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Abstracts

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Abstract Nr.:7

Titel: Synaptic activation of newly formed granule cells in the adult hippocampus

Autoren: Schwarzacher S.(1),Jungenitz T.(1),Deller T.(1),

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Abstract:
Hippocampal granule cells maintain the ability to generate new neurons through adulthood. Increasing evidence show, that adult neurogenesis is advantageous for hippocampus-dependent forms of learning and memory. Here, we studied at which maturation stage newly formed granule cells are integrated into the dentate gyrus network.

We induced LTP of granule cells with high frequency stimulation (HFS) of the perforant path in rats. Mature and maturing granule cells were detected with different immunocytochemical markers. Following HFS, almost 100% of mature Calbindin-positive granule cells were labelled with c-fos and zif268, two markers for synaptic activation, as well as for Arc, a marker for synaptic plasticity. Unexpectedly, both c-fos and Arc were absent in immature Doublecortin-positive granule cells, whereas zif268 was found to be upregulated in a subset of immature neurons after HFS. Stimulation-induced expression of zif268 correlated well with dendritic growth in immature neurons, revealing prominent staining only when granule cell dendrites reached the outer molecular layer, the termination zone of stimulated afferents. P-CREB133, a general activity marker, was strongly expressed in mature granule cells following HFS, but again was not upregulated in immature neurons. In contrast, the level of endogenously expressed P-CREB133 correlated to the stage of dendritic growth in immature neurons.

Since the expression of marker molecules for maturation and synaptic activation correspond to the structural development of granule cells, we conclude that maturing granule cells show an increasing synaptic integration into the dentate gyrus network but lack the ability to respond to synaptic potentiation at the transcriptional level.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:8

Titel: Aromatase activity in hippocampal neurons and ltp

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Abstract:
Hippocampus-derived estradiol is essential for synapse maintenance in the hippocampus. Inhibition of aromatase induces spine synapse loss in organotypic hippocampal slice cultures, which originated from female animals and in hippocampi of female cyclic and ovariectomized mice after systemic treatment with the potent aromatase inhibitor letrozole, for periods of seven days and four weeks. In acute slices of these animals LTP was heavily impaired in the presence of letrozole. After seven days of treatment, LTP could not induced any more by TBS, although NMDA synaptic transmission was not affected and the impairment of LTP was totally rescued when we applied letrozole together with estradiol. This was also true in hippocampal slice cultures. A reduction in the magnitude of LTP by 60% was already found after 24 hours of treatment. Interestingly enough, the effects were not seen in male animals. Impairment of LTP was also seen in aromatase deficient mice. Our results indicate that systemic inhibition of aromatase in mice affects synaptic plasticity in the hippocampus.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:9

Titel: Role of clock genes in the hippocampal learning and behaviour of mice

Autoren: Jilg A.(1),Ried C.(1),Lautenschütz B.(1),Utech L.(1),Schwegler H.(2),Dehghani F.(3),Stehle J.(1),

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Abstract:
Purpose: The role of clock gene expression was analysed in the hippocampus of mice, to dissect their role within learning and memory formation.
Methods: Subfield-specifically, the expression of all major clock genes was analyzed by RT-PCR, and immunhistochemistry in cryostat-cut hippocampal sections of wildtype (WT) and Per1-/- mice, killed at 8 diurnal time-points. Additionally, learning associated proteins were analysed in mouse hippocampus, following an eight-arm radial arm maze test.
Results: mRNA of clock genes mPer1,2, mCry1,2, mClock, mBmal1 exhibited a rhythmic expression in a 24-h time-locked fashion in both, WT and Per1-/- mice. Clock gene proteins PER1, PER2, CRY1, CRY2, CLOCK and BMAL1 could be observed in cell nuclei of neurons in the Stratum pyramidale and Stratum granulosum of the mouse hippocampal formation. In WT, a diurnal rhythm in protein expression was detected for PER1, CRY2, CLOCK and BMAL1. Notably, in Per1-/- mice, circadian phase and amplitude of clock gene protein rhythms were greatly altered. In the eight-arm radial arm maze test, Per1-/- mice exhibit a higher number of repeated arm re-entries (errors), as compared to WT. On the basis of comparative microarray analyses the Per1-related differential expression of hippocampal structurally to plasticity related genes is currently investigated.
Conclusions: Presented data show a time-of-day-dependent rhythmic expression of clock genes and their corresponding protein products in hippocampal neurons of WT mice. The loss of Per1 severely affects animal behaviour and the coordinated clock gene expression in the hippocampus, implicating a trigger function for PER1 in time-dependent mnemonic processes.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:10

Titel: Eralpha and erbeta in estrogen-regulated spinogenesis

Autoren: Fester L.(1),Zhou L.(1),Hagshenas S.(1),Behem C.(1),Rune G.(1),

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Abstract:
In previous reports, we showed that hippocampus-derived estradiol or aromatase activity in hippocampal neurons resp. regulate spinogenesis. We also addressed the question as to which of the estrogen receptors (ERs), ERalpha and/or ERbeta;, are involved in this process using specific agonists and antagonists for either ERalpha and ERbeta. We found that estradiol and PPT, the ERalpha agonist, rescued spine synapse number and upregulated synaptophysin and spinophilin in cultures, in which estradiol synthesis and as a consequence spine synapse number was downregulated in response to aromatase inhibitors. This increase was abolished in the presence of specific ERalpha antagonists. Vice versa, after inhibition of aromatase activity, the ERbeta agonist DPN further promoted letrozole-induced spine synapse loss and downregulated synaptophysin and synaptopodin. This effect, in turn, was not found when the cultures were treated with a specific ERbeta antagonist. These findings suggested that ERalpha mediates spine growth, whereas retraction of spines is mediated by ERbeta in estrogen-regulated spinogenesis. Our hypothesis was further confirmed by our findings using siRNA against ERalpha and ERbeta. Spine density was reduced after knock down of ERalpha and increased as compared to the control after knock down of ERbeta.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:11

Titel: Developmental plasticity in perforant path involves changes in presynaptic ion channel expression

Autoren: Bender R.(1),Klapetke H.(1),Wilkars W.(1),

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Abstract:
Purpose: The probability of transmitter release at perforant path (PP) synapses is largely determined by the ensemble of ion channels at the axon terminals. This ensemble includes HCN1 and Kv1.2 (potassium) channels. We previously showed that the transport of HCN1 to presynaptic sites in PP is developmentally regulated, but little is known about a time-dependence of Kv1.2-expression. Here we studied the time course of Kv1.2-appearance, and also of its beta-subunit, Kvbeta2, in PP. In addition, we examined the expression of a newly discovered HCN1 beta-subunit, TRIP8b.
Methods: Developmental time courses of HCN1, Kv1.2, Kvbeta2 or TRIP8b expression in PP were determined with immunohistochemistry and Western Blots, using commercially available (HCN1, Kv) or individually developed (TRIP8b) antisera. Analysis of TRIP8b further involved in-situ-hybridization.
Results: HCN1 and Kv1.2 show an inverse time course of expression in PP terminals: Kv1.2-expression increases, while HCN1-expression decreases with maturation. Kvbeta2 increases in parallel with Kv1.2. TRIP8b-expression also increases with maturation, but thus shows a time course opposite to its alpha-subunit, HCN1.
Conclusions: The inverse time courses of presynaptic HCN1- and Kv1.2-expression suggest that maturation of PP axon terminals involves an expression shift of these ion channels that likely affects transmitter release probability. The parallel expression of Kv1.2 and Kvbeta2 is concordant with a presumed role of Kvbeta2 for Kv1.2 axonal transport. In contrary, the inverse time courses of HCN1- and TRIP8b-expression suggest that TRIP8b does not support, but rather prevents HCN1 axonal transport.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:12

Titel: Distribution of dendritic a-type k+ channels determines plasticity in sensory and associative synapses in the piriform cortex

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Abstract:
Two spatially distinct pathways project onto the apical dendrites of layer 2/3 pyramidal cells in layer 1 of the piriform cortex. Direct synaptic inputs from mitral cells in the olfactory bulb project onto the distal portion of the dendrite, constituting the sensory layer 1a. Associational synapses from within the piriform cortex and other brain areas cluster in the proximal layer 1b. This laminar structure allows a clear spatial distinction between sensory and associative inputs. A key functional difference between the two synaptic inputs is their plasticity.
During LTP induction, Ca2+ signals in postsynaptic spines are a key determinant of plasticity outcome. Here, we analyse Ca2+ signals in proximal (layer 1b) and distal (layer 1a) dendritic spines within pyramidal neurons of the piriform cortex. We demonstrate that the laminar distribution of different synaptic inputs is a prerequisite for differences in synaptic plasticity induction based on the electrical compartmentalization of the dendritic tree. We propose that the distribution of intrinsic conductances mediating electric compartmentalization of the dendritic tree may serve as an alternative structural mechanism regulating synaptic plasticity.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:13

Titel: Fast intracellular trafficking and surface motility of the synaptic cell-adhesion molecules neurexins

Autoren: Niesmann K.(1),Heine M.(2),Schneider R.(2),Brinkhaus L.(1),Missler M.(1),

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Abstract:
Purpose: To analyse the dynamics of intracellular trafficking and surface diffusion of the synaptic proteins alpha- and beta-neurexin, and a putative regulation by their differential binding behaviour.
Methods: Full-length neurexin-1alpha and -1beta with various fluorophore-tags were expressed in primary hippocampal neurons. Intracellular trafficking was analysed in live-imaging experiments, complemented by immunocytochemical analysis. Surface diffusion of single neurexin molecules was tracked using quantum-dot labelled antibodies.
Results: Our data demonstrate that both neurexin-isoforms are transported throughout the neuron in transport vesicles. We observed fast axonal and dendritic intracellular transport of alpha- and beta-neurexins with the two isoforms being transported anterogradely and retrogradely at different velocities. Both co-transport and separate transport of alpha- and beta-neurexins was observed in co-transfection experiments. Surface tracking illustrated high motility of both neurexin-isoforms with differential behaviour on synaptic and extrasynaptic membranes. Mobility was more confined in synapses. Removing ambient calcium by EGTA increased the diffusion coefficient of both isoforms, indicating that the limiting factor for diffusion may be the calcium-dependent neurexin-neuroligin interaction.
Conclusions: Our data illustrate that transport of alpha- and beta-neurexin within neuronal cells and on the neuronal surface is a surprisingly dynamic process. They suggest that neurexin-isoforms are transported along the same intracellular routes but that their trafficking is independently modulated. Furthermore, the results reveal a differential, calcium-dependent behaviour of alpha- and beta-neurexins on the synaptic surface. Together, our findings present the first description of alpha- and beta-neurexin dynamics that may form the basis for their distinct functional role in synaptic transmission and synapse formation.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:14

Titel: Developmental stabilization and activity-dependent regulation of gephyrin scaffolds at hippocampal gabaergic synapses

Autoren: Vlachos A.(1),Reddy-Alla S.(2),Papadopoulos T.(2),Deller T.(1),Betz H.(2),

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Abstract:
Gephyrin is a scaffolding protein essential for synaptic clustering of inhibitory glycine and GABAA receptors. Here, we investigated the dynamics of gephyrin at individual synapses of CA1 pyramidal neurons in organotypic entorhino-hippocampal slice cultures prepared from a newly generated mouse line, which expresses green fluorescent protein-tagged gephyrin under the control of the Thy1.2 promoter. Slice cultures were prepared at postnatal day 4-5 and analyzed either at an early developmental stage (week1) or after 4 weeks of in vitro differentiation using confocal microscopy and whole cell patch-clamp recordings. At both stages, individual GFP-gephyrin clusters were clearly discernable in a subset of CA1 pyramidal neurons and mainly associated with dendritic shafts and the somatodendritic region. Fluorescence recovery after photobleaching (FRAP) revealed a developmental stabilization of postsynaptic gephyrin scaffolds that was accompanied by an increase in gephyrin cluster size and inhibitory synaptic strength. Treatment of mature slice cultures with a GABAA receptor antagonist or diazepam revealed a homeostatic regulation of gephyrin cluster stability and size by inhibitory synaptic activity. Our results indicate an important role of gephyrin scaffold dynamics in inhibitory synapse differentiation and plasticity.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:15

Titel: Developmental plasticity in reeler mutant mice leads to columnar activation of functional cortical modules

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

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Abstract:
Cortical lamination is regarded as a necessary pre-requisite for the orderly establishment of long-distance intra- and subcortical projections. Recently it became evident that also local short-distance connections (forming the “canonical microcircuit”) are tightly correlated to the long-distance projection target of the respective neurons. We now ask whether a functional canonical microcircuit can be found in reeler mutant mice, a model system for severe developmental disturbance of layered structures? The whisker-to-barrel-pathway presents a somatotopic map of the sensory periphery at all processing stations, including the somatosensory (barrel) cortex. Exploration of a novel environment resulted in expression of the inducible transcription factor c-Fos at all stages of the pathway, surprisingly including columnar modules in the barrel cortex. We now determined whether laminar identity of individual neurons, which express c-Fos, is different in reeler compared to their wild type controls. Toward this end, we combined immunocytochemical detection of c-Fos with in situ-hybridization of Rgs8 as a marker for layer II/III, RORB as a marker for layer IV and ER81 as a marker for layer V neurons. Cell counts revealed that layer IV located (in wild type) or fated (in reeler) neurons are statistically indifferent whereas the relative numbers of layer II/III and even more layer V fated cells are significantly less strongly expressing c-Fos in reeler as compared to wild type. We conclude that an intact thalamic input into a disordered cerebral cortex promotes a developmental plasticity mechanism to ensure the formation of barrel-related columns with a largely preserved canonical microcircuitry

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:16

Titel: Clinical l1cam mutations cause neuronal l1 trafficking defects by divergent mechanisms

Autoren: Schaefer M.(1),Nam Y.(1),Keglowich L.(1),Bouché E.(1),Kueffner M.(1),Bock H.(2),Frotscher M.(1),

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Abstract:
Mutations of the neural cell adhesion molecule L1 cause neurodevelopmental disorders such as X-linked hydrocephalus, spastic paraplegia and mental retardation. Here, we investigated the cellular pathomechanisms of two clinical L1 missense mutations, R184Q and W1036L, which are associated with severe disease phenotypes. Expression of these mutations in neuronal NSC-34 cells causes partial retention of mutated L1 protein in the endoplasmic reticulum and reduced cell surface expression. In primary neuronal cultures, we find that both L1 mutations impair axonal targeting of mutated L1. However, the R184Q mutation restricts mutated L1 to cell bodies whereas the W1036L mutation leads to aberrant localization of mutated L1 to dendrites. Similar effects were observed following single-cell electroporation in organotypic hippocampal slice cultures. As a functional consequence, axon growth and arborisation are reduced. These results indicate that different clinical L1CAM mutations cause neuronal L1 trafficking defects by divergent mechanisms that interfere with L1-mediated axon growth and arborisation.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:17

Titel: Mice differ from rats: detection of tyrosine hydroxylase containing neurons in the mouse striatum after 6-ohda-injection

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Abstract:
We established a mouse model of Parkinson's by transferring the well known 6-OHDA-injection protocol from rats to mice. C57BL/6-mice were lesioned by stereotactic 6-OHDA-injection into the right medial forebrain bundle. One, 2 and 3 months after lesion apomorphine- and amphetamine-induced rotations were evaluated and forepaw preferences of lesioned mice were compared with age matched intact controls. Three months after lesion, mice were perfused with 3.7% PFA and immunohistochemistry to visualize tyrosine hydroxylase (TH) immunoreactive neurons in brain sections was performed. In the apomorphine test we observed significantly different rotational behaviours during one to three months post lesion: one group demonstrated rapidly increasing numbers of high contralateral rotations over time, whereas the other group showed low and stable amounts of rotations. The high rotating animals used their right forepaws significantly more often as compared to the low rotating or intact mice. However, to our surprise the ipsilateral rotations in the amphetamine test were not as high as expected. TH-immunohistochemistry revealed a nearly complete unilateral dopaminergic cell loss in the substantia nigra and a dopaminergic deafferentiation, resulting in loss of TH-ir nerve terminals, in the ipsilateral striatum. However, in all lesioned animals we observed respective numbers of TH-containing neurons in the dopaminergic deafferentiated striatum. In Hemiparkinsonian rats we never observed such TH-ir perikarya. We conclude, that plasticity of striatal neurons in the mouse brain considerably differs from that of the rat. The underlying mechanisms are yet unknown.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:18

Titel: Pterygopalatine diabetic neuropathy: evidences of cell death presence and possible etiology of the diabetic dry eye syndrome

Autoren: Rusu M.C.(1),Pop F.(2),Mirancea N.(3),

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Abstract:
The pterygopalatine ganglion (PPG) occupies the pterygopalatine fossa, distally to the anterior opening of the vidian canal. In such deep location it was not so consistently approached for detailed structural studies in humans. Moreover, there are few, if not missing, available data on the parasympathetic diabetic neuropathy. So, we aimed to investigate in humans the ultrastructural features of the pterygopalatine diabetic neuropathy, in order to evaluate whether or not diabetes mellitus is consistent with a dysfunction of that ganglion. For this, we dissected free 4 human adult pterygopalatine ganglia, at autopsy, and we prepared the specimens for electron microscopy (TEM). Toluidin blue was used for the semithin cuts that evaluated as positive the presence of cell death processes within the PPG, evidencing apoptotic bodies and apoptotic syncitia within the ganglia and also the vidian nerve diabetic neuropathy. The ultrastructural studies evidenced a series of alterations, reactive and specific for the diabetic cell death: presumably both, apoptosis and necroptosis, are the processes that occur within the diabetic pterygopalatine ganglion, determined by or determining the vidian neuropathy. As the PPG is the main provider of the autonomic stimuli for the lacrimal gland, it seems reasonable to consider that the diabetic cell death and neuropathy of the PPG may represent the, surprisingly yet undetermined, etiology of the dry eye syndrome that occurs frequently with diabetes. Funding: Grant UEFISCSU (Executive Unit for Financing Higher Education and University Scientific Research), 317/2007

Kategorie: Lecture

Rubrik: 3.Methods/Teaching
Abstract Nr.:19

Titel: Embedding in a sac: a new technique in the casting and curing stage of plastination.

Autoren: Steinke H.(1),Kürtül I.(2),Suganthy R.(3),

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Abstract:
Plastination is a unique process of preservation and storage of body components used in teaching and research purposes. However, block plastination is a relatively expensive and time consuming process. To reduce the cost and to finish polymerization in a shorter time, we established an embedding technique called the sac embedding. After CT and MRI, the specimens were subjected to pre-cooling, shock freezing, dehydration and degreasing in the acetone series. Then, forced impregnation process was applied with the resin mixture E12 + E6 + E600. Instead of using a box to make a block during the casting and curing stage of the process, we embedded the specimen in the same mixture in a sac made of polyester foil. An ‘old’ polymerized plastic or wooden block was attached to the specimen and the sac was wrapped with tape. This limits the usage of resin to the inner volume of the plastinate. Then, the ‘plastination sac’ was put in the warming device for further polymerization and curing. When the sac was removed from the plastinated specimen, the block of old plastinated wood was removed and the specimen was mounted on a socket for sawing. Consequently, a significant amount of the resin was saved and the remaining resin was frozen for further reuse. The sections acquired by sawing the sac plastinate provide a high degree of gross details, while preserving in situ structural reliability of the specimens for cross-sectional anatomy, thus contributing to the understanding of modern diagnostic imaging procedures including MRT and CT.

Kategorie: Lecture

Rubrik: 3.Methods/Teaching
Abstract Nr.:20

Titel: Are combined professional and didactical trained student tutors an effective support for students in the dissection course?

Autoren: Shiozawa T.(1),Lammerding-Koeppel M.(2),

Adressen: (1)Experimental Embryology and Tissue Engineering|Institute of Anatomy, Eberhard-Karls-University Tuebingen|Tuebingen|Germany; email:thomas.shiozawa@uni-tuebingen.de; (2)Competence Center for University Teaching in Medicine|Competence Center for University Teaching in Medicine, Eberhard-Karls-University Tuebingen|Tuebingen|Germany

Abstract:
Background: Student tutors have long been employed as peer teachers in the dissection course, but their qualification and performance varied widely. We devised a curriculum for combined professional and teaching training and tested its effectiveness. For this survey students of the dissection course were randomly assorted to 10 trained and 10 non-trained tutors, and blinded concerning his or her training.
Research question: Can trained tutors coach the students better in terms of learning and dissection? What influences have trained tutors on the learning behaviour of their students?
Methods: The students evaluated their tutor regarding the 11 learning goals of the combined professional and teaching training. Furthermore the students were surveyed concerning their learning behaviour with the LIST questionnaire (77 items in 11 categories).
Results: 188 questionnaires for the quality of coaching were returned (95.67% return rate). Trained tutors were evaluated better in 7 of 11 categories in comparison to the control group. Significantly (p<0.05) better ratings were attained for conveying basic dissection techniques (4.31 vs. 3.89), positive group atmosphere (4.69 vs. 4.44), learning support (4.24 vs. 3.79), visualising (3.99 vs. 3.56) and use of activation techniques (4.11 vs. 3.82).
For their learning behaviour, students coached by trained tutors state that they learn more with their fellow students (3.25 vs. 2.96, p<0.05).
Conclusion: The combined professional and teaching training program is effective, trained tutors are perceived significantly better concerning their professional and teaching quality. Students coached by trained tutors learn significantly more often in teams than their colleagues.

Kategorie: Lecture

Rubrik: 3.Methods/Teaching
Abstract Nr.:21

Titel: Tuebingen's "sectio chirurgica" – an innovative, transdisciplinary teaching concept

Autoren: Hirt B.(1),Shiozawa T.(2),

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Abstract:
Background: A telemedical surgical broadcasting was established to transmit live surgeries on especially embalmed cadavers. In a transdisciplinary approach the medical directors of different surgical disciplines performed surgical approaches under an almost realistic surgical environment. The moderation was done by an anatomist. In 12 evening events around 5000 medical students were counted at the Tuebingen's 'Sectio chirurgica'.
Research question: Is the "Sectio chirurgica" able to interlock preclinical with clinical content? Can this program raise the students' learning motivation and interest for pursuing a career in the surgical field?
Methods: The course objectives were evaluated with an especially designed questionnaire (21 items, 5 point Likert scale and open answers).
Results: 213 questionnaires were returned (60.2% return rate). The course was rated 3.41±1.1 for the improvement of the motivation in surgical disciplines, and 3.73±0.9 for the connection of clinical and pre-clinical content.
[2.61±0.76 for motivating to learn anatomy, 2.42±1.2 for reducing first anxiety of working in the OR, 2.87±1.3 for starting a doctoral thesis in surgery // Overall score 2.05±0.88 // 95.8% would recommend the course]
Conclusion: The new program achieves good grades and is in student's view well able to interlock pre-clinical with clinical content. Furthermore, the course can raise the motivation for surgical disciplines. The 'Sectio chirurgica' is appropriate to overcome the strict delineation between the preclinical and clinical part in non-reform medical degree programs.

Kategorie: Lecture

Rubrik: 4.Gross Anatomy/Clinical Anatomy
Abstract Nr.:22

Titel: Anatomy of a mermaid

Autoren: Matei A.(1),Schmidt N.(1),Muresan M.(2),Rotar I.(3),Branzaniuc K.(4),

Adressen: (1)Anatomy Departament|University of Medicine and Pharmacy|Cluj-Napoca|Romania; email:adelamatei@gmail.com; (2)Pathological Anatomy|Country Hospital|Cluj-Napoca|Romania; (3)1st Departament of Obstetric and Gynecology|University of Medicine and Pharmacy|Cluj-Napoca|Romania; (4)Anatomy Departament|University of Medicine and Pharmacy|Targu-Mures|Romania

Abstract:
Sirenomelia is a rare malformation, characterized by the presence of a single inferior member. This condition represents the result of the fusion of the inferior members, but there have been described situations when one of the members is missing. Other anomalies are usually present such as renal agenesis and severe urinary tract dysplasia, the absence of the external genitalia, anorectal agenesis, single umbilical artery, anomalies of the lumbosacral spine. Some authors consider sirenomelia as the most severe form of the caudal regression syndrome.We report a case of a 10 gestational weeks fetus with a crown-rump length of 6 cm, with sirenomelia; the fetus had only the left bony side of the inferior member. The dissection has revealed the muscles and the bones of a single member of the thigh represented by a single coxal bone (malformed), the right femur, patella and only the proximal epiphysis of the tibia. No anomalies have been found at the level of the lumbosacral spine. The analysis of the musculature and the posterior position of patella showed that the member was rotated with approximately 180 degrees. We consider that this is the consequence of a lack of normal medial rotation of the member.

Kategorie: Lecture

Rubrik: 4.Gross Anatomy/Clinical Anatomy
Abstract Nr.:23

Titel: Combining the players into a team: the trigeminal-autonomic system.

Autoren: Rusu M.C.(1),Niculescu V.(2),

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Abstract:
The strictly unilateral headaches, more commonly known as trigeminal autonomic cephalalgias (TACs), are characterised by severe, strictly unilateral pain in the territory of the distribution of the trigeminal nerve, associated with autonomic manifestations. The pathophysiologies of the TACs have not yet been adequately clarified. Moreover, the anatomical scaffold for the TACs is usually separately considered in traditional textbooks and thus it cannot offer a suitable image for understanding the clinical and patophysiological patterns of these cephalalgias. That is why we considered suitable to address this issue and to develop an integrative anatomical concept that brings together the trigeminal and the autonomic systems of the head, based upon anatomical dissections/microdissections and immunohistochemistry. The concept of the trigeminal-autonomic system (TAS) is supported by complex peripheral neural wiring patterns, traditional and/or neglected, and by various phenotipic patterns of the ganglionic neurons. The centers of the TAS are mainly but not exclusively located at the level of the: (1) cavernous sinus; (2) pterygopalatine fossa; (3) infratemporal fossa; (4) orbit. To suport the TAS we bring here evidences resulted from dissections./microdissections in 40 human adult cadavers and also immunohistochemistry performed on the trigeminal, pterygopalatine, otic, ciliary and cavernous sinus ganglia/microganglia, for various peptides and transmitters. Funding source: Grant UEFISCSU (Executive Unit for Financing Higher Education and University Scientific Research), 317/2007

Kategorie: Lecture

Rubrik: 4.Gross Anatomy/Clinical Anatomy
Abstract Nr.:24

Titel:Enterocolic volvulus through common mesentery – case report

Autoren: Neagos C.(1),Graur F.(2),Pop C.(3),Blidaru D.(1),Litean O.(4),Nagy C.(1),

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Abstract

This paper presents the case of a 63 year old male patient, hospitalized in the “3rd Surgical Clinic”, Cluj-Napoca, with symptoms suggesting an intestinal obstruction. Emergency surgery was performed during which the diagnosis of bowel obstruction was confirmed. The intraoperative findings were a common mesentery with the volvulation of the last enteral loops and of the ascending colon, the latter being located on the left side of the median line. The surgical treatment consisted of intestinal devolvulation and ascending colon colopexy to the right colic flank.
This case illustrates the clinical picture and the surgical treatment options of a complex anatomical malformation of the small bowel, ascending colon and mesentery, caused by rotation abnormalities of the midgut during the intrauterine life.
These anomalies of the midgut rotation, including the common mesentery mentioned above, have been described in the literature as a cause of intestinal occlusion

Kategorie: Lecture

Rubrik: 4.Gross Anatomy/Clinical Anatomy
Abstract Nr.:25

Titel: Statin therapy affects the expression of genes that regulate calcium homeostasis and membrane repair in skeletal muscle

Autoren: Draeger A.(1),Sanchez-Freire V.(1),Monastyrskaya K.(1),Hoppeler H.(1),Mohaupt M.(2),Babiychuk E.(1),

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Abstract:
In skeletal muscle of patients with clinically-diagnosed statin-associated myopathy, discrete signs of structural damage predominantly localize to the T-tubular region and are suggestive of a calcium leak. The impact of statins on structurally intact skeletal muscle of non-myopathic patients is not known. We analyzed the expression of selected genes implicated in the molecular regulation of calcium and membrane repair, in lipid homeostasis, myocyte remodelling and mitochondrial function. Microscopic and gene expression analyses were performed using validated TaqMan® custom arrays on skeletal muscle biopsies of 72 age-matched subjects who were receiving statin therapy (n=38), who had discontinued therapy due to statin-associated myopathy (n=14), and who had never undergone statin treatment (n=20). In structurally intact skeletal muscle, obtained from statin-treated, non-myopathic patients, statins cause extensive changes in the expression of genes of the calcium regulatory and the membrane repair machinery, whereas the expression of genes responsible for mitochondrial function or myocyte remodelling was unaffected. Discontinuation of treatment due to myopathic symptoms led to a normalization of gene expression levels, the genes encoding the ryanodine receptor 3, calpain 3 and dystrophin being the most notable exceptions.
Hence, even in clinically asyptomatic (non-myopathic) patients, statin therapy leads to an upregulation in the expression of genes that are concerned with skeletal muscle function and membrane repair.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:26

Titel: Are there anatomical adaptations to bipedalism in the human lumbar perivertebral musculature?

Autoren: Hesse B.(1),Schilling N.(1),Fischer M.(1),Fröber R.(2),

Adressen:(1) Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum|Friedrich-Schiller-Universität|Jena|Germany; email:bettina.hesse@uni-jena.de; (2) Institut für Anatomie I|Friedrich-Schiller-Universität|Jena|Germany

Abstract:
Purpose: Human bipedalism is characterised by a permanent upright trunk posture as well as tilting motions contributing to body propulsion. Therefore, the demands on the back musculature differ between humans and quadrupedal mammals. Because the topography of the lumbar perivertebral muscles as well as their innervation and activation patterns are surprisingly similar in humans and quadrupedal mammals, we explored other potential adaptations of the muscles to human bipedalism.
Methods: 1) We examined the anatomical cross sectional areas (ACSA) of the muscles of 5 humans and 17 species of quadrupedal mammals. 2) We investigated the distribution pattern of the two main muscle fibre types over the muscular cross section in three male donated bodies in comparison to different quadrupedal mammals using immune-histochemistry.
Results and Discussion: 1) Except from the lateral dorsovertebral tract, no difference in the ACSA of humans and quadrupedal mammals was observed. Surprisingly, the ACSA of the lateral tract in humans was more comparable to other quadrupedal non-primates and monkeys than to other great apes. 2) In contrast, the fibre type distribution pattern showed clear differences in humans compared to quadrupedal mammals, but was to some extend similar to the pattern of the other great apes.
Conclusions: Adaptations to the upright trunk posture and hence to bipedalism are found in the human lumbar perivertebral musclesin in part at the cellular level but not in gross anatomy.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:27

Titel: Trabecular bone architecture in human thoracic vertebrae – a microct-study

Autoren: Doberauer J.(1),Wurzinger L.(1),Müller-Gerbl M.(2),Putz R.(1),

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Abstract:
Purpose: Later stages of osteoporosis are often complicated by vertebral fractures. In order to improve prevention as well as select the most suitable surgical treatment, a better understanding of the local mechanical situation is needed. Therefore, our study aimed at investigating regional differences in the trabecular structure inside thoracic vertebral bodies. Due to the kyphotic shape of the thoracic spine we expect an adaptation to higher mechanical stress in ventral regions of the vertebral bodies.
Methods: We used four female cadavers (aged 57-80, average 64). Four thoracic vertebrae (T2, T5, T8, T11) of each spinal column were prepared for micro-tomographic imaging. Following structure parameters of the cancellous bone were assessed: bone-volume-fraction, connectivity density, structure-model-index, trabecular number, trabecular separation, trabecular thickness, and degree of anisotropy.
Results: Five structural parameters showed clearly perceptible tendencies from cranial to caudal. Resultively, Conn.D., SMI, and Tb.N. showed high values near the endplates, decreasing towards the midtransverse section. In reverse, Tb.Th. and Tb.Sp. were low near the endplates, increasing towards the midtransverse section. BV/TV, Conn.D. and Tb.N. were higher in the dorsal regions than in the ventral ones.
Conclusions: Our analysis showed clear patterns of distribution of the trabecular structure in all thoracic vertebral bodies we investigated. The dorsal zones of the vertebral bodies showed a structure substantially more capable of withstanding compressive strength than other regions. Against our hypothesis we have to interpret these findings as an adaptation to higher load transmission by the dorsal regions of the adjacent vertebrae.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:28

Titel: The occipital somatic lateral plate mesoderm – a novel source for vertebrate neck musculature

Autoren: Theis S.(1),Valasek P.(2),Otto A.(2),Harel I.(3),Tzhor E.(3),Tajbakhsh S.(4),Christ B.(1),Huang R.(5),Patel K.(2),

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Abstract:
The cucullaris muscle of birds and its mammalian homologues the trapezius and sternocleidomastoid muscles enable an extensive range of head movements. Their origin and development remains controversial. We show for the first time that not only does the somatopleure at the occipital level have myogenic properties but also that it is the source of the musculature of the cucullaris, a muscle previously believed to be of somitic origin. We present molecular and genetic evidence to show that the muscle is not only unique in its origin, but additionally that its temporal development is distinct; forming later than any other muscle group described to date. At the molecular level, we show that the cucullaris muscle and its mammalian homologues, which are found in the body of the animal, develop like a head muscle rather than deploying the programme used by the trunk muscles. In concordance with this conclusion, we present experimental and genetic evidence demonstrating that they contain connective tissue that is neural crest in origin. Finally, we provide evidence that the mechanism by which the neck muscle develops is conserved in vertebrates.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:29

Titel: Ectodermal wnt6 is an early negative regulator of limb chondrogenesis in the chicken embryo

Autoren: Geetha-Loganathan P.(2),Nimmagadda S.(2),Christ B.(3),Huang R.(4), Scaal M.(1)

Adressen: (1) Dept. of Molecular Embryology|Institute of Anatomy and Cell Biology, University of Freiburg|Freiburg|Germany; email:martin.scaal@anat.uni-freiburg.de; (2) Department of Oral Health Sciences|Life Sciences Institute, University of British Columbia|Vancouver|Canada; (3) Dept. of Molecular Embryology|Institute of Anatomy and Cell Biology|Freiburg|Germany; (4) Dept. of Neuroanatomy|Insitute of Anatomy|Bonn|Germany

Abstract:
Pattern formation of the limb skeleton is regulated by a complex interplay of signaling centers located in the ectodermal sheath and mesenchymal core of the limb anlagen, which results, in the forelimb, in the coordinate array of humerus, radius, ulna, carpals, metacarpals and digits. Much less understood is why skeletal elements form only in the central mesenchyme of the limb, whereas muscle anlagen develop in the peripheral mesenchyme ensheathing the chondrogenic center. Here, we present evidence that in the chicken embryo, ectodermal signals inhibit limb chondrogenesis at an early stage upstream of Sox9, thus restricting skeletal development to the central core of the limb mesenchyme. We identify Wnt6 as a candidate mediator of ectodermal inhibition of chondrogenesis in vivo. Our results argue for a role of ectodermal Wnt signaling in centripetal limb pattern formation.

Kategorie: Lecture

Rubrik: 9.Developmental Biology
Abstract Nr.:30

Titel: Vegf is important for the early vascularization of the long bone epiphysis

Autoren: Allerstorfer D.(1),Michael B.(1),

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Abstract:
Purpose: To evaluate the spatio-temporal distribution of two angiogenic cytokines, Vascular Endothelial Growth Factor (VEGF) and Secretoneurin (SN), during early bone development in the epiphysis. Furthermore, the moment of mineralization of the cartilage matrix and the fate of hypertrophic chondrocytes were examined.
Methods: Tissue samples from postnatal mice aged D4 – D20 were used. VEGF and SN protein was localized via immunohistochemical staining. In-situ-hybridization was performed to detect expression of VEGF-A mRNA. In addition, ultrastructure and histochemistry (von Kossa staining) were carried out.
Results: At D4 the presence of both VEGF on mRNA and protein was detected in the perichondrium and in certain resting chondrocytes below it. By D6 chondrocytes adjacent to the invading cartilage canal were VEGF positive and this result was supported by mRNA expression of VEGF in the corresponding area. Findings also correlate at D20 were both the protein as well as the molecular transcript could be found in the hypertrophic as well as the resting chondrocytes surrounding the SOC. Surprisingly there was no staining of SN at any moment. Hypertrophy of the chondrocytes was first detected 7 days after birth and this was accompanied by the mineralization of the cartilage matrix.
Conclusions: We conclude that VEGF is the critical molecule for the generation of an early vascular network in the epiphysis of murine long bones. SN which is considered to induce postnatal vasculogenesis does not play a role in this event. Hypertrophic chondrocytes undergo cell death by a mechanism interpreted as chondroptosis.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:31

Titel: Hrem for visualizing gene expression patterns in early embryos

Autoren: Geyer S.(1),Chipman A.(2),Rasskin-Gutman D.(3),Weninger W.(1),

Adressen: (1) Center for Anatomy and Cell Biology|Medical University of Vienna|Vienna|Austria; email:stefan.geyer@meduniwien.ac.at; (2) Alexander Silberman Inst. of Life Sci.|The Hebrew University of Jerusalem|Jerusalem|Israel; (3) Inst. Cavanilles for Biodiversity and Evolutionary Biology|University of Valencia|Valencia|Spain

Abstract:
Purpose: We aimed at exploring the capacities of the high resolution episcopic microscopy (HREM) technique for generating three dimensional (3D) computer representations of gene expression patterns in the context of the tissues of early embryos of various species.
Methods: We explored the capacities of the HREM technique to examine 3D expression patterns of mlc2a, krp, hunchback, QIK, Tbx5, and Nkx2.5 mRNAs in whole mount stained mouse, chick, zebrafish, and milkweed bug embryos of various developmental stages. The specimens were processed according to standard HREM-data generation protocols. Digital volume data, consisting of 1 000 to 2 000 single sections were created within 4-8 hours in a highly automated way. Their voxel sizes ranged between 0.54x0.54x1.5 µm3 and 3x3x3 µm3. The software Amira® was used for 3D visualization and 3D analysis.
Results: Comprehensive comparisons of our 3D models with photographs of the whole mount stained specimens prior to embedding and with histological sections demonstrate that HREM is capable of generating authentic digital volume data. In contrast to histological sections and to whole mount examinations with the aid of dissection microscopes, HREM data reveal the true 3D arrangement of gene expression patterns in the context of embryonic tissues.
Conclusions: Our results show that HREM allows for systematic comparisons of normal and abnormal embryo anatomy and of normal and abnormal gene expression patterns. Thus it recommends itself as a tool for researching the evolution of development as well as for researching the etiology of congenital malformations in humans.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:32

Titel: Endogenous anti-lymphangiogenesis

Autoren: Wilting J.(1),Becker J.(1),Pavlakovic H.(1),Albuquerque R.(2),Weich H.(3),Ambati J.(2),

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Abstract:
Purpose: A number of years ago, endogenous pro-lymphangiogenic factors have been detected. These are Vascular Endothelial Growth Factor-C (VEGF-C) and VEGF-D. They bind and activate VEGF receptor (R)-3, which is a transmembrane receptors of lymphatic endothelial cells (LECs). The proteolytically processed form of VEGF-C also possesses affinity, though weaker, to VEGFR-2, which also is a transmembrane receptors of LECs. Endogenous anti-lymphangiogenic factors have not been detected yet.
Methods: Cloning of sVEGFR-2, production of antibodies, immunostaining, transgenic mouse models, cell culture, real-time RT-PCR, immunoprecipitation, proliferation assays.
Results and Conclusions: Here we describe a secreted (soluble) splice variant of VEGFR-2, called sVEGFR-2, which is produced by various cell types, e.g. in the cornea and in the epidermis. The knock-out of sVEGFR-2 induces i) ingrowth of lymphatics into the cornea of early postnatal mice and ii) hyperplasia of dermal lymphatics. The sVEGFR-2 binds VEGF-C but not VEGF-A, inhibits VEGF-C-induced activation of VEGFR-3 and reduces proliferation of lymphangioma-derived LECs. Down-regulation of sVEGFR-2 in progressed neuroblatoma, which is characterized by lymphogenic spread of tumor cells, indicates a function for tumor lymphangiogenesis and lymphogenic metastasis. We present the first endogenous anti-lymphangiogenic factor and correlate its loss-of-function with lymphangioma and lymphogenic spread of tumor cells.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:33

Titel: Intrinsic patterning properties of the neural tube regulating the nerve root pattern of the branchiomotor nerve

Autoren: Zhongtian Bai[1, 2], Qin Pu[2], Michael Hans[4], Dieter Swandulla[4], Jianlin Wang[1], Ruijin Huang [2, 3]

Adressen: [1] MOE Key Laboratory of Arid and Grassland Ecology, School of Life Science, Lanzhou University, Lanzhou, Gansu 730000, China, [2] Department of Neuroanatomy, Institute of Anatomy, University of Bonn, Nussallee 10, 53115 Bonn, Germanym, [3] Department of Molecular Embryology, Institute of Anatomy and Cell Biology, Albert-Ludwig-University Freiburg, P.O. Box 111, D-79104, Freiburg, Germany, [4] Institute of Physiology II, University of Bonn, 53111 Bonn, Germany

Abstract:
Nerve exit points have been shown to be a key guidepost to regulate cranial motor axon projection. Branchiomotor neurons migrate dorsally and send their axons to exit points formed by late migrating neural crest cells. However, there is only little evidence implementing the role of the neuroepithelium in the patterning of the branchiomotor nerve. In this study, we investigated whether the neuroepithelium of the rhombomere or the motor neuron determine the nerve root pattern, by means of neural tube transplantation in the avian embryo. To change the axial position of motor neurons, we transplanted the ventral neural tube from rhombomere 8 (r8) to trunk levels and vice versa. We found that the transplantation of neurons of the accessory nerve was not able to induce an accessory specific pattern in the trunk region. The graft of the dorsal neural tube from the trunk level into r8 disturbed the accessory pattern in the occipital region. We furthermore perfomed transplantations between rhombomeres. Grafting of the dorsal neural tube from r8 to r2-r5 showed that the trigeminal and facial neural axons straightly extend along the neural tube without outgrowing to the first and second branchial arches. In contrast, the dorsal neural tube grafted from r2-5 to r8 allowed accessory axons to grow vertically into the periphery. Our results suggest that the nerve root pattern of branchiomotor neurons lies within the neuroepithelium itself.

Kategorie: Lecture

Rubrik: 8.Cell Biology
Abstract Nr.:34

Titel: New insight into the role of prp in fracture healing

Autoren: Tohidnezhad M.(1),Breuer F.(1),Beckmann R.(1),Lippross S.(2),Varoga D.(2),Bornemann J.(3),Bovi M.(3),Herrmanns Sachweh B.(4),Wruck C.(1),pufe T.(1),

Adressen: (1) Anatomy und Cellbiology|RWTH|Aachen|Germany; email:mtohidnezhad@ukaachen.de; (2) Trauma surgery|UKSH Campus Kiel|Kiel|Germany; (3) Pathology|RWTH|Aachen|Germany; (4) Pathology|RWTH|Aachen|g

Abstract:
Purpose: Oxidative stress is detrimental to cellular health and can harm cells. Expression of genes, which reduce cellular stress, protect cells from oxidative damage. Transcription of these enzymes is regulated through antioxidant response element (ARE). NF-E2-related factor 2 (Nrf2) plays a major role in transcriptional activation of ARE-driven genes. Platelet rich on growth factors (PRGF) is a mixture of autologous proteins and growth factors, prepared from a determined volume of Platelet rich plasma (PRP) and can be used in in vitro experiments. Platelate concentrates are used for various surgical procedures and have a beneficial effect on fracture healing. The exact mode of action of PRP is not investigated so far.
The aim of the study was to elucidate if the platelets are able to regulate the activity of ARE and expression of Nrf2 in an in vitro model of fracture healing.
Methods: Platelets and subsequently PRGF were obtained from healthy human donors.
SAOS-2, humane osteoblasts cell line, were used for this study. A luciferase reporter plasmid containing an ARE-side regulating the firefly luciferase reporter gene was used for Dual Luciferase assays in order to determine activity of ARE. The amount of nuclear translocated Nrf2 was measured by Western blotting.
Rsults: In this study we could demonstrate that PRGF regulates the activity of Nrf2 and leads to an upregulation of antioxidative Enzymes, in Osteoblasts.
Conclusions: Nrf2-activation may has an beneficial effect on fracture healing via defending oxidative stress.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:35

Titel:Inhibition of cgmp-specific pde5 induces relaxation of the epididymal duct

Autoren: Mietens A.(1),Feuerstacke C.(1),Tasch S.(1),Müller D.(1),Middendorff R.(2),

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Abstract:
Purpose: Contraction and relaxation of the epididymal duct appears to be a finely regulated process ensuring transport and maturation of spermatozoa. Components of cGMP-related signalling pathways have been shown to modulate epididymal contractility, but the occurrence and role of cGMP-specific phosphodiesterase 5 (PDE5) in epididymal tissue is unclear. The use of the PDE5-specific inhibitor sildenafil for the treatment of pulmonary hypertension exposes a growing number of young patients to chronic PDE5 inhibition and the issue of possible side effects related to epididymal function and male fertility needs to be addressed.
This investigation specifies occurrence and role of PDE5 in epididymal tissue.
Methods: PDE5 expression was analyzed in human and rat tissue using laser capture microdissection (LCM)-assisted RT-PCR, Western blot and immunohistochemistry techniques, the functional role of PDE5 was investigated by organ bath studies.
Results: PDE5 was detected at mRNA and protein level and localized to contractile cells of the epididymis. Staining was observed in peritubular and vascular smooth muscle cells. Organ bath studies confirmed a functional role for PDE5 in regulating epididymal duct contractility. Sildenafil elicited relaxation of spontaneous and norepinephrine-induced contractions of epididymal duct segments.
Conclusion: cGMP-specific PDE5 participates in the regulation of epididymal duct contractility and may thus influence transport and maturation of spermatozoa suggesting a role for cGMP-related pathways in the context of male fertility. Moreover, sildenafil, a competitive inhibitor of PDE5, could unfold unknown effects or side effects within the epididymis.
(KFO 181)

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:36

Titel: Localization and function of carbonic anhydrase 2 in the murine male reproductive tract

Autoren: Wandernoth P.(1),Mannowetz N.(1),Raubuch M.(1),Wennemuth G.(1),

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Abstract:
Cytosolic carbonic anhydrase II (CA II) catalyzes the reversible reaction of carbon dioxide into bicarbonate, this regulating the intracellular bicarbonate concentration. An increase in bicarbonate in sperm induces early activation, which is established by an enhanced beat frequency. We demonstrate that CA II is one way to generate bicarbonate, which, in turn, stimulates the sperm-specific soluble adenylyl cyclase (sAC) to produce high levels of cAMP, which initiate essential pathways, such as early sperm activation and protein phosphorylation.
The presence of CA II in the epididmal duct and in sperm was shown by immunoblotting and immunohistochemical techniques. CA II is located in epithelial cells lining the epididymal duct and also in sperm. Functional tests to assess the flagella beat frequency of wt and CA II ko sperm were carried out with CASA measurements and waveform analysis. CASA measurements show that the average velocity of ko sperm is reduced by 52%. Also the fast linear progressive motility shows a significant decrease by 45%. With waveform analysis of CA II ko sperm, we show a reduced carbon dioxide induced acceleration of sperm beat frequency. KO sperm reach a maximum beat frequency of 5.01±0.54 Hz after 40 sec treatment with 2% carbon dioxide only. In comparison, hereto wt sperm reach a maximum of 6.63±0.6 Hz. These results suggest that CA II is involved in early activation of spermatozoa.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:37

Titel: The effect of a sertoli cell-specific knockout of connexin 43 on testicular gene expression in prepubertal mice

Autoren: Giese S.(1),Hossain H.(2),Brehm R.(3),Bergmann M.(1),Tchatalbachev S.(2),Izar B.(2),Chakraborty T.(2),Guillou F.(4),Willecke K.(5),

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Abstract:
Purpose: Comparison of the gene expression pattern between homozygous Sertoli cell-specific Connexin43 knockout mice (SCCx43KO) and wildtype (WT) mice together with the initiation of spermatogenesis on day 8 post partum (pp), identification and analysis of candidate genes to explain the observed altered testicular phenotype in adult KO mice.
Methods: For comparison of mRNA expression, a CodeLink Whole Genome Bioarray was applied. Normalisation and statisical evaluation of data sets were performed. Results of the microarray analysis were validated using Real-Time PCR. Immunohistochemistry (IHC) and immunofluorescence (IF) were used to investigate possible alterations at protein level.
Results: Microarray analysis revealed 301 significantly regulated genes. 250 of these genes were down- and 51 were up-regulated. Among these genes some are known to play essential roles in the onset and maintenance of spermatogenesis as Stra8 and Dazl, established as germ cell-specific genes and required for progression through meiosis. Real-Time PCR confirmed the microarray data for selected genes as did IHC and IF.
Conclusions: Our data show that the deletion of Cx43 in Sertoli cells leads to an alteration of gene expression as early as day 8 pp., probably due to impaired Sertoli cell-germ cell crosstalk.

Kategorie: Lecture

Rubrik: 10.Reproductive Biology
Abstract Nr.:38

Titel: Distribution of basigin (cd147) and monocarboxylate transporters in the male reproductive tract

Autoren: Mannowetz N.(1),Raubuch M.(1),Wandernoth P.(1),Wennemuth G.(1),

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Abstract:
Lactate is an essential substrate for both germ cell differentiation and sustained motility of mature spermatozoa. During spermatogenesis, Sertoli cells provide L-lactate, which in turn is taken up by spermatogenic cells. Transport of lactate anions and protons is driven by monocarboxylate transporters (MCTs) in an electroneutral manner. Basigin (CD147) was found to act as a chaperone for MCTs in rat erythrocytes. For germ cell differentiation, this protein is of importance because basigin-/- mice are infertile due to an arrested spermatogenesis. We focus on both the distribution of CD147, MCT1 and MCT2 in the male reproductive tract, the interactions of basigin with MCT isoforms and the changes in pHi through stimulation of sperm with different isomers of lactate. We show that both MCTs and basigin are present in the sperm tail, with basigin additionally localized in elongating spermatids. In epididymal sperm, basigin co-immunoprecipitates both MCT isoforms and co-localizes with MCT1 in the acrosome of corpus and cauda sperm and with MCT2 in the principal piece of caput sperm. pHi measurements elucidate that the decrease is more pronounced with L-lactate than with D-lactate (0.2 vs. 0.08 units). Half-time analysis of the decrease during stimulation with lactate results in 92.5±2 s (L-lactate) and 167.3±54.8 s (D-lactate). During recovery, the mean half-time values are 119.8±9.8 s (L-lactate) and 39.9±2.7 s (D-lactate). Our results suggest that in mature spermatozoa, basigin interacts with MCTs, which are more involved in the transport of L-lactate than D-lactate.

Kategorie: Lecture

Rubrik: 10.Reproductive Biology
Abstract Nr.:39

Titel: The oxldl-dependent responses in granulosa cell subtypes is cell type-specific in respect to specific lipoprotein receptors and antioxidant activity

Autoren: Serke H.(1),Bausenwein J.(1),Hirrlinger J.(2),Vilser C.(1),Jogschies P.(3),Hmeidan F.(3),Blumenauer V.(3),Spanel-Borowski K.(1),

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Abstract:
The response of human granulosa cells from different origins on oxidative stress is still unknown. We currently investigated the oxLDL-induced and stress-related responses of granulosa cell subtypes by studying specific lipoprotein-receptors and antioxidants. A possible relationship between cell death and enzyme activity was also validated for supernatants. Cultures of cytokeratin-positive (CK+) and -negative (CK-) granulosa cells and of cumulus cells taken from women undergoing in vitro fertilization (IVF) therapy were treated with 150 µg/ml oxLDL or native LDL (nLDL) under serum-free conditions for 36 h. The percentage of dead cells was determined by uptake of propidium-iodide. The protein expression levels in lysates were examined by Western blots for lectin-like oxidised low-density lipoprotein-receptor-1 (LOX-1), toll-like receptor 4 (TLR4), and CD36. The activities of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) were determined in lysates and in cell-conditioned supernatants. The oxLDL-dependent increase of lipoprotein-receptors and of antioxidant activity was cell type-specific: TLR4 in CK+ cells, LOX-1 in CK- cells, and CD36 in cumulus cells. This finding was associated with a cell-specific antioxidant ranking in lysates: SOD activity in CK+ cells, total glutathione in CK- cells, and catalase activity in cumulus cells. In supernatants, oxLDL-treated CK+ cell cultures showed a significant increase of catalase activity, whereas a moderate increase was noted for GPx in CK- and cumulus cells. We conclude that the increase of catalase/GPx activity in the supernatant may be to be due to cell death or to secretion (supported by the DFG Sp232/12-1).

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:40

Titel: Maternal diabetes influences the igf system in the preimplantation rabbit blastocyst

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

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Abstract:
In humans, type 1 or insulin-dependent diabetes has been found to negatively affect pregnancy by causing early miscarriage and poor prenatal outcomes. Also diabetogenous embryopathies are known complications of diabetes during pregnancy. The reasons are not fully understood. We established a rabbit model for diabetes mellitus chemically induced by the agent alloxan and have analysed reproductive and developmental effects during early pregnancy. The alloxan treatment results in a complete loss of endogenous insulin (hypoinsulinaemia) followed by elevated blood glucose levels about 14mmol/L (hyperglycaemia).
The focus of current report lies on diabetogenous effects on the maternal and embryonic insulin/insulin growth factor (IGF) receptor system during the preimplantation period. The expression and activation of the IGF ligands and receptors were characterised by quantitative PCR, Western blot and phosphorylation assays for receptor signalling. In the uteri and blastocysts from diabetic females the expression pattern of IGF1, 2, insulin receptor (InsR), IGF1 and IGF2 receptor was distinctly different compared with normoglycaemic controls. While maternal insulin concentration was decreased, both IGF1 and 2 RNA were increased in diabetic uteri and blastocysts. Contrary to the ligands the expression of the InsR and IGF1R were downregulated in blastocysts from diabetic females. The downregulation of both receptors led to reduced phosphorylation of the downstream kinases (Akt and Erk), indicating a loss in IGF growth factor sensitivity. The dysfunction of the embryonic IGF system may potentially be involved in the mechanism of diabetogenous subfertility and embryopathies.
Supported by the German Research Council (DFG; NA 418/4-2)

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:41

Titel: Activation of nrf2/are pathway by vegf in the human trophoblastic bewo cell line.

Autoren: Kweider N.(1),Rosen C.(1),Fragoulis A.(1),Tohidnezhad M.(1),Brandenburg L.(1),Pufe T.(1),Wruck C.(1),

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Abstract:
Purpose: Preeclampsia (PE) is a multisystem disorder peculiar to human pregnancy, characterised by widespread endothelial damage. Increasing evidence suggests that oxidative stress may play a key role in the aetiology of PE. Recently, we have shown that PE is associated with increased NF-E2 related factor 2 (Nrf2) activity within cytotrophoblast. Nrf2 controls the cellular defence against oxidative stress via binding of the antioxidant response element (ARE) within the promoter region of genes coding for anti-oxidative enzymes like Heme oxygenase 1. Also the expression of VEGF is significantly higher in Placenta biopsies with Preeclampsia. An interrelation is both factors are not examined.
Methods: Quantitative analysis was performed to measure mRNA levels (RT-PCR) and protein levels (Western blot) in VEGF stimulated BeWo cells. WST assays were performed to measured cell-viability and ARE-Luciferase assays to quantify Nrf2-activty.
Results: We showed an Nrf2-activation after VEGF treatment in BeWo cells. These activation leads to an up-regulation of Nrf2-target genes like Heme oxygenase 1.
Conclusion: We propose a direct relationship between VEGF expression and the defence against oxidative stress via Nrf2 activation. This is somewhat surprising because Nrf2-activity is known to be regulated in response to oxidative stress. VEGF mediated up-regulation of Nrf2-activity is therefore rather a prevention of oxidative stress than a reaction on it.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:42

Titel: Growth hormone (gh) differentially acts on the gh/igf-system in adult tilapia immune organs

Autoren: Shved N.(1),Berishvili G.(2),Baroiller J.(3),Mazel P.(2),Reinecke M.(1),Eppler E.(2),

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Abstract:
In mammals, the GH/IGF-I and the immune system have been shown to interact. The stimulatory significance of GH for the immune system is indicated by the presence of the GH-receptor (GHR) and IGF-I. However, little is known on their local regulation in immune organs. There is evidence for interactions also in bony fish since the GHR, IGF-I and IGF-II are also found in different fish species. Knowledge on immune modulation is important for aquaculture and sustainment of wild-life fish. To investigate the local regulation of IGF-I, IGF-II and GHR by GH in spleen and head kidney, we challenged adult tilapia with intraperitoneal GH injections and measured the expression of hepatic GHR and IGF-I and IGF-I in serum as indicators for GH action. GHR mRNA was downregulated and IGF-I mRNA elevated as well as IGF-I serum levels in the GH-injected fish. IGF-I gene expression in head kidney was raised, even stronger than in liver. In accordance, using in situ hybridization, more head kidney macrophages and leukocytes expressed IGF-I mRNA in GH-treated than control fish. In contrast, in spleen IGF-II mRNA but not IGF-I mRNA was elevated. Thus, head kidney and spleen reacted differently to GH treatment indicating different roles of GH and the IGFs in growth, differentiation and sustainment in the immune system of the tilapia. Whether this also has implications for immune response demands for further studies using bacteria and other pathogens.

Supported by Swiss National Foundation and Hartmann Müller-Foundation for Medical Research.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:43

Titel: Sulforaphane suppresses inflammation and induces apoptosis in tnfa stimulated synoviocytes

Autoren: Laufs J.(1),Tohidnezhad M.(1),Brandenburg L.(1),Varoga D.(2),Pufe T.(1),Wruck C.(1),

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Abstract:
Rheumatoid arthritis (RA) is characterized by progressive inflammation associated with rampantly proliferating synoviocytes. In this study, we investigated whether sulforaphane (SF), a naturally occurring isothiocyanate, could inhibit the inflammatory process and the synovial hyperplasia in an in vitro model of RA.
Cultured Synoviocytes were stimulated with TNFa with or without SF pre-treatment. The activity of NFkB and AP 1 were investigated via Dual-Luciferase promoter-assays. Cytokine secretion levels were detected using ELISA. Cell proliferation of synoviocytes was determined by CyQuant assay and apoptosis by DNA-laddering. Both TNFa induced NFkB and AP 1 activation and expression of Interleukin (IL)-6 and IL-1 were reduced by SF in a dose-dependent manner. In addition, SF could significantly inhibit proliferation and induce apoptosis only in TNFa stimulated but not in unstimulated synoviocytes.
Our results demonstrate that SF has antiphlogistic properties and it may inhibit pannus formation in RA. All these properties make SF to a promising lead structure for the development of a novel therapeutic agent for rheumatoid disorders.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:44

Titel: Nrf2 induces il-6 expression via an antioxidant response element within the il-6 promoter

Autoren: Wruck C.(1),Tohidnezhad M.(1),Brandenburg L.(1),Varoga D.(2),Kan Y.(3),Eickelberg O.(4),Pufe T.(1),

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Abstract:
IL-6 gene expression is controlled by a promoter-region containing multiple regulatory elements such as NF-kB, NF-IL6, CRE, GRE and TRE. In this study, we demonstrated that TRE, found within the IL-6 promoter, is embedded in a functional antioxidant response element (ARE) matching an entire ARE consensus sequence. Further, point mutations of the ARE consensus sequence in the IL-6 promoter construct selectively eliminate ARE but not TRE activity. Nrf2 is a redox-sensitive transcription factor which provides cytoprotection against electrophilic and oxidative stress and is the most potent activator of ARE-dependent transcription. Using Nrf2 knockout mice we demonstrated that Nrf2 is a potent activator of IL-6 gene transcription in vivo. Our findings suggest a possible role of IL-6 in oxidative stress defence and also give indication about an important function for Nrf2 in the regulation of hematopoietic and inflammatory processes.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:51

Titel:T he activation pattern of human natural killer (nk) cells is modulated by adipokines

Autoren: Hübner L.(1)

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Abstract:
Purpose: The evaluation of the number and activation pattern of human NK cells by supernatant adipokines of 3T3L1 adipocytes.
Methods: Investigations were performed by fluorescence activated cell sorting after challenging with different dosages of adipocyte supernatant. After 24h of stimulation the number of NK cell subsets and leptin receptor (Ob-R) positive cells was evaluated. Furthermore, the expression of TRAIL (TNF-related apoptosis-inducing ligand), intracellular granzyme A and Interferon-gamma was measured. Finally, the ability of NK cells to form conjugates with tumour cells was determined.
Results: The number of NK cells was not significantly altered by the challenge with adipokines. Interestingly, the CD56bright (cytokine-expressing NK cells) and the CD56dim (cytolytic NK cells) reacted differently towards the challenge: The TRAIL expression of CD56bright was significantly higher after adipokine stimulation as compared to CD56dim NK cells. Furthermore, numbers of Ob-R expressing cells were significantly higher in the CD56bright subset group.
Conclusions: Our results show that, effects of adipokine stimulation of human NK cells differ in a dose- and subset-specific manner. As NK cells are an integral component of the innate immune system to regulate tumour progression and metastasis, the present data support a relationship between adipose tissue and immune cells.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:52

Titel: Genetic disruption of nrf2 impairs articular destruction in a mouse model of rheumatoid arthritis

Autoren: Fragoulis A.(1),Gurzynski A.(2),Varoga D.(3),Brandenburg L.(1),Pufe T.(1),Wruck C.(1),

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Abstract:
Objectives. Increasing evidence suggests that oxidative stress may play a key role in joint destruction due to rheumatoid arthritis (RA). The aim of this study was to elucidate the role of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that maintains the cellular defence against oxidative stress.
Methods. RA was induced in mice lacking the gene for Nrf2 (Nrf2-KO) and in wild-type control (Nrf2-WT) mice. The severity of cartilage destruction was evaluated by histopathology of HE-staining. The extent of oxidative stress in the joints was measured via TBARS-assay and 4HNE-immunohistochemistry. Nrf2-activation was analyzed in synovial explants from healthy donors and patients suffering from RA. We used a Xenogen-imaging-system to measure Nrf2-activity in an ARE-luciferase transgenic mouse during RA.
Results. Nrf2 was significantly higher expressed and active in the joints of arthritic mice, as well as in the joints of RA patients compared to control groups. The histopathology of RA included erosion of the cartilage at the joint margins. Nrf2-KO mice suffer higher rates of clinical signs and more severe knee and paw injury as compared to Nrf2-WT. The degree of oxidative damage due to RA was significantly higher in Nrf2-KO mice than in wild-type littermates, as indicated by elevated malondialdehyde levels.
Conclusion. These data demonstrate that Nrf2 exerts a protective role against oxidative stress during antibody-induced arthritis. These finding indicate that the use of Nrf2-activators might be considered as an adjunct therapeutic strategy to combat joint destruction in rheumatoid arthritis.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:53

Titel: Involvement of scavenger receptor marco and formyl peptide receptor like-1 in neisseria meningitidis-induced antimicrobial peptide rcramp expression by glial cells

Autoren: Brandenburg L.(1),Braun B.(1),Leib S.(2),Jansen S.(1),Lucius R.(3),Wruck C.(1),Pufe T.(1),

Adressen: (1) Department of Anatomy and Cell Biology|RWTH Aachen University|Aachen|Germany; email:lbrandenburg@ukaachen.de; (2) Institute for Infectious Diseases|University Bern|Bern|Switzerland; (3) Department of Anatomy|Christian-Albrechts-University|Kiel|Germany

Abstract:
Recent studies have suggested that the scavenger receptor MARCO (Macrophage receptor with collagenous structure) mediate activation of the immune response after infection of the central nervous system (CNS) with Neisseria meningitidis (NM). We could show that NM induced antimicrobial peptide rCRAMP expression and MARCO interacts with the chemotactic G-Protein-coupled receptor (GPCR), formyl-peptide-receptor-like-1 (FPRL1) in glial cells.
Using a rat meningitis model, we investigated the influence of MARCO and FPRL1 for the immune response in form of rCRAMP expression of the CNS to NM infection in glial cells. We therefore analyzed the FPRL1 and MARCO expression by immunofluorescence and real-time RT-PCR in the rat meningitis model. Furthermore we examined the receptor involvement by real-time RT-PCR, extracellular-signal regulated kinases 1/2 (ERK1/2) phosphorylation and cAMP level measurement in glial cells (astrocytes and microglia) and transfected HEK293 cells using receptor deactivation by antagonists.
Receptor deactivation by antagonists verified the importance of MARCO for NM-induced rCRAMP expression in glial cells. Furthermore, we demonstrated a functional interaction between FPRL1 and MARCO in NM-induced signalling by real-time RT-PCR, ERK1/2 phosphorylation and cAMP level measurement. In addition, we show an NM-induced increase of receptor expression by immunofluorescence and real-time RT-PCR.
We propose that the induction of MARCO and FPRL1 by innate immune stimulation enhances recognition and uptake of pathogenic organisms such as NM, thus contributing to host defence against infection.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:54

Titel: Cxcr7 is a major component of sdf-1 signalling in astrocytes

Autoren: Ödemis V.(1),Engele J.(1),

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Abstract:
It was originally assumed that the chemokine, stromal cell–derived factor 1 (SDF-1/CXCL12), would exclusively signal through CXCR4. This view was only recently challenged by the identification of CXCR7 as a second SDF-1 receptor, which turned out to affect metastasis, growth and survival of different tumor types. However, studies undertaken so far failed to demonstrate CXCR7-dependent signalling in several cell types, including endothelial cells and lymphocytes. Here, we have analyzed the role of CXCR7 in SDF-1-dependent signalling in astrocytes. We found that cultured cortical astrocytes express CXCR4 and CXCR7 receptors on their cell surface. Stimulation of astrocytes with SDF-1 resulted in the temporary activation of Erk1/2, Akt, and PKCzeta, but not of p38 and PKCalpha. Blocking experiments with either CXCR4 or CXCR7 antagonists inhibited SDF-1 induced signalling pathways. Interestingly, the signalling pattern remained fully inducible in astrocytes derived from a CXCR4-deficient mouse line, but was completely abrogated following depletion of astrocytic CXCR7 by RNAi. Furthermore, we show that the mitogenic effects of SDF-1 on cultured astrocytes are abolished upon depleting CXCR7. Together, these findings identify CXCR7 as an active component of SDF-1 signalling in astrocytes, and hence point to CXCR7 as a putative novel target for cancer therapy.
Supported by the DFG

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:55

Titel: Mp4-, mog:35-55- and plp:178-191-induced experimental autoimmune encephalomyelitis (eae) of c57bl/6 mice – three models, three different patterns of demyelination and axonal damage

Autoren: Kuerten S.(1),Javeri S.(1),Kirch C.(1),Lehmann P.(2),Addicks K.(1),

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Abstract:
Purpose: Myelin and axonal damage are considered main histopathological features of multiple sclerosis (MS). Still, it is unclear how exactly structural damage can account for functional clinical deficits. Here, we systematically study demyelination and axonopathy in MP4-, MOG:35-55- and PLP:178-191-induced EAE of C57BL/6 mice and elucidate how three different neuroantigens can trigger three different patterns of CNS pathology.
Methods: Spinal cord demyelination was evaluated by luxol fast blue and SMI-99/MHC-II immunostaining, axonal damage and motoneuron atrophy by analysis of SMI-32 reactivity. Tract pathology was evaluated on methylene-blue stained semi-thin sections.
Results: While the MP4 model was characterized by persistent demyelination, in MOG:35-55-induced EAE demyelination was transient, PLP:178-191 did not trigger myelin loss. Axonal damage was present in all three models and a possible correlate for the chronic course of EAE. However, evidence for axonal transection was only found in the MP4 and MOG peptide model. Strikingly, in MP4-induced EAE deficits in motor function resided in motor neuron atrophy while in the MOG peptide model pyramidal tract degeneration was the prominent pathological feature. PLP peptide-induced EAE showed both perikaryal changes and fiber loss. Degeneration of sensory tracts was manifest in all models with a general attack on the anterolateral and more gradual changes in the posterior system.
Conclusion: The differential changes in CNS pathology triggered by MP4, MOG:35-55 and PLP:178-191 suggest that these models are powerful tools for reflecting MS heterogeneity, fuelling research as to the mechanisms underlying the complexity of the human disease.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:56

Titel: Deficiency in bdnf alleviates autoimmune encephalomyelitis by affecting peripheral immunity to neuroantigen

Autoren: Javeri S.(1),Rodi M.(1),Lehmann P.(2),Addicks K.(1),Kuerten S.(1),

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Abstract:
Purpose: In previous studies on multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) brain-derived neurotrophic factor (BDNF) has been described to play a conflicting role involving both neuroprotective and neurodestructive effects. Here we investigate the impact of BDNF on clinical outcome, peripheral immune response and CNS pathology in MP4-induced EAE using gene-modified mice as a novel approach. Methods: BDNF-deficient B6.129S4tmJae/J and wild-type mice were immunized with the MBP/PLP fusion protein MP4 and clinical disease outcome was assessed. ELISPOT and ELISA assays determined the antigen-specific cytokine response. CFSE analysis established the proliferative capacity of peripheral immune cells. Employing immunohistological studies CNS inflammation and demyelination were evaluated. Results: BDNF-deficient mice showed a significantly milder disease in terms of clinical onset and disease severity compared to wild-type mice. Remarkably, IFN-gamma, IL-2 and IL-17 producing T cells were present in wild-type, but absent in BDNF-deficient mice. Immunization with ovalbumin triggered a vigorous TH1 response in both groups. The overall proliferation and cytokine secretion patterns upon mitogen stimulation were comparable. On pathological examination inflammatory lesions demyelinative plaques were significantly diminished in BDNF-deficient mice. The cellular composition of infiltrates differed showing less B cells and CD8+ T cells in the BDNF-deficient mice. Conclusion: We show that BDNF influences the EAE on the level of peripheral and central immunity in response to low-affinity self-antigen. Our data do not support BDNF as a neuroprotective factor, but suggest an immunologically more complex and disease enhancing role of BDNF in MS/EAE.

Kategorie: Lecture

Rubrik: 11.Immune Biology
Abstract Nr.:57

Titel: Induction of antimicrobial peptide psoriasin by bacterial components in glial cells

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Abstract:
Induction of antimicrobial peptide Psoriasin by bacterial components in glial cells
Antimicrobial peptides are part of the innate immune system in epithelial and non-epithelial surfaces, and may also have important functions in the brain. However, little is known about the expression of antimicrobial peptides in the CNS and whether glial cells can secrete these peptides. We have used cell cultures, real-time RT-PCR, immunohistochemistry, agar diffu¬sion test, ELISA, Western Blot and an animal model to get more information about the role of antimicrobial peptides in the CNS. In detail, we have investigated the expression of the antimicrobial peptide Psoriasin also known as S100A7, which was first identified as an over-expressed peptide in psoriatic skin, in rat glial cells (astrocytes and microglia) after incubation with bacterial components. Furthermore, we used cerebrospinal fluid (CSF) and serum from patients with bacterial meningitis to detect Psoriasin. Finally, we investigated the occurrence of Psoriasin in an animal model of bacterial meningitis. We demonstrate (i) the detection and the (not only for the first time the expression but also) secretion of biological active Psoriasin in glial cells, and (ii) the occurrence of antimicrobial peptides in the cerebrospinal fluid of meningitis patients. Moreover, we could show an involvement of Psoriasin in the rat meningitis model pointing to a role of Psoriasin in the pathogenesis of this disease. Our results suggest that Psoriasin is an important part of the innate immunity in the brain against bacterial CNS pathogens.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:58

Titel: Local progenitors in the vascular wall: msc-derived pericytes in vascular morphogenesis

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Abstract:
Purpose: Vascular wall-resident mesenchymal stem cells (VW-MSCs) may represent an important source of pericytes/smooth muscle cells during angiogenesis in physiological and pathological processes. The experimental program of these studies aims at better defining vascular wall-resident MSCs and the functional processes of the potential to differentiate into pericytes and SMCs as well as the contribution of the VW-MSCs to vascular injury and vascular remodelling and thereby identifying and validating molecular targets.
Methods and Results: We recently succeeded in isolation of MSC derived from adult human blood vessels and established the cell culture conditions. We applied different cell culture conditions including treatment with growth factors and tumor-derived factors (VEGF, PDGF, TGFbeta) as well as supernatant of cultivated tumor cells in order to asses the angiogenic capacities of the VW-MSCs and to achieve cultivation-induced differentiation into pericytes/SMC. The angiogenic capacity of VW-MSC to form functional, perfused blood vessels in vivo was further addressed in an in vivo endothelial cell xenotransplantation assay employing different combinations of angiogenic cytokines (modified matrigel plug assay).
Conclusions: Our results show that a part of cells derived ofVW-MSCs behave like pericytes. In vitro these cells associate with endothelial cells and form functional blood vessels in vivo. Thus, MSC are good candidates for supplying a reserve function within the vessel wall: MSCs are usually involved in physiological vascular homeostasis, but might also act as a reservoir of undifferentiated cells ready to supply the cellular demands for the local capacity of neovascularization in disease processes.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:59

Titel: A novel truncated ceacam1 form appears due to turnover processes in aging human epithelial and endothelial cells

Autoren: Muturi H.(1),Scheffrahn I.(1),Derya T.(2),Ergün S.(1),Singer B.(1),

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Abstract:
Purpose: Carcinoembroyic antigen-related cell adhesion molecule 1 (CEACAM1) is a cell adhesion receptor molecule, which plays important roles in cell morphogenesis, tumorigenesis, insulin metabolism, T-cell regulation and neovascularisation. Recently, we reported a soluble form of CEACAM1 in human urine. However, the origin of the truncated CEACAM1 remained unclear. Thus we aimed to discover the exact form of truncated CEACAM1 and its cellular origin.
Methods: In human urine we found a variant of CEACAM1 with a molecular weight of 72 kDa. Sandwich-ELISA and western blotting analysis revealed that this novel CEACAM1 variant lacks the N-domain and part of the A2 domain.
Results: Investigating the origin of truncated CEACAM1 revealed that cells expressing CEACAM1 endogenously (A549, T102/3, HT29, AS-M.5) and those stably transfected with CEACAM1 (Hela-CEACAM1) contained both native and truncated variant of CEACAM1. Further studies showed that truncated CEACAM1 appeared not due to differences in glycosylation and was not caused by apoptosis. Furthermore, we found that the truncated form emerged due to CEACAM1 turnover processes in aging, contact inhibited cells. Additional studies revealed that an age-related increase of truncated CEACAM1 could be observed in cell lysates and supernatant of aging cells due to protein turnover. Thus our data showed the likely source of the truncated CEACAM1 variant found in human urine.
Conclusion: Here we show a novel truncated form of CEACAM1 generated during cell aging.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:60

Titel: Curcumin promotes differentiation of mesenchymal stem cells to chondrocytes by suppression of nf-kb in a 3-dimensional co-culture model of osteoarthritis

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Abstract:
Objective:
Articular cartilage contains a population of resident mesenchymal stem cell (MSC)-like progenitors but despite this in osteoarthritis cartilage exhibits a poor regeneration capacity. Recently, we demonstrated that the chondrogenic differentiation of MSCs is enhanced by the phytochemical curcumin. Curcumin inhibits the activation of NF-kappaB and the expression of apoptotic and pro-inflammatory genes in chondrocytes. Therefore, the aim of the present study was to evaluate the influence of curcumin on IL-1beta-induced NF-kappaB signalling pathway in MSCs during chondrogenic differentiation.
Methods:
MSCs were either cultured in a ratio of 1:1 with primary chondrocytes in high-density culture or cultured alone in monolayer with/without curcumin and/or IL-1beta;. The morphology of the high-density co-cultures was evaluated by electron microscopy and the expression of proteins was assessed by western blotting.
Results:
At the ultrastructural level, co-cultures treated with IL-1beta and curcumin, developed into well-organised, cartilage-like structures. In these co-cultures western blotting demonstrated high quantities of collagen type II, CSPGs, integrins, activation of the adaptor protein Shc, of ERK1/2 and of the chondrogenic transcription factor Sox-9. Curcumin inhibited IL-1beta-induced activation of Ikappa-Balpha and NF-kappaB translocation to the nucleus, induction of apoptosis and inflammation in a concentration- and time-dependent fashion in MSCs as it did in chondrocytes.
Conclusion:
Curcumin antagonizes the IL-1beta induced activation of NF-kappaB signalling in MSCs thus promoting chondrogenic differentiation of MSCs in a microenvironment shared with primary chondrocytes in high-density culture. This might be an exciting new therapeutic approach for the prophylactic treatment of OA.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:61

Titel: Pattern of er-specific chaperones in inflamed tissue

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Abstract:
Formation of granulation tissue is crucial for wound closure and part of successful wound healing. The granulation tissue is composed of tissue matrix supporting a variety of cell types, such as fibroblasts, inflammatory cells, and endothelial cells. Cell culture experiments revealed that all these cell types react most sensitive towards severe hypoxia and chemically induced ER-stress by upregulating Mdg1/ERdj4 and BiP/Grp78, chaperones that are located in the endoplasmic reticulum (ER). In order to investigate the involvement of these chaperones in restoration of wounded tissue, we examined human granulation tissue and analysed the expression of the ER-specific chaperones, Mdg1/ERdj4, BiP/Grp78, and Grp94. Immunohistochemical stainings and subsequent quantification revealed similar distribution patterns and significantly elevated protein levels of all three chaperones in fibroblasts, inflammatory, and endothelial cells. Protein levels were low in control tissue. Further investigations of how these chaperones are regulated during wound repair may spur new therapeutic concepts to improve the healing process.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:66

Titel: Mutation of the desmosomal protein desmoglein 2 induces cardiac alterations that are also found in human arrythmogenic right ventricular cardiomyopathy

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Abstract:
Purpose: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is considered to be a disease of the desmosome. To examine the impact of desmosomal cadherin desmoglein 2 (Dsg2) mutations in ARVC pathogenesis, mice harbouring mutant desmoglein 2 gene were generated. The mutated gene lacks exons 4-6 which code for the adhesion-mediating extracellular domains 1 and 2.
Methods: Cardiac morphology, histology and mRNA expression were analysed in homozygous mutated animals, heterozygous and wild type mice at the age of 2, 8 and 12 weeks. To assess cardiac function echocardiography was performed at the age of 8 weeks.
Results: Homozygous mutated animals developed normally, but 2 weeks after birth fibrotic lesions could be detected. Echocardiographic examination revealed that a) both heart chambers were dilated and b) left heart performance was significantly compromised compared to wild type animals. mRNA expression of GDF15, a novel biomarker for impaired heart function, was significantly upregulated in 2, 8 and 12 week old homozygous mutated mice. Furthermore, the mRNA expression of the cardiac stress marker c-myc and the heart failure markers ANF and BNF were significantly upregulated in 8 and 12 week old homozygous mutated animals. At all time points the assessed parameters of heterozygous animals did not differ from those of their wild type littermates.
Conclusion: Homozygous mutated mice develop an ARVC-like phenotype. Consequently, our mutant mice will serve as a valuable tool a) to unravel the molecular disease mechanisms of ARVC in the context of desmosomal adhesion-mediated cell function and b) to develop therapeutic strategies.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:67

Titel: Granulocyte-derived soluble ceacam8 is a potential therapeutic agent with pleiotropic effects triggered by its cell surface interaction with ceacam1

Autoren: Singer B.(1),Scheffrahn I.(1),Klaile E.(2),Mueller M.(2),Öbrink B.(2),Ergün S.(1),

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Abstract:
Purpose: Granulocytes (PMNs) are usually known as the leukocyte subpopulation involved in acute inflammatory responses. These short-lived cells were thought of as terminally differentiated without considerable de novo synthesis of proteins. However, recent progress has shown, that PMNs are able to synthesize proinflammatory cytokines [e.g., TNF, IL-1, IL-8, MIP-1] and angiogenic factors [e.g., VEGF] in response to certain stimuli.
Results: Here we present soluble CEACAM8 as so far underestimated PMN-derived factor with pleiotropic effects on leukocytes. De novo synthesize could be provoked by GM-CSF/G-CSF treatment and was naturaly found in patients suffering e.g. psoriasis As member of the CEA-related cell adhesion (CEACAM-) family it represents a highly glycosylated protein that revealed in contrast to cytokines an extensive half-life. Interestingly, we found that soluble CEACAM8 binds to the cell-adhesion receptor CEACAM1, which is expressed in most leukocytes, activated endothelia and epithelia. Analyzes of the interaction between soluble CEACAM8 and membrane bound CEACAM1 revealed various co-stimulatory effects on T- and B-lymphocytes (e.g. proliferation, cytokine secretion).
Conclusions: These findings place soluble CEACAM8 at a pivotal position where it orchestrates inflammatory responses, immune regulation and wound healing processes. Thus, an increase of the amount or the inhibition of PMN-derived soluble CEACAM8 could be viewed as potentially effective strategy for therapeutic immune-intervention.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:68

Titel: The contribution of desmoglein 3 depletion to loss of keratinocyte cohesion in pemphigus vulgaris is dependent on keratinocyte differentiation

Autoren: Spindler V.(1),Endlich A.(1),Waschke J.(1),

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Abstract:
In the autoimmune disease pemphigus vulgaris (PV) intraepidermal blister formation due to loss of intercellular adhesion is mediated by autoantibodies directed against the desmosomal Ca2+-dependent cadherin-type adhesion molecules desmoglein (Dsg) 1 and Dsg3. Although Dsg3 depletion is believed to be involved in PV pathogenesis, we did not detect reduction of Dsg3 levels in keratinocytes (HaCaT) cultured for longer time periods in previous studies. Therefore, we hypothesized that loss of cellular Dsg3 depends on keratinocyte differentiation. Indeed, Dsg3 depletion in response to PV-IgG was strong in primary keratinocytes (NHEK) in which desmosome formation typically is induced by switch to high Ca2+ for 24 h and was significantly increased when HaCaT cells were cultured in high Ca2+ for 3d compared to 8d. Because protein kinase C (PKC) signaling was shown to be involved in keratinocyte differentiation we studied whether loss of Dsg3 was PKC-dependent. Both, Dsg3 depletion and loss of cell adhesion in response to PV-IgG were reduced by Gö-6976-mediated PKC inhibition in NHEK cultures. In contrast, PKC inhibition had no significant effect on cell dissociation in 8d old HaCaT monolayers indicating that in differentiated cells Dsg3 depletion plays a minor role for loss of cell cohesion in pemphigus. Thus, it is possible that the typical suprabasal epidermal cleavage formation in PV may in part be explained by a higher susceptibility of basal keratinocytes for Dsg3 depletion compared to the more differentiated cells in superficial layers.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:69

Titel: Proteinuria activates insulin- and igf1 r signalling, a mechanism of enac-mediated volume retention

Autoren: Kaminski H.(1),Peters H.(2),Bachmann S.(1),Franziska Theilig(1),

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Abstract:
Proteinuria is a symptom of many glomerular renal diseases which result in nephrotic or nephritic syndrome. Signs of volume retention such as edema formation or hypertension are observed frequently. In the collecting duct a dysregulation of ENaC is assumed to be causative and hormonal activation of aldosterone and vasopressin was excluded.
Therefore, we hypothesized an activation of the insulin and IGF receptor signalling to account for the volume retention under proteinuria.
For the induction of an experimental glomerulonephritis (GN; type Thy1) and puromycin-induced nephritic syndrome (PAN) Wistar rats were injected with either OX-7, puromycin or vehicle. After 6 days, kidneys were prepared for histochemical or biochemical analysis.
Urinary excretion of insulin (control 2.19 ± 0.5; GN 25.1 ± 9.9 und PAN 18.6 ± 6.4 ng/ 24h) and IGF-1 (control 11.9 ± 1.7; GN 264.6 ± 65.5 und PAN 431.9 ± 58.24 ng/ 24h) were strongly increased. Insulin and IGF-1 R were localized to the apical and basolateral membrane of the collecting duct. Proteinuria induced an increased phosphorylation of the apical insulin/IGF-1 receptor. Furthermore, activation of the insulin/ IGF-1 signalling was determined and increased expression of WNK1 (GN +131.4 ± 19.3 und PAN +239.9 ± 36.9%), phospho-AKT (GN +122.3 ± 7.6 und PAN +106.1 ± 29.4%) and phospho-SGK1 (GN +204.7 ± 82.9 und PAN 297.2 ± 22.5 %) were observed favouring an activation of ENaC.
In summary, our results show that proteinuria activated the apical insulin/ IGF-1 signalling which may account for ENaC mediated volume retention.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:70

Titel: Transcription factor fra-2 and its possible role in invasion and metastasis of breast cancer

Autoren: Schroeder C.(1),Janke S.(2),Richter U.(3),Streichert T.(4),Wirtz R.(5),Milde-Langosch K.(6),Schumacher U.(7),

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Abstract:
Fra-2 is a member of the Fos family of AP-1 transcription factors which are often up-regulated in breast cancer. The results of previous studies suggested that it might be involved in the regulation of tumor invasion and metastasis in breast cancer.

To analyze the impact of Fra-2 on the aggressive behavior of breast cancer cells, we established stable transfectants of the weakly invasive MCF-7 cells and as well in the highly invasive MDA-MB231 breast cancer cells with Fra-2 overexpression. The consequences of Fra-2 upregulation on the biology of the breast cancer cells were analysed by MTT assays and Matrigel invasion assays. In addition, possible target genes which were differentially regulated in stable transfectants with Fra-2 overexpression were identified by microarray analysis and, partly Western blots.

Cell proliferation was not influenced by Fra-2. In contrast, the invasive potential of the cells was increased by Fra-2 in MDA-MB231 and MCF7 cells. By using the GeneChip Human Genome U133A 2.0 array (Affymetrix), we identified several genes which are known to be involved in cell adhesion or invasion and which were up- or downregulated in stable transfectants, i.e. ICAM-1, L1CAM, ALCAM, and CX43.
In clinical breast cancer samples, upregulation of Fra-2 protein expression has been observed and was shown to be associated with a more aggressive phenotype. Our data of the experimental studies with breast cancer cell-lines indicates that Fra-2 may play an important role in tumor progression by transcriptional regulation of genes which are involved in cell adhesion and invasion.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:71

Titel: Acquired resistance to etoposide triggers

Autoren: Diaz-Carballo D.(1),Acikelli A.(1),Banaz-Yasar F.(1),Ergun S.(1),

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Abstract:
Purpose: Our studies aimed characterizing the cancer stem cell patterns in neuroblastoma cell lines resistant to chemotherapy such as to etoposide.
Methods: Resistance and cross-resistance to cytostatics were measured by MTT proliferation assay. The gene expression of some cancer stem cell (CSCs) markers like CD34, CD44, CD117 and p75NTR was studied using both conventional RT-PCR and qPCR. The protein expression of these markers was corroborated applying immunostaining on cells and tissue sections. The resistance to ionizing radiation was analyzed by exposing cells to gamma radiation.
Results: Ours studies revealed that some tissue-specific stem cell markers are significantly upregulated in etoposide-resistant sublines which also showed crossresistance to doxorubicin and radiation treatments. In addition, a tight association between resistance to etoposide and overexpression of CXCR4 isoforms with a concomitant downregulation of its ligand SDF1alpha was found. Cell hypertrophy was a common morphologic change detected in our studied, probably due to an up regulation of an alpha motif and leucine zipper containing kinase (ZAK). Furthermore, we corroborated that many of these pleiotropic effects were maintained when cells were xenographted into nude mice.
Conclusions: In view of the fact that etoposide and doxorubicin are commonly used clinical agents for neuroblastoma treatment, our data suggest potential induction of selection of so called “cancer stem cells” by these cytostatics, a phenomenon which might account for an eventual progression to intractable tumors. Consequently, improved cure rates may only be achieved via identification and therapeutic targeting of remanent chemotherapy resistant cancer stem cells.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:72

Titel: Detection of surfactant proteins in human testes and its regulation during different types of testicular cancer

Autoren: Schicht M.(1),Claassen H.(1),Wagner W.(2),Matthies C.(2),Ackermann P.(1),Schob S.(1),Paulsen F.(1),Bräuer L.(1),

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Abstract:

Surfactant Proteins (SP), originally known from lung tissue and meanwhile detected in a bulk different extrapulmonal human tissues play a major role in lowering surface tension of interfaces. Beyond this SP's are important players of the innate immune system. The surfactant proteins A and D are e.g. featured to immobilize microorganisms by binding to bacterial surfaces and due to this triggering opsonization processes. SP-B and SP-C are able to lower surface tension of fluid-gas-interphases and also to increase rheology and disassembly of mucous fluids. Here, we present the first detection of surfactant proteins A, B, C and D in human testicular tissues by means of RT-PCR, Western blot analysis and immunhistochemistry. All investigated tissue samples show expression of all four surfactant proteins mainly in Sertoli cells of testes and slight expression in spermatogonia and spermatocytes. We further investigated the quantitative distribution of the four surfactant proteins by means of ELISA in tissue samples of different types of testicular carcinoma. In case of testicular cancer the expression of SP-A, SP-C and SP-D is strongly downregulated which possibly indicates immunmodulatory effects of SP-A and SP-D. In contrast the protein expression of the surface active surfactant protein SP-B is significantly upregulated.
In conclusion all known surfactant proteins seem to be physiological part of the male reproductive system and might be regulated in malignancies suggesting a potential role during tumor formation.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:73

Titel: Av, b1 and b3 integrins mediate opn mechano-protection in podocytes

Autoren: Schordan S.(1),Schordan E.(1),Endlich K.(1),Endlich N.(1),

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Abstract:
Podocyte damage and failure is the main cause of chronic kidney disease. Increased mechanical load in podocytes due to glomerular hypertension is one of the important factors leading to podocyte damage. In previous studies we have shown that mechanical stretch increases osteopontin (OPN) expression in podocytes, and that exogenous OPN is mechano-protective via facilitating cytoskeletal reorganization of podocytes. In the present study we asked whether the mechano-protective effect of OPN in podocytes is mediated through specific integrins, and whether endogenous OPN of podocytes is required for mechano-protection. Conditionally immortalized mouse podocytes and primary podocytes (PP) from OPN-/- and OPN+/+ mice were used. Cyclic biaxial mechanical stretch (0.5 Hz, 7% linear strain) was applied for up to 3 d. Stretch-induced cell loss was about 30% higher in OPN-/- PP as compared to OPN+/+ PP. Increased cell loss of OPN-/- PP was rescued by OPN coating. OPN+/+ PP, but not OPN-/- PP, exhibited complete stretch-induced reorganization of the actin cytoskeleton consisting of radial stress fibers and an ARC (actin rich center). Analysis of integrin expression by RT-PCR, screening with anti-integrin antibodies, application of RGD and SLAYGLR peptides, and siRNA knockdown of integrins identify the AlphaV Beta1 and AlphaV Beta3 integrins as receptors responsible for OPN mediated mechano-protection.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:74

Titel: Regulation of force transmission by formin mediated stress fiber elongation at focal adhesions in podocytes

Autoren: Schordan E.(1),Schordan S.(1),Schulz S.(2),Spatz J.(2),Endlich N.(1),Endlich K.(1),

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Abstract:
The filtration of blood is depending on the intact morphology of podocyte foot processes around the capillaries. The actin cytoskeleton and focal adhesions (FA) are responsible for the complex morphology of interdigitating foot processes. Recently, we have demonstrated that the connection between actin filaments and FA is highly dynamic due to polymerization of actin at FA with a constant velocity of 0.2-0.4 µm/min. This centripedally movement is depending on force and on actin polymerization. Further, we show that stress fiber assembly at FA is highly influenced by mDia1. Our hypothesis was that elongation of actin filaments uncouples force at FA. Contraction induced by calyculin A shows that podocytes transfected with the dominant negative (DN) form of mDia1 fail to resist to cell contraction whereas wild type (WT) and constitutively active (CA) forms prevent rapid cell collapse. To study traction forces and dynamics of the cytoskeleton at a subcellular resolution, podocytes were cultured on two-dimensional arrays of pillars. We found that different constructs of mDia1 did not affect mean force development per pillar, i.e. 57.7+/-5.4 nN/pillar for WT, 55.4+/-3.6 for DN and 55.8+/-4.9 for CA but contraction of podocytes on pillars revealed a correlation between force and GFP intensity of the WT mDia1 which was absent for DN. Taken together, these data describe a novel mechanism mediated by mDia1 that uncouples force at focal adhesions to facilitate adaptation of podocytes in response to contraction which might be important for the maintenance of foot processes in vivo.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:75

Titel: Expression of tetraspanin cd151 in cultured podocytes

Autoren: Blumenthal A.(1),Endlich N.(1),Endlich K.(1),Giebel J.(1),

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Abstract:
Podocytes play an important role in supporting the glomerular filtration barrier in the kidney. Their function is based on the formation of foot processes and integrin-mediated adhesion to the glomerular basement membrane. Podocytes are sensitive to mechanical stress. Loss of podocytes is associated with proteinuria and renal diseases. Recently, some tetraspanins were shown to modulate integrin function. Tetraspanins are enriched in microdomains possibly regulating membrane fluidity and signal transduction. As CD151 knock-out mice develop a renal phenotype and glomerulosclerosis this tetraspanin seems to be essential for maintaining the glomerular filtration barrier. The aim of the present study was to get insight in the expression of CD151 in cultured podocytes under normal and stretched conditions.
We investigated CD151 in cultured podocyte cell lines SVI and E11 by Western Blot, RT-PCR, transfection experiments and immunocytochemistry. CD151 mRNA and protein were detected in both cell lines. CD151 was localized to cell membranes, cell-cell contacts and perinuclear vesicles. At sites of cell-cell contact CD151 was colocalized to actin and actin-associated proteins, occasionally. SVI and E11 showed delicate membrane protrusions positive for CD151. In most of these protrusions no actin-associated proteins were seen. After stretching real-time PCR showed no changes of CD151 mRNA levels compared to static cells but a translocation of CD151 into clusters was evident. Migration assays revealed an impaired motility of podocytes after transfection with pEGFP-N3-CD151.
As CD151 is translocated after mechanical stress and impairs podocyte motility after overexpression an involvement in regulation of adhesion and membrane fluidity could be assumed.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:76

Titel: Induction of apoptosis and dna damage via line-1 mediated retrotransposition events

Autoren: Banaz-Yasar F.(1),Gedik N.(1),Hanilce S.(1),Bongartz B.(1),Schumann G.(2),Ergün S.(1),

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Abstract:
Long interspersed nuclear element-1 (LINE-1) retrotransposons are mobile elements that insert into new genomic locations via reverse transcription of an RNA intermediate. The mechanism of retrotransposition is not entirely understood. The integration of these elements occurs by target primed reverse transcription (TPRT). TPRT initiates double strand breaks during the integration of LINE-1. It is also discussed that the retrotransposition process could be affected by the cellular response to DNA damage. The aim of this study was to evaluate the potential effects of LINE-1 mediated retrotransposition events on DNA damage and apoptosis in human endothelial cells. The induction of apoptosis and DNA double strand breaks (DSB) were analysed in human hybrid endothelial cell line EA.hy926 which were stably transfected with human LINE-1 element. Since the induction of DNA damage is also induced following gamma irradiation, we determined the expression level of phosphorylated p53 and phosphorylated histone H2AX protein levels upon X-ray irradiation with 5 Gy for 24 hours. Our results show that EA.hy926 LINE-1 cell clones react with a strong upregulation of phosphorylated p53 protein already 15 min after irradiation compared to the WT cells. Also the expression of phosphorylated histone H2AX protein was elevated in the cell clones with retrotransposition events 15 min after irradiation whereas the WT cells have a delayed expression of phosphorylated histone H2AX protein. Taken together, our data show that LINE-1 retrotransposition events can have negative consequences like induction of apoptosis and DSB in EA.hy926 cell line.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:77

Titel: Identification of the corticosterone binding site of organic cation transporter 3

Autoren: Volk C.(1),Gorboulev V.(1),Müller T.(2),Koepsell H.(1),

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Abstract:
Organic cation transporters from the SLC22 protein family are polyspecific transporters for various organic cations including monoamine neurotransmitters and cationic drugs as metformin or cisplatin. It is therefore presumed that OCTs contain a complex binding pocket with distinct interaction sites for different substrates. Inhibitors as the steroid corticosterone also interact within the binding pocket. Corticosterone inhibits all OCT subtypes, but with quite different affinities. The Ki value for human OCT3 is ~ 0.2 µM while rat OCT3 is inhibited with a 20fold lower affinity. The aim of our study was to understand the molecular basis of corticosterone binding. Using mutational analysis we found out that an amino acid in position 222 in the 4th transmembrane helix (TMH) is involved in binding. In rOCT3 a phenylalanin is located in this position, while it is tyrosine in hOCT3. Exchanging the Phe222 for Tyr, the affinity of rOCT3 to corticosterone was significantly increased, but still lower than the affinity of hOCT3. Furthermore, the affinity to the substrate tetraethylammonium was also increased by this exchange. Using a 3D model of rOCT3 which was derived from the crystal structure of the related protein LacY permease, we could model the binding of corticosterone to rOCT3 from both sides of the membrane. Four additional amino acids in TMH 1, 4, 8 and 10 could be identified which are in close contact to the corticosterone molecule and therefore might also be involved in binding.

Kategorie: Lecture

Rubrik: 8.Cell Biology
Abstract Nr.:78

Titel: The cell biology of bone marrow stimulating surgery in osteoarthritis

Autoren: Breuer F.T.(1),Hartz C.(2),Pries F.(3),Varoga D.(2),Pufe T.(1),

Adressen: (1) Department of Anatomy and Cell Biology|RWTH Aachen University|Aachen|Germany; email:fbreuer@ukaachen.de; (2) Department of Trauma Surgery|University Hospital of Schleswig-Holstein, Campus Kiel|Kiel|Germany; (3) Department for Arthroscopic Surgery and Sports Traumatology|Mare-Klinikum|Kronshagen|Germany

Abstract:
Purpose: Characterisation of hemarthros-derived cells in orthropadic surgery such as arthroscopic abrasion arthroplasty (AAP) and microfracture surgery.
Methods: Postoperative hemarthros from patients after arthroscopic knee procedures, taken 5, 22 and 44 hours after surgery, were collected. The practices of AAP and microfracture surgery were compared to abrasion chondroplasty (ACP).The aim was to characterise the isolated cells by ELISA and Luminex®-technique, focussing on the cytokines and growth factor expression, as well as characteristic markers of mesenchymal stem cells (MSCs), e.g. CD44. The differentiation to chondrocytes (e.g. collagen type II, chondroitin-4-sulfate) determines the quality of cartilage regenerates.In addition hemarthros-derived MSCs were cultured in high density. These 3D-cultures were stimulated with hemarthros-serum obtained from AAP or ACP. The immunhistochemical results give clues to the potential cartilage regeneration.
Results: The immigration of MSCs increases after opening the bone marrow cavity in contrast to the solely remodelling ACP practice. The morphologic differentiation of MSCs to a chondrocyte-like cell shape, producing an extracellular matrix in high density culture indicates a regeneration of degenerated hyaline-like cartilage in arthrotic joint. The ELISA shows a doubled concentration of TGFbeta1 22 hours after AAP. Equally are the results for VEGF and IGF-1 after 44 hours.
Conclusions: AAP and microfracture surgery reveal a source of MSCs. The morphological convergence of regenerates to hyaline cartilage and the different levels of chondrogenetic factors like TGFbeta1 and cartilage-protective IGF-1, dependent on the kind of arthroscopic practice, underline the benefit of bone marrow stimulating surgery methods in orthopaedic therapy.

Kategorie: Lecture

Rubrik: 8.Cell Biology
Abstract Nr.:79

Titel: Role of cytokeratin retraction and actin reorganization in pemphigus acantholysis

Autoren: Gliem M.(1),Heupel W.(1),Hübner S.(1),Waschke J.(1),

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Abstract:
In human autoimmune blistering skin disease pemphigus vulgaris autoantibodies are mainly directed against keratinocyte cell adhesion molecules desmoglein (Dsg) 3 and 1 and cause keratinocyte cell dissociation (acantholysis). Early ultrastructural work revealed cytokeratin (CK) retraction to be a characteristic hallmark of acantholytic keratinocytes and recent studies reported profound alterations of the actin cytoskeleton. Nevertheless, the temporal sequence and relevance of these cytoskeletal phenomena in pemphigus pathogenesis compared to other events such as inhibition of Dsg3 function or induction of cellular signaling are only poorly understood. Therefore, we examined roles of CK and actin in PV-IgG-mediated keratinocyte dissociation by using different approaches such as pharmacological inhibition or stabilization of cytoskeletal components, live cell imaging and overexpression studies. Both, PV-IgG-induced CK retraction and cell dissociation were found to be p38MAPK-dependent. Similarly, pharmacological p38MAPK activation caused CK retraction and reduced keratinocyte adhesion, however without fragmentation of Dsg3 localization. Since overexpression of Dsg3 prevented PV-IgG-induced cell dissociation and CK retraction, CK retraction appeared to result from Dsg3 depletion. Parallel to CK retraction, PV-IgG treatment resulted in striking changes in actin cytoskeleton organization. Pharmacological stabilization of actin filaments partly blocked acantholysis, whereas actin depolymerization strongly increased pathogenic effects of PV-IgG. Furthermore, Rho GTPase-mediated stabilization of the junction-associated actin belt resulted in protection against PV-IgG-induced acantholysis. Taken together, these experiments indicate that actin reorganization is critical for PV-IgG-induced acantholysis. CK retraction may also contribute to p38MAPK-dependent keratinocyte dissociation in pemphigus but appears to be secondary, at least to Dsg3 depletion.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:80

Titel: Rab11b regulates trafficking of v-atpase in salivary ducts during acid-base disturbances.

Autoren: Oehlke O.(1),Osterberg N.(1),Roussa E.(1),

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Abstract:
Redistribution of acid-base transporters is a crucial regulatory mechanism for many cells to cope with extracellular pH changes. Moreover, in epithelial cells, translocation of acid-base transporters ultimatively leads to changes in vectorial transport of H+ and HCO3-. The specific proteins involved in regulated vesicular traffic of acid-base transporters in epithelial cells are largely unknown. In the present study we have investigated the impact of Rab11b in the acidosis-induced trafficking of the vacuolar type H+-ATPase (V-ATPase) in salivary duct cells in vitro using the human submandibular cell line of ductal origin HSG as an experimental model. We show that Rab11b is expressed in salivary duct cells in vitro and in vivo, where it co-localizes and interacts with the E subunit of V-ATPase. Extracellular acidosis up-regulates Rab11b mRNA expression in HSG cells and causes the translocation of V-ATPase-containing vesicles via the trans-Golgi network towards the plasma membrane, a process mediated by Rab11-family interacting protein RIP11. Loss of function experiments using specific siRNA against Rab11b or RIP11 prevents acidosis-induced V-ATPase translocation. These data introduce Rab11b as a crucial regulator of acidosis-induced V-ATPase traffic in duct cells of SMG.

Kategorie: Lecture

Rubrik: 14.Central nervous system/signal transduction and connections
Abstract Nr.:81

Titel: Interplay at the synaptic cleft - neurexins binding to neuroligins, dystroglycans and neurexophilins

Autoren: Reissner C.(1),Klose M.(1),Stahn J.(1),Missler M.(1),

Adressen: (1) Department of Anatomy and Molecular Neurobiology|Institute of Anatomy|Münster|Germany; email:creissn@uni-muenster.de

Abstract:
Purpose: To evaluate the function and binding properties of neurexins to neuroligins, dystroglycans and neurexophilins and their interplay at the synapse.
Methods: Identification of the exact binding sites by biochemical (pulldown and surface plasmon resonance assays) and cell biological methods (cell culture and imaging studies) through site directed mutagenesis.
Results: While alpha-neurexins bind to all three putative partners, beta-neurexins bind exclusively to neuroligins. All neuroligins bind to the identical binding epitope on the LNS6 domain of alpha-Neurexins 1, 2 and 3, and to the single LNS domain of beta-Neurexins. Alpha-dystroglycan interacts with an corresponding epitope at the calcium binding site of LNS2 of alpha-Neurexin. The third ligand, neurexophilin, binds to an hitherto uncharacterized epitope on the LNS2 domain. Surprisingly, the neurexin/neurexophilin complex destined for the presynaptic membrane is preformed early in the secretory pathway, and appears very stable. Finally, interaction between these partner proteins is not mutually exclusive, and may form a quadruple transsynaptic complex.
Conclusions: Our results support the role of alpha-neurexins as a major center piece in the organisation and maturation of synapses. Identification of point mutations that selectively prevent binding to neuroligin, dystroglycan or neurexophilin will now allow the functional analysis of these various interactions.

Kategorie: Lecture

Rubrik: 14.Central nervous system/signal transduction and connections
Abstract Nr.:82

Titel: Regulation of the expression of the clock gene bmal1 in the ht22 hippocampal cell line

Autoren: Maronde E.(1),Boulaaouin S.(2),

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Abstract:
Learning and memory processes depend on circadian time. However, the different cell types in primary hippocampal preparations are unduly difficult. We therefore used the immortalized mouse hippocampal HT22 cell line as a model system to study clock gene activity and clock protein regulation under the influence of various agents (Dexamethasone [Dex], Sp-cAMPS, acidic fibroblast growth factor [FGF-1], and norepinephrine), relevant to learning and memory processes. We investigated the glucocorticoidreceptor-, PKA-, MEK- and PI3K-pathway signal transduction pathways, using a Bmal1-promoter-driven luciferase reporter gene construct (Bmal1-luc). Bmal1 shows circadian oscillations in both, mRNA and protein level and produces a strong circadian phenotype, when deleted in mice. Bmal1-luc activity varied spontaneously in vitro in a circadian manner. Bmal1-luc was enhanced by Dex, a glucocorticoid receptor agonist, to the culture medium. When coapplied, FGF-1 exerted synergistic effects on the amplitude of the Bmal1-luc compared to Dex alone. Selective inhibition of the glucocorticoid receptor- and the PI3K-pathway reduced the effect of Dex plus FGF-1 on Bmal1-luc amplitude to control levels, whereas inhibition of the MEK-pathway only inhibited the synergistic effect of Dex plus FGF-1. A circadian clock is present in hippocampal HT22 cells and regulated by glucocorticoid receptor-, PKA-, PI3K- and MEK signalling pathways. Thus, next to endogenous circadian oscillations, acute signal transduction pathways also influence Bmal1-expression in HT22 cells. Interestingly, stimuli that have been shown before to influence memory processes seem to alter clockwork properties, possibly affecting the temporal gating of hippocampal learning.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:83

Titel: Modification of fibroblast growth factor receptor 1 trafficking induces changes in axon growth

Autoren: Hausott B.(1),Vallant N.(1),Rietzler A.(1),Schlick B.(1),Klimaschewski L.(1),

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Abstract:
Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) promote axon growth during development and repair of the nervous system. FGFR1 exhibits the highest expression in adult sensory neurons from dorsal root ganglia. FGF-2 is up-regulated in response to a peripheral nerve lesion and enhances axonal elongation of pre-lesioned neurons by activation of the Ras/ERK and the PI3K/Akt pathway.
We analyzed the effects of inhibition of FGFR1 endocytosis or inhibition of lysosomal FGFR1 degradation on axon regeneration by adult sensory neurons in vitro. Overexpression of FGFR1 enhanced FGF-2-induced elongative axon growth, which was strongly increased by co-treatment with the lysosomal inhibitor leupeptin. Furthermore, leupeptin increased co-localization of FGFR1-EGFP with lysosomes and endosomes, and enhanced receptor membrane levels by stimulating recycling. Inhibition of FGFR1 endocytosis by methyl-beta-cyclodextrin promoted FGF-2-induced axon growth through enhanced axonal branching and increased phosphorylation of ERK and Akt.
Our data indicate that trafficking of FGFR1 controls axon growth by adult peripheral neurons. Inhibition of FGFR1 endocytosis by methyl-beta-cyclodextrin promotes axonal branching, whereas the lysosomal inhibitor leupeptin stimulates elongative axon growth, which is required for regeneration.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:84

Titel: The neuroprotective efficacy of the cannabinoid win 55,212-2 is determined by a novel cross-talk between cannabinoid 1 receptor, transient receptor potential (trp)a1 and n-type voltage-gated ca2+ channels

Autoren: Koch M.(1),Kreutz S.(2),Böttger C.(2),Grabiec U.(1),Ghadban C.(1),Korf H.(2),Dehghani F.(1),

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Abstract:
Pathological events like inflammatory or neurodegenerative processes are regulated by cannabinoid (CB)1 and CB2 receptors. The mechanisms behind cannabinoid effects show a high variability and may involve additional transient receptor potential (TRP) and/or N-type voltage-gated Ca2+ channels. Organotypic hippocampal slice cultures were excitotoxically lesioned using NMDA (50µM) and subsequently incubated with the synthetic cannabinoid WIN 55,212-2 (0.001µM-10µM). WIN exerted neuroprotective effects in an inverse concentration-dependent manner, most effectively at 0.01µM. The CB1 receptor antagonist AM251 blocked neuroprotection mediated by WIN whereas the CB2 receptor antagonist AM630 showed no effects. The TRPA1 channel blocker HC-030031 enhanced the neuroprotective efficacy of high (10µM) WIN concentrations and the number of degenerating neurons became equal to that seen after application of the most effective WIN dose (0.01µM). In contrast to the TRPV1 channel blocker 6-iodo-nordihydrocapsaicin, the N-type voltage-gated Ca2+ channel blocker omega-conotoxin (GVIA) completely blocked neuroprotection shown by 10µM WIN. GVIA and HC-030031 displayed no effects at WIN concentrations lower than 10µM. Our data implicate that WIN protects dentate gyrus granule cells in a concentration dependent manner by acting upon CB1 receptor. At high (10µM) concentrations WIN additionally activates TRPA1 and N-type voltage-gated Ca2+ channels that both interfere with CB1 receptor mediated neuroprotection. This leads to the conclusion that physiological and pharmacological effects of cannabinoids strongly depend on the concentration and the neuroprotective efficacy of cannabinoids may be determined by a cross-talk between signaling cascades activated by CB1 receptor, TRPA1 and N-type voltage-gated Ca2+ channels.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:85

Titel: Reelin as an attractive signal for cortical neurons

Autoren: Zhao S.(1),Chai X.(1),Bouche E.(2),Bock H.(2),Frotscher M.(1),

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Abstract:
Ordered neuronal migration is an essential step in brain development, particularly in highly laminated brain stuctures, such as the cerebral cortex, hippocampus and cerebellum. Reelin is a large extracellular matrix glycoprotein secreted by Cajal-Retzius cells in the marginal zone of neocortex and hippocampus. It has been shown that Reelin is required for the proper neuronal positioning and correct layer formation in these regions. In reeler mutant mice deficient in Reelin, neuronal migration is disrupted and the normal inside-out layering of the cerebral cortex is reversed. However, the mechanism of Reelin function in these processes has remained unclear. Using slice co-cultures of wildtype hippocampus with the neocortex of reeler mice and mutants for the two Reelin receptors, Apolipoprotein receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR), we have shown that Reelin plays divergent roles in neuronal migration and layer formation in the cortex. While binding of Reelin to ApoER2 promotes neuronal migration, binding of Reelin to VLDLR terminates the migration process. We hypothesize that different Reelin fragments diffuse for different distances in the cortex and bind to ApoER2 and VLDLR at different time points and in different cell compartments, thereby controlling proper layer formation in the cortex.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:86

Titel: Maturation and differentiation of secondary radial glial cells in the reeler dentate gyrus

Autoren: Brunne B.(1),Zhao S.(1),Herz J.(2),Frotscher M.(1),Bock H.(2),

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Abstract:
Maturation and differentiation of secondary radial glial cells in the reeler dentate gyrus
Purpose: The secondary radial glial scaffold of the developing dentate gyrus is morphologically severely altered in reeler mutants. As these radial glial cells function as precursor cells, the present study investigates to which extent their maturation and differentiation is affected.
Methods: An immunohistochemical marker profile that delineates the maturation process of secondary radial glial cells was established and used to compare wild type and reeler mice. BrdU injections combined with NeuN and S100beta triple-labeling were used to determine the time course of neurogenesis and astrogliogenesis. Quantitative immunocytochemical studies of dissociated precursor cells supplemented the morphological in vivo analysis.
Results: Immunohistochemical profiling with antibodies against nestin, vimentin and GFAP characterized the maturation of secondary radial glial cells, with nestin being highly expressed in early, vimentin in intermediate, and GFAP in late phases of development. With these markers, the maturation of the secondary radial glial scaffold appears normal in reeler mutants. Differentiation studies revealed that there is a slight shift toward increased astrogliogenesis without any overt effect on the overall time course of astrogliogenesis and neurogenesis.
Conclusions: Although secondary radial glial cells are morphologically severely altered in reeler mutants, their immunocytochemical maturation is only slightly affected.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:87

Titel: Assembly of sympathetic preganglionic neurons in the intermediolateral column is controlled by reelin-induced cofilin phosphorylation

Autoren: Krüger M.(1),Zhao S.(1),Chai X.(1),Brunne B.(1),Bouché E.(2),Bock H.(3),Frotscher M.(1),

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Abstract:
In the mature mammalian spinal cord, sympathetic preganglionic neurons (SPN) are located in the intermediolateral column (IMLC). This specific localization results from primary and secondary migratory processes during the development of the spinal
cord. Thus, following neurogenesis in the neuroepithelium, SPN migrate first in a ventrolateral direction and then, in a secondary step, dorsolaterally to reach the IMLC. These migratory processes are controlled, at least in part, by the glycoprotein Reelin known to be important for the development of laminated brain structures. In
reeler mutants deficient in Reelin, SPN migrate ventrolaterally as normal. However, most of them then migrate medially to become eventually located near the central canal. Here, we provide evidence that in wild-type animals this aberrant medial migration towards the central canal is prevented by Reelin-induced cytoskeletal stabilization, brought about by phosphorylation of cofilin. Cofilin plays an important role in actin depolymerization, a process required for the changes in cell shape during migration. Phosphorylation of cofilin renders it unable to depolymerize F-actin, thereby stabilizing the cytoskeleton. Using immunostaining for phosphorylated
nonmuscle cofilin (p-cofilin), we demonstrate that SPN in wild-type animals, but not in reeler mutants, are immunoreactive for p-cofilin, suggesting that Reelin near the central canal induces cofilin phosphorylation in SPN, thereby preventing them from additional migration towards the central canal. The results extend our previous studies on cortical neurons where Reelin in the marginal zone was found to stabilize the leading processes of migrating neurons and terminate the migration process.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:88

Titel: Mitochondrial dynamics and its role in neurodegeneration and protection

Autoren: Arnold S.(1),Beyer C.(1),

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Abstract:
Purpose: Proper regulation of mitochondrial dynamics is vital to neural cells. It supports mitochondria to provide a myriad of services to the cell including energy production and regulation of apoptosis. The overall morphology of the mitochondrial population in a given cell is determined by a dynamic equilibrium between organelle fusion and fission and depends on the relative activities of fusion (Mfn1, Mfn2) and fission (Fis1, Drp1) proteins.
Methods: Quantitative RT-PCR, viability analysis, immunofluorescence and electron microscopy analyses of cortical astrocytes of female and male mice were performed.
Results: Cortical astrocytes treated with 100 nM estradiol for 3 h showed gender-specific and significant changes in mitochondrial fusion gene (Mfn1) transcription which were paralleled by rectified changes of anti-apoptotic Bcl-2 transcription. The ratio of proliferation (Pcna) to pro-apoptotic (Bax) gene transcription was greater than 1 in female and less than 1 in male astrocytes. This was reflected by the number of viable to apoptotic cells and indicates a higher cell survival rate of female vs. male astrocytes after estradiol treatment. Similar observations were made when astrocytes were co-treated with staurosporine (100 nM) and estradiol, whereas staurosporine alone caused an increased transcription of apoptosis indicator genes Bax and Fis1.
Conclusions: Our findings suggest a pronounced sensitivity of male astrocytes for apoptosis. This may serve as an explanation for the higher incidences of certain neurodegenerative diseases in males.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:89

Titel: Steroid hormones are neuroprotective in an experimental brain ischemic model

Autoren: Beyer C.(1),Dang J.(2),Kipp M.(2),

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Abstract:
Purpose: Stroke represents one of the most frequent neurological disorder with only limited therapeutic opportunities such as immediate thrombolysis. 17ß-estradiol (E) and progesterone (P) have been effectively proven to be neuroprotective under different neuropathological conditions using in vitro and in vivo approaches. In this report, we demonstrate the efficiency of both steroids under stroke-like conditions.
Methods: The intra-luminal thread model of middle cerebral artery occlusion in male rats was used to obtain focal cerebral ischemia. During a 24h recovery period, animals were exposed to steroids by subcutaneous neck depots. After that, senso-motoric behavioral test were performed, the size of the infarct areal was quantified by stereotactic measures, and the expression of genes was analyzed by Affimetrix and real-time PCR.
Results: The application of both hormones resulted in a greater than 70% behavioral recovery and a significantly reduced infarct area in the cerebral cortex but not in the basal ganglia. Single hormone applications were less effective. A set of inflammatory genes and protective factors appear to be positively regulated by the steroids within the penumbra region.
Conclusions: Our data clearly reveal the potency of both steroid hormones to protect against neuronal death and to restore behavioral performance after focal ischemia. Future studies have to address the exact cellular and cell-cell specific responses within the penumbra and to develop therapeutic strategies for stroke units.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:90

Titel: Transgenic mice expressing mutant a53t human alpha-synuclein show disturbed sleep/wake rhythms and reduced homer 1bc immunoreaction in the suprachiasmatic nucleus.

Autoren: von Gall C.(1),Pfeffer M.(1),Markova Z.(1),Gispert S.(2),Korf H.(1),

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Abstract:
Many neurodegenerative disorders including Parkinson's disease (PD) are associated with circadian sleep disturbances. Alpha-synuclein is implicated in PD as missense mutations in the alpha-synuclein gene are found in autosomal dominant PD and alpha-synuclein is a major constituent of protein aggregates in different synucleinopathies. In the present study, we investigated locomotor activity rhythms in transgenic mice expressing mutant A53T human alpha-synuclein (A53T). In addition, immunoreaction for Homer proteins involved in synaptic scaffolding was analyzed in the suprachiasmatic nucleus (SCN) of A53T. Wildtype (WT) mice showed high locomotor activity during the dark phase and low activity during the light phase. This behaviour (entrainment) is controlled by an endogenous rhythm generator in the SCN receiving retinal input. In addition, light suppresses locomotor activity in night active animals (masking). However, A53T showed a significant increase in locomotor activity during the light period as compared to WT littermates. This suggests a reduced masking effect of light in A53T. Moreover, the re-entrainment of A53T to a 6 h phase shift in the light regime was significantly slower as compared to WT. These data suggest an impairment of circadian light perception in A53T. Importantly, immunoreaction for Homer1bc was significantly reduced in the SCN of A53T as compared to WT. This might indicate a disturbance in the synaptic integrity within the SCN of A53T. Our data provide evidence that transgenic animals expressing mutant alpha-synuclein represent an interesting model to assess the effects of synucleinopathies on the circadian system.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:91

Titel: Motor activity impairment of go2alpha deletion mutants is overcome by repeated amphetamine treatment

Autoren: Brunk I.(1),Sanchis-Segura C.(2),Blex C.(3),Perreau-Lenz S.(4),Bilbao A.(4),Birmbaumer L.(5),Spanagel R.(4),Ahnert-Hilger G.(1),

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Abstract:
Background: Go2alpha resides on plasma membranes and synaptic vesicles. Vesicular Go2alpha inhibits vesicular monoamine accumulation. Deletion of Go2alpha abolishes cocaine induced behavioural sensitization due to disturbance of the striatal dopamine system. Here we provide evidence that deletion of Go2alpha has a different impact on amphetamine induced effects.

Methods: Brain fractions of untreated and psychostimulant treated wild type and Go2alpha-/- mice were analyzed by Western-blot, vesicular monoamine uptake and release. Motor activity and behavioural sensitization were tested using amphetamine.

Results: Go2alpha-/- mice develop behavioural sensitization following repeated injections of amphetamine, but at reduced rates compared to wild type littermates. Amphetamine differentially affects the dopamine system in wild type and Go2alpha-/- mice: D1 receptor amount is lower, D2 receptor amount is higher in Go2alpha-/- mice compared to wild type littermates. Cocaine treatment caused a comparable difference in D1/D2 receptor amounts, nevertheless it failed to provoke behavioral sensitization in Go2alpha-/- mice. In contrast to cocaine amphetamine treatment yielded higher amounts of the NMDA-receptor subunit NR2B in Go2alpha-/- compared to wild type mice. Amphetamine but not cocaine treatment maintains the balance between the glutamate receptor GluR1/5 interacting proteins homer and homer1a in the mutants.

Conclusion: Amphetamine provokes behavioral sensitization in Go2alpha-/- mice by an enhanced inhibition of the indirect pathway without disturbing the direct pathway thereby overcoming the disturbance in the dopaminergic system. The data shed new light on the various modulatory mechanisms involved in the control of motor activity.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:92

Titel: Role of nuclear factor erythroid 2-related factor 2 (nrf2) in the pathogenesis of amyotrophic lateral sclerosis (als)

Autoren: Rosen C.(1),Tohidnezhad M.(1),Brandenburg L.(1),Fragoulis A.(1),Pufe T.(1),Wruck C.(1),

Adressen: (1) Department of Anatomy and Cell Biology|University Hospital of Aachen|Aachen|Germany; email:crosen@ukaachen.de

Abstract:
INTRODUCTION: Mutations in Cu/Zn superoxide dismutase 1 (SOD1) are a cause of motor neuron death in about 20 % of cases of familial amyotrophic lateral sclerosis (ALS). There is significant evidence that oxidative stress makes a major contribution to the selective death of motor neurons in this disease. Nuclear factor erythroid 2-related factor 2 (Nrf2) is known as the major regulator of a battery of genes encoding detoxifying and antioxidative enzymes via binding to the antioxidant response element (ARE). The aim of the current in vitro study was to elucidate the protective role of the Nrf2/ARE-System against oxidative stress in the motor neuron-like cell line NSC34.
METHODS: NSC34 cell were exposed to oxidative stress via H2O2 treatment or stably transfected with hSOD1G93A gene. The Nrf2/ARE-System in the motor neuron-like cell line was genetically activated via the transfection of shRNA against the mRNA of the Nrf2-Inhibitor Keap1 or by stimulation with andrographolide. Nrf2-activation was measured via Western blot, Real time-PCR and ARE-Luciferase assay in NSC34 cells. The protective potential of Keap1-RNAi knock down and andrographolide was measured via cell viability and toxicity assays.
RESULTS: We demonstrate that shRNA against Keap1 and andrographolide activates the Nrf2/ARE-System. An upregulated Nrf2/ARE-System yields to a lower vulnerability to oxidative stress in the motor neuron-like cell line NSC34.
CONCLUSIONS: These findings indicate that both the use of purified andrographolide and Keap1-shRNA might be considered as an adjunct therapeutic strategy to combat neural demise in amyotrophic lateral sclerosis.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:93

Titel: Chemokine expression during early demyelination

Autoren: Kipp M.(1),Neumann H.(2),Linnartz B.(2),Beyer C.(1),

Adressen: (1) Institute of Neuroanatomy|RWTH Aachen University|Aachen|Germany; email:mkipp@ukaachen.de; (2) University Bonn|Institute of Reconstructive Neurobiology|Bonn|Germany

Abstract:
Purpose: Chemokines play an important role in the brain. Under pathological conditions, these factors become critical modulators of microglia chemotaxis. During active demyelination microglia attraction is followed by myelin debris phagocytosis, a prerequisite for successful remyelination. In this study, we have investigated the regulation of chemokines in distinct brain areas of a multiple sclerosis animal model.
Methods: Acute demyelination was induced in mice by cuprizone administration. The complex regulation of chemokines was investigated using Affymetrix gen chip analysis and quantitative real-time PCR of candidate genes. Invasion of inflammatory cells was additionally investigated in CCL2- and CCL3-deficient animals. Since oligodendrocytes represent a possible source of chemokines, we have studied chemokine expression in cultured oligodendrocytes (OLN-93 cells).
Results: Gene expression analysis disclosed a complex dynamic regulation of chemokines. CCL2 expression followed a biphasic pattern with peaks after week 1 and 5. In contrast, CCL3 levels peaked at week 1. Microglia cell invasion was reduced in animals, deficient for CCL2 or CCL3, compared to their wild-type littermates. LPS and TNFα promoted chemokine secretion of cultures oligodendrocytes, whereas glutamate and hydrogen peroxide did not. Increased migration of microglia toward the conditioned medium of stimulated oligodendrocytes was detected suggesting functional relevance of oligodendroglia derived chemokines.
Conclusion: These results demonstrate that chemokines are selectively regulated during acute brain demyelination in an experimental MS model. Besides brain resident inflammatory cells, oligodendrocytes appear to be an additional important source of local chemokine production during early demyelination. Thereby, spoilt oligodendrocytes might initiate remyelination by early microglia attraction.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:94

Titel: Analysis of remyelination failure in an experimental multiple sclerosis model

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Abstract:
In multiple sclerosis, spontaneous remyelination becomes limited after repeated episodes of demyelination. This is believed to be a major cause of neurologic disability. We used the cuprizone mouse model to investigate differences in demyelinated white matter lesions showing spontaneous or lack of remyelination.
Acute and chronic demyelinated lesions were generated by cuprizone feeding for 5 or 13 weeks, respectively. The corpus callosum was analyzed for endogenous remyelination capacity by IHC and gene expression analysis. Differences in expression levels of genes were determined by Affymetrix analysis and real-time PCR. In addition, glial cells, axonal condition and precursor cells were investigated.
Withdrawal of cuprizone after acute demyelination (5 weeks) resulted in a robust endogenous remyelination, whereas remyelination was limited after chronic cuprizone exposure (13 weeks). Both lesions are characterized by a loss of mature oligodendrocytes and astrocyte/microglia accumulation. Quantification of cell parameters revealed lower numbers of IBA1+ microglia within the chronic demyelinated lesion but comparable numbers of GFAP+ astrocytes. After acute demyelination, the expression of well-known pro-myelination genes (e.g. IGF1) was increased. The expression of a set of unanticipated factors, e.g. the radial glia cell marker FABP7, and proteins involved in lipid metabolism (e.g. ADFP, HEXA, HEXB) was induced after acute but not chronic demyelination.
Remyelination appears to fail due to proper oligodendrocyte-axon interactions. Our results suggest that the diminished activity of glial cells might account for remyelination failure due to a reduced expression of pro-myelination factors.

Kategorie: Lecture

Rubrik: 13.Pheripheral and vegetative nervous system
Abstract Nr.:95

Titel: Guidelines for histopathological work-up, evaluation and diagnostics of human enteric neuropathies

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Abstract:
Purpose: A large spectrum of gastrointestinal motility disorders is associated or caused by enteric neuropathies. However, significant methodical discrepancies in reliably delineate normal from abnormal conditions of the enteric nervous system require standardized tissue acquisition and processing for establishing an unequivocal diagnosis.
Methods: Different approaches regarding tissue harvesting and processing, visualization of the enteric nervous system, and quantification of enteric ganglia, nerve and glial cells were compared based on own experiences and data from the literature. Enteric neuropathies were classified according to their histopathological pecularities and the underlying aetiologies.
Results: Adequate tissue processing requires (1) sufficient specimen size (> 2 cm), (2) orthograde orientation, (3) HE staining plus immunohistochemistry using pan-neuronal (HuC/D, PGP 9.5), pan-glial (S-100), and lymphocyte (CD3) markers. Quantitative analysis should include (1) determination of density of ganglia, nerve and glial cells per intestinal length, (2) all ganglionated nerve plexus layers (myenteric and submucosal plexus). Enteric neuropathies are classified in (1) primary neuropathies including developmental disorders, degenerative, inflammatory and inclusion body neuropathies, and (2) secondary neuropathies due to systemic disorders.
Conclusions: Standardized tissue processing, optimized staining protocols and both qualitative and quantitative analysis of the enteric nervous system allow to more precisely determine the histopathological features and nature of enteric neuropathies underlying human gastrointestinal motility disorders. Classification of the different histopathological entities provide helpful prognostic and therapeutic information and should therefore be performed in severe cases of gastrointestinal motor disturbances.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:96

Titel: Ctgf is a key modulator of the actin cytoskeleton in trabecular meshwork cells and intraocular pressure in the mouse eye

Autoren: Fuchshofer R.(1),Kressel S.(2),Junglas B.(2),Köstler J.(3),Wagner R.(3),Bösl M.(4),Tamm E.(5),

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Abstract:
Purpose: The acto-myosin system in the human trabecular meshwork (HTM) plays an important role in modulating intraocular pressure (IOP). The information on factors that modulate the HTM actin cytoskeleton is incomplete. Since Connective Tissue Growth Factor (CTGF) is expressed in high amounts in HTM cells, we analyzed if CTGF-signaling affects HTM actin cytoskeleton and IOP.

Methods: Cultured HTM cells were stably transfected with an overexpression or a knockdown construct for CTGF. CTGF overexpression in the TM of mouse eyes was achieved via adenoviral-mediated gene transfer (Ad5-CTGF) and via generation of transgenic mice with ocular overexpression of CTGF. Cells and eyes were analyzed by RT-PCR, immunoblotting, immunohistochemistry and microscopy.

Results:
CTGF overexpression via Ad5-CTGF or via transgenic overexpression caused a substantial increase in CTGF in the anterior eye, and a significant increase in IOP. By immunohistochemistry, a substantial increase of fibronectin and of alpha-smooth muscle-actin in the chamber angle was detected. HTM cell lines with CTGF overexpression formed more actin stress fibers than control cells. In contrast, actin stress fibers were rare or completely absent in cells with CTGF knock-down, an effect that could be reversed upon treatment recombinant CTGF.

Discussion: Our results strongly indicate that CTGF is a key modulator of the HTM actin cytoskeleton and of IOP. Modification of CTGF signaling appears to be a promising strategy to treat high IOP and glaucoma.

Support: DFG FOR 1075

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:97

Titel: Effects of stanniocalcin 2 on vessel formation in human neuroblastoma

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Abstract:
Purpose: Neuroblastoma (NB) is the most frequent solid extracranial tumor in children. Despite aggressive multimodal therapy, average mortality rates remain in the range of 40% and increase to 75% for advanced stages. Recently we showed that in keratoepithelin (TGFBI/BIGH3) -overexpressing NB cells tumor growth is inhibited, and analysis of these cells revealed enhanced expression of stanniocalcin 2 (STC2). STC2 is a secreted phosphoprotein, expressed in various embryonic and adult tissues as well as in several tumor cell lines. STC2 expression in mice results in pre- and postnatal growth restriction and is associated with favorable prognosis in estrogen receptor-positive breast cancer. In contrast, inhibition of STC2 renders mouse NB cells more vulnerable to apoptotic stimuli, and in renal cell carcinoma Stc2 expression is associated with poor outcome. To elucidate the function of Stc2 in progression and vascularization of NB, we analysed a large panel of cell lines and primary tumors and investigated effects of STC2 on proliferation, invasion and tumor formation of NB cell lines in vitro and in vivo.
Results: Stc2-transfected NB cells show inhibition of proliferation, but increased invasiveness. In chorio-allantoic-membrane tumor formation assays we observed an increased tendency of bleeding in tumors of Stc2-transfected NB cells.
Conclusions: Our results suggest that STC2 is a tumor promoting factor in NB and may, due to its vessel destructive properties, also facilitate tumor cell emigration and metastasis formation.

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Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:98

Titel: Longterm investigation of morphologic changes in the rat brain after Botulinum toxin A application

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Abstract:
Recently we described the stereotactic injection of Botulinum neurotoxin A (BoNT) into the striatum of rats. Interestingly, the motor abilities of 6-hydroxydopamine (6-OHDA) lesioned rats (hemiparkinsonian rats) are improved by blocking overregulated cholinergic transmission by injection of BoNT into the ipsilateral striatum. Three doses of BoNT were investigated: 100 pg, 1 ng and 2ng. The brains were examined 2 weeks, 1, 3 and 6 months post injection. Nissl staining and immunohistochemical visualization of cholinergic neurons by choline acetyle transferase (ChAT), dopaminergic neurons by tyrosine hydroxylase (TH) and immunohistochemical stainings of synaptic proteins were performed.
In the injected striata ChAT- and TH-immunoreactive vesicle-like round structures of different size (2 - 10 micrometer diameter) were observed. The BoNT-induced varicosities (BiVs) appeared at each time point after a BoNT injection and their number decrease over time. The volume of the injected striata was slightly decreased compared to the control hemisphere. The number of cholinergic neurons, however, was not changed. The motoric behaviour of the BoNT animals revealed a clear therapeutic effect of intrastriatal BoNT in hemiparkinsonian rats up to 3 months post injection. The apomorphine-induced rotations disappeared from about 8 to 0 per min. Later on, the pathological apomorphine-induced rotations reappear.
In conclusion, intrastriatal applied BoNT can counteract consequences of experimental 6-OHDA-induced hemiparkinsonism by modulating neuronal transmission of striatal circuits.

Kategorie: Lecture