucleoli von Interphase Glioma-Zellen deutet auf eine neue Rolle der eNOS hin.

POSTER 89

Die Phosphorylierung der eNOS in Zellen der enterischen Mukosa und des Plexus myentericus während der embryonalen Entwicklung

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Während eine Phosphorylierung der eNOS an Ser1177 zu einer Aktivitätssteigerung des Enzymes führt, kommt es bei der Phosphorylierung der eNOS an Thr495 und an Ser116 zu einer Runtermodulierung der eNOS-Aktivität. Trotz der bekannten Lokalisation der eNOS in Epithel- und Nervenzellen ist wenig über die Phosphorylierung der eNOS während der embryonalen Entwicklung in diesen unterschiedlichen Typen der Zellen bekannt. Um erste Rückschlüsse auf eine regulative Rolle der eNOS-Phosphorylierung während der embryonalen Entwicklung in Zellen der enterischen Mukosa und des Plexus myentericus zu erhalten, wurden 7 µm dicke Paraffinschnitte der Embryonalstadien E13.5, E14.5, E16.5, E18.5, E20.5 und des postnatalen Stadiums P3 der Maus auf die Lokalisation der Gesamtform der eNOS und der phosphorylierten Formen des Enzyms immunhistochemisch untersucht. In Epithelzellen der enterischen Mukosa wurde eine Lokalisation für eNOS, p-eNOS an Ser1177 und an Ser116 von E14.5 bis P3 identifiziert. In Zellen des myenterischen Plexus von E18.5 bis P3 wurde eine Immunreaktivität für eNOS und p-eNOS an Ser116 detektiert. In Zellen der enterischen Mukosa war p-eNOS an Thr495 nicht detektierbar. Aus diesen Lokalisationen kann geschlossen werden, dass die Entwicklung der Epithelzellen der enterischen Mukosa über die Phosphorylierung der eNOS an Ser1177 und an Ser116 moduliert wird, während die Entwicklung der Zellen des myenterischen Plexus über die Phosphorylierung der eNOS an Ser116 moduliert wird. Es gibt eine embryonale entwicklungsstadienspezifische und zelltypspezifische Phosphorylierung der eNOS.

POSTER 90

Bradykinin induziert ERK1/2-unabhängige Phosphorylierung der eNOS am Thr495 in Odontoblasten

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Die Aktivität der endothelialen Stickstoffmonoxidsynthase (eNOS) kann über die phosphorylierte Akt/Protein Kinase B (Akt/PKB) vermittelte eNOS Phosphorylierung am Ser1177 hochmoduliert und über eine extrazellulären signalregulierte Kinase 1/2 (ERK1/2) vermittelte Phosphorylierung am Ser116 und am Thr495 runtermoduliert werden. Die Phosphorylierungsformen der eNOS und der an ihrer Regulation beteiligten Kinasen sind bisher in Odontoblasten nach der Bradykinin (BK)-Behandlung nicht bekannt. Zur Klärung der Regulation der eNOS in Odontoblasten nach der BK-Behandlung für 1, 3, 5 und 10 Minuten, untersuchten wir die Phosphorylierung von eNOS, Akt/PKB und ERK1/2 in Odontoblasten von Rattenmolaren mittels Organbadexperimenten. Die BK-Behandlung (10^{-7} M) induzierte eine transiente Phosphorylierung der eNOS am Ser1177. Neben der Induktion der Phosphorylierung der Akt/PKB durch BK in Odontoblasten, führte die BK-Behandlung zu einer signifikanten Phosphorylierung der eNOS am Thr495 während der gesamten BK-Behandlungsperiode. In Odontoblasten bewirkte der BK-Rezeptor-2-Antagonist HOE 140 (10^{-6} M) eine signifikante Reduktion der Signalintensitäten von p-eNOS am Ser1177, am Thr495 und p-Akt/PKB. Die Ergebnisse weisen auf duale Effekte von BK auf die Aktivierung der eNOS in Odontoblasten hin: zum Einen auf eine Akt/PKB-abhängige Hochregulierung der Aktivität von eNOS durch die vorübergehende Phosphorylierung am Ser1177 und zum Anderen auf eine ERK1/2-unabhängige Runterregulierung der Aktivität von eNOS durch die Phosphorylierung der eNOS am Thr495.

POSTER 91

Immunzytochemische Studie zur Verteilung von EMMPRIN, Caveolin-1 und Matrix-Metalloproteinasen während der Zahnentwicklung

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Matrix-Metalloproteinasen spielen eine wichtige Rolle in der Biomineralisation der Zahnhartgewebe. Aus verschiedenen Untersuchungen an anderen zellulären und Organsystemen ist bekannt, daß an der Expression von MMPs das Transmembranprotein EMMPRIN (Extracellular Matrix-Metalloproteinase Inducer, CD147) beteiligt ist (Gabison et al. 2005).
Ziel dieser Untersuchungen war deshalb der immunhistochemische Nachweis von EMMPRIN sowie der Matrixmetalloproteinasen MT1-MMP (MMP-14) und MMP-2 in den Zahnanlagen neugeborener Ratten. Mittels Fluoreszenz-Doppelmarkierungen sollten mögliche Kolokalisationen zwischen EMMPRIN und Caveolin gezeigt werden.
EMMPRIN wies in Ameloblasten sowie in Zellen des inneren Schmelzepithels eine dichte und membranständige Verteilung besonders im apikalen Drittel der Zellen sowie an der basalen Kontaktfläche zu den Zellen des Stratum intermedium auf. In Odontoblasten konnte EMMPRIN erst mit Beginn der Dentinbildung nachgewiesen werden.
Besonders im Schmelzorgan zeigten EMMPRIN und die untersuchten MMPs ein ähnliches Verteilungsmuster. Das besonders in Ameloblasten gut nachweisbare Caveolin-1 war nur geringfügig mit EMMPRIN kolokalisiert.
Aus den Ergebnissen kann geschlußfolgert werden, daß EMMPRIN an der Regulation der MMPs in Ameloblasten, möglicherweise über Interaktionen mit den Zellen des Stratum intermedium, beteiligt ist. Die Funktion von Caveolin-1 in der Amelogenese bedarf weiterer Untersuchungen.

POSTER 92

Isolation und osteogenes Differenzierungspotential von mesenchymalen Stammzellen aus der Pulpa humaner Zähne

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Da mesenchymale Stammzellen die Fähigkeit besitzen sich in Nerven-, Fett-, Knorpel- oder Knochenzellen zu differenzieren, besteht die Möglichkeit, sie auch im Rahmen der zellgesteuerten Regeneration von Knochengewebe im Mund-, Kiefer- und Gesichtsbereich zu ersetzen. Ziel der Untersuchung war es, mesenchymale Stammzellen aus der Pulpa von Zähnen der ersten und zweiten Dentition zu isolieren und eine osteogene Differenzierung zu induzieren. Dazu wurde die Pulpa von exfoliierten Milchzähnen, Weisheitszahnkeimen und überzähligen Zähen der zweiten Dentition steril entnommen und nach Zerkleinerung kultiviert. Nach 10-15 Tagen waren die Zellen zu 80% konfluent, Die Isolierung der Stammzellen wurde mit Hilfe der Magnet assoziierten Zellsortierung (MACS) unter Aufreinigung der CD106-positiven Zellen durchgeführt. Anschließend wurde mit Hilfe der FACS Analyse der Anteil der CD105 und CD106 positiven Zellen in der Suspension bestimmt. Eine osteogene Differenzierung der Zellen wurde ab der 5. Passage mit einem Differenzierungsmedium, bestehend aus Dexamethason, Ascorbinsäure und Glycerolphosphat, induziert. Die bisherigen Daten zeigen, dass in allen Fällen eine Proliferation der von zellen aus Pulpagewebe nachweisbar war. Der Anteil an CD105 und CD106 positiven Zellen in der FACS-Analyse betrug durchschnittlich 57%. Nach MACS-Aufreinigung der Zellsuspension und Zugabe des Differenzierungsmediums konnte nach 3- und 6-wöchiger Inkubation eine Zunahme der alkalischen Phosphatase-Aktivität im Vergleich zur Kontrolle nachgewiesen werden.
Damit bietet sich die Möglichkeit autologe Stammzellen zu gewinnen und durch Induktion in die osteogene Differenzierungsrichtung zu erreichen.

POSTER 93

Interleukin-10 modulates pro-apoptotic effects of TNF-α in human articular chondrocytes

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In the pathogenesis of osteoarthritis pro-inflammatory cytokines such as TNF-α promote chondrocyte apoptosis and induce plenty of catabolic and pro-inflammatory mediators leading to cartilage degradation. Additionally, elevated levels of the immunoregulatory cytokine interleukin (IL)-10 have been previously demonstrated in osteoarthritic cartilage (Iannone et al., 2001). Since the particular function of IL-10 remains still enigmatic in cartilage, the aim of the present study was to analyze the interplay between TNF-α and IL-10 on chondrocytes survival *in vitro*.
Human articular chondrocytes were either stimulated with 10 ng/mL recombinant TNF-α or IL-10 alone or co-treated with 10 ng/ml IL-10 and TNF-α for 48 hours. Biochemical markers associated with apoptosis such as activities of the initiator caspases-9, and the effector caspases-3/-7, the mitochondrial apoptotic inducer Bax, and the suppressor Bcl-2 were evaluated by caspase activity assays, western blot analysis and flow cytometry. The production of IL-10 by chondrocytes was revealed by ELISA and immunofluorescence microscopy.
Chondrocytes, stimulated with 10 ng/mL TNF-α raised significantly their endogen IL-10 secretion. Despite stimulation with IL-10 alone had no significant effect on caspase-3/-7 activity in chondrocytes, co-treatment with IL-10 and TNF-α for 48 hrs inhibited significantly caspase-3/-7 activity ($p= 0,031$) compared to the non stimulated controls. The effects of these cytokines on caspase-9 activity were not significant. Additionally, a decreased Bax/Bcl-2 ratio was evident in chondrocytes which were co-stimulated with IL-10 and TNF-α as shown by western blot analysis.
The study suggests that TNF-α upregulates the IL-10 expression in chondrocytes *in vitro* and may therefore, amplificate effects of IL-10. IL-10 seems to modulate the pro-apoptotic capacity of TNF-α in human articular chondrocytes as shown by the decrease in caspase-3/-7 activity. The changed Bax/Bcl-2 ratio by IL-10 and TNF-α implicates an interplay of IL-10 and TNF-α on mitochondrial pathways.

Titel in deutscher Sprache:

Posterbeitrag:
Interleukin-10 moduliert pro-apoptotische Wirkungen von TNF-α in menschlichen Chondrozyten

POSTER 94

Resveratrol blocks IL-1β-Induced Stimulation of Caspase3 and Apoptosis in Human Articular Chondrocytes *in vitro*

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Resveratrol (*trans*-3,4´-trihydroxystilbene) is a polyphenolic phytoalexin that is present in various fruits, in the skin of red grapes, peanuts and root extracts. Recent studies have shown that resveratrol exhibits potent antioxidant properties and is able to exert anti-inflammatory and anti-catabolic properties in several cell types. The pro-inflammatory cytokine interleukin 1 β (IL-1β) plays a pivotal role in the pathogenesis of osteoarthritis in humans and animals. In this study we investigated whether resveratrol is able to block the effects of IL-1β, specifically the activation of caspase3 and subsequent induction of apoptosis in chondrocytes.

Cultures of human articular chondrocytes were pre-stimulated with 10 ng/ml IL-1β for 1, 12 and 24 h before being co-treated with IL-1β and 100 μM/ml resveratrol or the caspase inhibitor Z-DEVD-FMK for 1, 12 and 24 h respectively *in vitro*.

Resveratrol significantly increased the IL-1β-induced inhibition the expression of collagen type II and signal transduction receptor integrin β1 in a time dependent manner. Incubation of chondrocytes with IL-1β resulted in activation of the caspase 3, PARP cleavage and apoptotic cell death. The treatment of chondrocytes with IL-1β induced mitochondrial swelling in a time-dependent manner. These changes were observed as early as 1 h after treatment of cells with IL-1β. These effects were abolished through the co-treatment with resveratrol. Furthermore, co-treatment of the IL-1β–stimulated cells with the caspase inhibitor blocked apoptosis as shown by electron microscopy and Western blotting, suggesting that this process is a caspase-dependent pathway.

In summary, our results confirm that resveratrol is an effective *in vitro* inhibitor of caspase 3 and apoptosis in human chondrocytes. These findings suggest that this dietary polyphenolic compound may have future applications in the nutraceutical based therapy of human and animal OA.

POSTER 95

The expression of insulin-like growth factor I and II messenger (m) RNA in liver and extrahepatic sites is greatly enhanced in a GH-overexpressing transgenic bony fish

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Numerous lines of growth-hormone (GH) overexpressing fish have been produced. However, these have been characterized only concerning fundamental growth parameters and not on the expression of the growth-promoting hormones IGF-I and IGF-II. The GH-overexpressing fish used here were produced from crosses between a wild type female tilapia (*Oreochromis niloticus*) and a transgenic male. This line of tilapia carries a single copy of a chinook salmon (s)GH gene spliced to an ocean pout antifreeze promoter co-ligated with a carp ß-actin/lacZ reporter gene construct, integrated into the tilapia genome. The transgenics displayed an approximately 1.4-fold increase in head-tail length and an about 2.2-fold higher weight than their non-transgenic siblings. Liver and extrahepatic organs, such as gills, heart, brain, skeletal muscle, kidney, spleen, intestine and testes, of 17 months old males of transgenic (n=10) and wild-type (n=10) fish were investigated by RT-PCR for the expression of IGF-I and IGF-II mRNA. No obvious organ abnormalities were observed in any of the organs. In all organs investigated higher amounts of both IGF-I and IGF-II mRNA were present in the transgenics. The higher expression of both IGFs in the transgenics was most pronounced in gut and testes and lowest in spleen. In most organs, i.e., brain, gills, liver, muscle and testes, the expression of IGF-II mRNA was more enhanced than that of IGF-I mRNA. Thus, the permanent high expression of GH in the transgenics not only increases IGF-I but also IGF-II both in liver and extrahepatic sites. Because the increase in liver was only moderate we assume that the growth enhancement is mainly due to the increased IGF expression in extrahepatic sites. Supported by the SNF (Grant No. 111028) and by the Hartmann Müller-Stiftung for Medical Research (Grant No. 1115).

POSTER 96

Comparative study of V2 vasopressin receptor expression along the nephron in mouse, rat, and human kidney

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Arginine vasopressin (AVP) exerts diverse effects in the kidney, promoting reabsorption of water and electrolytes. The actions of AVP are mediated through V1a, V1b and V2 receptors. Activation of V2 receptor has prominent heterogenous effects on tubular epithelia, raising intracellular cAMP levels. Biosynthesis rate and phosphorylation of target ion transporters and water channels are activated hereby. In line with the high prevalence of this issue a number of studies have been performed to localize the V2 receptor mRNA and protein synthesis, however, results have been inconclusive among species and target cells. Therefore detailed analysis of the V2 receptor distribution in the kidney is important for better understanding of the urine concentrating mechanism. We have applied non-radioactive *in situ* hybridization on well preserved tissue samples to compare V2 receptor distribution along the renal tubules in adult rat, mouse, and human kidney. To identify the nephron segments we labeled consecutive sections with antibodies against segment-specific proteins. In rat, mouse, and human tissues alike, strong expression was found in medullary portions of thick ascending limbs of the loops of Henle (TAL) and in both, medullary and cortical collecting ducts. In cortical TAL V2 mRNA was barely detectable except for macula densa cells in rat which showed a prominent expression. Distal convoluted and connecting tubules were moderately expressing V2 mRNA. Immunostaining of consecutive serial sections or double labeling using antisera against Tamm-Horsfall protein, Na^+-K^+-$2Cl^-$-co-transporter type 2, Na^+-Cl^--co-transporter or to aquaporin 2 confirmed the expression patterns. We conclude, beside the known V2 receptor expression in the collecting duct, an equal or even stronger synthesis was detected in the medullary TAL. These findings indicate the prominent role of V2 receptor signaling in TAL function in all three examined species.

POSTER 97

Pathohistologie eines transgenen Herzmodells für Familiäre Hypertrophe Kardiomyopathie

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Veränderungen im herzspezifischen Troponin I (TnI) tragen mit etwa 5 % zur Gesamtinszidenz der Familiären Hypertrophen Kardiomyopathien (FHC) bei, wobei die Deletion des Lysins 183 die häufigste Mutation darstellt. Ein entsprechendes transgenes Herzmodell aus der Maus wurde makroskopisch, lichtmikroskopisch und ultrastrukturell charakterisiert.
Alle transgenen Tiere hatten ein im Verhältnis zum Körpergewicht verkleinertes Herz (trangsgen: 5,32 mg/g Körpergewicht, nicht transgen: 6,34 mg/g Körpergewicht). Auffällig waren die stark vergrößerten Atrien, die wesentlich mehr Interzellularsubstanz und hypertrophierte Herzmuskelzellen aufwiesen. Das Verhältnis der Wanddicken der linken zur rechten Herzkammer war bei den transgenen Tieren größer als bei den nicht transgenen Geschwistertieren (transgen: 3,1; nicht transgen: 2,3). Die für die FHC typischen Hypertrophiemerkmale wie eine exorbitante Zunahme der Zellgröße und eine großflächige Einlagerung von Kollagen wurde nicht beobachtet. Die ebenfalls für die FHC typische Fehlanordnung der Kardiomyozyten war nur in einer milden Form vorhanden, allerdings hatte der überwiegende Teil der Herzmuskelzellen eine eckige bis irreguläre Form und wies häufig Einbuchtungen auf. Die morphometrische Auswertung ergab für den rechten Ventrikel eine gegenüber den nicht transgenen Herzen verringerte Zellflächen (transgen: 324,0 μm^2; nicht transgen: 378,1 μm^2), für den linken Ventrikel hingegen leicht vergrößerte Zellen (transgen: 417,5 μm^2: nicht transgen: 373,6 μm^2). Ultrastrukturell zeigte sich bei den transgenen Tieren eine Häufung von Fehlanordnungen der Sarkomere, Kontraktionsbanden, die oft von angeschwollenen Mitochondrien begleitet werden, bis hin zu Kontraktionsbandnekrosen. Auffällig waren die Zunahme des Interstitiums um die Kapillaren herum und ein erhöhter Anteil kollabierter Kapillaren. Fazit: Die Deletion des Lysins 183 im herzpezifischen TNI verursacht im Mausmodell einen gegenüber der FHC im Menschen abweichenden Phänotyp.

POSTER 98

Characterization of peroxisomes in alveolar type II cells of the adult mouse and human lung.

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Peroxisomes in the lung are most abundant in alveolar type II cells, the specific cell type synthesizing and secreting the lung surfactant. To date nothing is known about peroxisomal metabolism in AECII, even though peroxisomes might exert a pivotal role in the homeostasis of surfactant lipids and in protecting the respiratory epithelium and the alveolar wall against high oxygen concentration and oxidative imbalance. In this study, we characterized the peroxisomal compartment in AECII by means of a) IHC and IF on Paraffin sections of mouse and human lung using several antibodies against marker enzymes of matrix and membrane proteins of peroxisomes, 2) cytochemical staining of catalase activity in mouse lung on the electron microscopic level with a modified alkaline DAB method, 3) specific isolation of mouse AECII with subsequent subcellular fractionation of organelles for enriched peroxisomal fractions and Western blotting, 4) primary culture of mouse AECII and characterization of peroxisomes under distinct culture conditions. As shown by IHC, IF, and EM all cell types in the mouse and human lung contain peroxisomes, however, with highest numerical abundance in AECII. Most peroxisomal marker antibodies selectively labelled AECII, even though with distinct labelling intensities. Experiments with primary AECII cultures revealed that the peroxisomal compartment is drastically down-regulated during dedifferentiation of AECII as monitored in parallel by the loss of the surfactant protein C (SP-C). Comparative Western blots of enriched peroxisomal fractions of pure AECII with relative quantitative assessment of bands revealed, that peroxisomes in AECII exhibit a distinct enzyme composition from liver peroxisomes and are selectively enriched in catalase (H_2O_2-degradation), thiolase (β-oxidation) and PMP70 (lipid transport), suggestive for a specific function of peroxisomes in the degradation of reactive oxygen species and in surfactant lipid metabolism in the lung.

POSTER 99

Autophagy is an important pathway for peroxisome degradation in higher eukaryotes

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Peroxisomes are organelles in eukaryotic cells that are involved in a variety of processes such as the metabolism of lipids and of reactive oxygen species. Their enzyme content can vary depending on the metabolic situation. For a flexible and dynamic adjustment of peroxisomal metabolism to changing metabolic situations, the turnover of these organelles has to be precisely controlled. In yeast, two distinct pathways, the macroautophagy and the microautophagy are involved depending on the degradation stimulus. In higher eukaryotes, a 15-Lipoxygenase-mediated (15-LOX) destruction of the peroxisomal membrane has been proposed as an alternative initial step in peroxisome turnover.
We have recently identified a mutated version of Pxmp2, the most abundant protein of the peroxisomal membrane that exerts a "toxic" effect on the peroxisomal compartment. A fusion protein of the Pxmp2 with the green fluorescent protein (GFP) was localized to peroxisomes when expressed at low levels. At high expression levels, however, peroxisomes could not be detected in these cells. To investigate this phenomenon in more detail, we have established a CHO cell line (BGL231) for inducible expression of the Pxmp2-GFP-fusion protein using the ecdysone-inducible gene expression system. This cell line represents the first experimental system to analyze the degradation of peroxisomes in higher eukaryotes in a synchronized fashion. In first experiments we could show, that the degradation process required initial steps in autophagosome formation. The typical peroxisome degradation process was altered in response to known inducers or inhibitors of autophagy. Inhibition of 15-LOX, however, did not affect the degradation process.

POSTER 100

Fluorescence intensity of green (EGFP) and red (DsRed) fluorescent proteins is affected by fixatives, pH and histological staining procedures.

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Fluorescent proteins such as EGFP or DsRed are established repoter molecules for analysis of promoter activities, of the subcellular localization of fusion proteins and of protein interactions. An increasing number of transgenic mice is being generated using fluorescent reporter molecules. However, after fixation and tissue processing, the fluorescence intensity is often so weak that the fluorescent reporter molecules have to be visualized by immmunohistochemistry with specific antibodies.
We have used transgenic EGFP- or DsRed-expressing cell lines to analyze conditions that affect their fluorescence intensity. Cells were subjected to various fixatives, detergents, pH conditions or histological staining solutions and fluorescence intensity was quantified by FACS (Fluorescence activated cell sorting) analysis. We could show, that fluorescence intensity of EGFP and DsRed was affected differently by pH. Treatment of cells with ethanol lead to a concentration dependent decrease in EGFP fluorescence. However, ethanol-mediated reduction of fluorescence could be partly prevented by fixation of cells with aldehydes prior to ethanol treatment. The effects of ethanol treatment were less pronounced for DsRed. Detergents did not affect fluorescence intensity whereas histological staining solutions such as haemalaun, giemsa or methylene blue abolished specific fluorescence and lead to diffuse autofluorescence instead. Our data support a combination of aldehyde fixation with differently propagated serial sections for analysis of EGFP expression in transgenic animals.

POSTER 101

abgesagt

POSTER 102

Anti-angiogenic gene transfer induces metabolic changes in rat hepatomas

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In vivo, human troponin I (TROP), the soluble receptor for vascular endothelial growth factor (sFLT) and angiostatin (ASTAT) represent potent inhibitors of angiogenesis and tumor growth. Therefore, transfer of these genes into tumors may induce changes in perfusion, but also more general ones in metabolism.
We established Morris Hepatoma (MH3924A) cell lines expressing TROP, sFLT or ASTAT and quantified ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake by dynamic positron emission tomography (PET) after tumor inoculation in ACI rats. Furthermore, expression of glucose transporter-1 and 3 (GLUT-1; GLUT-3) as well as hexokinase-1 and -2 were investigated by RT-PCR and immunohistomorphometry. In addition, gene array analyses were performed.
In vivo, the FDG uptake (SUV and Ki) was significantly higher in TROP (n = 7), sFLT (n = 6) and ASTAT (n = 7) in comparison to wild type (WT; n = 9) MH3924A. Modeling of the dynamic FDG datasets showed that the vascular fraction and distribution volume was higher in all tumors. Immunohistomorphomtry revealed an increase of percentage of hexokinase-1 and –2 as well as GLUT-1 and -3 immunoreactive cells in most genetically modified tumor groups. Using gene arrays and comparing all three groups of genetically modified tumors, we found changes in expression of several genes related to apoptosis, signal transduction / stress or metabolism / synthesis.
We conclude that TROP, sFLT or ASTAT expressing MH3924A tumors show enhanced ^{18}F-FDG uptake, mainly attributable to an enhanced vascular fraction, distribution volume and increased ^{18}F-FDG transport.

POSTER 103

The Significance of Precancerous Damage in the Development of Malignant Gastric Tumors

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The incidence of gastric cancer is extremely elevated in the western part of Romania, holding one of the top positions within the overall neoplastic pathology. The present study revealed once again the late diagnosis of cancer. The malignant tumoral pathology followed between 1995-2000, consisted of 449 cases, mainly belonging to the age groups of the fifth and seventh decade. As for the distribution of the cases according to factors of aggression on the gastric mucosa, precancerous conditions are extremely important. Atrophic chronic gastritis is nothing but the adaptive response to long-term inflammation, found in 54 cases. Intestinal metaplasia was present in 104 cases, more commonly in the antrum and along the lesser curvature. Gastric dysplasia, present in 104 cases, was associated to carcinoma, being diagnosed as severe or moderate dysplasia. Just like in the case of all precancerous damage, the basal membrane is intact. The glandular lumen sometimes contains mucin secretion material. As a consequence, the study also focused on the presence, density and composition of the inflammatory infiltrate in the lamina propria, an aspect which has not been referred to in literature.

Keywords: gastric cancer, precancerous damage, malignant gastric tumors

POSTER 104

The role of Vascular Endothelial Growth Factors A, C, D and their receptors in progression and metastasis of Barrett's adenocarcinoma

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Background & Aims: Angiogenesis, the formation of new blood vessels from preexisting vasculature, is a prerequisite for tumor growth and metastasis. VEGFR-1-mediated signalling via VEGF-A has been identified to critically influence tumor-related angiogenesis. Recently it became clear that basic mechanisms of hemangiogenesis may also apply to the lymphatic system and that VEGF-C and VEGF-D are intimately involved in the regulation of lymphangiogenic processes by activating VEGF receptor (VEGFR-) 2 and VEGFR-3. The adenocarcinoma of the distal esophagus (Barrett's carcinoma) develops from a specialized, glandular, intestinal metaplasia, the Barrett's esophagus, and follows a metaplasia-dysplasia-carcinoma sequence. We investigated neovascularization and expression of VEGF-A, -C and -D along with their receptors in these sequence and correlated the findings with clinico-pathological parameters.
Methods: VEGF-A, -C, -D, VEGFR-1, -2 and -3 were analyzed in 88 Barrett adenocarcinomas, 23 cases of Barrett's dysplasia, 43 cases of Barrett's epithelium and 38 non-cancerous esophageal squamous epithelia employing immunohistochemistry. Blood and lymph vessel densities were assessed after staining with CD31- and LYVE-1-specific antibodies.
Results: VEGF-A- and VEGF-D-expression on epithelial cells significantly increased in Barrett's dysplasia, whereas VEGF-C-expression on epithelial cells stayed constant in the course of the metaplasia-dysplasia-carcinoma sequence. VEGFR-2-positive vessels were predominant in Barrett's dysplasia, whereas VEGFR-3-positive vessels significantly increased in Barrett's dysplasia and adenocarcinoma. Presence of VEGF-D in relapse of Barrett's adenocarcinoma was correlated with non-curative resection (R1).
Conclusions: The increase of VEGF-A-expressing epithelial cells and VEGFR-2-expressing vessels in Barrett's dysplasia indicates that an angiogenic switch (between avascular and vascular growth of neoplastic cells) may exist, but also that it occurs late in the metaplasia-dysplasia-carcinoma sequence. VEGF-D on the other hand may stimulate lymphangiogenesis by activating VEGFR-3 in Barrett's dysplasia. This indicates that angiogenesis and lymphangiogenesis are possibly induced simultaneously.

POSTER 105

Morphological comparison of IPEC-1 and IPEC-J2 cells as model to study the barrier function of the intestinal epithelium.

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The intestinal mucosa is continuously exposed to a wide variety of luminal antigens, i.e. food, microbial and environmental antigens. Therefore the intestinal mucosal immune system must be capable to discriminate between harmless and dangerous antigens as well as respond to those antigens appropriately without damaging intestinal tissue. The integrity of the intestinal epithelial cell barrier is important for the entry of antigens from the intestinal lumen to the underlying lymphoid structures.
The aim of this work was the comparison of the morphological characteristics of two porcine intestinal epithelial cell lines IPEC-1 and IPEC-J2. Both cell lines were analysed for their surface molecule expression by flow cytometry. Furthermore the cells were tested for the expression of the intermediate filament protein cytokeratin 18.
IPEC-1 cells were negative for CD45, MHC class I, MHC class II, CD80/86, CD1, CD14 and cytokeratin 18, whereas IPEC-J2 cells were MHC class I positive and a marginal upregulation of CD1 and CD14 compared to PBMC was determined. Immunhistocytochemic staining revealed that undifferentiated as well as differentiated cells of both cell lines highly express cytokeratin 18. Both cell lines were also screened by routine histological procedures.
Current morphological and functional analyses focus on the establishment of an in vitro co-culture systems of intestinal epithelial cells with cells of the gut associated lymphoid tissue to study their cross-talk and their influence on the barrier function of the intestinal epithelium.

POSTER 106

Inselzellentwicklung des fetalen humanen Pankreas

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Typ 1- und Typ 2-Diabetes sind mit einem signifikanten Verlust insulin-sezernierender Beta-Zellen assoziiert. Der Nachschub von körpereigenen Vorläuferzellen stellt daher für eine zukünftigeTherapie des Diabetes eine vielversprechende Option dar. Grundlage hierfür ist die Kenntnis der Beta-Zell-Entwicklung und deren Differenzierung. Ziel der vorliegenden Untersuchung war es, den Verlauf der Inselzellentwicklung an fetalem humanem Pankreas zu verfolgen.
Insgesamt wurden 42 Pankreasproben von Feten (16.-38.Schwangerschaftswoche, SSW) und zwei Proben von Erwachsenen als Vergleich für die Untersuchungen verwendet. Bei keinem der untersuchten Fälle war anamnestisch eine Stoffwechselerkrankung bekannt. Paraffinschnitte wurden mit Hilfe immunhistochemischer (IHC) Färbungen für Insulin, Glukagon, Ki67 (Replikation) und aktivierte Kaspase 3 (Apoptose) angefärbt. Das Verteilungsmuster der Zellen wurde lichtmikroskopisch semiquantitativ ausgewertet.
In der 16.-20.SSW konnte mittels IHC- Doppelfärbung von Insulin und Glucagon ein gemeinsames Auftreten von Alpha- und Beta- Zellen in Form eines Inselzellkomplexes gezeigt werden. Die Beta-Zellen lagen zentral und wurden ringförmig von Alpha-Zellen umgeben. In der 20. SSW kam es zu einer Umstrukturierung dahingehend, dass die Alpha-Zellen in die Peripherie abwandern und ab jetzt unregelmäßig nebeneinander liegen. Von der 16.-25.SSW dominierten neben den Inselzellkomplexen ubiquitäre vereinzelte Beta-Zellen das Erscheinungsbild. Ab der 26.SSW war eine deutliche Größenzunahme des Inselkomplexes erkennbar, während die vereinzelt gelegenen Beta-Zellen im Verhältnis abnahmen. Darüber hinaus war zwischen der 16.-25.SSW eine extrem hohe Proliferationstätigkeit (KI67) der Beta-Zellen nachzuweisen, die ab der 26.SSW deutlich abnahm. Aktivierte Kaspase 3 konnte in allen untersuchten Stadien in geringgradiger Anzahl nachgewiesen werden.
Das endokrine Pankreas unterliegt in der Fetalperiode mehreren Umbauprozessen, in denen es sich von einem geordneten Inselzellkomplex in ein ungeordnetes Zellbild wandelt. In diesen praenatalen Umbauphasen findet eine vermehrte Zellproliferation statt, die nicht von Apoptosen begleitet wird. Am Ende der Praenatalperiode gleicht das Pankreas dem des adulten Menschen. Um sich der Regeneration der Beta-Zellen bei Patienten mit Diabetes weiter anzunähern, ist in weiterer Folge die Klärung der Interaktion zwischen den fetalen Alpha- und Beta-Zellen notwendig.

POSTER 107

The role of Wnt11 in dermis development

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The Wnts are a large family of secreted proteins that regulate a variety of developmental processes in vertebrates and invertebrates. The Wnt11 gene encodes a protein composed of 354 aa. It plays a central role in tissue morphogenesis during vertebrate gastrulation. It has also been described to stimulate proliferation, migration, cytoskeletal rearrangement and contact independent growth by a beta-catenin independent mechanism. In the chick embryo starting from stage HH19, the expression of *Wnt11* is restricted to the dermomytome and from stage HH24 *Wnt11* is expressed in the subectodermal mesenchyme of the limb and feather buds. This unique expression pattern of *Wnt11* in the paraxial mesoderm and dermomyotome suggests that it may play a role in dermis development. We are interested in elucidating the role of Wnt11 in dermis development and for this reason we constructed a shRNA construct coupled with a EGFP sequence. The *Wnt11* shRNA-EGFP construct was injected into the somites of stage HH16-17 embryos and subsequently electroporated. After 24 hrs of reincubation, the embryos were analyzed in ovo under fluorescence to detect the transfected region indicated by EGFP. The transfected embryos were submitted for in situ hybridization using *Wnt11* specific RNA probes. We noticed that the transfection site or GFP fluorescent site co-related with the silencing seen in the in situ samples. Further, we analyzed what happens with the expression of other markers involved in dermis and muscle development when *Wnt11* is silenced. In situ hybridisation for *c-Dermo1* and myogenic markers like *MyoD* and *Myf5* were downregulated following RNAi targeting of *Wnt11*. We now work further to analyze the effects of the silencing on other genes that are active in dermis development.

POSTER 107A

Maintenance of *Hox* gene expression does not depend on the size of isolated cell groups

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Previous studies have shown that presomitic mesoderm is already determined with respect to the segment specific morphological pattern. The genetic information for this segmental identity is coded in the *Hox* genes. In the hindbrain and cranial neural crest, *Hox* expression has been shown to be modulated by cell community effects. It is unknown whether the *Hox* expression in the paraxial mesoderm is stable or does also depend on the number of cells. In this study we grafted somitic cell groups of different size from thoracic to cervical segments. After transplantation of the lateral compartment of one somite, ectopic rib formation can be observed in the cervical region. Thus, the transplant is able to interpret extrinsic signals in the new cervical environment correctly, but is irreversibly committed to form ectopic structures according to its original position. When a small cell group isolated from the somite core was grafted, they maintain their original *Hox*-expression. Moreover, we also show these small part of somitocoel cells grafted from a thoracic somite into the wall of the cervical neural tube also maintain their *Hox*-expression. Our data shows that the maintenance of *Hox* gene expression in the somite does not rely on the cell number.

POSTER 108

Transcription of ADAM13 during chicken embryonic development

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ADAMs (a disintegrin and metalloprotease) are transmembrane proteins that have a disintegrin and a metalloprotease domain as well as multifunctional regions. ADAM proteins mediate adhesive cell-cell or cell-matrix interactions and proteolysis. They are involved in morphogenesis and tissue formation during embryonic development. In the present study, we investigated the expression of ADAMs during chicken embryonic development. Our results show that at least one member of the ADAM family, ADAM13, is expressed in a temporospatially regulated pattern in cranial neural crest-derived structures, such as the head mesenchyme and the craniofacial skeleton, the branchial arches, and the meninges surrounding the brain. Furthermore, ADAM13 is also transcribed in structures derived from the trunk mesoderm, such as the somites and derived muscles, the meninges surrounding the spinal cord, the dorsal aorta, the developing kidney and several digestive organs. Our data suggest that ADAM13 plays a role in morphogenesis and tissue formation during chicken embryonic development.

POSTER 109

Detection of Hypoxia and HIF-1α in the developing chick embryo

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The term hypoxia describes the deficiency in the normal oxygen supply to cells resulting either from an environmental reduction in oxygen delivery such as high altitude or serious ischemia due to reduction of blood flow to a given tissue. Cells undergo a number of biological responses when placed in hypoxic condition, associating with their signalling pathways that regulate proliferation, angiogenesis and apoptosis. Hypoxia inducible factor 1 alpha (HIF-1α), a bHLH transcription factor is a master regulator in hypoxic conditions and regulates hypoxia-associated homeostatic processes. Physiological hypoxia is also found to occur during normal embryonic development. In this study, we detail the presence of hypoxia in normal developing embryos by using a hypoxia marker, pimonidazole, and its related antibody. Furthermore, we also analysed the expression pattern of HIF-1 α at mRNA and protein level using RNA probes and specific antibodies, respectively.
Hypoxia marker labelled region of high proliferation in developing embryos. In this regard, we also found that hypoxia inducible factor (HIF 1α) and proliferative cell nuclear antigen (PCNA) were co-localized with possible hypoxic regions in embryos. Thus we suggest that proliferation may be one of the inducing factors of hypoxia. Hypoxia in turns could be one of the major driving force of morphogensis.

POSTER 110

Hypoacetylation Impacts the Gene Expression Patterns during Chick Limb Development

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Acetylation and deacetylation represent key modifications in the control of gene transcription during embryonic development and tumorigenesis. However, it is not fully clear whether the hypoacetylation affects the spatiotemporal expression patterns of developmental genes implicated in embryonic limb development, and which genes are more sensitive to such modification *in vivo*. Taking the chick limb bud as an experimental model, we have investigated the reaction of a batch of genes in the limbs treated with Trichostatin A (TSA), a histone deacetylase (HDAC)-inhibitor. The results show that TSA changes the expression levels of some genes, which have important functions during limb development. Among them, *BMP4, SF/HGF* and *Twist1* were up-regulated; *BMP2, FGF8, Shh, Scleraxis, Myf5* and *MyoD* were down-regulated. In contrast to that, the *Pax3*, *Paraxis, Msx1*, *CREB,* and *PCNA* were still expressed at the same levels as controls. Our results indicate that chick limb development can serve as a convenient *in vivo* model for studying the epigenetic regulation on gene expression. It may be useful for improving our understanding of the role of chromatin remodelling and epigenetic control of gene expression patterns during embryonic development and gene control.

POSTER 111

Asymmetrical expression patterns of genes involved in the pregastrulation development in the rabbit

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Anterior-posterior axial differentiation of the mammalian embryo is apparent first immediately prior to gastrulation by the expression patterns of several genes involved in the development of the body plan: At least signalling molecules such as *Dickkopf1(Dkk1)*, *Cerberus related 1(Cer1)* and *lefty1* and transcription factors such as *Hex* play a role in this process. To answer the question if these genes are involved in the establishing left-right asymmetry as well, in-*situ* hybridisation experiments were carried out in late pregastrulation rabbit embryos to reveal complex topographical expression patterns to indicate an assumed area of highest effectivity. While all genes examined show anterior-posterior asymmetry, *Dkk1* and *Cer1* show additionally lefty-right asymmetrical expression patterns in the early rabbit embryo. The *Dkk1* expression domain forms a “clasp-like” shape which lies along the anterior margin but has differently shaped end regions on the left and the right side. *Cer1* is described to show a mushroom-like patterning with two differently shaped “shoulders” on either side. Because further investigations showed no preference of this asymmetrical distribution for one body side, the idea of an oscillating gene expression as described for the cyclic development in somite formation was analysed: Embryonic discs were longitudinally cut into two equal halves and selectively cultured in a “New type culture” to examine a possible role of cyclic *Dkk1* expression for establishing laterality prior to gastrulation.

POSTER 112

Pax8 is essential for uterine development

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Absence of the Pax8 gene results in congenital hypothyroidism in mice and mutations of the Pax8 gene have been associated with thyroid hypoplasia in humans. As in humans, treatment of congenital hypothyroid Pax8 null mice with thyroxine normalizes the known deficits. However, we report here that thyroxine-substituted female $Pax8^{-/-}$ mice are infertile since they lack a functional uterus revealing only remnants of myometrial tissue. In addition, the vaginal opening is absent. Interestingly, oviduct, cervix and upper parts of the vagina are not affected, although Pax8 expression has been described in the entire Müllerian duct before. Since the natural outflow of the oviduct is impaired, a hydrosalpinx develops frequently. Folliculogenesis, ovarian hormone production, and transcription of pituitary hormones are in a normal range. Thus, infertility in $Pax8^{-/-}$ mice is rather due to a defect in development of the Müllerian duct than to hormonal imbalance, pointing to a direct morphogenic role for Pax8 in uterine development. Since we demonstrated Pax8 expression not only in the uterine epithelium of mice but also in the human endometrium, it remains to be elucidated whether adequate development of the uterus may also be affected in congenital hypothyroid female patients with mutations in the Pax8 gene.

POSTER 113

Growth characteristics and angiogenesis of human endometrium after subcutaneous and intraperitoneal implantation in Rag-1-knockout and nude mice

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Endometriosis is a common gynaecological disease characterized by the presence of ectopic endometrial tissue outside the uterine cavity causing pain and subfertility. Little is known about the cell biological mechanisms of its pathogenesis, and current therapeutical treatments are associated with high recurrency rates. Because endometriosis occurs only in humans and some non-human primates, there is a need to develop animal models to develop new therapeutic approaches. We established an in-vivo model by transplanting premenopausal human endometrial tissue into recombinase-activating gene (RAG)-1 deficient mice revealing a severe immunodeficiency by lacking functional B- and T-lymphocytes. Growth characteristics of and angiogenesis in those endometrial fragments was analysed for up to 8 weeks in regard to the site of subcutaneous and intraperitoneal implantation, respectively. These results were compared to the established nude mouse model. In both types of mice, more than 80% of the human tissue fragments could be recovered after 3 weeks, and more than 50% after 8 weeks, independent from the site of implantation. However, size of lesions decreased after 4 weeks of culture. We could show that concerning intraperitoneal fragments this in part is due to an integration of the human endometrial tissue into the abdominal wall of the host. Microscopic evaluation revealed a well preserved morphology as well as the establishment of a blood supply in subcutaneous as well as intraperitoneal human endometrial lesions up to 8 weeks of culture in both types of immunodeficient mice with an increase in dilatation of glands with increasing time after implantation. We here show that in regard to morphological preservation and angiogenesis there are no obvious differences between subcutaneous and intraperitoneal growing human endometrial tissue nor between culture in Rag-1-KO-mice compared to commonly used nude mice.

POSTER 114

Modellversuch der Gebärmutterhalspseudoerosien

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Wir haben Wirkung der überschißenden Menge der Hormonalstoffe auf die strukturelle Bestandteile der Gebärmutterschleimhaut von den Kaninnchweibchen untersuht, damit das Versuchmodell der Pseudoerosien zu konstruieren. Die serienweise verfertigten histologische Schnitte wurden nach den klassische Methoden von Van-Gison, Hämotoxilin-Eosin und mit dem Mukarzin nach Meier gefärbt. Die halbdünne und ultradünne Schnitte wurden extra mit der Hilfe der electronen Mikroskop verfertigt, nachdem wurde die morphometrische Analyse der erhaltenen Material mit der weiteren statistischen Auswertung durcheführt. Die Ergebnisse der durchgeführten Untersuchungen weisen darauf, daß der Epithelgwebe seine Kennzeichnungsmerkmale verliebt. Bei der morphologischen Untersuchungen wurde eine intensive Kernvolumenvergrößerung konstatiert, sie beträgt durchschmittlich bis 108 mkm^3 (kontrollgruppe 31 mkm^3) und bei der Zytoplasma bis 236 mkm^3 (in der kontrollgruppe - 48 mkm^3). Besonders deutlich wurden diese Veränderungen während des gemischten Eisatzes der Hormonen, die eine gleichgelfende Wirkung haben, bestimt. Auf solcher Weise, sind die Zellendyskompensation und Zellendesorganisation der Epithelschicht besondere Kernzeichen, die bei der Hormonalhomöostasestörungen der Gebärmutterhalsschleimhaut entstehen, die strukturelle Veränderungen sind aber im höherem Grad dem erosiven Prozeß sehr ähnlich.

POSTER 115

Blastocyst-mediated induction of early response genes in the receptive endometrium

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The successful implantation of an embryo is dependent on the cellular and molecular dialogue between uterus and the embryo. Defects in this interaction may lead to failure of implantation. Although to date many specific factors have been identified during this critical period, the molecular mechanism of embryo implantation is still unknown. To evaluate early genes regulated prior and during the implantation reaction in the receptive endometrium by the blastocyst, tubal ligation was carried out unilaterally in rats at least two weeks before mating. On 4 dpc, of each animal single oligonucleotide microarray analyses were performed of the isolated endometrium of one uterine horn containing blastocysts and of that of the contralateral uterine horn without contact to blastocysts. Data obtained by microarray analysis were verified using quantitative RT-PCR. We demonstrated that the nuclear receptors Nr4a1 (Nur77) and Nr4a3 (Nor1) as well as the transcription factors aft3 and msg1 were significantly upregulated in the receptive endometrium only in the presence of blastocysts. These transcription factors are early response genes and could be at the beginning of a signal cascade in the receptive endometrium leading to successful implantation.

POSTER 116

Influence of in-vitro culture of bovine embryos on the structure of the zona pellucida

Der Einfluß der in-vitro Kultur boviner Embryone auf die Struktur der Zona Pellucida

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The zona pellucida is an extracellular structure at the direct interface between maternal and embryonic side which all signals of the embryo maternal dialogue as well as nutritional factors have to pass. Up to now it has not been investigated whether the in-vitro culture is influencing the structure of the zona pellucida compared to in-vivo embryos. Therefore, in-vitro (oocyte, zygote, 2-, 4-, 8-, 16-cell, morula, blastocyst) and in-vivo (zygote, 4-cell, morula, blastocyst) embryos have been prepared for microscopical investigation. Araldit embedded embryos have been sectioned semi thin and stained with haematoxilin. A morphometrical evaluation was performed to determine the percentage of the more reticulare outer part compared to the total thickness of the zona. Furthermore the total thickness of the zona pellucida has been compared between in vitro and in vivo embryos. In parallel embryos have been by scanning electron microscopy. Up to the 16-cell stage the zona of in-vivo and in-vitro embryos is similar, but in vivo morulae as well as blastocysts show a significant thicker zona than in vitro. The reticular part of the zona is thicker in in-vivo embryos (30.2$\pm$2.1% vs 12.4$\pm$1.8%). Investigating the pores of the zona pellucida in vivo morulae/blastocysts show smaller sized than in vitro. Most of the in vivo morulae/blastocysts are totally covered by secret granules, wherefore the pores could not be investigated. Furthermore, 30-50% of in vitro embryos show partly degenerated outer layers of the zona pellucida. This investigation demonstrates, that in-vitro and in-vivo zonae pellucidae are significantly different which may negatively influence embryonic development.

POSTER 117

CYR61 (CCN1) and NOV (CCN3), two novel angiogenic and migratory regulators in the human placenta: Implications in preeclampsia.

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One of the most favoured hypotheses is that preeclampsia is caused by shallow invasion of the extravillous trophoblast leading to uteroplacental insufficiency and hypoxia. In this study we have focused on CYR61 (CCN1) and NOV (CCN3), members of the CCN family, which are discussed as key players in angiogenesis and migration, processes which are affected in this disorder. CYR61 and NOV mRNA and protein expression was investigated in human placenta during normal pregnancy compared to early- and late-onset preeclamptic placentae. During normal pregnancy, both CCN members demonstrated elevated expression levels and were strongly coexpressed in endothelial cells of vessels, stromal cells and in non-proliferating interstitial extravillous trophoblast (EVT) giant cells. However, NOV showed an earlier onset of expression in villous endothelial cells during gestation compared with CYR61, which may signify distinct roles of these proteins in placental angiogenesis. No obvious differences in the localization of CYR61 and NOV in preeclamptic placentae were detected but a change in the intracellular distribution in trophoblast giant cells. Interestingly, we found decreased levels of CYR61 and NOV in early-onset but not in late-onset preeclamptic placentae compared to matched controls. First analyses of serum samples of preeclamptic patients pointed to a downregulated expression of both molecules in serum and therefore they might be useful as attractive prognostic clinical markers. It is well documented that trophoblast invasion is regulated by oxygen. Using trophoblast cell lines, studies on the regulatory mechanisms of both CCN molecules and their physiological consequences for proliferation or invasion under hypoxia are in progress. In conclusion, our data show that CYR61 and NOV are involved in the pathogenesis of preeclampsia through their effect on placental angiogenesis and extravillous trophoblast migration.

POSTER 118

Korrelation von Invasivität und Integrinexpression in Chorionkarzinomzellen

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Im Rahmen der Invasion des Trophoblasten spielen Integrine eine große Rolle (Damsky et al, 1994). Einerseits bieten sie den Zellen ein Substrat zur Verankerung im Gewebe, andererseits wird über die differentielle Expression in Abhängigkeit von der Position der Trophoblastzellen in den Zellsäulen der Haftzotten die Invasivität des Trophoblasten reguliert. In einer früheren Arbeit war gezeigt worden, dass die Invasivität von malignen Trophoblastzellen (Chorionkarzinomzellen) durch die Modulation der Differenzierung je nach verwendetem Agens reduziert oder gar gesteigert werden kann. In der vorliegenden Studie wurde überprüft, ob mit den gleichen Agenzien die Expression von Integrinen in JAr-Chorionkarzinomzellen moduliert werden kann und ob die Korrelation mit Veränderungen der Invasivität bestimmte Regeln erkennen lässt. Die Zellen wurden behandelt mit Phorbol-12-myristoyl-13-acetyl-diester (PMA), Retinsäure (RA), Methotrexat (Mtx) oder Dibutyryl-cAMP (dbcAMP). Die Invasion wurde mit Gelen aus rekonstituierten Gelen aus Matrigel in einem Boyden-Kammer-System erfasst. Die Expression der Integrin-Untereinheiten □5, □6, □v, □1 und □4 wurde per Northern und Western Blot untersucht.

Die Behandlung der Zellen mit PMA steigerte die Invasivität auf das Fünffache, hatte aber keinen Einfluss auf die Expression der untersuchten Integrine. RA steigerte die Invasivität auf das ca. Zweifache. Dabei wurde die Expression von □1 deutlich und die von □4 schwach reduziert, die von □6 nahm leicht zu. Unter Mtx wurde die Invasivität drastisch gegen null reduziert und die Expression von □1 und □4 deutlich gesenkt. dbcAMP senkt die Invasivität um ca. 50%, ähnlich klar die Expression von □1 und □4. Dabei wurde die Expression von □5 signifikant gesteigert. Es ergab sich also keine klare direkte Korrelation zwischen Invasivität und Integrinexpression. Man muss eher davon ausgehen, dass Invasion und Integrin-Expression unabhängig von einander reguliert werden und die verschiedenen Agenzien beide Prozesse unterschiedlich beeinflussen.

POSTER 119

Exposure to dioxin and hypoxia affects estrogen-mediated cellular functions in MCF-7 cells

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Lipophilic environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), known as promoter of tumorigenesis, accumulate in mammary gland tissue. Tumor growth leads to hypoxic stress by high proliferation rates and insufficient vascularization. Because TCDD interferes with estrogen signalling in steroid hormone sensitive cells, we studied potential competitive effects of TCDD, hypoxia and estrogen in a well established tumor model, the mammary cancer cell line MCF-7. Cell viability, cell cycle progression, cell migration and anchorage-independent growth were examined. Our data show that TCDD treatment acted anti-estrogenic on cell cycle progression and anchorage-independent growth. AhR activation by TCDD promoted motility of MCF-7 cells independently of the ER pathway. Additional hypoxic conditions abolished the TCDD effect on cell migration. Hypoxic cultured cells did not exhibit higher motility. ERα protein amount was reduced by TCDD or hypoxia. Simultaneous exposure diminished this decrease and could be correlated with an increase in ERE-mediated reporter gene activity. Taken together our results demonstrate that the functional behaviour of MCF-7 cells is clearly different following simultaneous treatment with TCDD and hypoxia if compared with treatment of each stressor alone. Notably, the TCDD effect on ERE-mediated gene expression depends on the oxygenation state of the cells and can be estrogenic and anti-estrogenic. The divergent reaction of MCF-7 cell to TCDD due to their oxygenation state might be an important reason for the heterogeneity in TCDD effects observed in tumor cells.
This work was supported by DFG GK 416/3 and the Wilhelm-Roux-Programme of MLU.

POSTER 120

Comparative evaluation of the three flavonoids Epigallocatechingallate, Quercetin, Rutin and their effects on human (prostate, cervix and breast) cancer cell lines LNCaP, PC-3, HelaS3, MCF-7.

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The flavonoids epigallocatechingallate, quercetin, rutin are known *inter alia* as antioxidants common in green tea and apples. It was recently shown, that they may have significant growth inhibiting effects on human cancer cell lines and in tumor xenografts in different animals like mice and rats.
The primary catechine in green tea is the flavanol (-)-epigallocatechin-3- gallate (EGCG) — a polyphenolic compound with a flavonoid structure. Quercetin is a flavonoid that forms the “backbone” for many other flavonoids, including the citrus flavonoid rutin. Quercetin and rutin are flavonols.
EGCG (50µM) inhibited cell proliferation significantly after 24 hours of all mentioned cell lines. Furthermore EGCG reduced tumor weight in PC-346C / PC-3 xenografts significantly after 6 weeks. Cell cycle analysis showed a significant S-phase arrest in LNCaP cells and a significant G2-phase arrest in PC-3 cells. Apoptotic cells were found in LNCaP but not in PC-3 cells. Similar data could be observed for quercetin using four human cancer cell lines. In spite of chemical relation to EGCG and quercetin, rutin showed no effects on cancer cell lines.
We are now engaged to elucidate the mechanistic differences by testing quercetin and rutin in cell cycle and apoptosis trials. This and further studies may validate the use of the three flavonoids as mild and natural agents for cancer prevention or even treatment.

POSTER 121

Glucocorticoids induce androgenic activity in prostate (cancer cells).

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Prostate cancer (PC) is the most common type of cancer found in men in Germany. It was recently shown, that glucocorticoids may interfere the conventional therapy, especially the chemotherapy of PC and other cancers.
Early stage PC is mostly androgen-dependent and therefore we analyzed the effects of glucocorticoids on androgen-receptor (AR) regulation in prostate and AR-RBA of currently applied glucocorticoids.
Furthermore, impact of glucocorticoids and pregnenolone on proliferation and PSA-secretion of LNCaP cells were evaluated. Finally, LNCaP xenografts in nude mice were performed to monitor the %-change of the tumor volumes between orchiectomized / vehicle-treated and orchiectomized / hormone-treated animals. Validation of AR regulation (prostate) in a modified Hershberger Assay was accomplished previously.
Preliminary data indicate a significant impact of glucocorticoids on binding to the AR in cell culture and a faint dose-independent indication of stimulation of established androgen-dependent parameters in the Hershberger Assay.
To draw a conclusion, it will be necessary to enforce further studies on cancer-related endocrine regulation to avoid contraindications and multiple or unmanageable intricacies in regard to therapy. Finally, these data may also explain the use of glucocorticoids as doping drugs in competitive sport.

Poster 122

(Anti-)androgen induced testosterone serum levels and androgen receptor (AR) expression in skeletal muscle and prostate in mice, rats and cynomolgus monkeys.

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The goal of this study is to elucidate the effect of androgens and androgen receptor antagonists on the effects of circulating testosterone serum levels and the correlation to the expression of the AR in skeletal muscles and prostate in mice, rats and cynomolgus monkeys. For the analysis of the expression of the ARs, immunohistochemical procedures were used (anti-AR rabbit polyclonal, Santa Cruz Biotechnology and anti-AR rabbit monoclonal, BD Pharmingen). The AR antagonists were applied in a modified Hershberger Assay, in which the animals were treated over two weeks subcutaneously. The inhibition of cyproterone acetate (3 mg) was 60%, casodex (1 mg) inhibited in the range of 40% and flutamide (1 mg) inhibited in the range of 38%. In rats the effects of the AR antagonists on circulating testosterone levels were as follows: in intact control animals, we analyzed a circulating testosterone level in the range of 4 ng/ml; in castrated animals the circulating level was not detectable, whereas flutamide induced a testosterone increase in the range of 37 ng/ml and finally casodex had no effect on testosterone levels. In monkeys we analyzed circulating testosterone levels from date zero up to day 11 of treatment: flutamide increased the circulating testosterone levels from 5 up to 15 nM/ml, whereas casodex had almost no effect on testosterone levels in monkeys. The testosterone serum levels were now correlated with the histo-chemical expression of the AR in skeletal muscle and prostate: it turned out, that the AR was down-regulated after orchiectomy, whereas a compound-specific expression pattern was detected for the skeletal muscle and prostate depending on the induced testosterone serum levels.

Poster 123

Abgesagt

POSTER 124

Peroxisomes in the human and mouse testis

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The vital importance of peroxisomal metabolism for regular function of the testis is stressed by the severe spermatogenesis defects induced in the absence of functional peroxisomes due to biogenesis defects of this organelle. However, only sparse information is available on the role and enzyme composition of this organelle in distinct cell types of the testis. In the present study, we characterized the peroxisomal compartment in testicular somatic and germ cells by 1) immunofluorescence stainings on Paraffin sections of human and mouse testis, 2) comparative analyses of expression levels of peroxisomal proteins by Western blotting of enriched peroxisomal fractions from isolated murine Leydig-, peritubular myoid- and Sertoli cells in primary culture, 3) comparative RT-PCR. Finally, a modified alkaline DAB-method was established to identify germ cell peroxisomes also in later stages of spermato-/spermiogenesis on the EM level. Formerly, peroxisomes were thought to be absent in late stages of spermatogenesis. Our results obtained with specific, highly sensitive antibodies to peroxisomal marker proteins are indicative for the presence of this organelle in all cell types in the testis, including late spermatids and residual bodies. In addition, we could show that peroxisomes in the human and mouse testis exhibit in addition to marked differences in abundance and structure, a strong heterogeneity in their enzyme content. Highest and selective enrichment of the important peroxisomal lipid transporters (ABCD1= ALDP) as well as ACOX 2, the key regulatory enzyme of the β-oxidation pathway for side-chain oxidation of cholesterol and possibly of steroids was found in Sertoli cells. In contrast, expression levels of the β-oxidation enzymes ACOX1, ACOX3, MFP1 and MFP2 were similar in Sertoli-, peritubular myoid- or Leydig cells. Our results are suggestive for an important and cell-type specific function of peroxisomes in the testis and point to a special role of Sertoli cell peroxisomes in testicular lipid metabolism.

POSTER 125

Genomisches Imprinting während der normalen und gestörten Spermatogenese

Genomic imprinting during normal and impaired spermatogenesis

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Imprinted genes are monoallelically expressed in a parent-of-origin dependent manner due to allele-specific differential DNA methylation of CpG dinucleotides that needs to be resetted in the course of the germ line. Aberrant resetting of imprinting marks may lead to developmental disorders, such as Prader-Willi/Angelman and Beckwith-Wiedemann syndrome. Imprinting disorders are discussed as potential genetic risk in assisted reproductive techniques, where most of the natural selection mechanisms are bypassed. As most testicular biopsies from infertile men contain some small areas with normal spermatogenesis which allow testicular sperm extraction to be carried out, therapeutic testicular biopsies will play an increasing role for the treatment of male factor infertility. We therefore analyzed the timing of the reestablishment of imprinting marks for the maternally imprinted gene SNRPN and the paternally imprinted gene H19 isolating different germ cell types by single cell microdissection from human testicular paraffin sections. SNRPN was investigated by methylation-specific PCR, whereas for the analysis of H19, a single strand conformation polymorphism (SSCP) based method has been used. During normal spermatogenesis, both SNRPN and H19 showed the correct imprinting state in all germ cells analyzed. Contamination by somatic Sertoli cells could be excluded due to Sertoli cell-specific vimentin immunohistochemistry prior to laser microdissection. In addition, our results demonstrated correct genetic imprints for these genes even in spermatocytes and spermatogonia selected from seminiferous tubules exhibiting spermatogenic arrest at the level of spermatocytes and spermatogonia, respectively. In conclusion, we present a method which allows the investigation of the methylation state of imprinted genes in germ cells without contamination by somatic Sertoli cells. Our data indicate that genetic imprinting is already established in spermatogonia both in normal and impaired spermatogenesis.

POSTER 126

Untersuchungen zum Spermatogenesephänotyp in transgenen Mäusen mit Sertoli Zell-spezifischem Knockout des Connexin43-Gens

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Im Keimepithel des Hodens kommt Connexin43 (Cx43) zwischen benachbarten Sertoli Zellen sowie zwischen Sertoli und Keimzellen vor und wird mit der pubertären Initiierung der Spermatogenese in Verbindung gebracht. Da Mäuse mit einem generalisierten Connexin43-Knockout aufgrund von Herz-Kreislauf-Missbildungen nicht lebensfähig sind, wurde eine konditionale Cx43-Knockout-Mauslinie (SCCx43KO) unter Einsatz des Cre/LoxP-Rekombinasesystems generiert, bei der die Deletion des Cx43-Gens auf Sertoli Zellen beschränkt ist. Ein Fertilitätsassay ergab, dass die homozygoten SCCx43KO Tiere dieser Linie steril, die heterozygoten SCCx43KO und Wildtyp-Mäuse jedoch fertil waren. Nach Genotypisierung wurde das erfolgreiche Ausschneiden des Cx43-Gens im Hoden mithilfe einer RT-PCR und dem indirekten immunhistochemischen Nachweis der nuklearen β-Galaktosidase bestätigt. Homozygote SCCx43KO-Mäuse zeigten ein vermindertes Hodengewicht und einen Arrest der Spermatogenese auf der Stufe der Spermatogonien. Interessanterweise fand sich in einzelnen Keimtubuli dieser Tiere eine zumindest qualitativ intakte Spermatogenese. Mittels Cx43-In-situ Hybridisierung sowie Cx43- und Androgenrezeptor-Immunhistochemie wurde der Differenzierungszustand der Sertoli Zellen in diesen Tubuli näher charakterisiert. Zusammenfassend deuten die bisherigen Ergebnisse auf das Vorliegen eines somatischen Mosaiks hin.

POSTER 127

Verzögerte Ausbildung des Lumens in Samenkanälchen bei Mäusen mit einer Sertoli-Zell-spezifischen Deletion des Transkriptionsfaktors Krüppel-like factor 4

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Der Transkriptionsfaktor Krüppel-like factor 4 (Klf4) spielt eine wichtige Rolle bei der terminalen Differenzierung verschiedener epithelialer Zelltypen wie z.B. der Keratinozyten. Klf4 bewirkt in Zellkultur einen Arrest in der G_0-Phase und passt die Genexpression entsprechend an: Zellzyklus-Gene werden herunter- und differenzierungsspezifische Gene heraufreguliert. Klf4 wird auch im Hoden in runden Spermatiden und den Sertoli-Zellen exprimiert, wobei die mRNA in den Sertoli-Zellen sehr stark durch FSH induziert werden kann. Die generelle Inaktivierung von Klf4 führt zum perinatalen Tod der Tiere, da die Haut keine Verdunstungsbarriere ausbildet. Um die Rolle von Klf4 bei der postnatalen Hodenentwicklung zu untersuchen, haben wir Klf4 mit Hilfe des Cre-loxP-Systems Sertoli-Zell-spezifisch deletiert und diese Mutanten histologisch, immunhistochochemisch, endokrinologisch und molekularbiologisch analysiert.
Sertoli-Zell-spezifisch Klf4-defiziente Mäuse weisen zum Zeitpunkt des Abschlusses ihrer proliferativen Phase einen signifikant höheren Anteil an Samenkanälchen auf, die noch kein Lumen ausgebildet haben. Das Zytoplasma der mutierten Sertoli-Zellen ist dabei stark vakuolisiert. Dies indiziert eine verzögerte Reifung der Sertoli-Zellen. Mittels Microarrayanalysen wurden mehrere stark differentiell exprimierte Gene identifiziert (z.B. Htatip2, Sec8l1; beide etwa 20-fach herunterreguliert), die diesen Phänotyp erklären könnten. Interessanterweise waren weder FSH- noch Testosteron- noch T3-Serum-Werte verändert. Zusammengefaßt wurde in vivo gezeigt, dass Klf4 bei der postnatalen Sertoli-Zell-Reifung und Tubulusformation eine wichtige Rolle spielt.

POSTER 128

Morphological features of apoptosis in human neutrophils, vaginal epitheliocytes and HeLa cells under the influence of conditionally pathogenic bacteria and their structural components

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The aim of present work is to study the morphological features of apoptosis in human neutrophils, vaginal epitheliocytes and HeLa cells under the influence of conditionally pathogenic bacteria and their components. It is established that Gram-positive and Gram-negative conditionally pathogenic bacteria and the structural components of their cell wall (such as peptidoglycan, lipoteichoic acids, protein A and lipopolysaccharide), can induce the apoptosis of neutrophils, vaginal epitheliocytes and HeLa cells. The stimulation of apoptosis under their influence manifests in appearance of apoptic process markers - CD95-specific and CD38-non specific receptors on cell membranes. The induction of CD95- and CD38-receptors expression in immune cells correlated with increase of immune cells count with significant apoptic morphological changes. Apoptic cells were characterized by disruption of plasma membrane integrity and structural changes of intracellular organelles; the cells, as a rule, became round-shapeded, contracted, and lost their microvilli. The nuclear chromatin was aggregated and fragmented. The certain number of apoptic cells transformed into so called ‘apoptic bodies’ - pieces of apoptic cells, which contained small parts of condensed nucleus, or lost the nuclear material at all. So, conditionally pathogenic bacteria are able to induce increased apoptosis of human neutrophils, vaginal epitheliocytes and HeLa cells. Capacity to initiate the apoptic program relatively to the studied cells possess different bacterial structural components, including peptidoglycans, teichoic acids and lipopolysaccharides. The manifestation of the apoptosis inducing activity depends on their concentration and species specificity.

POSTER 129

Upregulation of vasoactive intestinal polypeptide expression and synaptophizin in human thymus after chronic thyroiditis and correlation with thymus hyperplasia.

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After the Chernobyl nuclear plant tragedy the problem of chronic thyroiditis became especially actual for the population of Belarus. It is known that chronic thyroiditis is one of principal causes of nontoxic goiters development. However influence and correlation of the given pathology with thymus hyperplasia are insufficiently investigated. Taking into account, that peptide hormones, cytokines and their receptors constitute a complete biochemical circle between immune and neuroendocrine systems, secretion disturbance in the thyroid gland and thymus can be related by a feedback mechanism, moreover it affects the activity of other various regulatory processes.Now there are no yet convincing theories proving correlation between chronic thyroiditis and thymus hyperplasia. Thus our main goal was to study the distribution patterns of vasoactive intestinal polypeptide in the human thymus hyperplasia. Thymus samples were obtained by operation biopsy from young men (age range between 17 and 28 years) with chronic thyroiditis and with thymus hyperplasia. Thymus and thyroid gland samples of patients of similar age after accidental trauma were used as control. Our results demonstrate a VIP and SYN substantial expression increase in the thymus of human with chronic thyroiditis and thymus hyperplasia. Substantial growth of expression of SYN as integral membrane glycoprotein which is generally associated with secretor vesicles, allows us to judge about rising secretory activity of organs, particularly in the thymus. Moreover thymus hyperplasia is capable to secrete a greater than normal quantity of biologically active substances, namely VIP, such abilities of which (enhancement of T cell production and survival in the thymus, controlling T cell and macrophage generation of cytokines and NO generation system, regulating T cell-dependent production of antibodies, protection of CD4+, CD8+ T lymphocytes from apoptosis), can be the reason of some pathological processes development, including chronic autoimmune thyroiditis.

POSTER 130

Effektor T-Zellen verändern während ihrer Wanderung durch die Milz das Zytokinmilleu

Bieber K, Speck U, Nachbar LB, Bergmann L, Schmidt P, Bode U, Kalies K und Westermann J

Lymphozyten zirkulieren im Blut und den lymphatischen Organen wie der Milz. Es gibt Hinweise, dass die Expression verschiedener Oberflächenmoleküle dieser wandernden Lymphozyten vom vorherrschenden Milieu in der Milz beeinflusst wird. Bis heute ist jedoch nicht geklärt, ob diese wandernden Zellen wiederum in der Lage sind, das Milieu in der Milz zu verändern. Um diese Fragestellung eingehender zu untersuchen, benutzen wir ein bestehendes Tiermodell, bei dem naïve Lymphozyten, Gedächtniszellen oder in vitro aktivierte Lymphozyten in kongene Ratten injiziert und deren Migration durch die Milz untersucht wurde. Immunologische Analysen erlaubten uns nicht nur, diesen unterschiedlichen T-Zelltypen zu folgen, sondern auch die Effekte zu studieren, die diese Zellen auf endogene Populationen ausüben. Hierbei zeigte sich, dass alle untersuchten Zelltypen das gleiche Wanderungsverhalten in der Milz aufwiesen, was bedeutet, dass die Migration unabhängig von verschiedenen Oberflächenmolekülen auf den unterschiedlichen T-Zelltypen ist. Im Gegensatz hierzu hatten die verschiedenen T-Zelltypen jedoch einen unterschiedlichen Einfluss auf das Mikromillieu in der Milz. Wohingegen naïve T-Zellen das lokale Zytokinmillieu nicht beeinflusst haben, bewirkten Effektor T-Zellen einen moderaten Anstieg der mRNA Expression verschiedener Zytokine und eine signifikante Proliferation endogener Zellen. Des Weiteren bewirkten sie die Ausbildung von Keimzentren, ein klarer Hinweise für eine T-zellinduzierte B-Zellproliferation selbst in Abwesenheit von Antigen. Unsere Untersuchungen zeigen somit klar, dass Effektor T-Lymphozyten in der Lage sind, das Milieu der Milz direkt zu beeinflussen.

POSTER 131

Human spleen ontogeny: From primordial arterial B cell lobules to a non-segmented organ

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Only few details of fetal spleen development are so far known in rodents and humans. We have thus performed an immunohistological analysis of lymphocytes, macrophages, endothelial and stromal cells in 29 human spleens from the 11th week of gestation to the early postnatal period, which revealed four developmental stages. At stage 0 the organ anlage contains erythrocyte precursors, few macrophages and almost no lymphocytes. At stage I fetal spleens start exhibiting arterial vascular lobules and lymphocytes just begin colonizing the organ. At stage II B and T lymphocytes form periarteriolar clusters. B cell clusters predominate, because B cells aggregate around the more peripheral branches of splenic arterioles, while T cells occupy the interlobular parts of the vessels located more centrally. The vascular lobules of stage I and II consist of central arterioles surrounded by B cells, peripheral venules and capillaries in between. The lobular architecture slowly dissolves at late stage II when sinuses apparently grow out of the peripheral venules into the centre of the lobule. Interestingly, the periarteriolar B cell accumulations do not represent the precursors of follicles, but obviously persist as periarteriolar B cell clusters in the adult splenic red pulp. Follicles containing follicular dendritic cells develop at late stage II from B cells in direct contact to the interlobular periarteriolar T cell clusters. At stage III before birth the lobular architecture is no longer recognized. In contrast to mice, the chemokine CXCL13 is already present in vascular smooth muscle cells and adjacent stromal cells at stage I before B cells immigrate. CCL21, on the contrary, is only demonstrated in fibroblast-like cells of T cell clusters from stage II onwards.

POSTER 132

MHC class II-enriched late endosomes - Origin of intestinal epithelial cell-derived exosomes

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Intestinal epithelial cells (IEC) are thought to stimulate CD4+ T cells and were demonstrated to release MHC class II/peptide loaded exosomes with immunogenic competence. Here we studied the subcellular compartments within IEC involved in antigen processing and class II peptide loading. Ovalbumin (OVA) was injected into jejunal or ileal loops of Tnf(Delta)(ARE) mice which spontaneously develop a Crohn′s like ileitis, and C57/129SvEv controls. Specimens were taken from OVA-incubated loops at different times up to two hours. MHC class II, invariant chain (Ii), the IEC membrane marker A33 and OVA were subcellularly localised using fluorescence light and cryo electron microscopy. Tissue levels of IL-12 and IFN-gamma were determined using quantitative real-time RT-PCR. Expression of MHC class II and Ii was found in the ileal epithelium of Tnf and control mice, but absent from the jejunal epithelium. In ileal IEC of both Tnf and control mice, the majority of MHC class II and Ii was localised in multivesicular late endosomes (MVLE). OVA accumulated in these MVLE one hour after injection. OVA targeting to MVLE was also detected in jejunal IEC, lacking MHC class II and Ii. Internal vesicles of MVLE were labeled for A33, and A33-positive exosomes were identified in intercellular spaces of the epithelium. Compared to control jejunum and ileum as well as Tnf jejunum, the inflamed ileum of Tnf mice showed significantly increased levels of IL-12 and IFN-gamma. Our *in vivo* findings suggest that MVLE might be crucially involved in class II-associated antigen processing and peptide loading within IEC. A33 labeling on internal vesicles of MVLE further indicates that these compartments are most likely the origin of MHC class II/peptide-loaded exosomes released from IEC. OVA targeting and accumulation in MVLE seems to be a uniform process within the small bowel and independent of mucosal inflammation. Further studies need to address modulations of antigen handling within MVLE which might be responsible for differences in the IECs′ capacity of antigen presentation to CD4+ T cells.

POSTER 133

Luminal antigens access MHC I and MHC II pathways in late endosomes of intestinal epithelial cells. Antigen uptake studied in vivo in Crohn's ileitis

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In Crohn's disease (CD), intestinal epithelial cells (IECs) are suggested to be involved in the stimulation of pro-inflammatory $CD4^+$ and $CD8^+$ T cells. The underlying mechanisms of MHC class I and II-associated presentation of exogenous antigens by IECs is still unknown. Our aim was to investigate in vivo the subcellular expression of MHC class I and II in IECs and its interference with the endocytic trafficking of luminal antigens. During ileoscopy, ovalbumin (OVA, in saline) was sprayed onto the ileal mucosa of CD patients (active ileitis and ileitis in remission) and healthy controls. Biopsies were taken at different times after OVA exposure. OVA, MHC class I and II were subcellularly localised by fluorescence light and cryo electron microscopy. MHC class I was seen in villus and crypt IECs in all subjects, whereas class II expression in crypt IECs was restricted to patients with ileitis. Cell surface expression of MHC class I and II proteins was predominantly found at basolateral and faintly at apical membranes. The majority of intracellular MHC class I and II was localised in late endosomes (LE) and a strong co-localisation of the two proteins was detected in these compartments. OVA was delivered into LE already 10 minutes after luminal exposure. OVA trafficking and labelling patterns for MHC class I and II within IECs showed no differences regarding the degree of mucosal inflammation. We provide first evidence that MHC class I and II pathways intersect within LE of IECs, which are efficiently accessed by luminal antigens. LE are likely to be responsible for class I and II-associated antigen processing in IECs. Our in vivo findings indicate that presentation of exogenous antigens by IECs is not restricted to MHC class II, but might also occur via MHC class I, a pathway recently described in professional antigen presenting cells as "cross presentation".

POSTER 134

Prävention der Diabetesentwicklung in der LEW.1AR1-*iddm* Ratte als Tiermodell des menschlichen Typ 1 Diabetes durch FTY720 als neues Immunmodulanz

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Durch die Gabe des neuen Immunmodulanz FTY720 soll in der LEW.1AR1-*iddm* (IDDM) Ratte eine offene Manifestation des Diabetes mellitus verhindert oder nach Krankheitsmanifestation ein Schutz der verbliebenen Betazellen vermittelt werden. FTY bewirkt durch Zurückhalten aktivierter und nicht aktivierter B- und T-Lymphozyten in den das Organ-drainierenden Lymphknoten über die Interaktion mit dem Sphingosin-1-Rezeptor eine Erniedrigung der Lymphozytenzahl im peripheren Blut.
Für die Primär- und Sekundärprävention wurden die Tiere mit FTY (1mg/kg Körpergewicht) über 40 Tage behandelt. Mittels *in situ* und *real time* RT-PCR wurden die Genexpressionen von Zytokinen (IL-1beta, IFN-gamma, TNF-alpha, IL-4, IL-10), des Chemokins MCP-1 und des Enzyms iNOS in Pankreasbiopsien und Pankreas-drainierenden Lymphknoten zum Zeitpunkt der Diabetesmanifestation, am Ende der Therapie sowie 30 Tage nach Therapiebeendigung analysiert.
Im Vergleich zu Kontrolltieren der IDDM-Ratte zeigten die Pankreasinseln der ab dem 50. Lebenstag mit FTY behandelten Tiere zu allen untersuchten Zeitpunkten keine Beta-Zellapoptose oder das Auftreten eines Immunzellinfiltrats. In den Pankreas-drainierenden Lymphknoten kann durch die Genexpression von proinflammatorischen Zytokinen und iNOS zwischen Tieren mit und ohne eine bereits bestehende Immunzellaktivierung unterschieden werden. Ohne eine Therapie wären die Tiere mit Immunzellaktivierungszeichen diabetisch geworden. Bei der Therapie bereits akut diabetischer Tiere konnte weder das Immunzellinfiltrat vermindert noch die restlichen Beta-Zellen geschützt werden.
Durch das Immunmodulanz kann auch nach Immunzellaktivierung eine Infiltration in die Pankreasinseln bei einer Primärprävention verhindert werden, wogegen nach bereits fortgeschrittener Immunzellinfiltration der Eingriff in das „Homing“ für einen Beta-Zellschutz nicht mehr ausreicht.

POSTER 135

Präklinisches Screening von DP4-Inhibitoren beim Asthma bronchiale*

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CD26/Dipeptidylpeptidase 4 (DP4) ist ein ubiquitär exprimiertes Transmembranmolekül und vermittelt neben seiner Peptidaseaktivität auch adhäsive Effekte und T-Zell-Aktivierungsprozesse. Da eine genetisch reduzierte CD26 Expression zu einem erniedrigten T-Zelleinstrom in die Lungen im OVA-induzierten Asthmamodell führt, wurden die Konsequenzen einer pharmakologischen Inhibition der DP4 als möglichen neuen therapeutischen Ansatz zur Behandlung des Asthma bronchiale untersucht. Dazu wurden unterschiedliche Konzentrationen des Inhibitors Isoleucyl-Thiazolidide (P32/98) zu verschiedenen Zeitpunkten verwendet (während der Immunisierung, unmittelbar vor Provokation, während der Provokation und über die gesamte Zeit von Start der Immunisierung bis Tötung über das Trinkwasser).
Die pharmakologische Inhibition von CD26 mittels systemischer Gabe (i.p.) während der Immunisierung oder vor der Allergen-Provokation zeigt keine signifikanten Änderungen in den Leukozytensubpopulationen in Lunge und BAL. Demgegenüber zeigte sich bei der chronischen Applikation des Inhibitors über das Trinkwasser (10mg/kg KG/24h) ein stärkerer Einstrom von Entzündungszellen in die BAL. Dieser basierte auf einer signifikant höheren Rekrutierung von T-Zellen als auch einem tendenziell höheren Einstrom von sowohl Makrophagen als auch neutrophilen und eosinophilen Granulozyten. Im Gegensatz dazu führte eine simultane Verneblung des Inhibitors mit dem Allergen zu einer signifikanten Reduktion von T-Zell-Subpopulationen und von $CD4^+$NKT Zellen im Lungeninterstitium und der BAL. In beiden Kompartimenten nahm die mittlere Expression sowohl des IL2-Rezeptors (CD25) als auch von CD26 signifikant ab. Die Daten belegen erstmals eine spezifische Modulation des Krankheitsverlaufs im OVA-induzierten Asthmamodell der Ratte über pharmakologische Inhibition von CD26.

*unterstützt durch SFB 587

POSTER 136

Dosisabhängige Rekrutierung von Entzündungszellen in die Lungen in einem neuen F344 Ratten Modell für Asthma bronchiale

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Die Ovalbumin-(OVA)-induzierte Atemwegsentzündung in Ratten ist ein etabliertes Modell für das allergische Asthma bronchiale. Die hierbei auftretenden entzündlichen Prozesse stellen eine spezielle zellvermittelte Immunreaktion dar, bei der insbesondere eosinophile Granulozyten und T-Zellen beteiligt sind. Die Stärke der Entzündungsreaktion ist dabei abhängig vom Rattenstamm. Brown Norway Ratten wurden bislang bevorzugt als Asthmamodell etabliert und eingesetzt. Vor kurzem konnte in der eigenen Arbeitsgruppe erstmals die wichtige Funktion von CD26 bei der Rekrutierung von T-Zellen in die bronchoalveoläre Lavage belegt werden. CD26 ist neben der Peptidaseaktivität bekannt für adhäsive und T-Zell-aktivierende Eigenschaften. Da CD26-Defizienz nur auf dem F344-Ratten-Hintergrund untersucht werden kann war daher das Ziel dieser Untersuchung, F344 Ratten als neues Modell für Asthma bronchiale zu etablieren. Für die allergische Provokation der Atemwege wurden unterschiedliche Dosierungen des Allergens (0%, 1%, 2.5%, 5% und 7.5%) verwendet. Bereits eine niedrige Allergen-Dosierung (1%) führte zu einer Bronchokonstriktion, aber erst Dosierungen ab 5% führten zu einer Mobilisation und Einwanderung von Eosinophilen, T-Zellen und dendritischen Zellen in das Inflammationsgebiet. Außerdem konnte ein dosisabhängiger Anstieg der aktivierten $CD4^{+}CD25^{+}CD26^{+}$ T-Zellen gezeigt werden. Diese Zellsubpopulation beinhaltet die zur Regulation der Entzündungsreaktion die T regulatorischen T Zellen. Die vorliegende Arbeit zeigt, dass F344 Ratten als ein neues allergisches Atemwegsmodell für Studien an der asthmatischen Erkrankung sehr gut geeignet sind.

POSTER 137

Die Bedeutung prolinspezifischer Peptidasen beim Asthma bronchiale
- Histochemischer Nachweis von DP4- und DP4-ähnlicher Enzymaktivität auf Lungenschnitten*

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Die Dipeptidylpeptidase 4 (DP4) ist eine prolinspezifische Protease, die eine wichtige Rolle im Rattenmodell für Asthma bronchiale spielt. Dabei ist über die Rolle anderer zur DP4 funktions- und/oder strukturhomologer Proteine, wie DP8 und DP9, wenig bekannt. Diese könnten aufgrund ihrer Homologie zu DP4 ebenfalls eine Rolle während solcher Immunreaktionen spielen.
Mittels einer von Dikov et al. 1999 publizierten histochemischen Methode haben wir die Expression und Verteilung von DP4, DP8 und DP9 auf Lungenschnitten untersucht. Dazu wurden Lungenschnitte von DP4-positiven Wildtyp-F344-Ratten und DP4-defizienten F344-Ratten nach Asthma-Induktion und in Ruhe angefertigt.
Mit Hilfe einer Modifikation dieser Methode konnten wir zeigen, dass die Enzymaktivität in den Lungen der DP4-positiven Ratten vorwiegend in den Alveolarsepten zu finden ist und weniger in den Bronchien. Auf den Lungenschnitten der DP4-defizienten Ratten ist die Aktivität von DP8 und DP9 nachweisbar. Diese DP4-ähnliche Aktivität befindet sich hauptsächlich in den Bronchien und weniger in den Alveolarsepten. Der Vergleich DP4-positiver und –defizienter Proben zeigt, dass die DP4-Aktivität in der Lunge deutlich stärker ist als die Aktivität von DP8 und DP9. Erste Ergebnisse deuten auf eine Hochregulation der DP4 nach Asthma-Induktion hin.
Der histochemische Aktivitätsnachweis von DP4, DP8 und DP9 zeigt erstmals die Aktivitätsverteilung dieser drei Peptidasen in den Kompartimenten der Lunge und ermöglicht die Beantwortung der Frage nach Aktivitätsänderungen bei Entzündungsmodellen wie dem Rattenmodell für Asthma bronchiale.
*Mit Unterstützung durch den SFB587/B11

POSTER 138

Expression der Surfactantproteine (SP) A und D in CD26 positiven und CD26 defizienten Fisher-Ratten im Asthma-Modell*

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Die Ektopeptidase CD26 (Dipeptidylpeptidase 4, DP 4) findet sich sowohl in löslicher Form als auch ubiquitär als Transmembranprotein auf Epi- und Endothelzellen sowie auf immunkompetenten Zellen. Aufgrund ihrer unterschiedlichen funktionellen Eigenschaften lässt sich eine wichtige Rolle von CD26 bei allergischen und entzündlichen Erkrankungen sowie bei Autoimmunprozessen vermuten. Für das Modell des Asthma bronchiale konnten wir dieses vor kurzen in den uns zur Verfügung stehenden CD26 positiven und defizienten Ratten zeigen. Diese entzündlich veränderten Zytokin- und Chemokinmetabolismen könnten direkt oder auch indirekt das pulmonale Surfactantsystem beeinflussen. Die SP-A und SP-D gehören zur Familie der „host defense lectins“ (Collectine) mit immunmodulierenden und opsonierenden Eigenschaften. Ziel dieser Untersuchung war es daher festzustellen, ob die beiden Substämme in Kontrolltieren vor und nach Asthma-Induktion Unterschiede in der SP-Expression zeigen. Nach zweimaligen Sensibilisierungen erfolgte 22 Stunden nach einer 5% OVA-Provokation die Gewinnung der BAL und die Organentnahme. Kontrolltiere wurden weder sensibilisiert noch provoziert. Die mittels qRt-PCR bestimmte mRNA und die mittels Western Blot bestimmte Expression der SP-A und SP-D zeigten in der Kontrollgruppe nur geringe Unterschiede. Nach Asthma-Induktion fanden wir in den CD26 defizienten Tieren eine tendenziell geringere m-RNA Synthese und Expression des SP-A. Stammunabhängig ist die SP-A-mRNA nach Asthma-Induktion signifikant erhöht, während die SP-D-mRNA keine signifikanten Unterschiede zeigt. Weitere Untersuchungen sind nötig, um einen möglichen Einfluß der CD26-Defizienz auf das Surfactantsystem aufzuzeigen.

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POSTER 139

CD26-abhängige Rekrutierung von Eosinophilen nach intradermaler Injektion von Eotaxin

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Der CCR3-Agonist CCL11/Eotaxin spielt eine wichtige Rolle bei der Rekrutierung eosinophiler Granulozyten im Rahmen allergischer Entzündungsreaktionen. Er wird durch die Dipeptidylpeptidase 4 (DP4/CD26) gespalten, wodurch er seine chemotaktische Aktivität verliert. Da dieses Enzym ubiquitär exprimiert wird, haben wir in der vorliegenden Arbeit den Einstrom eosinophiler Granulozyten in die Haut nach intrakutaner Eotaxin-Gabe (0, 10, 100, 1000 pmol) in jeweils DP4-positiven Wildtyp- und DP4-defizienten F344 Ratten verglichen. Zusätzlich applizierten wir DP4-positiven Wildtyp-Ratten Eotaxin in Kombination sowohl mit unspezifischen als auch spezifischen Inhibitoren der DP4 und der DP8 und 9.
Mit aufsteigender Eotaxin-Konzentration kam es zu einem vermehrten Einstrom eosinophiler Granulozyten in die Haut. Dieser Effekt ist bei DP4 defizienten Ratten stärker ausgeprägt als beim Wildtyp. Die zusätzliche Gabe des unspezifischen Inhibitors Isoleucyl-Thiazolidide (P32/98) in Wildtyp-Tieren führt zu einer signifikanten Erhöhung der intradermalen Eosinophilenzahl. Der hochspezische DP4-Inhibitor UG92 erhöhte ebenfalls signifikant die Akkumulation der eosinophilen Granulozyten, wohingegen keine Effekte durch die Inhibition der DP8 und 9 durch den spezifischen Inhibitor UG93 zu sehen waren.
Die intrakutane Gabe von CCL11/Eotaxin zeigt deutlich die Wirkung dieses CCR3-Agonisten für die Rekrutierung von eosinophilen Granulozyten. Seine Wirkung unterliegt dabei der Modifikation durch die DP4, nicht jedoch im signifikanten Maße der durch DP8 und 9.