

Lecture Abstract Booklet

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Abstracts are arranged in the same order as lectures are presented during the congress

1

Title:Oncological surgery in urology: How to reduce morbidity –
Anatomical approach.

Authors: Bartsch G

Addresses: Innsbruck, Austria

No abstract available.

2

Title: Prostatic stem cell regulation in health and disease.

Authors: Risbridger GP, Taylor R, Cunha GR

Addresses: Melbourne, Australia

No abstract available.

3

Title:Embryologic development, tumor spread and the new principle of radical surgical treatment. Total mesometrial resection

Authors: Höckel M

Addresses: Leipzig, Germany

No abstract available.

Title: Homeobox genes: Linking embryogenesis and tumorigenesis of "epithelial cancers"

Authors: Naora H

Addresses: Houston, Texas, USA

No abstract available.

Titel: Regulation of human testicular peritubular cells by TNFalpha

Authors: Albrecht M.(1), Schell C.(1), Mayer C.(1), Schwarzer J.(2), Frungieri M.(3), Mayerhofer A.(1),

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Abstract:

Purpose: To explore the nature of human testicular peritubular cells and mechanisms of their regulation.

Methods: Human testicular peritubular cells (HTPCs) were isolated from adult human testes and cultured under standardized conditions (Albrecht et al., J Clin Endocrinol Metab, 2006). Complementary approaches, including genearray/RT-PCR studies, Western blotting/immunocytochemistry and ELISA techniques were employed to study phenotypic characteristics of HTPCs and actions of TNFalpha.

Results: HTPCs are positive for TNFalpha receptors 1 and 2 and respond to recombinant human TNFalpha by a rapid phosphorylation of ERK1/2. They express the NGF gene and TNFalpha stimulated mRNA levels and secretion of NGF in a dose- and time- dependent manner. Similarly, MCP-1 was identified as a product of HTPCs, which was regulated by TNFalpha in a concentration and time-dependent way. TNFalpha furthermore strongly enhanced expression and/or synthesis of COX2 and prostaglandin D secretion. In addition, ICAM-1, which was not detected at protein level in the absence of TNFalpha, was induced upon TNFalpha stimulation.

Conclusions: Our results provide novel insights into the nature of human testicular peritubular cells, which, besides their structural function, are able to secrete potent signalling molecules and are regulated by TNFalpha.

Grant support: DFG MA 1080/16-3

Category: Lecture

Titel: Mechanisms of generation of Leydig cell toxic H₂O₂ by ascorbic acid

Authors: Kosova V.(1), Müller D.(1), Bargheer O.(1), Middendorff R.(1),

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Abstract:

Recent findings that ascorbic acid (Asc) and other compounds, regarded as antioxidants, induce cell death selectively in cancer cells suggest an unanticipated therapeutic potential. How these agents can act in a cytotoxic manner remains to be established.

In MA-10-Leydig tumor cells, Asc led to a loss of cell viability that could be reversed by catalase, indicating an involvement of H₂O₂. In agreement, administration of exogenous H₂O₂ resulted in a dose-dependent decrease of cell number. Using a newly developed assay based on light emission from luminol in response to H₂O₂-mediated oxidation, H₂O₂-generating, i.e. prooxidative, activity of Asc was found in the absence of cells and serum at low (<1 mM) concentrations. Superoxide was shown to be involved in this process. Reducing activity of Asc, indicated by use of the tetrazolium salt MTT, was found at concentrations >1 mM and could be inhibited by a heat-resistant low-molecular-weight serum protein. In the presence of either this protein or serum, Asc-induced H₂O₂ generation dose-dependently increased, indicating an involvement of the protein in uncovering prooxidative Asc effects. Asc-induced cytotoxicity could be abrogated by NO in a cGMP-independent manner, thereby excluding direct interactions of H₂O₂ and cGMP pathways.

Taken together, Asc-induced generation of H₂O₂, resulting in cytotoxic effects on Leydig cells which can be prevented by NO, can take place outside of cells, and a low-molecular-weight protein is responsible for a switch of the vitamin from a predominantly antioxidative to a prooxidative, H₂O₂-generating, cytotoxic molecule.

Category: Lecture

Titel: Peroxisome deficiency in Sertoli cell-specific PEX13-knockout mice leads to complete loss of germ cells and azoospermia.

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Abstract:

Sertoli cells play a central role in fetal gonad development and postnatal spermatogenesis. They provide physical support for the seminiferous epithelium and create an impermeable and immunological barrier (blood-testis barrier) in favour of normal germ cell development and maturation in adult testis. As shown recently by our group, peroxisomes are present in high abundance in Sertoli cells, exhibiting high levels of lipid transporters and beta-oxidation enzymes.

In order to understand Sertoli cell-specific functions of peroxisomes on spermatogenesis in vivo, we generated a conditional PEX13 knockout mouse using the Cre/loxP recombination system. Sertoli cell-specific (Scs) PEX13-deletion leads to complete disruption of peroxisomal metabolic pathways in this cell type due to defective peroxisome biogenesis. The ScsPEX13-knockout resulted in a gradual deterioration of spermatogenesis until arrest of spermatogenesis and complete loss of germ cells. Already in P15-P30 animals lipid accumulation and vacuolation of Sertoli cells was observed. At later time points (P90) seminiferous tubules looked spongy and showed reduced numbers of germ cells. At this stage animals were already less fertile. A complete loss of germ cells was observed in adult testis after 130 days in ScsPEX13-KO animals. In seminiferous tubules of P130 knockout mice only Sertoli cells were present and peritubular myoid cells were proliferated, surrounding seminiferous tubules in double layers. In the interstitial space Leydig cells increased excessively in number, forming big clusters.

Our data demonstrate that peroxisomes in Sertoli cells are an absolute requirement for normal spermatogenesis, the integrity of Sertoli cell and sperm cell functions.

Category:

Lecture

Titel: Differentiation of the blood-testis barrier in conditional Sertoli cell connexin43 knockout (SCCx43KO) mice

Authors: Zeiler M. (1), Winterhager E. (2), Willecke K. (3), Guillou F. (4), Steger K. (5), Bergmann M. (1), Brehm R. (1)

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Abstract:

Purpose: Connexin(cx)43, which is the predominant gap junctional protein in testis, occurs between neighboring Sertoli cells (SC) and between Sertoli and germ cells (GC). In our conditional knockout mouse model, in which only SC lack a functional cx43-gene, the initiation of spermatogenesis has been shown to be inhibited. Additionally, a significant increase in SC number and a significant reduction of GC (spermatogonia) per seminiferous tubule was observed resulting in infertility of adult SCCx43KO^{-/-} males. As cx43 in SC is believed to play a role in the regulated formation of the blood-testis barrier (BTB), we hypothesized that deletion of this cx leads to an alteration of BTB assembly and disintegration of the SC junctional complexes providing a possible explanation for the reported GC deficiency and altered spermatogenesis.

Methods: For that purpose, the murine BTB was examined in SCCx43KO^{-/-} mice in comparison with their wild type and heterozygous littermates using immunohistochemistry, immunofluorescence and/or western blot analysis for cx43, N-cadherin, Zonula occludens (ZO)-1 and occludin, and Real-time PCR for cx43, N-cadherin, occluding and claudin11. Additionally an electron microscopy study with lanthanum tracer and hypertonic glucose fixative was carried out to investigate the functional status of the barrier.

Results: Although first results show a modified protein localization for N-cadherin and occludin in seminiferous tubules of SCCx43KO^{-/-} mice and quantitative changes in mRNA expression, the BTB was closed and functional.

Conclusions: Preliminary results indicate that cx43 in SC is not essential for a functional BTB formation in mouse testis.

Category: Lecture

Titel:Estrogenic regulation of cyr61 in endometriotic lesions

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Abstract:

Endometriosis is an estrogen dependent benign gynaecological disease of women in the reproductive age. Routinely, endometriosis is medicated using hormonal treatments such as GNRH agonists or progestins. Like in tumors, local growth and maintenance of the ectopic endometriotic lesions are dependent on neoangiogenesis. Several angiogenic factors such as CYR61, VEGF and angiopoietins are known to be upregulated in endometriosis.

Here, we have investigated estrogenic regulation of CYR61 in endometrial epithelial HES cells and in cells transiently transfected with the estrogen receptors ESR1 and ESR2. In addition, we evaluated CYR61 regulation in ectopic endometriotic tissues from 18 patients considering the use of hormonal contraceptives (containing estradiol/gestagens) or Enantone®, a GNRH agonist, treatment.

Incubations with 17 β -estradiol significantly increased CYR61 mRNA and protein levels in parental as well as in ESR1 overexpressing HES cells. This effect was abolished by a simultaneous application of an anti-estrogen. CYR61 in ESR2 transfected cells did not respond to estrogen.

In patients, CYR61 was highly elevated in ectopic lesions, when compared to corresponding eutopic endometrium from the same women. The protein was strongly expressed in uterine epithelial cells as well as in endothelial cells. Pill users displayed still increased CYR61 levels in ectopic sites but enantone treatment abolished exclusively the local upregulation. Moreover, CYR61 immunostaining disappeared from endothelial cells in lesions from the enantone-treated group. In conclusion, endometrial estrogenic up-regulation of CYR61 expression is mediated by ESR1 and a GNRH agonist leading to a systemic estradiol deficiency is very effective in reducing angiogenesis in endometriosis.

Category: Lecture

Titel: Regulation of CCN proteins CYR61 (CCN1) and NOV (CCN3) in human trophoblast cells under hypoxia

Authors: Wolf N.(1), Schmidt M.(2), Dunk C.(3), Frede S.(4), Winterhager E.(1), Gellhaus A.(1),

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Abstract:

Purpose: The prevalent theory of the pregnancy disease preeclampsia is a shallow invasion of the extravillous trophoblast into the decidua and maternal vessels, which leads to hypoxia and uteroplacental insufficiency. Since angiogenesis and/or migration of the trophoblast are affected in this disorder we have focused on molecules that are discussed as key players in these processes, CYR61 (CCN1) and NOV (CCN3), which are expressed in placental vessels and in the invasive extravillous trophoblast.

Methods: Since invasion is known to be regulated by oxygen tension we investigated the expression of CYR61 and NOV in the invasive trophoblast cell line JEG3 under hypoxia using qRT-PCR and western blotting. Cells were cultured up to 24h under normoxia (20% O₂) or hypoxia (1% O₂).

Results: Our results showed an increase of CYR61 mRNA and intracellular as well as secreted protein upon hypoxia. Whereas NOV mRNA and intracellular protein were upregulated, secretion of NOV seems to be downregulated. The elevated protein level of CYR61 and NOV are accompanied by the stabilization of the HIF-1 α protein. To confirm these observations we treated JEG3 cells with Dimethylxalylglycine (DMOG) to mimic the hypoxia induced HIF-1 α stabilization under normoxia. Stabilization of HIF-1 α upon DMOG treatment leads to a similar increase of CYR61 and NOV transcript and intracellular protein expression.

Conclusions: CYR61 and NOV are hypoxia-inducible genes in the cell line JEG3. An imbalance in the production of both CCN molecules at the maternal-fetal interface, resulting from a low oxygen supply, could be the reason for migration impairment.

Category: Lecture

Title: Morphogenesis in colorectal cancer

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No abstract available.

Titel: Epithelial development of the human anorectum: conventional histologic and immunohistochemical findings

Authors: Zehm S.(1), Aigner F.(2), Fritsch H.(3),

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Abstract:

Background: The epithelial lining of the anorectum still raises discussions concerning the levels of transition between the various sections. Since the expression of cytokeratins depends on the epithelial cell-type, period of development and stage of differentiation, it has been proposed that the anorectal epithelia can be discriminated and characterised by their specific patterns of cytokeratin expression.

Methods: In the present study the differentiation and spatiotemporal distribution of the different epithelial sections of the human anorectum were examined by means of conventional histology and immunohistochemistry. Monoclonal antibodies were directed against cytokeratins 18, 7 and 14. Thirteen human embryos and fetuses as well as sections of a two-year-old child and of three adults were studied microscopically.

Results: Distinct age-related changes concerning the morphology and the expression of cytokeratins within the different epithelial sections lining the anorectum were observed. In all specimens of all stages it was possible to differentiate the epithelial sections by their cytokeratin expression profile. These findings in normal anorectal epithelia were compared to the cytokeratin expression profile in 17 untreated distal rectal carcinoma.

Conclusion: Our histomorphological study of developmental processes provides first evidence for the cellular origin and differentiation of the anorectal epithelia. Our results may help to understand the occurrence and characteristics of different carcinoma within the distal rectum.

Category: Lecture

Titel: The vascular nature of hemorrhoids

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Abstract:

Purpose: The arterial blood supply of the corpus cavernosum recti (CCR) is commonly believed to be associated with the pathogenesis of hemorrhoids. Over decades several anatomical studies have revealed the complex topography of the anorectum and its vascularization. Still there is scarce knowledge about the exact mechanisms of filling and drainage of the arteriovenous network within the anorectal submucosa with regard to hemorrhoidal disease.

Methods: 41 patients (17 female, 24 male; mean age 48 years) with hemorrhoids of Goligher grade I to IV were compared to 17 healthy volunteers (nine female, eight male; mean age 29 years) by means of transperineal colour Doppler ultrasound (CDUS) with regard to the superior rectal artery branching pattern and the venous drainage of the CCR. **Results:** The mean calibre of the arterial branches in the study group with hemorrhoids was 1.9 ± 0.7 mm (range 0.6 to 3.6 mm) and 0.9 ± 0.2 mm (range 0.6 to 1.2 mm) in the control group ($p < 0.001$). The arterial blood flow was significantly higher in patients with hemorrhoids than in the control group (mean 33.9 vs. 11.9 cm/sec, $p < 0.01$). Significant changes in the venous drainage of the CCR in patients with different grades of hemorrhoids were detected by transperineal CDUS.

Conclusions: Transperineal CDUS is introduced as a sensitive method to assess the filling and drainage of the internal hemorrhoidal plexus. Hypervascularization of the anorectum is proposed to contribute to the growth of hemorrhoids rather than being a consequence of hemorrhoids. Pre- and postoperative assessment of its vascularization helps to judge the success of a technique for treatment of different grades of hemorrhoids.

Category: Lecture

Titel: Morphologic conditions for nerve-sparing surgery of pelvic organs

Authors: Kneist W.(1), Herrmann M.(2),

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Abstract:

Purpose: A basic understanding of clinical anatomy and functional neuroanatomy of the inferior hypogastric plexus (IHP) is essential to avoid iatrogenic injury.

Methods: We review the pelvic topography with respect to nerve sparing surgery.

Macroscopic data were acquired from fixed cadavers of 13 male and 12 female donors. Various non-surgical and surgical approaches were used. Components of the IHP were documented by photography and hand drawings. By analyzing the experience of confirmed nerve sparing mesorectal excision in rectal cancer patients' discussion was focused on the efferent branches of the IHP in order to explain the heterogeneity of postoperative functional disturbances.

Results: We identified efferences from IHP to the pelvic organs. Three clusters of ganglia in different segments of the IHP were found in all specimens. Secondary plexuses are located at the lateral aspect of the middle rectum, paracervix, seminal vesicle and bladder. Ventral and lateral wall of excavatio vesicorectalis, and excavatio rectouterina, seminal vesicles and ureter can be used as landmarks for a complete or selective nerve sparing surgery. Function disturbances after mesorectal excision occurred in up to 27 percent of patients, in 55 percent of the affected patients either as genital or as urinary dysfunction. Therefore, mainly the damage of different plexus segments is responsible for the different combinations of function disturbances.

Conclusions: Respecting identifiable key zones of the IHP during surgical procedures seems reliable and could lower clinically relevant function disturbances. However, in patients with specific resection limits, a selective nerve sparing technique may be an option.

Category: Lecture

Titel: Unilateral ischiadic nerve lesion after pelvic floor reconstruction (Prolift GyneCare Total®): case report and anatomical study

Authors: Shiozawa T.(1), Skutella T.(1), Wallwiener D.(2), Reisenauer C.(2),

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Abstract:

Objective: To present a case report and to discuss the anatomical conditions for posterior pelvic floor reconstruction in relation to the rare complication of an unilateral ischiadic nerve lesion in this case. **Study Design:** Retrospective case review and prospective cadaveric dissection. **Methods:** The medical record and consultant reports were reviewed from a patient who reported postoperative paresthesia and pain after posterior Prolift® placement. In addition, four ethanol-preserved cadaver pelvis specimens were operated with the Prolift Gynacare Total® implant and dissected to identify the topographic relations of the implant in the small pelvis and gluteal fossa. The distances between nerves (ischiadic, pudendal) and implant were measured. **Results:** The patient was diagnosed with postoperative paralysis of the ischiadic nerve and required physiotherapy and medication postoperatively. The mean distance from the ischiadic nerve to the implant in the small pelvis was 20±2 mm. In the gluteal fossa 28±3 mm lay between nerve and the implant passage through the sacrotuberal ligament. Shorter distances (11±1 mm) were measured between implant and pudendal nerve on level with the sacrospinal ligament. **Conclusions:** Direct nerve injury can be most possibly excluded as an explanation for this rare complication; other causes e.g. positioning damage during the operation can be discussed. However, the proximity of the implant to the nerval structures in the gluteal fossa emphasizes the importance of maintaining the correct landmark-based direction during surgery.

Category: Lecture

Titel: Anatomical conditions for pelvic floor reconstruction with polypropylene implant and its application for the treatment of vaginal prolapse

Authors: Reisenauer C.(1), Shiozawa T.(2), Kirschniak A.(3), Drews U.(4), Wallwiener D.(1),

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Abstract:

Objective: The purpose of the surgical treatment of vaginal prolapse is not only the restoration of the anatomy but also of the visceral functioning. To maintain the quality of life for patients with recurrent vaginal prolapse, to avoid colpectomy or colpocleisis at the same time, synthetic materials have been introduced in transvaginal reconstructive surgery of the pelvic floor. The aim of this study is to determine the anatomical position of the polypropylene implants after reconstruction of every compartment of the pelvic floor and to determine the relation of the implants to the major neighbouring neurovascular structures on the basis of corpse dissections.

Methods: Following the technique of the TVM Group from France we present the pelvic floor reconstruction using Prolift Gynecare. To reach the aims of the study, anatomical dissections of the pelvic floor on three specially preserved anatomical specimens are performed after the placement of the implants.

Results: The anatomical dissections show that every defect in all three compartments of the pelvic floor can be repaired by using polypropylene implants. Between the implants and the major neighbouring neurovascular structures a safe distance exists with slight individual differences. Consequently neurovascular injuries will be avoided if the described surgical technique is performed.

Conclusion: The pelvic floor reconstruction using polypropylene implants is a treatment option especially for the surgical correction of the recurrent vaginal prolapse. If the surgeon has thorough anatomical knowledge and performs the surgical technique in the recommended manner, injuries of the major neighbouring neurovascular structures will be avoided.

Category: Lecture

Titel:Revaluation of the fetal muscle development of the vesical trigone

Authors: Oswald J.(1),Richter E.(2),Longato S.(3),Brenner E.(3),Radmayr C.(4),Bartsch G.(5),Fritsch H.(3),

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Abstract:

Purpose: The fetal development of the interureteric muscle, a precondition of a sufficient opposite anchoring of the ureterovesical junction (UVJ) and the muscle architecture of the bladder neck were investigated in an immunohistochemical study.

Methods: In 38 (16 female, 22 male) fetal specimens and 7 newborns (2 female, 5 male) ranging from 9 to 40 weeks, the ureterotrigonal units were investigated. Histology was based on serial consecutive sections of the urinary bladder base. Anti-human α -smooth muscle actin immunostaining was used to demonstrate the time course of muscle development and arrangement, respectively.

Results: A much earlier developmental stage of the trigone muscle configuration during fetal life was noticed than reported to date. The condensation of myoblasts located mainly in the dorsal wall of the trigone and at the bladder outlet was present as early as at 12 weeks of gestation. The trigone develops continuously as a single circular muscular layer corresponding to the posterior part of the vesical sphincter muscle. Muscle fibres forming the interureteric junction were demonstrable as early as at 14 weeks of gestation.

Conclusions: There is a close connection of the trigonal smooth muscle layer and the vesical sphincter muscle forming the main part of the trigone by 12 weeks of gestation. The fetal development of the trigone, in detail the muscle architecture of the bladder neck which consists only of a ring-shaped muscular layer as well as the transversal oriented interureteric muscle, respectively forms a functional entity representing the anatomical basis for a competent UVJ.

Category: Lecture

Titel: Gender-related development of the internal urethral sphincter and bladder outlet in human fetuses provides evidence of a transient fetal infravesical obstruction in male fetuses

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Abstract:

Introduction

To examine the fetal development of the internal urethral sphincter, in particular a possible gender related difference of the bladder outlet which may be associated with bladder emptying at a different voiding pressure.

Materials and Methods

In all, 37 (14 female, 23 male) normal fetal bladder neck specimens with the smooth muscle complex of the internal sphincter were investigated. (mean gestational age 19.4 weeks) After immunostaining of sagittal serial sections the internal sphincter volumes and bladder outlet diameters were measured and correlated with gender and age of gestation.

Results

Between the 20th and the 40th week of gestation significant higher internal sphincter muscles volumes could be demonstrated in male fetuses compared to females. As a result of this gender difference the bladder outlet was significantly ($p < 0.001$) greater in females compared to male fetuses. (1077.5 vs 694.7 μm).

Conclusions

From the 20th week of gestation until the 40th weeks of gestation male fetuses demonstrate a significant higher internal urethral sphincter volume compared to female fetuses. The possibility of a functional fetal infravesical obstruction in male fetuses may play a role in this significant narrowed bladder necks.

Category: Lecture

Titel:Shape of the ciliary processes in the mouse eye

Authors: May C.(1),May A.(1),

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Abstract:

In contrast to most mammalian species, the ciliary processes in the mouse eye form an irregular pattern. To characterize the arrangement of the processes, adult C57BL/6J animals were studied using scanning electron microscopy. The different quadrants of the eye showed typical characteristics: in the superior quadrant, large and radial oriented processes were present. In the inferior quadrant, the processes were small but still mainly radial oriented. In the temporal quadrant, the processes showed radial and longitudinal courses, some of the processes being L-shaped. In the nasal quadrant, the few processes were oriented longitudinal.

During aging the ciliary processes maintain their shape while a mild shift in the relation pars plana to pars plicata occur. This shift starts early and is more pronounced in animals with retinal degeneration (rd/rd mouse).

Category: Lecture

Titel: Orofacial functional compartments- Background and experimental evidence

Authors: Engelke W.(1),Knösel M.(2),Fanghänel J.(3),Kubein-Meesenburg D.(2),Nägerl H.(2),

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Abstract:

Background: Functional anatomy of the oral cavity still provides unresolved problems. In particular the mechanism of posterior displacement of intraoral structures causing mesopharyngeal obstruction during sleep is primarily associated with neuromuscular factors but recently has been advocated to be dependent on physical anatomical factors also.

Functional Model: The newly developed functional model of oronasopharyngeal structures defines besides the nasoepipharyngeal and the oropharyngeal compartment two functional oral compartments which are different from the clinical anatomic approach. Based on functional aspects during rest position and swallowing, a peridental compartment A lined by the floor of the mouth and buccal mucosa and surrounding the dentoalveolar structures is defined and a subpalatal compartment B between the hard and soft palate on one hand and the tongue surface on the other is postulated. Consequently, the cavum oris and vestibulum oris only form parts of the functional compartments in case of open mouth observation.

Method: Monitoring of atmospheric pressure was performed on 50 subjects during a period of 3 minutes during rest and during swallow of saliva and with the tongue repositioning manoeuvre carried out.

Results: Pressure diagrams showed that in both compartments negative pressure was formed after swallowing indicating closed systems. Furthermore the majority of volunteers exhibited differences in timing and altitude of negative pressure formation in both compartments measured.

Conclusion: The results give evidence that after swallowing, two different intraoral functional anatomical compartments can be formed which may have a large impact on stabilisation of the oronasopharyngeal system as well on the developing dentition.

Category: Lecture

Titel: The function of the lumbar perivertebral musculature – Implications by the distribution of muscle fibre types

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Abstract:

Purpose: Interpretations of muscle topography and EMG data concerning the function of the lumbar perivertebral musculature are conflicting. Based on topography, the m. multifidus is thought to stabilise and the mm. longissimus et iliocostalis to mobilise the vertebral column. Likewise the mm. quadratus lumborum et psoas major are referred to as mobilisers, the former additionally stabilising the last rib during inspiration. Based on EMG data, all muscles are suggested to stabilise as well as to mobilise the vertebral column. To get further implications on back muscle function, we investigated the distribution pattern of the two main muscle fibre types.

Methods: The proportion of type I and type II fibres was determined over the whole muscular cross section along the lumbar spine of three donated male bodies. For this, the fibres were marked on consecutive serial sections of the perivertebral muscles using immune-histochemistry.

Results & Discussion: The more or less equal distribution of muscle fibres of both types over the muscles' cross sections confirms the results obtained by EMG data. All muscles may stabilise and mobilise the vertebral column. In particular, an unexpected high proportion of type I fibres in the mm. longissimus et iliocostalis as well as the m. psoas major assigns them a more stabilising function than anticipated based on topography. This is consistent with studies using mathematical models to examine the muscles' function. Thus, muscle topography is not sufficient to infer a muscle's function and can lead to misinterpretations if functional parameters and internal muscle characteristics are unknown.

Category: Lecture

Titel:Antigen uptake by dendritic cells - impact on lung inflammation in asthma

Authors: Tschernig T.(1),Hartwig C.(2),Mazzega M.(2),Constabel H.(3),Gessner E.(3),Behrens G.(3),

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Abstract:

Mechanisms of antigen uptake by dendritic cells (DC) determine the outcome of immune responses. One pathway of phagocytosis are Fc-receptors. To identify the impact of Fc-gamma-receptors (FcγR) on the antigen uptake and antigen presentation of dendritic cells, FcγR-deficient mice were sensitized and challenged with a typical allergen (ovalbumin/OVA) inducing an asthma-like inflammation in the lung. The numbers of eosinophils – a key marker in allergic inflammation - were significantly reduced in FcγR-deficient mice, whereas the levels of IgE and IgG were not affected. Furthermore, the impact of FcγR on antigen presentation and T cell proliferation was determined in vitro and in vivo. In vitro T cell proliferation was strongly stimulated by immune complexes in wild-type mice. In contrast, the FcγR-deficient mice showed a reduction in T cell proliferation. These findings have been confirmed in vivo demonstrating that FcγR are involved in the outcome of asthma-like lung inflammation.

Supported by the German Research Foundation: SFB587, B5

Category: Lecture

Titel: Severe metabolic disturbances in Clara cells and alveolar epithelial cells due to peroxisome deficiency in the lung of PEX11beta-knockout mice.

Authors: Karnati S.(1), Baumgart-Vogt E.(2),

Addresses: (1) Department of Anatomy and Cell Biology II | Institute for Anatomy and Cell Biology | Giessen | Germany; (2) Department of Anatomy and Cell Biology II | Institute for Anatomy and Cell Biology | Giessen | Germany; email: Baumgart-Vogt.Eveline@anatomie.med.uni-giessen.de

Abstract:

Peroxisomal abundance is controlled by metabolic demand and is regulated by proteins of the peroxin 11-family (Pex11p), involved in peroxisomal division and proliferation. In the lung peroxisomes are most abundant in AECII cells and Clara cells, both cell types involved in surfactant synthesis. We have used PEX11^{-/-}-KO mice to investigate the pathological consequences of peroxisome deficiency in the lung. We characterized lungs of wild type and PEX11beta-KO mice by means of 1) IHC and double-IF for lung cell types, anti-oxidative enzymes, peroxisomal proteins 2) RT-PCR analysis and 3) Western blot analysis of enriched peroxisomal fractions. Our data show a reduced abundance of peroxisomes in all lung cell types. A compensatory increase of beta-oxidation enzymes and Pex13p was noted in Clara cells and AECII cells of PEX11^{-/-}-KO mice. Significant reductions of the Clara cell protein 10 (CC10) and the T1⁻-protein/podoplanin were noted. In addition, a marked increase of mitochondrial SOD2 and significant down regulations of extracellular SOD3, glutathione S-transferase 1, glutathione peroxidase, peroxiredoxin V and hemoxygenase 1 were noted in PEX11beta-deficient mouse lungs, suggesting an accumulation of reactive oxygen species in PEX11beta-KO animals. As suggested by our data, peroxisomes might play an important role in the control and regulation of ROS metabolism in the lung as well as in airway homeostasis. Peroxisomal malfunction might be involved in lung diseases, such as asthma, chronic obstructive pulmonary dysplasia and idiopathic pulmonary fibrosis. Further studies have to prove which relevance peroxisomal ROS metabolism has in the protection against chronic-inflammatory lung diseases.

Category: Poster

Titel: Expression and regulation of antimicrobial peptide rCRAMP after bacterial infection in primary rat meningeal cells

Authors: Brandenburg L.(1), Varoga D.(2), Nicolaeva N.(3), Leib S.(4), Lucius R.(3), Podschun R.(5), Wruck C.(1), Pufe T.(1),

Addresses: (1) Anatomy and Cell Biology|University Hospital|Aachen|Germany; email: lbrandenburg@ukaachen.de; (2) Department of Trauma|University Hospital Schleswig-Holstein|Kiel|Germany; (3) Anatomy|Christian-Albrechts-University|Kiel|Germany; (4) Institute for Infectious Diseases|University Bern|Bern|Switzerland; (5) Institute of Infection Medicine|University Hospital Schleswig-Holstein|Kiel|Germany

Abstract:

Antimicrobial peptides are part of the innate immune system in many organ systems. But, little is known about the expression of these peptides in the brain. To address this question we examined the expression and regulation of the antimicrobial peptide rCRAMP in cell culture of primary rat meningeal cells as the important part of the blood brain barrier (BBB) after incubation with different bacterial supernatants of meningitis pathogens by real time RT-PCR and Western Blotting. To explore the occurrence and function of rCRAMP, Wistar rats were infected with *Streptococcus pneumoniae* as an animal model of bacterial meningitis in vivo.

We demonstrate here (i) the expression, secretion and bactericidal properties of rCRAMP, and (ii) in experimental pneumococcal meningitis we localized rCRAMP to primary rat meningeal cells by fluorescence microscopy. Moreover, (iii) our results demonstrate the modulation of rCRAMP expression by inflammatory signal transduction pathways triggered through the action of NF- κ B and MAPK in neuroinflammatory processes.

The results suggest that rCRAMP is an important part of the innate immunity in the brain by the meninges against pathogens.

Category: Lecture

Titel: A Clostridium botulinum C3 protein-derived peptide exerts neurotrophic activity by promoting neurite outgrowth and synaptic connectivity

Authors: Höltje M.(1), Hofmann F.(2), Münster-Wandowski A.(1), Just I.(2), Ahnert-Hilger G.(1),

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Abstract:

Purpose: To investigate enzyme-independent neurotrophic effects of Clostridium botulinum C3 proteins on hippocampal cultures

Methods: Using primary hippocampal cultures we performed morphometrical measurements of axonodendritic growth as well as immunocytochemical detection of synaptic marker proteins to analyse synaptic connectivity after incubation with the short peptide C3bot aa154-182.

Results: We could demonstrate that C3 botulinum (C3bot) possesses an additional neurotrophic function independent from its enzymatic activity. In the present study, we localized the region of C3bot responsible for the neurotrophic effect to the amino acids 154-182. By applying this short peptide in nanomolar doses we detected and quantified promoting effects both on axonal and dendritic arborisation. Furthermore, we investigated the question whether the enhanced neurite outgrowth was also accompanied by alterations in synaptic connectivity. Cultures treated with C3bot aa154-182 exhibited an increased number of both glutamatergic (VGLUT1/2) and GABAergic (VGAT) terminals. Moreover, VGLUT1 was found to predominate over VGLUT2 immunoreactivity in glutamatergic terminals under either control conditions or after treatment with C3bot peptide.

Conclusions: A short peptide of 29 amino acids derived from C3bot is responsible for the enzyme-independent effect of the full length protein. It is sufficient to promote neurite outgrowth and enhances the number of both excitatory and inhibitory terminals.

Category: Lecture

Titel: Organotypic cerebellar slice cultures of ankyrinG-deficient mice as a tool to study molecular and cellular mechanisms of neuronal polarity

Authors: Schultz C.(1), Sobotzik J.(1), Del Turco D.(1), Deller T.(1),

Addresses: (1) Institute of Clinical Neuroanatomy, Dr. Senckenbergische Anatomie | J. W. Goethe-University | Frankfurt am Main | Germany; email: Schultz@em.uni-frankfurt.de

Abstract:

AnkyrinG is a membrane-associated adapter protein which is involved in establishing a membrane-associated diffusion barrier in the axon initial segment (AIS). This AIS-specific diffusion barrier presumably contributes to the maintenance of axo-dendritic polarity. We have recently studied mice with a cerebellum-specific deficiency for ankyrinG. Strikingly, a subset of Purkinje cell axons in these mice exhibit hallmark features of axons and dendrites. We have designated these axo-dendritic hybrids as "axodendrites". To determine whether axodendrites can be also studied in vitro, we prepared organotypic slice cultures from the cerebellum of ankyrinG^{-/-} mice at postnatal day 8-10. Parasagittal cerebellar slices were cultured for 14-21 days, fixed, and stained for calbindin as a marker for Purkinje cells. A subset of Purkinje cell axons exhibited typical features of axodendrites. Most importantly, they possessed cytoplasmic protrusions closely resembling dendritic spines. Similar to dendritic spines, a fraction of these axodendritic spines were contacted by boutons immunopositive for VGlut1, a marker for glutamatergic terminals. These boutons most likely correspond to aberrant parallel fiber synapses, since all other candidate glutamatergic systems, including climbing fibers and mossy fibers, are abolished in cerebellar slice preparations. These findings suggest that cerebellar slice cultures of ankyrinG^{-/-} mice are a suitable in vitro model to study the impact of ankyrinG-deficiency on neuronal polarity.

Category: Lecture

Titel:EGFT-tagged borna disease virus – a new tool for monitoring structural alterations of infected neurons in the hippocampus

Authors: Heimrich B.(1),Gülzow T.(2),Ackermann A.(3),Staeheli P.(3),Schneider U.(3),

Addresses:(1)Dept. Neuroanatomy|Institute of Anatomy & Cell Biology|Freiburg|Germany; email:bernd.heimrich@zfn.uni-freiburg.de; (2)Dept. Neuroanatomy|Inst. Anatomy &Cell Biology|Freiburg|Germany; (3)Dept. Virology|Univ. Freiburg|Freiburg|Germany

Abstract:

Newborn rats that have been infected with the neurotropic Borna disease virus (BDV) develop a widespread infection of neurons in the hippocampus. After several weeks post infection dentate granule cells undergo progressive cell death whereas infected pyramidal neurons survive. A detailed time course analysis of this neurodevelopmental damage has not been performed so far.

We generated an EGFP-tagged BDV mutant to study morphological changes of identified infected hippocampal neurons even in living tissue. Already at 14 days post infection pyramidal cells and dentate granule cells showed strong EGFP-BDV labelling. Entire dendritic arbors and axonal processes of infected neurons were stained in a Golgi-like manner. Beside an infection of principal neurons in the hippocampus we also observed BDV-infected Cajal-Retzius cell in the dentate gyrus. Using parvalbumin immunostaining we could demonstrate that at least this population of inhibitory interneurons in the hippocampus is susceptible to BDV infection. Hippocampal slice cultures infected with BDV immediately after explantation showed similar replication of EGFP-BDV and living infected neurons could be monitored over time.

Our data demonstrate that we could establish a green fluorescent BDV as a tool which allows us to record and to analyze morphological changes of neurons that had been infected with BDV. (Supported by the DFG: He 1520; SCHN 765/1-5).

Category: Lecture

Titel:Gonadotropin-releasing hormone regulates spine density via its regulatory role in hippocampal estrogen synthesis

Authors: Prange-Kiel J.(1),Jarry H.(2),Schoen M.(1),Kohlmann P.(1),Lohse C.(1),Zhou L.(1),Spergel D.(3),Rune G.(1),(4),

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Abstract:

Spine density in the hippocampus depends on the activity of local aromatase, the final enzyme in estrogen synthesis. In view of the abundant gonadotropin-releasing hormone receptor (GnRH-R) mRNA expression in the hippocampus and the direct effect of GnRH on estradiol (E2) synthesis in gonadal cells, we tested GnRH as a potential regulator of hippocampal E2 synthesis. In hippocampal cultures, E2 synthesis, spine synapse density, and immunoreactivity of spinophilin, a reliable spine marker, were consistently upregulated in a dose-dependent manner at low doses of GnRH, but decreased at higher doses. GnRH was ineffective in the presence of GnRH antagonists or aromatase inhibitors. Conversely, GnRH-R expression was increased after inhibition of hippocampal aromatase. As we found estrus cyclicity of spine density in the hippocampus but not in the neocortex and GnRH-R expression to be five-fold higher in the hippocampus compared to the neocortex, our data strongly suggest that estrus cyclic synaptogenesis in the female hippocampus results from cyclic release of GnRH.

Category: Lecture

Titel: Estradiol stimulates expression of reelin in hippocampal Cajal-Retzius cells

Authors: Bender R.(1), Zhou L.(1), Paysen D.(1), Lanowski J.(1), Rune G.(1),

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email: rbender@uke.uni-hamburg.de

Abstract:

Hippocampus-derived 17-beta-estradiol (E2) has been shown to regulate neuronal function in mature hippocampus (Rune et al., 2006). However, in immature hippocampus, where aromatase activity is also detectable, the role of endogenously synthesized estradiol is largely unknown. To examine the function of E2 during development, we characterized estrogen receptor (ER) distribution in the hippocampi of early postnatal rats, and determined the effects of estradiol on reelin expression in Cajal-Retzius (CR) cells, using organotypic hippocampal slice cultures. Reelin is essential for neuronal migration during development. Methods: ER (alpha, beta) expression was detected using previously described antisera and cRNA-probes (Rune et al., 2002). For culture experiments, hippocampal slices from 5-day-old rats were cultured for 14 days, and treated with either E2, E2 + ER blocker (ICI182,780) or the aromatase-inhibitor letrozole. Matched (untreated) slices served as controls. Reelin immunoreactivity was analyzed using a cell imaging system. Results: ERalpha, but not ERbeta, was expressed in neurons of immature hippocampus, including CR cells. Culturing hippocampal slices in the presence of E2 resulted in an increase (>50%) of reelin expression in CR cells, that was abolished if ICI182,780 was co-applied. Application of letrozole caused a decrease of reelin expression. Conclusions: 1) CR cells express estrogen (alpha) receptors, which are functional. 2) Estradiol stimulates the expression of reelin in CR cells. 3) Reduced expression of reelin in the presence of aromatase inhibitor indicates that hippocampus-derived estradiol contributes to the regulation of reelin. These data suggest a modulatory influence of estrogen levels on hippocampal development via regulating reelin production in CR cells.

Category: Lecture

Titel: Estrogen and progesterone prevent demyelination and affect oligodendrocyte function in an experimental multiple sclerosis mouse model

Authors: Kipp M.(1), Acs P.(2), Komoly S.(2), Beyer C.(1),

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email: mkipp@ukaachen.de; (2) Neurology Department|University of Pecs|Pecs|Hungary

Abstract:

Purpose: Sex hormones, e.g. estrogen and progesterone, are thought to affect and delay progression of multiple sclerosis in pregnant women. Although both steroid hormones are regarded as neuroprotective factors in the brain and elevated during pregnancy, only estrogen was tested experimentally and in clinical trials.

Methods: Adult male mice were fed with cuprizone for a defined time interval. Cuprizone induces demyelination of distinct brain areas such as the corpus callosum (CC). Animals were exposed to estrogen or progesterone (dissolved in sesame oil) or a combination of both steroids by repeated injections into the neck region. The status of myelination was analyzed by MRI and conventional histological stainings. Functional markers of oligodendrocytes were additionally analyzed.

Results: The individual application of estrogen and progesterone resulted in an apparent but moderate prevention of demyelination in the CC as demonstrated by MRI as well as in histological sections. A combined treatment with both steroids nearly completely counteracted the process of demyelination. Furthermore, oligodendrocyte markers were significantly increased after hormone application.

Conclusions: These data support the concept that sex steroids can protect the brain from demyelination caused by multiple sclerosis. It appears remarkable that the simultaneous administration of both hormones was most effective. The findings also suggest that the beneficial steroid effects require interactions with oligodendrocytes by either preventing their cell death or recruiting new cells for myelin formation.

Category: Lecture

Titel: Estrogen protects neural cells by regulating mitochondrial function

Authors: Arnold S.(1), Singh S.(1), Wright de Araújo G.(1), Misiak M.(1), Römgens A.(1), Beyer C.(1),

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Abstract:

Purpose: Mitochondria play an essential role in the regulation of cellular energy metabolism, apoptotic processes, and neurodegeneration. This makes mitochondria a perfect target for neuroprotection. We have analyzed the effect of estrogen, a neuroprotective steroid hormone, on structural and functional aspects of astrocytic and neuronal mitochondria.

Methods: Quantitative RT-PCR analysis and polarographic measurements of mitochondrial respiratory chain enzymes of primary mouse astrocytes/neurons were performed.

Results: Estrogen exerted short-term effects by decreasing and increasing proton-pumping activity of mitochondria from cortical and mesencephalic astrocytes, respectively. These differences may account for region-specific efficiencies of short-term neuroprotection by estrogen. Long-term estrogen exposure influenced the expression of fusion/fission and respiratory chain proteins, thereby affecting mitochondrial morphology and function: (1) Estrogen increased mitochondrial gene expression of respiratory chain complexes, thereby promoting energy production. (2) Estrogen reduced the expression of mitochondrial fission proteins in astrocytes challenged with apoptotic stimuli, thereby promoting cell survival. (3) Cytochrome c oxidase (COX) isoform IV-2 expression was increased during hypoxia and 3-nitropropionic acid treatment (3-NPA, in vitro M. Huntington model) in cortical and striatal astrocytes, respectively. Increased COX IV-2 caused elevated COX activity, abolition of COX sensitivity towards the cellular energy level, elevated ROS production, and decreased cell viability. Estrogen suppressed the increase of COX IV-2 expression and its functional consequences, thus promoting cell survival.

Conclusions: Estrogen affected structural and functional properties of mitochondria, thereby supporting survival of astrocytes and neurons under hypoxia and 3-NPA treatment. Supported by DFG (Emmy Noether-Program, SA), START (RWTH Aachen, SA).

Category: Lecture

Titel:Therapy of breast cancer with aromatase inhibitors: implications for cognition?

Authors: Rune G.(1),Zhou L.(2),Fester L.(1),von Bittersdorf B.(1),von Hacht R.(1),Hassu B.(1),Nogens H.(3),Jarry H.(4),

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Abstract:

Therapy of breast cancer with aromatase inhibitors: implications for cognition?

Gabriele M. Rune¹, Lepu Zhou¹, Lars Fester¹, Breda von Blittersdorf¹, Regine von Hacht¹, Basel Hassu¹, Henning Nogens¹, Hubertus Jarry²

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Aromatase inhibitors such as letrozole, are commonly used in the therapy of postmenopausal women who suffer from hormone-dependent breast cancer (Geisler et al., 2002; Puddefoot et al., 2002). Pilot studies have demonstrated that aromatase-inhibitors affect cognition and memory deficits in women treated with these inhibitors (Dowsett et al., 2005). Based on our in vitro findings of letrozole-induced spine loss and impairment of LTP in hippocampal cultures, we studied spine synapse density in mice after systemic application of letrozole and in aromatase knock-out mice. Both the treatment with letrozole and the knock-out of aromatase resulted in reduced spine synapse density specifically in the hippocampus, but was found neither in the cortex nor in the cerebellum. With letrozole, spine synapse loss was strongest in cycling female mice and less dramatic in ovariectomized and male animals. In aromatase knock-out mice the effects were highly significant in the dorsal hippocampus, in males as well as in females. Our results show, that letrozole is easily transported across the blood-brain barrier, and very likely exerts an inhibitory influence on cerebral estrogen synthesis. The findings point to the necessity of further clinical studies on the effects of letrozole after systemic application in women.

Category: Lecture

Title: Vagus all over? But be careful!

Authors: Neuhuber W

Addresses: Erlangen, Germany

No abstract available.

Title: Transmitter specification in the sympathetic nervous system

Authors: Dechant G

Addresses: Innsbruck, Austria

No abstract available.

Title: The DRG neuron: a model to study somatosensory transduction and transformation

Authors: Kress M

Addresses: Innsbruck, Austria

No abstract available.

Title: The impact of the immune system in genetically-mediated demyelination

Authors: Martini R

Addresses: Würzburg, Germany

No abstract available.

Titel:Nerve damage and repair of the hindlimb – it is all about walking

Authors: Neiss W.F.(1),Barham M.(1),Andermahr J.(2),Slawiska U.(3),

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Wiederherstellungschirurgie|Klinikum der Universität zu Köln|D-50924 Köln|Germany;
(3)|Nencki Institute of Experimental Biology|PL-02-093 Warszawa|Poland

Abstract:

Purpose: Review on experimental sciatic nerve repair.

Results: The sciatic nerve is one of the thickest mixed nerves in mammals. In rats it conveys the axons of about 2000 motoneurons and 10500 DRG cells (J.E. Swett 1986, 1991) and thus represents the most complex commonly used model on peripheral nerve repair, regeneration and recovery of function. Own experiments and data from the literature agree that motoneurons and sensory neurons survive nerve transection and regenerate quite well after many types of nerve repair: direct end-to-end suture, interposed fresh or predegenerated nerve transplants or guiding tubes made from blood vessels, collagen, silicone or resorbable synthetic materials, and possibly seeded with matrix proteins and/or engineered Schwann cells. Sciatic motoneurons regenerate – as proved by retrograde labeling – even after transplant delayed for 12 months after the nerve transection. Training of the rats by treadmill walking improves the electromyogram of the reinnervated musculature (muscle action potential up, latency of contraction down). However, the animal must be re-enabled to walk, run, jump and climb, but such functional recovery does not occur. In all our experiments the walking track analyses yielded no difference between denervation and reinnervation.

Conclusion: Due to misdirection of reinnervation walking does not improve after sciatic nerve repair despite of successful motoneuron regeneration (retrograde labeling) and muscle reinnervation (restoration of EMG). – Supported by EU COST B30 “Neural Regeneration and Plasticity”.

Category: Lecture

Titel:An overview on alternative cell based strategies to increase peripheral nerve regeneration across long gaps

Authors: Haastert K.(1),Chaturvedi S.(1),Haile Y.(1),Grothe C.(1),

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Abstract:

The technique of autologous nerve grafting for reconstruction of transected peripheral nerves after substantial tissue loss is accompanied with sacrifice of healthy nerves and mismatch in size and function. Development of biohybrid nerve transplants (BNT) is of high interest. During the last years we investigated several ways to increase peripheral nerve regeneration (PNR) across long gaps. We used a rat model in which sciatic nerve gaps from 10-15 mm lengths were bridged by silicone tubes including differentially modified matrices and Schwann cells (SC). We demonstrated before that regeneration promoting growth factors like fibroblast growth factor-2 can be increased in concentration at the site of nerve reconstruction by ex vivo gene therapy(1,2). Here results will be presented regarding the investigation of the fate of transplanted cells with a specific focus on adult SC from rat and human(3). Furthermore, we analysed the contribution of these cells to the myelination of regenerating host axons. Therefore, we transplanted adult SC from green fluorescent gene expressing rats. In addition results will be presented demonstrating that polysialic acid (polySia), a homopolymer of alpha-2,8-linked sialic acids, which is involved in axonal pathfinding, supports survival of grafted SC and peripheral nerve tissue regeneration when incorporated into acellular and cellular nerve grafts. In conclusion, ideal BNT composition should include SC, growth factors and polySia as scaffold material.

1)Haastert, K.; Lipokatic, E.; Fischer, M.; Timmer, M.; Grothe, C. Neurobiol Dis 2006, 21, 138-153. 2)Haastert, K.; Grothe, C. Curr Gene Ther 2007, 7, 221-228. 3)Haastert, K.; Mauritz, C.; Chaturvedi, S.; Grothe, C. Nat Protoc 2007, 2, 99-104.

Financial support : DFG-FOR-548/1

Category: Lecture

Title: Polysialic acid is critical for structural and functional recovery after sciatic nerve crush in dependence to the expression level in different cell types

Authors: Jungnickel J, Hellriegel H, Bronzlin P, Timmer M, Grothe C, Eckhard M

Addresses: Hannover/Bonn (Germany)

No abstract available.

Title: Effects of enhanced FGF signaling on axonal growth

Author: Hausott Barbara

Address: Medical University Innsbruck, Department of Anatomy and Histology, Division of Neuroanatomy, Muellerstrasse 59, A-6020 Innsbruck

Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) are involved in a variety of biological processes in the nervous system including survival, proliferation, differentiation and neurite outgrowth. Furthermore, FGFs are relevant for neuronal repair processes following lesions of the brain and of the peripheral nerve. They act via 4 types of FGFRs which are coupled to signaling pathways including the MAP kinase, the Akt/protein kinase B and the phospholipase C pathway. Negative feedback regulators such as Sprouty or Sef inhibit FGFR signaling at different levels of the MAP kinase pathway and are at the same time regulated themselves by FGF. The ubiquitin ligase c-Cbl tags the receptor with ubiquitin for endocytosis followed by its degradation in the lysosome, which leads to the termination of the signal.

FGFR1, -2 and -4 are expressed in adult sensory neurons obtained from dorsal root ganglia, albeit at different levels with FGFR1 being expressed at much higher levels than FGFR2 or FGFR4. Enhanced signaling of FGFR1 improves axonal regeneration. FGFR1 overexpression promotes FGF-2-induced axonal growth by adult sensory neurons which is further increased by lysosomal inhibition of receptor degradation.

The FGFR negative feedback inhibitors Sprouty and Sef are differentially expressed in central and peripheral neurons. Different Sprouty isoforms are up-regulated in adult sensory neurons by FGF-2 and NGF. Down-regulation of Sprouty2 enhances FGF-2 induced axonal growth by adult sensory neurons.

The positive effects of FGFR1 overexpression, lysosomal inhibition of FGFR1 degradation and down-regulation of negative FGFR feedback inhibitors demonstrate the significance of FGFR signaling not only for developmental but for adult regenerative axonal growth as well.

Category: Lecture

Titel: Experimental inflammation promotes the regeneration of skin nerves

Authors: Hendrix S.(1), Picker B.(1), Peters E.(2),

Addresses: (1) Institute for Cell Biology and Neurobiology, Center for Anatomy|Charité - Universitätsmedizin Berlin|Berlin|Germany; email: sven.hendrix@charite.de;
(2) Neuroscience Research Center|Charité - Universitätsmedizin Berlin|Berlin|Germany

Abstract:

Purpose: Previously, we have demonstrated that neuronal plasticity in skin is substantially influenced by inflammatory mediators. Here, we tested the hypothesis whether experimental inflammation may be a useful tool to promote the regeneration of skin nerves after lesion.

Methods: Skin was denervated by axotomy of all skin nerves, which innervate the dorsal back skin of mice. Sham-operated mice without axotomy were used as controls. Experimental dermatitis was induced either by sensitizing mice with ovalbumin and complete Freund's adjuvant to provoke a contact dermatitis-like skin inflammation or with ovalbumin and aluminium hydroxide to provoke an atopic dermatitis-like skin inflammation. The numbers of immune cells and nerve fibers were determined by standard immunohistochemistry: Mast cells (FITC-avidin, GIEMSA), macrophages (F4/80), T cells (CD3), nerve fibers (PGP9.5, GAP-43).

Results: Skin re-innervation in all compartments was detectable at day 28 after axotomy. Both types of experimental inflammation had no significant influence on mast cell numbers or macrophages (F4/80). Surprisingly, axotomy significantly reduced T cell infiltration in atopic dermatitis skin, while there was no influence on T cell infiltration in contact dermatitis skin. Interestingly, in contact dermatitis skin the number of regenerating nerve fibers was significantly increased in the epidermis, dermis and subcutis as well as in selected hair follicle compartments.

Conclusions: These data suggest that mast cell-dependent dermatitis is suppressed by axotomy, while mast cell-independent skin inflammation develops normally and significantly promotes the regeneration of skin innervation after lesion.

Category: Lecture

Titel:Manually-stimulated recovery of motor function after facial nerve injury requires intact sensory input

Authors: Pavlov S.(1),Grosheva M.(2),Guntinas-Lichius O.(3),Irintchev A.(3),Sinis N.(4),Dunlop S.(5),Angelov D.(6),

Addresses:(1)Department of Anatomy, Histology, Embryology|Medical University Varna|Varna|Bulgaria; (2)Department of Oto-Rhino-Laryngology|University of Cologne|Cologne|Germany; (3)Department of Oto-Rhino-Laryngology|Friedrich-Schiller University|Jena|Germany; (4)Department of Hand-, Plastic-, and Reconstructive Surgery|University of Tuebingen|Tuebingen|Germany; (5)School of Animal Biology and Institute for Medical Research|The University of Western Australia|Perth|Australia; (6)Department of Anatomy I|University of Cologne|Cologne|Germany; email:angelov.anatomie@uni-koeln.de

Abstract:

We have recently shown in rat that daily manual stimulation (MS) of denervated vibrissal muscles enhances functional recovery following cut and anastomosis of the facial nerve (FFA). This novel MS strategy promotes full recovery of whisking function by reducing poly-innervation at the neuro-muscular junction (NMJ). Here, we examined whether the accurate re-innervation patterns and improved functional recovery was mediated by sensory input. First, we quantified the extent of synaptic input to motoneurons in the facial nucleus using synaptophysin immunocytochemistry following FFA with and without subsequent MS. Manual stimulation restored the total synaptic input to levels in intact animals and appeared to do so by stabilizing the normal contingent of trigeminal sensory afferent dendritic synaptic terminals. We therefore directly tested whether MS affected axotomized facial motoneurons via the monosynaptic intrafascicular trigemino-facial projections (trigeminal loop). FFA was performed and, in addition, the ipsilateral trigeminal infraorbital nerve (ION) was cut and sensory fiber regeneration prevented by extirpation (IONex). Animals received daily MS; controls were handled in the same way but lacked MS. Vibrissal motor performance and patterns of motor end-plate reinnervation were examined and compared to animals in which ION was intact. When the sensory system is intact, MS restores normal vibrissal function and reduces the degree of polyinnervation but when it is abolished, MS had the reverse effect; that is, functional recovery was worse than after ION without MS. We conclude that rehabilitation strategies must be carefully designed to take into account the extent of motor and/or sensory damage.

Category: Lecture

Title: Gap junctions in the Schwann cell lineage

Authors: Meier C¹, Li J¹, Rash JE², Dermietzel R¹

Addresses: ¹ Institut für Anatomie, Abteilung Neuroanatomie, Ruhr-University Bochum, Germany; ² Dept. of Biological Sciences, Colorado State University, Ft. Collins, CO

Gap junctions are composed of connexins or pannexins, which are transmembrane proteins forming inter- and intracellular channels for direct communication. In myelin-forming cells of the peripheral nervous system, the expression of multiple connexins, i.e. Connexin (Cx) 43, Cx29, Cx32, and Cx46 (after nerve injury), has been detected. Functional implications came from the finding that mutations / deletion of the Cx32 gap junction gene cause the demyelinating neuropathy X-linked Charcot-Marie-Tooth (CMT-X). Information on a) the subcellular localization of gap junction proteins in Schwann cells, and b) their expression during Schwann cell development might, in turn, contribute to the understanding of CMT-X pathomechanisms.

Using the high resolution method of freeze-fracture replica immunogold labeling, Cx32 and Cx29 were detected in ultrastructurally defined gap junctions in distinct areas of non-compact myelin in mature Schwann cells. To elucidate the developmental expression of connexins in the Schwann cell lineage, we employed histochemical methods on cultured neural crest, precursor, and immature Schwann cells, as well as on whole mount embryos. Our data demonstrate that in the mouse Cx43, Cx29, and Cx32 protein expression is activated in a distinct sequence that clearly correlates with major developmental steps in the lineage.

The coordinated expression and specific localization of connexin proteins point to a distinct function in the inter- and intracellular transport of molecules in cells of the Schwann cell lineage, and, thus, to a possible involvement in essential processes of peripheral nerve development, Schwann cell maturation, and myelination.

Category: Lecture

Title: Sympathetic neuronal and chromaffin progenitors of the neural crest: how similar, how different?

Authors: Unsicker K

Addresses: Heidelberg, Germany

No abstract available.

Title: Sympathetic innervation of sweat glands is pioneered by cholinergic neurons in the mouse

Authors: Schuetz B, von Engelhardt J, Goerdes M, Schaefer M, Eiden L, Monyer H, Weihe E

Addresses: Marburg, Heidelberg, Germany; Bethesda, USA

No abstract available.

Titel:Heteropentameric alpha7alpha10-nicotinic acetylcholine receptors are modulated via binding of lynx1 to the alpha10-subunit

Authors: Lips K.(1),Wesch D.(2),Clauss W.(2),König P.(3),Pfeil U.(1),Krasteva G.(1),Spies M.(1),Haberberger R.(4),Miwa J.(5),Heintz N.(5),Ibanez-Tallon I.(6),Kummer W.(1),Fronius M.(2),

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Abstract:

Apha9alpha10 nicotinic acetylcholine receptors (nAChR) are well characterized ion channels expressed by inner ear hair cells. Alpha10-subunits can neither build functional homopentamers nor heteropentamers with alpha2- to alpha6- or beta-subunits. Still, the occurrence of alpha10-subunits without alpha9-subunits was described for a variety of tissues. We asked whether the alpha10-subunits form functional receptors together with alpha7-subunits and if such receptors are modulated by lynx1, a modulator of brain alpha7-homopentameric nAChR.

We addressed this issue in rat sympathetic ganglia where we detected mRNA of lynx1, alpha7- and alpha10-subunits without alpha9-subunit. Using immunohistochemistry we demonstrated the colocalization of lynx1, alpha7- and alpha10-subunits in the plasma membrane of sympathetic neurons. Double-labelling immunofluorescence with subsequent fluorescence resonance energy transfer (FRET) analysis using confocal laser scanning microscopy revealed distinct FRET efficiencies for the combination of subunits alpha7/alpha10, lynx1/alpha10, and lynx1/alpha7. After coinjection of alpha7- and alpha10-subunit cRNAs into *Xenopus laevis* oocytes robust nicotine-induced currents were measured that were smaller than those produced by alpha7-subunits alone. Furthermore significant reduction of the nicotine-induced current was detected after additional expression of lynx1 whereas this modulator had no influence upon alpha7-homomers. Thus, we conclude that alpha7alpha10-nAChR occur in rat sympathetic neurons. The functional data suggest, that in heteropentameric alpha7alpha10-nAChR the alpha10-subunit modulates the receptor properties, e.g. by conferring sensitivity to the endogenous prototoxin lynx1.

Category: Lecture

Titel: In vitro approaches to the enteric nervous system

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Abstract:

Purpose: In vitro approaches deliver useful models to study effects of all kinds of factors be it trophic or toxic within a standardized environment. Due to the integration in the gut wall the access to the enteric nervous system (ENS) is not as easy. So i.e. the myenteric plexus has to be removed from its muscular surrounding to obtain a pure neuronal tissue culture.

Methods: A combination of enzymatical digestion (Collagenase) and mechanical agitation allows the dissection of pure myenteric plexus. Myenteric plexus from various species has been isolated (mouse, rat, pig, human) and cultured in 2 and three-dimensional culture systems. The cultures could be used as a model for neurotrophic or toxic influence, migration, differentiation or the expansion of neuronal stem cells from the ENS.

Results: The in vitro approach delivers objective data about neurotrophic or toxic influence which was measured in terms of neuronal survival or neurite outgrowth and branching. So GDNF increased and ethanol reduced survival and neurite outgrowth. The three-dimensional culture system delivers secondary ganglia, similar to those found in vivo.

Enteric neuronal stem cells can be isolated from the pre- and postnatal gut, expanded and differentiated in vitro. These cells can also be used to be transplanted in cell therapeutical approaches.

Conclusions: The in vitro approach to the ENS delivers data which cannot be obtained by other techniques and is so a valuable tool in the investigation of the plasticity and dynamics of this largest part of the peripheral nervous system

Category: Lecture

Title: Dietary-dependent changes in the enteric nervous system of premature piglets. A potential model for necrotizing enterocolitis

Authors: Els Van Haver, Chris Van Ginneken

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With the initiation of enteral feeding after birth, intestinal morphology and function has to adapt at a high pace. This remodelling is the more challenging in preterms, where it may result in necrotizing enterocolitis (NEC).

We hypothesized that in the preterm piglet, feeding-induced maladaptations of the enteric nervous system (ENS) and vasculature could be part of the etiopathogenesis of NEC.

We quantified certain inhibitory neurons, glial cells and the endothelium on immunohistochemically stained preterm small intestinal sections of 1) unfed piglets, 2) piglets receiving total parenteral nutrition (TPN) for 2-3 days and 3) piglets fed 2 days sow's colostrum (SOW) or formulated milk (FOR) following TPN.

After enteral feeding, the ENS and vascular endothelium grew in the same order as the intestine. However, a subpopulation of neurons, glial cells and endothelial cells, showed feed-type dependent alterations. Feeding formula lowered the density of nNOS and VIP^{ergic} myenteric neurons and of eNOS in the endothelium and resulted in a reactive gliosis. In addition, formula feeding caused a hypoxic, pro-inflammatory condition of the intestine, as shown by an increased HIF-1 α -immunoreactivity and elevated levels of IL-1 β . In conclusion, formula induces destructive changes in the immature small intestine, which affect the microvasculature and ENS, whereas colostrum prevents their occurrence and TPN postpones these changes. These conditions may be among the factors that predispose to NEC.

Category: Lecture

Titel: Interaction of the enteric nervous system and the gastrointestinal mucosal barrier

Authors: Schwarz L.(1), Klotz M.(2), Schäfer K.(2),

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Zweibrücken | Germany; (2) Life Science | University of applied science Kaiserslautern | 66482
Zweibrücken | Germany; email: karl-herbert.schaefer@fh-kl.de

Abstract:

Purpose: Growth and regeneration of the mucous layer is a very important prerequisite for the undisturbed function of the gastrointestinal tract. While in most in vitro studies merely enterocyte cell lines were used to model the mucosal barrier, the enormous influence of the intrinsic nervous system, the enteric nervous system, upon the gastrointestinal regeneration is mostly neglected.

Methods: In vitro experiments were performed using various enterocytic cell lines alone and in coculture with isolated myenteric plexus, or under the influence of supernatants from the corresponding culture. Enterocyte proliferation was measured using BrdU-, supernatant content was screened by neurotrophin ELISA. Enterocyte culture were partially processed for scanning electron microscopy. To evaluate the proximity of enteric glia towards the enterocytes in vivo, histological samples were processed for immunohistochemistry, evaluating the expression of the glial marker S100 and GFAP.

Results: The enterocyte proliferation in vitro could significantly be increased by using neuronal supernatants. These supernatants contained various neurotrophic factors such as NT-3 or CNTF. The surfaces of the enterocytes, as revealed by scanning electron microscopy, showed varying densities and quality of microvilli depending on the culture conditions. The immunohistochemically stained enteric glia in the intestinal villi were often to be found in a close opposition to the enterocytes. They were strongly S-100 positive and showed a less prominent GFAP staining.

Conclusions: The enteric nervous system and the enterocytes do strongly influence each other. This influence might be used to stimulate mucosal growth and regeneration by influencing the ENS directly using various neurotrophic factors.

Category: Lecture

Titel:Frizzled-4: A cell-surface marker for isolation of human neural cells from the enteric nervous system

Authors: Bareiss P.(1),Marco M.(2),Haeussler E.(1),Obermayr F.(3),Wagner S.(4),Skutella T.(1),Just L.(1),

Addresses:(1)Anatomy, Center of Regenerative Biology and Medicine|University of Tuebingen|Tuebingen|Germany; (2)Neural Development Unit|Institute of Child Health, University College London|London|UK; (3)Department of Pediatric Surgery|Children's Hospital, University of Tuebingen|Tuebingen|Germany; (4)Department of General-, Visceral- and Transplantation Surgery|University of Tuebingen|Tuebingen|Germany; email:ljust@anatom.uni-tuebingen.de

Abstract:

The enteric nervous system (ENS) is part of the PNS and regulates the blood flow and the peristaltic and secretory activity of the gut. There is evidence that the ENS is also involved in the function of the mucosal immune system and influences the epithelial stem cell compartment of intestinal mucosa.

Several groups have demonstrated that multipotent, self-renewing ENS progenitor cells persist in the fetal and postnatal gut of humans and rodents. These neural progenitors can be expanded and differentiated in neurons and glia cells under appropriate cell culture conditions.

An important role for the regulation of stem cells and progenitor cells has been attributed to the canonical Wnt pathway. The Wnt signalling cascade is initiated upon binding of the secreted Wnt ligand to a member of the family of Frizzled (Fzd) transmembrane receptors and a specific co-receptor of the family of low-density lipoprotein receptors.

We have recently identified Fzd-4 expressing cells in the ENS of human small and large intestine. We characterized positive cells from human perinatal und adult gut samples using RT-PCR, immunohistochemistry and FACS. Additionally, Fzd-4 positive cells were separated by MACS-Sorting to investigate the proliferation and differentiation capacity of selected cells in vitro. Further we proved the influence of different Wnts on the proliferation and differentiation capacity of cultured neural progenitor cells.

Our results indicate that Fzd-4 could be an interesting marker to isolate and enrich enteric progenitors and neural cells of the enteric nervous system.

Category: Lecture

Titel:Wnt4 expression depends on insulin and IGF1 in the rabbit blastocysts

Authors: Thieme R.(1),Ramin N.(1),Fischer B.(1),Navarrete Santos A.(1),

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Abstract:

Maternal hormones such as insulin and IGFs regulate preimplantation embryo metabolism and development. Their detailed effects, however, are largely unknown. We investigated the influence of insulin (17nM) and IGF1 (1.3nM) on mesoderm differentiation in rabbit day 6 p.c. blastocysts. The planar morphology of the embryonic disc of these blastocysts allows subtle analysis of early gastrulation processes. Mesoderm formation is initiated by Brachyury expression in the posterior gastrula extension (PGE). We previously described that insulin increases Brachyury expression. Here we report on the analysis of insulin signaling. Wnts are likely candidates for intermediated signaling molecules between insulin and Brachyury. Wnt4 is a member of the Wnt gene family and is known to stimulate Brachyury expression in mice. We quantified Wnt4 expression in in vivo day 6 p.c. and in vitro cultured blastocysts by quantitative real time PCR using SYBR green.

Supplementation with Insulin or IGF1 increased the amount of Wnt4 transcripts in cultured stage 0/1 and 2 blastocysts after 1 and 6 hours, respectively. The timely controlled expression of Wnt4 and Brachyury indicate that the effect of insulin and IGF1 on Brachyury is mediated via regulation of Wnt4 expression.

Supported by DFG FI306/13-1

Category: Lecture

Titel: Presence of different KIT receptor forms in the bovine corpus luteum (bCL)

Authors: Koch D.(1), Ricken A.(1), Hummitzsch K.(1), Erdmann S.(1), Sakurai M.(2), Spänel-Borowski K.(1),

Addresses: (1) Department of Anatomy|University of Leipzig|Leipzig|Germany; email: albert.ricken@medizin.uni-leipzig.de; (2) Department of Molecular Biology and Immunology|National Institute of Agrobiological Sciences|Tsukuba|Japan

Abstract:

Purpose: We have recently given evidence that tyrosine kinase KIT receptor positive cells change in distribution in the bCL during the estrous cycle. A detailed knowledge about KIT iso- (GNNK-/+), truncated (tr-KIT), and soluble forms (s-KIT) in the bCL is wanted.

Methods and Results: We performed RNA and protein extraction with bCL from the early, mid and late luteal phase and with cultures of thecal cells (TC), granulosa cells (GC), luteal granulosa-like cells (GLC), and luteal endothelial cells (EC). RT-PCR analysis revealed transcripts for both GNNK isoforms in all samples. 5'-RACE showed no tr-KIT. Additional RT-PCR with intron specific primers revealed multiple distinct mRNA sequences. Western blot analysis of the bCL lysates with an antibody against the cytoplasmic part of KIT demonstrated bands at 160, 130 and 50 kDa, respectively. Antibody preabsorption with an excess of immunizing peptide abolished the bands. Lysate predigestion with endoglycosidases converted the 160 and 130 kDa bands to a single 110 kDa band, probably due to loss of glycosylation. The three KIT positive bands were seen in cell lysates, too. Of note, EC and TC lysates showed strong bands at 160 and 130 kDa, whereas bands were moderate for GC and GLC. The enriched conditioned media of the four cell types revealed a soluble KIT form at 110 kDa when using an antibody against the extracellular part of bovine c-kit.

Conclusions: Soluble and membrane bound c-kit forms are present in the bCL. Intensity of KIT protein expression differs in follicle- and bCL-derived cells.

Category: Lecture

Titel: OxLDL induces apoptosis or autophagy in granulosa cell subtypes

Authors: Hueller H.(1), Vilser C.(1), Nowicki M.(1), Blumenauer V.(2), Hmeidan F.(2), Jogschies P.(2), Spaniel-Borowski K.(1),

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Abstract:

Purpose: Recently, we identified the lectin-like low density lipoprotein receptor (LOX-1) on cytokeratin-negative (CK-) granulosa cells derived from patients under in vitro fertilization therapy. The oxLDL dependent LOX-1 activation caused autophagy in CK- granulosa cells. The question was whether cytokeratin-positive (CK+) granulosa cells produce LOX-1 also.

Methods: We first established pure CK+ and CK- granulosa cell cultures, which were validated by immunostaining for CK filaments and by phalloidin-FITC staining for actin filaments. Cultures were treated without and with 150 µg oxLDL/ml under serum-free conditions for 12, 24 and 36 h. Samples were embedded for electron microscopy and protein extracts studied by Western blotting for LOX-1, microtubule-associated light chain protein 3 (LC3) as autophagic marker, cleaved caspase-3 and apoptosis inducing factor (AIF) as markers of apoptosis.

Results and Conclusions: Both cultures types showed a basal LOX-1 expression under serum-free conditions. Yet only the CK- granulosa cells increased LOX-1 protein 24 h after oxLDL treatment also compared to nLDL application. The finding correlated with a shift from the cytosolic LC3-protein towards the membrane-bound LC3-II protein. At the ultrastructural level, autophagosomes with the characteristic double membrane were noted. In CK+ granulosa cells, the LC3-II protein was absent 24h after oxLDL-treatment. The presence of AIF and the striking cell death at the 36-h-time point indicated apoptosis in CK+ granulosa cells. We conclude that morphological and functional heterogeneity of granulosa cells is involved in autophagic and apoptotic cell death. They might mediate two ways of follicular atresia (supported by the DFG Sp232/12-1).

Category: Lecture

Titel:Spheroids of bovine granulosa cells as in vitro model for luteolysis

Authors: Ricken A.(1),Hummitzsch K.(1),Kloß D.(2),Erdmann S.(1),Nowicki M.(1),Rothermel A.(2),Robitzki A.(2),Spaniel-Borowski K.(3),

Addresses:(1)Department of Anatomy|University of Leipzig|Leipzig|Germany; email:albert.ricken@medizin.uni-leipzig.de; (2)Centre for Biotechnology and Biomedicine|University of Leipzig|Leipzig|Germany; (3)Department of Anatomy|Leipzig|Leipzig|Germany

Abstract:

Purpose: Luteal cells are difficult to maintain in vitro. An appropriate technique to obtain fully luteinized granulosa cells (GC) is wanted.

Methods and Results: GC were harvested from bovine antral follicles (up to 2.5 cm in diameter) and grown to confluency in 250-ml flasks. One million dislodged cells were cultured in serum-containing medium in 35-mm Petri dishes on a rotary shaker at 72 rpm for up to 4 days. The GC formed spheroids of 165 µm in average diameter. They showed a two-layered periphery of lighter staining than the center in semi-thin sections. At day 1, mitotic activity was randomly distributed, as confirmed by BrdU uptake. At day 2, a few mitotic figures were seen at the spheroid's periphery and cessation of BrdU uptake was noted. Few apoptotic bodies appeared all over. Up to day 4, an increase in TUNEL labelling and in propidium iodide uptake was noted. Cells with "vacuoles" became striking, which related to different stages of autophagosomes and autophagolysosomes at the ultrastructural level. An amorphous core developed containing cell remnants and fibrils. To our surprise, the conspicuous signs of cell death were accompanied by increased luteinization of the spheroids. Progesterone levels (immunoassay) were high as well as the steroidogenic enzymes StAR and P450scc (immunoblots).

Conclusions: GC spheroids are generated in rotary culture. Spheroids are active in progesterone synthesis in spite of cell death. These findings are not reminiscent of a corpus luteum at the secretory stage, but at the onset of luteolysis.

Category: Lecture

Titel:Placental transcription factor Nrf2 expression is altered in preeclampsia

Authors: Wruck C.(1),Pufe T.(1),Kaufmann P.(1),Huppertz B.(2),Kadyrov M.(1),Kadyrov M.(1),

Addresses:(1)Anatomy and cell biology|University-Hospital RWTH Aachen|Aachen|Germany; (2)Medical University of Graz|Institute of Cell Biology, Histology, and Embryology|Graz|Austria

Abstract:

Objectives: Nrf2 is a key player in the cellular stress response. The function of the Nrf2 transcription factor system in the placenta is not known. Here we analyzed Nrf2 expression throughout normal pregnancy and compared to placentas from late-onset preeclampsia.

Methods: Samples from normal placentas (33-40 weeks gestation, n=10) and from placentas of preeclamptic women (35-40 weeks gestation, n=10) were embedded in paraffin, and stained using anti-Nrf2, anti-vimentin and anti-cytokeratin 7 and MIB-1 antibodies.

Results: Compared to normal term placentas late-onset preeclampsia more cells are stained for Nrf2. This staining appears in the cytoplasm of stromal cells together and also in some cytoplasm and nuclei of the vessel wall in stem villi. In severe preeclampsia nearly all nuclei of stroma and endothelial cells are stained as well, whereas the syncytiotrophoblast were negative and cytotrophoblast cells were few positive.

Conclusions: Alterations of villous tissues during preeclampsia have so far mostly been attributed to the villous stroma. Nrf2 immunohistochemistry reveals that also the villous stroma is heavily affected and demonstrates an abnormal accumulation of transcription factor. This may be used as markers for the development of oxidative stress in preeclampsia.

Category: Lecture

Titel:Complex patterns of ADAM12 mRNA and protein variants in the human placenta

Authors: Kokozidou M.(1),Kadyrov M.(1),Wruck C.(1),Bradenburg L.(1),Kaufmann P.(1),Pufe T.(1),

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Abstract:

Purpose:The up to now proposed candidate molecules for the fusion event in the placenta are not sufficient for the complete procedure.ADAM12 a new candidate expressed in two splice variants, a short ADAM12S and a long ADAM12L.Our aim is to localise both splice variants in the placenta and to explore effects of expression inhibition in 1st and 2nd trimester human placenta.

Methods:Expression of mRNA patterns was analysed via Northern blot and whole mount in situ RNA hybridisation in 1st and 3rd trimester placenta.Immunohistochemistry was performed using antibodies constructed in our lab, from collaborators and commercial ones, raised against both and/or either splice variant.Denuded placenta explants were treated with antisense oligonucleotides blocking both and/or either splice variant.The syncytium repair in a time lapse of 72-120 hours was followed up.

Results:Both ADAM12S and ADAM12L are present in stable cell lines derivating from placenta as well as the placenta itself. The in situ hybridisation revealed the presence of mRNA in both cyto- and syncytiotrophoblast layers of the organ and in immunohistochemistry ADAM12S was mainly found in the syncytiotrophoblast and the ADAM12L in the cytotrophoblasts.Interestingly the treatment of the denuded explants with oligonucleotides resulted to complete inhibition of the syncytial layer repair and accumulation of the cytotrophoblasts.

Conclusions:Our results show that both ADAM12S and ADAM12L are expressed in the human placenta and localise quite differently and functionally it seems that it is involved in syncytial fusion.

Category: Lecture

Titel:Mitochondria and mammalian germ cells: endosymbiosis in a bottleneck?

Authors: Viebahn C.(1),Püschel B.(1),

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Abstract:

Mitochondria appear to lead quite an independent life from the host cell, with their own DNA (mtDNA), asynchronous reduplication, a pivotal role in apoptosis, successful companionship with the maternal germ line only, and a rapid switch between mtDNA variants of close relatives observed in mammals. The latter feature sparked off the bottleneck theory which calls for a minute mitochondrial founder population ($n=10$) in premigratory primordial cells (pPGCs) to give new individuals a "clean start" with a functionally intact set of mitochondria. However intriguing, direct bottleneck evidence rests on an ultrastructural meta-analysis and still lacks molecular genetic support. Additionally, it is unclear how dysfunctional mtDNA is detected and how pPGCs are then singled out to die. Amongst a range of mitochondria-associated structures in germ cells (e.g. Balbiani bodies and nuage) the present study chose the mitochondrial PG2 epitope, which is expressed in the earliest primordial germ cells and has highly dynamic subcellular expression characteristics during germ cell development, to search for direct quantitative bottleneck proof in embryonic germ cells. Immunofluorescent colocalisation and confocal laser microscopy on both whole-mount preparations and semithin frozen sections of early rabbit gastrulation stages using the PG2 antibody to identify all pPGCs and the MTC02 antibody to count all mitochondria in every pPGC revealed mitochondria numbers at a range of 40 to 100 per pPGC. This does not yet fulfill bottleneck expectations as such but, at least, provides a quantitative basis for a functional analysis of the putative bottleneck in the mammalian mitochondrial cross-generation life cycle.

Category: Lecture

Title: Biochemical maturation of the chorioallantoic membrane is essential for emergence and stability of bifurcations

Authors: Kurz H et al.

Addresses: Salzburg, Zuerich, Switzerland; Freiburg, Germany

Lecture retracted

Titel:Phenotyping mutant mouse embryos with the microMRI-HREM pipeline

Authors: Pieleles G.(1),Geyer S.(2),Szumska D.(1),Schneider J.(1),Neubauer S.(1),Clarke K.(3),Dorfmeister K.(2),Franklyn A.(1),Brown S.(4),Bhattacharya S.(1),Weninger W.(2),

Addresses:(1)Welcome Trust Centre for Human Genetics|Oxford University|Oxford|United Kingdom; (2)Centre for Anatomy and Cell Biology|Medical University|Vienna|Austria; email:stefan.geyer@meduniwien.ac.at; (3)Department of Physiology, Anatomy and Genetics|Oxford University|Oxford|United Kingdom; (4)MRC Mammalian Genetics Unit|Oxford University|Oxford|United Kingdom

Abstract:

Mutagenesis screens are undertaken to investigate the causality of hereditary diseases. They randomly produce mutant mouse lines with unknown genetic defects. The first step in characterising these defects is to produce a precise description of the morphology of mutant mice and their embryos respectively. In this talk we present a recently developed high throughput phenotype-screening pipeline, which combines microMRI and the HREM (High resolution episcopic microscopy) volume data generation technique and permits large scale phenotype analysis of mutant mouse embryos. To demonstrate the capacity of our method, we used 15.5 dpc mouse embryos produced in a large scale ENU mutagenesis study. Embryos were harvested, fixed in formalin containing the MRI contrast agent dimeglumine gadopentetate and subjected to microMRI screening. Data of 32 embryos with a voxel size of 25.4 x 25.4 x 24.4 microns were generated in one run. Embryos in which MRI scanning revealed an indistinct phenotype were further analysed with HREM (voxel size: 1.07 x 1.07 x 2 microns). Many malformations, such as large ventricular septal defects could be diagnosed with certainty in microMRI data. But in some embryos microMRI could only provide hints on the presence of vascular malformations. These embryos were subjected to HREM analysis. With the aid of the HREM the malformations could be confirmed and further characterised. Minor cardiovascular malformations or malformations of small blood vessels sometimes escaped microMRI detection, and could be only diagnosed in HREM data. In summary, the combination of microMRI and HREM results in a powerful phenotyping pipeline fitting for large scale analysis of mutant mouse embryos.

Category: Lecture

Titel:Osteogenesis through high-density co-culture of osteoblasts with mesenchymal stem cells

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

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

Abstract:

Purpose: Tissue engineering of bone grafts with osteogenic progenitor cells represents an innovative and promising treatment strategy for large bone defects. Mesenchymal stem cells (MSC) sourced from adult donors possess osteoprogenitor potential.

Methods: The aim of this study was to evaluate the osteogenic potential of primary osteoblasts (POS) on MSCs in a co-culture system, at various cell ratios including 10%/90%, 30%/70% and 50%/50%. The co-cultures were treated with or without specific induction media in monolayer and high density cultures.

Results: In monolayer co-culture, MSC and POS actively searched for cell-cell contact leading to strong cell proliferation. In high density co-cultures ultrastructural evaluation and immuno-electron microscopy demonstrated osteogenesis, with no clear difference between with induction medium treated, untreated or pure POS cultures. Immunoblotting confirmed the presence of collagen type I, beta1-Integrin, Cbfa-1 and induction of the MAPKinase pathway in the co-cultures. The degree and quality of osteogenesis was proportional to the quantity of osteoblasts in the co-cultures. 50% POS in the co-culture markedly increased osteogenesis, comparable to pure POS cultures or pure MSC cultures treated with induction medium. The presence of a 10% POS in the co-cultures increased osteogenesis compared to pure MSC cultures without POS. Treatment with the induction media enhanced osteogenesis in the co-cultures even further.

Conclusion: We conclude that co-culture of MSC with POS combined with the three dimensional environment acts as a strong promoter of osteogenesis. The culture conditions described may improve future tissue engineering and regenerative medicine approaches for the clinical treatment of bone defects.

Category: Lecture

Titel:Apoptosis in limb development

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email:ata_carmen@yahoo.com

Abstract:

Purpose: to demonstrate the presence of the apoptosis in limb development.

Methods: human embryos were used to investigate the apoptosis during limb development. Histological and immunohistochemical techniques were used to detect the apoptotic cells.

Results: the microscopic study pointed out the specific features of the apoptosis during limb development. All the methods of colouring reflected the location of the cell death near the structure which will be adapt for future configuration of the entire limb. Conclusions: the observations reported here are in line with the general view that apoptosis has a key role in many crucial biological processes, especially those related to the development and turnover of tissues and organs. This study demonstrated that apoptosis during the development of limb has a role in the general shape of the limb and the development of different structures.

Category: Lecture

Titel: The formation and role of cartilage canals in the mice femur

Authors: Blumer M.(1), Longato S.(1), Schwarzer C.(2), Fritsch H.(1),

Addresses: (1) Anatomy, Histology & Embryology|Innsbruck Medical University|Innsbruck|Austria; email: michael.blumer@i-med.ac.at; (2) Pharmacology|Innsbruck Medical University|Innsbruck|Austria

Abstract:

A first and an essential event for the formation of the secondary ossification centre (SOC) is the early generation of vascularized cartilage canals. This process requires the proteolytic cleavage of the cartilaginous matrix which in turn will allow the vessels and mesenchymal cells to grow into the epiphysis. In this study, we examined the machinery of cells involved in canal formation and bone development. To achieve this, the femur of mice postnatal stages was investigated using various approaches.

Cartilage canals appeared the first time 5 days (D) after birth and only a few canals were present at this stage. At D 8 the number of canals increased some of which penetrating deeper into the chondroepiphysis exclusively made up from resting cartilage until this point of time. Macrophages and tartrate resistant acid phosphatase positive (TRAP) cells were encountered at various locations within the canals. At D 10 the epiphysis comprised resting, proliferating and hypertrophic cartilage. The canals were now highly branched within the hypertrophic zone and the first signs of endochondral bone formation were detectable. Macrophages and TRAP cells were still detectable. In addition, numerous canals' mesenchymal cells expressed type I collagen, and several small ossification centres were formed. At D18 the ossification nuclei coalesced into a large SOC.

Our results provide evidence that in mice macrophages as well as TRAP positive cells are important tools for formation of the cartilage canals. Furthermore, canals' mesenchymal cells have an osteogenic potential and are essential for establishment of the bone matrix.

Category: Lecture

Titel: The bHLH transcription factor ATOH8 is involved in early myogenesis

Authors: Dai F.(1), Runkel E.(1), Morosan-Puopolo G.(1), Rehim R.(1), Zhao W.(1), Yusuf F.(1), Bonafede A.(1), Gamel A.(1), Brand-Saberi B.(1),

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Abstract:

Purpose: Previously we have reported that a bHLH transcription factor, ATOH8, is down-regulated in a patient who suffered from severe myopathy. To understand whether this transcription factor is involved in myogenesis, the gene needs to be investigated in detail.

Methods: The ATOH8 gene expression pattern has been investigated by in situ hybridization in embryogenesis. Vector-based RNAi for knock-down of the ATOH8 gene has been constructed and delivered into chicken embryos in vivo after gene transfection via in ovo electroporation. The knock-down effects of ATOH8 on several myogenesis related genes have been investigated. The predicted promoter of the ATOH8 gene has been cloned into a reporter vector and tested in chick embryos in vivo.

Results: The results from in situ hybridization show that ATOH8 is expressed not only in the retina, neural tissues, but also in the myotomes of somites during the development of the chicken and mouse embryo. Knock-down of cATOH8 mRNA by RNAi resulted in down-regulated expression of ATOH8, MyoD, Myf5 and up-regulated expression of Myogenin. We have constructed an EGFP reporter plasmid with the predicted human ATOH8 promoter. Our results show that the EGFP reporter gene driven by the hATOH8 promoter can be expressed both in the neural tube and myotomes of chicken embryos.

Conclusions: Our results show that early myogenesis requires the bHLH transcription factor ATOH8, and our data suggests that more bHLH transcription factors join the network controlling gene expression in myogenesis.

Category: Lecture

Titel: Postnatal spine development: Biomechanical meaning

Authors: Wachowski M.M.(1), Spiering S.(2), Lee C.(2), Mansour M.(2), Endlich KH.(3), Kubein-Meesenburg D.(2), Nägerl H.(2),

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Abstract:

Purpose:

Right after birth the facets of the vertebral joints (VJ) mesh like roofing tiles in the entire spine. During postnatal growth, the lumbar facets transform into a vertical-sagittal alignment, two additional uncovertebral joints (UJ) emerge in the cervix, thoracic segments remain largely unchanged.

Methods:

Spatial motions of fresh and preserved human C3/C4, Th6/Th7, Th7/Th8, L3/L4, L4/L5 segments without pathological signs were measured with position and amount of an axial preload as parameter.

Results:

Thoracic segments: Preload position or amount did not influence the motion.

Lumbar segments: The preload position strongly influenced axial stiffness and IHA migration.

C3/C4-segments: The preload position or amount controlled segmental stiffness. The IHA and applied torques were not parallel aligned.

Conclusions:

Because of the roofing tile alignment of the thoracic VJ their guidance hardly influence segment motion for geometrical reasons.

The postnatal development in the cervical and lumbar region enables the autochthon musculature to control parametrically segmental stiffness and motion in a large range (factor 2 up to 30).

The corresponding mechanisms are traced back to the musculature controlled guidance of the joints which is made possible by the postnatal altered geometrical alignment of the joint facets or the postnatal arising additional joints. Quantitative anatomically based models are presented which describe the segmental mechanics.

Category: Lecture

Titel: The spinal nerve's dorsal three branching in the lumbar region

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Abstract:

Purpose: Three ramifications of the dorsal spinal branch has been recently discussed in the lumbar region, but not introduced to textbooks. This may be due to a controversial literature.

Materials and Methods: 2 postmortem specimens were embalmed with Thiel's method, 2 were dissected without fixation and 6 cadavers with alcohol or formaldehyde fixation at Institute of Anatomy Leipzig and the Tokyo Medical University.

The lumbar vertebral columns were dissected with surrounding muscles by using a ventral approach, bones were partly removed during the preparation of dorsal nerves. The ramification pattern was validated with the data of the Visible Human Project (VHP) and in 0.8 mm thin plastinated slices.

Results: The dorsal branch of the spinal nerve always showed delicate triple ramifications. First, a medial branch always took a medial-dorsal and caudal run. The second branch spread laterally and remained ventrally. This branch innervated the ventrolateral side of the dorsal muscles. Next, a thick intermedial branch was released from the main dorsal spinal branch, crossed the ventrally positioned nerves on the course to the dorsal integument, sometimes comprising five segments towards. Thicker nerves could be seen in VHP, and, additionally, thinner one in the plastinates, for verification of the topography.

Conclusions: By the ventral approach, which requires removing bones from the specimen, the dorsal nerve ramification system was described. Due to the intermediate branch's long way caudally, a strict segmental innervation of the skin is questionable. The different systems of "muscle carpets" appear to correlate with the nerve ramification system.

Category: Lecture

Titel: The lingual nerve revisited – a pictorial review

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Abstract:

The present study was designed in order to bring evidence on the course, detailed relationships and morphology of the lingual nerve LN. Probably the most spectacular branch of the mandibular nerve, the LN was studied on 34 human adult cadavers, fixed and unfixed, bilaterally, by dissections. Evidence was brought on the LN at the level of the pterygoid space, where it may be separated of the inferior alveolar nerve (IAN) by a well-developed pterygospinous process. In the pterygomandibular space it can be followed by the artery of Juvara; the IAN nerve descend between the sphenomandibular ligament and the mandibular branch (a specimen presented a thickened interpterygoid fascia that totally isolated the IAN and the LN, representing an anatomical obstacle for anesthesia). In the retromolar region the LN courses medially to the pterygomandibular raphe – important landmark for identification of the LN. In the third mandibular molar region nerve passes at the level of the alveolar crest and may be damaged during extractions of the third mandibular molar, normal or impacted. Internal to the mandibular lingual plate in the region of molars, the LN appears highly branched; it links the submandibular ganglion, sends off sublingual nerves, branches for the fauces and for the hypoglossal nerve and it may present the anastomosis of Sappey. The LN terminal branches continue on the genioglossus. By this study we try to bring an update to the knowledge of the LN anatomy addressed to a wide range of medical and surgical specialists dealing with it in their practice.

Category: Lecture

Titel: Venous valves near the saphenofemoral junction

Authors: Mühlberger-Reisinger D.(1), Morandini L.(1), Brenner E.(1),

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Abstract:

Purpose: To find out both, the frequency and exact position of terminal and preterminal valves in the great saphenous vein and of valves in the femoral vein near the saphenofemoral junction.

Methods: The exact position of valves in the great saphenous vein was studied macroscopically in a total of 217 veins. The measurement was performed in situ with a tape measure from the opened confluence of the great saphenous vein into the femoral vein to the nodule of the respective valve. The exact positions of valves in the femoral vein were measured above and below the saphenofemoral junction in a total of 61 femoral veins.

Results: Great saphenous vein: A terminal valve exists in 89.4% within the range of 0.0 to 1.4 cm distally to the saphenofemoral junction and a preterminal valve is present in 90.3%. Femoral vein: Valves proximally to the saphenofemoral junction exist in 74% with a mean distance of 3.8 cm. Venous valves distally to the saphenofemoral junction are present in 90% with a mean distance of 5.0 cm and in 56% of the cases there exists a second distal valve.

Conclusions: Due to the fact that a terminal valve in the great saphenous vein doesn't exist in about 10% and its non-existence should consequently be accompanied by varices, which was not the case, we believe that insufficient or missing valves near the saphenofemoral junction can't be the only reason for varices. This seems to be confirmed by results of valves positions in the femoral vein.

Category: Lecture

Titel: Morphological basics of a positive Kaposi-Stemmer-sign

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Abstract:

Purpose: In 1976, Robert Stemmer described a “clinical sign for the early and differential diagnosis of lymphoedema”: “A thickened longitudinal skinfold when pinching the toes is a clinical sign for early diagnosis of a lymphoedema, and delimits it from a pure venous oedema.” Since this first description it is used consistently in diagnostics of lymphoedema. Furthermore, the original definition was extended or changed several times by other authors. Nevertheless, the underlying morphology of this clinical sign was not investigated by now.

Materials and methods: The second toes of both a patient with lymphoedema (stage III; iL5VxF5) and a healthy subject were compared sonographically, macroscopically and microscopically.

Results: In lymphoedema, both cutis and subcutis were thickened, the structure of the dermal layers destroyed. Neither an accumulation of oedematous fluid in free spaces within the subcutaneous tissue nor lymphatic vessels could not be found.

Discussion: Different definitions and graduations of the Kaposi-Stemmer-sign complicate the diagnosis of lymphoedema and concomitantly the clinical and scientific comparability. Morphologically, a subcutaneous accumulation of oedematous fluid could not be proven, as it can be seen in other areas of the body. These accumulations occur normally along stronger fibrous structures within the subcutaneous tissue. We suppose that both subcutaneous and dermal architecture might be different in toes (and fingers) compared to other regions of the human body. We could show that the Kaposi-Stemmer-sign is based on a distinct morphological alteration of the dermis, which can also be visualized by ultrasound.

Category: Lecture

Titel: Small saphenous vein – valves and topographical anatomy at the saphenopopliteal junction

Authors: Schweighofer G.(1), Mühlberger-Reisinger D.(2), Brenner E.(1),

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Abstract:

Background: Venous valves play a role in the pathogenesis of varices. Although the system of the small saphenous vein has been studied by many authors the exact position of valves at the saphenopopliteal junction has not been studied up to now. Moreover, the topographical relation of the small saphenous vein to the neural trunks within the popliteal fossa remains unclear. An own saphenous fascia at distal levels has not been demonstrated anatomically.

Material and methods: The small saphenous vein was studied macroscopically in situ on 13 cadavers with a total of 25 veins at the dissection course of our institute.

Results: Only in 4 legs we have been able to measure the position of valves. A first one was at 0.0 to 2.8 cm from the junction. A second one could be identified between 4.5 and 6.8 cm. In 21 legs it was not possible to examine the valves due to following reasons: surgery (2), diameter too narrow for opening (6), dissection by students from the anterior approach (4), doubled small junction (2), netlike junction (5), no junction to the popliteal vein (2). All veins presented with an own saphenous fascia. In approximately two thirds of the inspectable cases the vein laid medial to the nerves.

Conclusion: In all our studied cases we found two valves at the saphenopopliteal junction. Nevertheless, the anatomical concept of a single lesser saphenous vein especially at the saphenopopliteal junction should be rethought. The existence of an own saphenous fascia can be confirmed.

Category: Lecture

Titel:Regional cartilage loss in patients with neutral, valgus and varus alignment of the knee – an in vivo MRI based study

Authors: Eckstein F.(1),Hudelmaier M.(1),Wirth W.(2),Cahue S.(3),Marshall M.(3),Sharma L.(3),

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Abstract:

Purpose: Malalignment is known to alter the load distribution between the medial and lateral femorotibial joint. Here we test the hypothesis that this load distribution determines the rate of cartilage loss (thinning) in various anatomical subregions of the femorotibial joint in persons with knee osteoarthritis.

Methods: A community-recruited cohort with radiographic knee OA (n = 174) had alignment measurement by full limb x-ray (74 neutral, 57 varus, 43 valgus malalignment). High resolution MR images of the knee were acquired at baseline and 27 ± 5 months later. Segmentation was performed by tracing the total subchondral bone (tAB) and cartilage surface area (AC) of the medial and lateral tibia (MT/LT) and the media and lateral weight-bearing femur (cMF/cLF) with blinding to acquisition order.

Results: Annualized changes in cartilage thickness were small in knees with neutral alignment, but were larger in the mechanically stressed compartment of knees with varus and valgus malalignment. Averaging over cartilage plates in each compartment, the rate of cartilage loss was 1.4:1 (medial vs. lateral) in participants with neutral alignment, 3.9:1 in participants with varus, and 1: 5.3 in participants with valgus malalignment. Regional changes were greatest in the central and external subregion in MT, in the central subregion of cMF, in the central and internal subregions of LT, and in the external subregion of cLF. Conclusions: The rate of cartilage loss in knee OA is strongly affected by alignment, with subregional analysis being able reveal the specific spatial patterns of cartilage loss in the femorotibial compartments.

Category: Lecture

Title:Lymphoscintigraphy for sentinel node detection in breast cancer

Authors: Fatu C, Fatu I, Huma M, Antohe D, Varlam H, Puisoru M

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No abstract available.

Titel:Dental and maxillary anomalies in a romanian sample of patients with cleft lip alveolus

Authors: Didilescu A.(1),Enache M.(2),Galie N.(1),Dimcevici - Poesina N.(3),Podoleanu E (4), David D.(5),Spinu T.(6),

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Abstract:

Clefts involving the lip and alveolus (CLA) are a significant congenital anomaly, requiring long-term treatments and having serious implications for affected individuals.

Purpose: To determine in 20 unilateral CLA subjects characteristics of the maxillary permanent teeth and maxillary arch development.

Methods: Clinical and radiographic examinations have been carried out to identify congenitally missing teeth and sagittal skeletal patterns. Study cast assessment was undertaken to evaluate mesiodistal widths of individual teeth, intercanine and intermolar distances for the maxillary arch, as well as dental relationships. The t- Student test and the ANOVA test of variance were used for data analysis. A p value less than 0.05 was considered statistically significant.

Results: The congenital absence of the upper incisors was higher on the cleft side than on the noncleft side. There was a statistically significant difference between the mesiodistal widths of cleft-side permanent upper lateral incisors, and their antimeres. The mean values for intercanine distance and intermolar distance were 27.95 mm and 34.13 mm, respectively. Most of the patients presented a class II dental relationship on the affected side comparing with class I/class III Angle on the unaffected side.

Conclusions: Patients with isolated CLA present perturbations in dental and maxillary arch development, a more severe disruption being recorded on the cleft side. Since both upper permanent teeth and maxillary arch are affected, it might be concluded that isolated CLA is not just a single anatomically localized disruption in development.

Category: Lecture

Titel: The anatomy of the orbit in multi slice spiral CT volume rendering 3D – reconstruction

Authors: SZABO B.(1), SZABO B.(2), SZABO I.(3),

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Abstract:

The communications between the orbit and the cranial cavity, nasale fossae, paranasal sinuses, viscerocranium are studied on 3D reconstructed models of the skull.

Material and Methods. Multislice spiral CT-scan (MSCT) was performed on a series of 48 normal and pathologic orbits. The examination was made on Light Speed Ultra Advantage, 8 row multidetector helical CT scan of the orbits and anterior skull base. In certain cases, the scan was performed only on the anterior skull base. Postprocessing and reconstructions were performed based on 512 / 512 matrix DICOM files with Materialise – Mimics software, trial version.

Results. The communications of the orbit were identified on the 3D-CT models, the optimal projection for the identification of the elements was studied and compared with the standard anatomical structure of the skull. The communications were represented in different sectional planes, standard AP, LL and axial. Special sectional planes for each communication were made.

Conclusions. A relation between 3D model and a prepared human skull is presented. The presentation shows a series of clinical cases with orbital trauma, before and after surgery.

Category: Lecture

Titel: Investigation of the alignment of the mandible and the cervical spine to the skull

Authors: Kubein-Meesenburg D.(1), Thieme K.(1), Weber S.(1), Gedrange T.(2), Fanghänel J.(2), Ihlow D.(1), Knösel M.(1), Nägerl H.(3),

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Abstract:

Purpose: Humans are able to move their mandible in almost plane sagittal motion cycles. The closed loops of mandible points can be classified by their area and path length. Are these kinematic parameters related to anatomical structures?

Methods: Active mandibular cyclic motions of 41 class-II-patients were recorded during therapy. The closed looped path of each mandibular point was valued by length L , sense of circulation, and enclosed area with regard to the absolute value A_{abs} and to A_{math} which considers the sense of circulation. L , A_{abs} , and A_{math} were related to anatomical structures by the respective X-ray radiograph.

Results: In each patient a single mandibular point P_{min} could be found whose absolute area A_{abs} was minimal, nearly zero: The path was almost circular. P_{min} lay partly inside, partly outside the bony structure of the condyle. In each case the point with the minimal path length lay below the condyle. The points with the area $A_{math} = 0$ formed a straight line which was mostly oriented to the cervical spine in parallel.

Conclusions: Classing kinematical pattern of mandibular motions with anatomical structures suggests that mandible, maxilla, and cervical spine work together as functional unit.

Category: Lecture

Title: Human incisor inclination adjustment according to natural craniofacial standards

Authors: Knösel M, Engelke W, Gripp-Rudolph L, Fanghänel J, Nägerl H, Kubein-Meesenburg D

Addresses: Göttingen, Greifswald

No abstract available.

Titel:Functional organization of Vidian nerve

Authors: ANTOHE I.(1),ANTOHE D.(1),VARLAM H.(1),MORARU M.(1),

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Abstract:

Aim. Our study aims to precise the mode of vidian nerve formation, the peculiarities of its traject and termination and to find a functional reason of deep, transpterygoid topography of its canal.

Material and methods. The anatomical material studied consist of :

- 10 cadaveric caphalic extremities midline sectioned (20 halves);
- 100 dry skulls (200 pterygoid canals);
- 100 CT images of the cephalic extremity of non-neurological patients.

Results and conclusions. Our study allowed us to describe new anatomical features concerning both the canal and the nerve. Thus the openings of the canal were named aperturae (posterior, quadrangular and anterior, estuary shaped) canalis pterygoidei. The traject of pterygoid canal was divided into crus anterior and crus posterior and the value of the in between angle was determined. The examination of CT images confirmed the existence of the described segments of pterygoid canal and its lateral angulation. Thoroughly dissection of the canal content allowed us to describe the multifascicular organization of vidian nerve its arterial sources and the presence of microganglions at the level of isthmus. The hystological examinations demonstrated that the microganglions are organised following the model of periferal nervous ganglia. We consider that the nerve could be interpreted as parasymphathetical hilus of nasopharynx and related pneumatized cavities of craniofacial junction.

Category: Lecture

Titel:Anatomical landmarks for sphenoidal sinus endoscopy

Authors: ANTOHE D.(1),ANTOHE I.(1),PUISORU M.(1),FATU C.(1),VARLAM H.(1),

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Abstract:

Introduction. The recent progresses of endoscopical surgery of the nasal fossae and the paranasal pneumatized cavities imposes reevaluation of local anatomy in order to establish precise landmarks of meatal regions and sinusal ostia.

Aim. Our study proposes to determine the topographical relations of the sphenoidal sinus with the main surrounding neuroarterial structures and to establish their endosinusal landmarks.

Material and methods. The anatomical material studies consists of 20 dry skulls from Anatomical Institute collection.

The extrasinusal observations were focused on left and right carotic sulci, sphenoidal lingula and middle and anterior clinoid processes. The endosinusal exploration was performed by direct light, by transillumination and by endoscopical examination passing the thin 3,5 mm flexible Storz endoscope through the sphenoid sinus orifice. Fifty 2D CT skull images from non neurological patients were also examined in order to establish the correspondence between anatomical and imagistic data.

Results and conclusions. Our study pointed out the extreme variability of the sphenoidal sinus and allowed us to establish five degrees of sinusal pneumatization. On the lateral walls there are demonstrated two carotic swellings, one inferior of the infracavernous part and one superior of the paraclinoid part of internal carotid artery superolaterally the optic canal may protrude the sinusal cavity. The incidence of this anatomical landmarks variations, studied comparatively on dry skulls and CT images confirmed the concordance between anatomical and imagistic data.

Category: Lecture

Titel:Anatomofunctional study of Glaserian fissure

Authors: MORARU M.(1),VARLAM H.(1),FATU C.(1),ANTOHE I.(1),ANTOHE D.(1),

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Abstract:

Aim. The goal of our study is to precise the mode of Glaserian fissure formation, to point out the functional interrelations between the tympanal bone, petrous part and squama of the temporal bone at middle part of skull exobasis and to describe the anatomical details that sustain the existence of an anterior hillus of the middle ear.

Material and methods. The anatomical material studied consists of 20 dry skulls from the collection of Iasi Institute of Anatomy. In order to expose the inferior procesus of tegmen tympani, the superior edge of tympanal bone as well as the squama neighbour to the fissure to be described were sculptured and thoroughly chiseled using a fine dental drill.

Results and discussions. Our study allowed to determine the variation of exocranial tympanic tegmen surface, the mode of Glasser fissure formation, the presence of sutural bones into the fissure, the relation of the upper border of tympanic bone and their interrelation with sphenoidal spine, the tubal process of tympanic bone and osseous part of auditory tube. Incidental absence of processus inferioris tegminis tympani was also demonstrated. The functional organization of this inferior processus that closes lateraly the isthmus of osseous auditory tube and, superiorly, the iter chordae anterior canal is also detailed. Clinical considerations include the relations with parotid gland, the possible involvement of chorda tympani nerve and during transmission of parotid gland infections to the middle ear.

Category: Lecture

Titel: A novel total knee replacement with natural roll-back - in vivo functional measurements

Authors: Nägerl H.(1), Frosch K.(2), Wachowski M.(2), Fanghänel J.(3), Kubein-Meesenburg D.(1),

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(3) Oral Anatomy|University Greifswald|Greifswald|Germany

Abstract:

Purpose: Comparison of the novel AEQUOS Total Knee Replacement with the natural knee as to roll-back.

Background: Roll-back in the natural knee makes the patella tendon to swivel posteriorly by 0.3° per 1° flexion (roll-back ratio). By that the flexural and the contact load onto the patella is reduced because the angle between the force line of the m. quadriceps and the line of the patella tendon is increased. In knees with common TKR the ratio was found to be $0.002^\circ/1^\circ$ flexion: hence the respective patella is exposed to increased loads since the m. quadriceps/patella tendon angle becomes more acute in flexion. The well-known anterior knee pain after TKR may be caused by the failing roll-back.

Methods: Lateral X-ray radiographs in various knee flexions and fluoroscopic measurements of patients with AEQUOS prosthesis.

Results: In eight patients the mean roll-back ratio was 0.26. This value is statistically not distinguished from 0.3. But it differed from 0.002 with high significance (t-test, $p < 0.001$, $df = 7$). One patient with series of fluoroscopic measurements during knee flexion up to 45° showed a ratio of 0.45.

Conclusions: The AEQUOS-TKR seems to get close to natural knee function. This behavior is traced back to the special prosthesis design, which shows a sagittally convexly curved lateral tibial plateau like in the natural knee.

Category: Lecture

Titel: Morphology of the proximal interphalangeal joint and impact of surface incongruity on joint function

Authors: Dumont C.(1), Kubein-Meesenburg D.(2), Albus G.(2), Endlich K.(3), Fanghänel J.(4), Stürmer K.M.(1), Dathe H.(2), Nägerl H.(2),

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Abstract:

Purpose: The objective of this study was to quantify both articulating joint surfaces of the proximal interphalangeal joint (PIPJ) and to correlate the collected data with joint kinematics. The anatomical data and the resulting view of joint function are presented.

Methods: 40 PIP joints of the fingers II-V in cadaveric hands, equally distributed right and left, without macroscopic signs of arthrosis were investigated. Highly precise dental replicas were used and the replicas were sliced in sagittal and transverse plane. The radii of curvatures and the centres of rotation were assessed. The data were evaluated by means of variance analysis and by paired Student's t-test.

Results: In the transverse plane the joint contacts on the inner slopes of both condyles of the proximal phalanx. 37 of 40 joints showed a small joint space in the intercondylar groove. In the sagittal plane the radius of curvature of the base of the middle phalanx was highly significant larger as the corresponding condyles of the proximal phalanx ($p < 0.001$). Comparing the cartilage surface, the base of the middle phalanx comes up to 40% of the cartilage surface of the head of the proximal phalanx, $64,3^\circ$ (SD: $10,5^\circ$) vs. $166,9^\circ$ (SD: $28,0^\circ$).

Conclusions: The difference of radii of the joint surfaces is precondition for movement of the contact areas on the articulating surfaces and precondition for joint lubrication and cartilage nutrition. The anatomical results were the base for the resultant development of a new prosthesis of the PIPJ.

Category: Lecture

Titel:Mathematical comparison of the shape of the adult and infant parasellar internal carotid artery (pICA)

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Abstract:

The internal carotid artery (ICA) of adults is heavily twisted inside the parasellar region. In infants it runs much straighter. However, due to the lack of adequate methods no objective definitions of the complexity of the parasellar segments of the ICA (pICA) or precise comparisons between the complexity of the adult pICA, and the complexity of the infant pICA do exist. Our presentation aims at presenting objective mathematical characterisations and comparisons of the shape of the infant and adult pICA. Using modern three dimensional (3D) reconstruction methods, we created 3D computer models of 27 infant and 60 adult pICAs. We skeletonised these models and calculated their curvature and torsion energy and total complexity, which we defined as the sum of curvature and torsion energy. Our calculations show that the total complexity of the adult pICA is much higher than the total complexity of the infant pICA. However, this is mainly caused by the amount of torsion energy, which is 16 fold higher in adults than in infants. Curvature energy in contrast, is only 4 times higher in adults than in infants. Our results suggest that extensive vascular remodelling of the pICA takes place during infancy and adulthood. Since this remodelling primarily leads to an increase in torsion energy, we hypothesise that spatial constraints in concordance with asynchronous growth and lengthening of the skull base and the pICA are responsible for the transformation of the straight shape of the infant to the corkscrew like form of the adult pICA.

Category: Lecture

Titel:Regional variations in microarchitecture, bone density and endplate mineralization within the cervical vertebrae

Authors: Müller-Gerbl M.(1),Eimannsberger A.(2),Fertmann-Matsuura M.(2),Weisser S.(2),Linsenmaier U.(3),

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Abstract:

Recent studies point to the fact, that cancellous bone density and structure present substantial variability inside the vertebral body. The objective of the current study was therefore a precise analysis of the microstructure at different, well defined locations within cervical vertebrae by means of microcomputed tomography and an assessment of the distribution of mineralization within the endplates.

Material and Methods: The material for micro-CT examination consisted of 8 cervical vertebrae (C4, 4 male, 4 female, age range 38-62 years). At 24 different well-defined locations structural and numerical bone parameters were determined and statistically analyzed. The mineralization patterns were displayed in 80 endplates (C3 – C7) of the same spines by means of CT-osteodensitometry (CT-OAM).

Results: Substantial site-dependent differences in bone density (BV/TV) and bone architecture were observed. The posterior areas presented a generally higher density than the anterior areas. Significant differences are also apparent between cranial and caudal portions. Furthermore, the caudal and dorsal parts tend to exhibit a plate-like structure, whereas the cranial and anterior parts tend to exhibit a rod-like structure (SMI). Also the distribution of subchondral mineralization revealed considerable topographic differences within each endplate. The zones of greatest density, in both the inferior and superior endplates, are localized over wide areas of the posterolateral surface.

Conclusions: A precise topographic differentiation in the cervical vertebral body reveals significant differences between the cranial and caudal portions and between the anterior and posterior regions. The structurally “strongest” area is found in the posterior caudal region.

Category: Lecture

Titel:VEG-Expression in Osteonecrosis of the Femoral Head

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Abstract:

Objective: This study was conducted to reveal VEGF expression in osteoblasts after glucocorticoid (GC) treatment in vitro and in vivo, and to explore VEGF expression during late stage femoral head necrosis.

Methods: Necrotic femoral heads were obtained during hip alloplasty surgery. VEGF-protein and VEGF receptor-2 (VEGFR-2) were localized by immunohistochemistry in sections of necrotic femoral heads. Expression of VEGF-, VEGFR-2- and VEGF splice variants-mRNA in cultured primary osteoblasts were analyzed by RTPCR. Regulation of VEGF protein was quantified in supernatants of cultivated osteoblasts by ELISA after exposure to glucocorticoids and in bone samples of necrotic femoral heads.

Results: Osteoblasts of necrotic bone areas of femoral heads revealed increased immunoreactivity to VEGF compared to those from non-necrotic areas. ELISA confirmed VEGF upregulation in necrotic bone areas in samples of necrotic femoral heads of ARCO stage IV. The splice variants with the strongest angiogenic potency (VEGF121 and VEGF165) were detectable in cultured osteoblasts. VEGF production was clearly downregulated in supernatants of cultivated osteoblasts after 24 hours of co-incubation with glucocorticoids, thus suggesting a direct influence of GC-induced VEGF decrease in the pathomechanism of early stage femoral head necrosis (ARCO stage I-II)

Conclusion: VEGF is produced in osteoblasts, and its concentration is strongly decreased after exposure to GC in vitro. In contrast, VEGF expression increases in osteoblasts of necrotic femoral heads with late stage disease. The observed increase of VEGF in later stage femoral head necrosis might stimulate the ingrowth of reparative arterioles into the necrotic femoral head.

Category: Lecture

Titel:LPS- induced endothelial barrier breakdown is caused by a decrease of cAMP leading to Rac 1 inactivation and loss of claudin 5

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Abstract:

Lipopolysaccharide (LPS)- induced breakdown of endothelial barrier functions contributes largely to the pathogenesis of sepsis. However, the underlying mechanisms are still unclear. In the present in vitro study, incubation of human dermal microvascular endothelial cells (HDMECs) with LPS led to a breakdown of endothelial barrier functions within 2- 2.5h as revealed by measurements of 70 kD FITC-dextran flux and of transendothelial electrical resistance (TER). This was associated with the formation of large intercellular gaps, stress fibers and fragmentation of VE-cadherin immunostaining. Moreover, claudin 5 immunostaining at cell borders was drastically reduced after LPS- treatment. Interestingly, activity of the small GTPase Rho A, which has previously been suggested to mediate the LPS-induced breakdown of the endothelial barrier was not increased after 2h. In contrast, activity of GTPase Rac 1, which is known to be important for maintenance of endothelial barrier functions, was significantly reduced to $65 \pm 9\%$ after 2h. All LPS- induced changes of endothelial cells were blocked by a Forskolin/Rolipram- mediated increase of cAMP. Consistently, ELISA-based measurements demonstrated that LPS led to a significant decrease of intracellular cAMP- levels to $41 \pm 2\%$ after 1h and to $46 \pm 8\%$ after 2h compared to controls. In summary, from our present data, we suggest that LPS disrupts endothelial barrier functions by decreasing intracellular cAMP-levels which in turn leads to an inactivation of Rac 1- activity. Moreover, this mechanism appears to be involved in the regulation of claudin 5.

Category: Lecture

Titel:Single cell-specific gene expression analysis in human skeletal muscle by combining laser microdissection and quantitative RT-PCR

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Abstract:

Human skeletal muscle is characterized by an enormous plasticity for adaptation to several stimuli. During exercise there are different homeostatic perturbations induced in recruited muscle cells due to mechanical, metabolic, neuronal and hormonal factors that lead to load-dependent skeletal muscle fiber type specific alterations.

The aim of this study was to investigate single cell-specific gene expression with regard to the existence of different chimera of myosin heavy chains (MHC).

Using Laser Microdissection and Pressure Catapulting (LMPC) several muscle fibers from M. vastus lateralis were precisely excised without contaminations. Subsequently, quantitative RT-PCR was performed and the MHC-I, -IIA, -IIX, and -embryonic genes were analysed in fast twitch fibers (FTF; IIA, IIX) and slow twitch fibers (STF; I) resulting from ATPase-staining. However the analysis of MHC gene expression in single FTFs and STFs shows that existence of a heterogenous distribution of MHC genes.

In conclusion, single cell-specific gene expression analysis demonstrates the existence of MHC-gene chimera. Thus, LMPC in combination with Real-Time PCR offers a reliable and reproducible way for fiber typ specific skeletal muscle research.

Category: Lecture

Titel:Dissecting the role of steric hindrance in pemphigus pathogenesis with peptides targeting the desmoglein adhesive interface

Authors: Heupel W.(1),Efthymiadis A.(1),Drenckhahn D.(1),Waschke J.(1),

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Abstract:

For autoimmune skin blistering in pemphigus, it was shown that pemphigus vulgaris (PV) but not pemphigus foliaceus (PF) autoantibodies inhibit desmoglein (Dsg) - mediated binding by steric hindrance. In contrast, for both PV and PF, autoantibody-induced cellular signalling is important. However, the relative contribution of the two mechanisms to pemphigus pathogenesis is unclear. Therefore, to explore the precise role of steric hindrance, a peptide binding to the predicted amino-terminal adhesive interfaces of Dsg 1 and Dsg 3 was designed. In AFM single molecule experiments the single peptide (SP) consisting of 10 amino acids blocked Dsg 1 homophilic transinteraction, whereas a tandem peptide (TP) including two SPs did not interfere with Dsg 1 transinteraction. In cell-based laser tweezer experiments, TP preincubation blocked PV-IgG-induced loss of Dsg 3 but not loss of Dsg 1 binding. Moreover, hampering of Dsg 3 or Dsg 1 binding by the respective monoclonal antibodies was also decreased by TP. However, TP treatment had no effect on PF-IgG-induced loss of Dsg 3 and Dsg 1 binding. Thus, TP was sufficient to block PV-IgG-induced steric hindrance of Dsg 3 binding but not to inhibit PV- and PF-IgG-mediated loss of Dsg 1 binding, which is supposed to be caused by cellular mechanisms. TP stabilization of Dsg 3 binding reduced PV-IgG-induced loss of desmosomes but did not abolish intercellular gap formation in keratinocyte monolayers indicating that steric hindrance significantly contributes to PV pathogenesis and that peptidomimetics could be a useful supplemental therapeutic approach in PV.

Category: Lecture

Titel:Localization and processing of the components of the renin angiotensin system (RAS) in renal epithelia

Authors: Pohl M.(1),Bader M.(2),Willnow T.(2),Hoffmann S.(3),Gretz N.(3),Harrison-Bernard4 L.(4),Bachmann S.(5),Theilig F.(6),

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Abstract:

Localization and processing of the components of the renin angiotensin system (RAS) in renal epithelia

Purpose: To localize the components of the RAS in kidney cells and to determine the role of the proximal tubule in their biosynthesis and metabolism.

Methods: We have analyzed the distribution of RAS components in the rodent kidney with a focus on their handling by the proximal tubule. We have used normal and transgenic rats with over-expression of hAo, and normal and transgenic mice with a mosaic defect of proximal endocytosis induced by megalin deficiency. Double staining in situ hybridization, immunohistochemistry, and biochemical techniques were applied.

Results: Earlier data on renin biosynthesis were confirmed. Angiotensinogen (Ao) was found to reach the proximal convoluted tubule (PCT, chiefly S1) by endocytotic uptake from the filtrate, whereas the straight part (PST) showed local Ao mRNA synthesis.

Overexpression of human Ao was restricted to PST as well. The endocytotic uptake of Ao and renin was shown to be megalin-dependent. Both components were identified as ligands of megalin. Their specific binding sites were mapped to its extracellular LDL binding domains. A role for the endocytotic apparatus in the transcytosis of Ao was highlighted.

Angiotensin converting enzyme was also expressed chiefly in PST in a megalin-dependent manner. Angiotensin receptor-1 was found in epithelia and vessel walls.

Conclusion: These results underline a prominent role for the proximal tubular handling of RAS components. A role for the renal RAS in volume regulation is further supported by our findings.

Category: Lecture

Titel: Short term effects of vasopressin (AVP) include modification of the turnover of essential membrane transport proteins

Authors: Mutig K.(1), Kahl T.(1), Nedvetsky P.(2), Paliege A.(1), Mengwasser J.(1), Böhlick A.(1), Bachmann S.(1),

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Abstract:

Purpose: Stimulation of trafficking and phosphorylation of the distal transport proteins is an accepted mechanism of the short term AVP-mediated activation of water and salt reabsorption in the kidney. Our purpose is to test if short term effects of AVP involve elevation of intracellular pools of the relevant distal salt and water transport proteins Na,K,2Cl-cotransporter (NKCC2), Na,Cl-cotransporter (NCC), and aquaporin 2 (AQP2).
Methods: Mice, AVP-deficient Brattleboro rats (DI), and cultured thick ascending limb (TAL) cells were stimulated with AVP (1h). Intracellular mRNA and protein pools of NKCC2, NCC, and AQP2 were quantified using Western blot and real time PCR. Double-staining histochemical protocols were used. Blockers of transcriptional and translational activation were used. Lysosomal and proteasomal parameters have been studied.
Results: Luminal trafficking and phosphorylation of NKCC2 were markedly increased after in vivo and in vitro application of AVP. Stimulation of DI rats with AVP significantly augmented intracellular protein pools of NKCC2 (+85%), NCC (+26%), and AQP2 (+26%) ($p < 0.05$) without simultaneous enhancement of the respective mRNA levels. Results obtained in mice and cultured TAL cells demonstrated similar increases of protein pools. Inhibition of protein translation did not abolish the AVP-induced increase (+55%; $p < 0.05$) of NKCC2. Similar data have been raised for NCC and AQP-2.
Conclusions: The results suggest that besides stimulation of phosphorylation and luminal trafficking the short term effects of AVP also involve modification of the turnover of NKCC2, NCC, and AQP2, probably by interfering with protein degradation. This may be a physiologically important mechanism.

Category: Lecture

Titel:Catecholamine biosynthesis and its hypoxic regulation in vascular cells

Authors: Rafiq A.(1),Paddenberg R.(1),Pfeil U.(1),Brüggmann D.(2),Kummer W.(1),

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Abstract:

Previously we demonstrated the presence of an intrinsic, non-neuronal dopaminergic regulatory system in the arterial wall. Functional studies showed an endothelium-dependent and independent vasodilatation in U46619-precontracted superior mesenteric artery under mild hypoxic conditions, which could be blocked by the specific dopamine D1 receptor antagonist SCH23990.

These observations led us to investigate catecholamine synthesis and hypoxic regulation in vascular cells (endothelial cells and smooth muscle cells).

Rat pulmonary and aortic endothelial and smooth muscle cells were isolated, cultured, and characterized by uptake of acetylated LDL and immunolabelling for factor VIII and smooth muscle actin. Cultured cells were exposed to hypoxia (1 % O₂) for 6, 12 and 24 hours. Real-time RT-PCR served to detect and quantify mRNAs coding for the complete enzymatic machinery of catecholamine synthesis, i.e. tyrosine hydroxylase (TH), L-aromatic amino acid decarboxylase, dopamine-beta-hydroxylase (DBH), and phenylethanolamine-N-methyl transferase (PNMT) in these cells. Hypoxic incubation for 6, 12 and 24 hours caused a 4-, 41- and 28fold increase in TH-mRNA, respectively, in pulmonary endothelial cells. Similarly, TH protein was upregulated in endothelial cells as shown by Western blotting. TH-mRNA and protein levels in smooth muscle cells were too low to allow quantification. DBH and PNMT were readily detected in both cell types. In endothelial cells, DBH- and PNMT-mRNA was also upregulated by hypoxia, albeit to a lesser extent than TH-mRNA.

In conclusion, vascular cells endogenously produce dopamine and potentially other catecholamines as well, and synthesis and release are regulated by hypoxia, thereby contributing to hypoxic vasodilation. (DFG GK 534)

Category: Lecture

Titel: Identification and functional characterization of novel soluble forms of human CEACAM1

Authors: Singer B.(1), Muturi H.(1), Scheffrahn I.(1), Tilki D.(2), Ergün S.(1),

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Abstract:

Here we demonstrate the identification of novel soluble variants of the carcinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1). CEACAM1 is a highly glycosylated protein at 120-160kDa. CEACAM1 is a multifunctional, homophilic cell adhesion receptor present in several epithelial, angiogenically activated endothelial and hematopoietic cells. Usually CEACAM1 is expressed as transmembrane protein. Extracellularly, CEACAM1 contains one N-terminal V-type Ig-like domain, followed by three C2-type Ig-like domains (A1-B1-A2). Additionally to the full-length form, twelve alternate splice forms have been reported. Nonetheless, in human urine we found a CEACAM1 form with a molecular weight of approximately 70kDa. Sandwich-ELISA and Western blot based epitope mapping using monoclonal antibodies specific for the N-domain (mAb#18/20), the A2 domain (mAb8G5) or the linker between the B1-A2 domain (4D1C2) revealed that this novel CEACAM1 form was lacking the N-domain and part of the A2-domain. Further analyzes revealed that cells expressing CEACAM1 endogenously (A549) or those transfected with CEACAM1 (Hela-CEACAM1) did show both, the full-length as well as the truncated form of CEACAM1 at 70kDa. Remarkably, the 70kDa CEACAM1 was not detectable in the feces but a 40kDa CEACAM1 form was found. According to our data, none of the so far described isoforms matched the 70kDa or 40kDa variant of CEACAM1. Sequencing of the 70 and the 40kDa CEACAM1 forms isolated from urine and feces, respectively will give us the chance to learn more about the origin and the biological functions of these truncated CEACAM1 forms, particularly in tumor growth and vascular biology.

Category: Lecture

Titel: The impact of LINE-1 mediated retrotransposition events in vascular endothelial cells and angiogenesis

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Abstract:

Long interspersed nuclear element (LINE-1 or L1) retrotransposons are mobile elements that insert into new genomic locations via reverse transcription of an RNA intermediate and play a significant role in shaping the mammalian genome. They are identified as active components of a mechanism involved in control of cell differentiation and proliferation. To analyse the role of LINE-1 in tumor angiogenesis we performed immunohistochemical staining on paraffin sections from normal and tumor tissues of human testis, prostate and urinary bladder. We generated cell clones with retrotransposition events using different cell types such as human dermal microvascular endothelial cell line (HMEC-1), porcine aortic endothelial cells (PAE), human bladder cancer cell line (RT4) and human dermal microvascular endothelial cells (HDMEC) and used them for proliferation assays. While endothelial cells of normal tissues of testis, prostate and bladder exhibited a clear immunostaining for ORF-2p, immature vessels of tumors of these organs were completely negative for LINE-1 ORF-2p. Our data show that LINE-1 encoded proteins are absent in endothelial cells of tumor blood vessels and LINE-1 mediated retrotransposition events result in a decreased endothelial cell proliferation in vitro in the used cell types. In comparison to the transient transfection in LINE-1 endothelial cell clones with retrotransposition events showed a significantly reduced proliferation activity. Double-immunostaining of LINE-1 ORF-2p and Ki67 revealed that particularly those cells show a strong Ki67 staining in which LINE-1 ORF-2p is not or only weak detectable. LINE-1 mediated retrotransposition events may have relevance for angiogenesis and tumor vascularisation.

Category: Lecture

Titel: Effects of interleukin-10 overexpression in two- and three-dimensional chondrocyte cultures

Authors: Mueller R.(1), John T.(1), Kohl B.(1), Schulze-Tanzil G.(1),

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Abstract:

Overexpression of the immunoregulatory cytokine interleukin (IL)-10 inhibits joint inflammation in animal models. However, the impact of high local concentrations of IL-10 on chondrocyte homeostasis remains unclear. Therefore, the aim of this study was to determine effects of adenoviral IL-10 overexpression on cartilage matrix production and matrix metalloproteinase (MMP) expression.

Human articular chondrocytes were adenovirally transduced with human IL-10 (adv/hIL-10), empty vectors (adv/empty) or treated with recombinant IL-10. Non transduced chondrocytes were used as controls. To simulate inflammatory conditions, some cultures were poststimulated with 10 ng/mL TNF α . Collagen type II and MMP-13 mRNA expression was measured 24 hours post transduction. To evaluate longterm effects of IL-10 on cartilage matrix synthesis, IL-10 overexpressing chondrocytes were also introduced into threedimensional (3D) cultures whereby IL-10 secretion and synthesis of extracellular matrix proteins were studied for 14 days post transduction using flow cytometry or immunohistochemistry.

mRNA analysis revealed a significant upregulatory effect of recombinant IL-10 and IL-10 overexpression on collagen type II expression whereas TNF α had a significant inhibitory effect. The TNF α induced MMP-13 expression could be modulated by IL-10 in a donor-dependent manner. adv/IL-10 transduced chondrocytes, embedded in 3D culture secreted IL-10 detectable for more than 2 weeks post transduction. No suppression of collagen type II or aggrecan synthesis was found in the 3D cultures investigated over 14 days.

IL-10 overexpression does not impair key features of chondrocytes differentiated phenotype (eg collagen type II and aggrecan expression) suggesting the potential use of IL-10 for gene therapeutic approaches in the joint and cartilage repair.

Category: Lecture

Titel:Adult human schwann cells (ahscs): Alternative cell-based strategy to enhance peripheral nerve regeneration across long nerve gaps

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Abstract:

Aim: We wanted to analyze the fate and function of ahSCs after implantation in a rat model of sciatic nerve repair.

Methods: Naive (physiological) ahSCs were isolated and enriched in vitro (1). 7×10^5 cells were either PKH-26-GL (fluorescent cell surface) labeled or left unlabeled prior to re-suspension in Matrigel and transplanted in silicone tubes bridging 10 mm sciatic nerve gaps. 2, 4 and 6 weeks after transplantation, PKH-26-GL labeled ahSCs were analyzed with regard to functional behavior (myelination of regenerated axons), survival, distribution and localization pattern within regenerated nerve tissue (n = 3, each).

3 and 7 weeks after transplantation of unlabeled ahSCs (n = 6, each), regenerated tissues were epon embedded and histomorphometrically analyzed for the number of regenerated myelinated axons in comparison to transplantation of acellular silicone tubes filled with Matrigel alone (n = 5, each).

Results: Implanted PKH-26-GL labeled ahSCs survived and were found to be heterogeneously distributed throughout the regenerated tissue cable in a distinct pattern. AhSCs displayed close association with the regenerating axons only 6 weeks after transplantation. Number of nerve transplants including gap-bridging tissue cables after 3 and 7 weeks was significantly higher after ahSC transplantation as compared to acellular control. Histomorphometric analysis displayed enhanced overall regeneration parameters 7 weeks after transplantation of ahSCs.

Conclusion: We demonstrate for the first time the survival and distribution pattern of ahSCs when implanted in a long peripheral nerve gap. Their close association with axons and enhanced tissue regeneration point towards a role in supporting axonal regeneration.

(1) Haastert, K., Mauritz, C., Chaturvedi, S. and Grothe, C., 2007. Nat Protoc, 2(1): 99-104.

Category: Lecture

Titel:Palisade Endings: Cholinergic Sensory Organs or Effector Organs

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Abstract:

Palisade Endings: Cholinergic Sensory Organs or Effector Organs?

Roland Blumer 1, Kadriye Z. Konakci 1, Christine Pomikal 1, Grazyna Wieczorek 2, Julius-Robert Lukas 3, Johannes Streicher 1

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Purpose: To analyze the molecular characteristics of palisade endings in extraocular muscles (EOMs) of a primate species (monkey) using cholinergic markers and classical markers for motor terminals.

Methods: Eleven monkeys (*Macaca fascicularis*) were analyzed. Whole EOM myotendons were immunolabeled using different combinations of triple fluorescent labeling. Labeling included antibodies against choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT), choline transporter (ChT), neurofilament, and alpha-bungarotoxin. Muscle fibers were counter-stained with phalloidin. Immunolabeled whole mounts were examined by confocal laser scanning microscopy and transmission electron microscopy.

Results: Nerve fibers forming palisade endings in monkey EOMs were ChAT immunoreactive and established alpha-bungarotoxin positive neuromuscular contacts outside the palisade complex. The palisade complex established nerve terminals with the tendon and with the muscle fiber. In fact, neurotendinous contacts were more frequent than neuromuscular contacts. Neurotendinous and neuromuscular contacts were immunoreactive for antibodies against ChAT, VAChT, and ChT. Neuromuscular contacts were alpha-bungarotoxin positive as well and exhibited structural features of motor terminals.

Conclusions: We show that palisade endings are supplied by nerve fibers that are unambiguously motor. Moreover, the data show that palisade endings contain all components necessary for the synthesis and storage of acetylcholine, the classical neurotransmitter of motor terminals. Thus, palisade endings are almost certainly effector organs.

Category: Lecture

Titel: Deletion of Go2alpha results in altered behavioural response following cocaine treatment due to imbalanced dopamine storage

Authors: Brunk I.(1), Blex C.(1), Sanchis-Segura C.(2), Sternberg J.(1), Perreau-Lenz S.(2), Bilbao A.(2), Hoertnagl H.(3), Juranek J.(1), Birnbaumer L.(4), Spanagel R.(2), Ahnert-Hilger G.(1),

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Abstract:

Background: Go2alpha is one of the two splice variants of Goalpha subunits. In contrast to mice lacking both subunits Go2alpha deletion mutants do not present any obvious pathological findings. Vesicular Go2alpha inhibits VMAT2 activity in neurons and may interfere with monoamine/dopamine balance. Using Go2alpha^{-/-} mice we studied the impact of Go2alpha on the dopaminergic system.

Methods: Brain fractions of wild type and Go2alpha^{-/-} mice were analysed by Western-blot, vesicular monoamine uptake assay, determination of monoamine oxidase activity and HPLC. Cocaine induced sensitisation was tested in order to detect behavioural impairments.

Results: G-protein mediated regulation of vesicular monoamine uptake was absent in synaptosomes of Go2alpha^{-/-} mice and uptake was significantly reduced, although VMAT2 expression was increased. In addition tyrosine hydroxylase expression was lower, both resulting in decreased striatal dopamine levels in Go2alpha^{-/-} mice.

In contrast to wild type animals Go2alpha^{-/-} mice did not show cocaine induced sensitization, which was reflected by a decrease in striatal expression of D1 receptor and its main down-stream signals Galpha and Golfalpha

Conclusion: Absence of Go2alpha affects vesicular monoamine/dopamine storage probably leading to an increase of toxic cytosolic dopamine. Reduction of tyrosine hydroxylase and increase of VMAT2 expression prevent cytosolic dopamine accumulation and ensure sufficient vesicular filling. Thus Go2alpha deletion mutants exhibit reduced striatal dopamine levels but appear to be healthy under normal conditions. Reduced dopamine level, however, attenuates striatal signal transduction via D1 receptors and prevents development of cocaine-induced behavioral sensitization.

Category: Lecture

Titel:Layer v/vi spiny inverted neurons

Authors: Bueno-Lopez J.(1),Mendizabal-Zubiaga J.(1),Chara J.(1),Reblet C.(2),

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Abstract:

We present research on spiny inverted neurons in rats, rabbits and cats. We have studied these cells with Nissl-staining, Golgi-impregnation, dye intracellular-filling and axon retrograde-track-tracing. Results show that spiny inverted neurons make up less than 8.5% of all cortical neurons in the visual cortex. Infragranular spiny inverted neurons constitute less than 15% infragranular neurons in the said cortex. Spiny inverted neurons congregate at layers V-VI in all studied species. Studies have also revealed that spiny inverted neurons are excitatory neurons which furnish axons for all sorts of cortico-cortical, cortico-claustral and cortico-striatal projections, but not for non-telencephalic centres. Spiny inverted neurons are conspicuous as a source of the backward cortico-cortical projection to primary visual cortex and from this to the claustrum. They constitute up to 82% of the infragranular cells that furnish these projections. Spiny inverted neurons may be classified into three subtypes according to the point of origin of the axon on the cell: the somatic basal pole, the somatic flank and the reverse apical dendrite. As seen with electron microscopy, the axon initial segments of these subtypes are distinct from one another in the number of received synaptic boutons. These features together may support a synaptic-input integration which is peculiar to spiny inverted neurons. Two differently qualified streams of axonal output may coexist in a projection which arises from any infragranular point within a given cortical area; one stream would be furnished by the typical pyramidal neurons, the other by the spiny inverted neurons. Work granted by 9/UPV00212.327-15837/2004.

Category: Lecture

Titel:Activation of astrocytes by CNTF-injection prevents development of recurrent seizures in a mouse model of temporal lobe epilepsy

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Abstract:

Purpose: To study if pre-activation of astrocytes by injection of CNTF can prevent the development of recurrent seizures elicited by an epileptogenic insult (intrahippocampal kainate injection) in a mouse model of temporal lobe epilepsy (TLE).

Methods: CNTF was injected stereotaxically into one hippocampus of C57Bl/6 mice 2 days prior to the injection of kainate at the same location. At various time points after kainate injection, animals were perfusion-fixed for morphological studies (measurement of granule cell dispersion, immunocytochemical staining, detection of degenerating neurons by Fluoro-Jade), or hippocampal tissue was prepared for determination of glial activation by quantitative PCR. In addition, recording electrodes were implanted for daily EEG-monitoring.

Results: Injection of CNTF into the hippocampus led to a rapid and robust activation of astrocytes (hypertrophy of cell bodies, up-regulation of GFAP mRNA expression). Both, CNTF- and saline-injected animals developed a status epilepticus immediately following kainate injection, indicating that activated astrocytes could not prevent kainate toxicity. However, morphological examination over a time course of 3 weeks following kainate injection showed, that CNTF-injected animals had reduced cell death in the CA3 region of the hippocampus, as well as strongly reduced granule cell dispersion in the dentate gyrus. EEG recordings revealed, that the rate of interictal spikes associated with fast ripples, which are indicative of epileptic activity, was strongly reduced in the CNTF-injected animals.

Conclusions: Our results show that astrocyte activation by CNTF triggers neuroprotective mechanisms, which prevent the development of chronic, recurrent seizure activity in the hippocampus. (Supported by the DFG: Transregio SFB TR3 and SFB 505)

Category: Lecture

Titel:Rhythmic expression of clock genes in the ependymal cell layer of the third ventricle: possible role for the photoperiodic response of gonads in mammals

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Abstract:

In several mammalian species living in temperate zones, timing of reproduction is regulated by the photoperiod decoded by melatonin. Melatonin regulates the gonadal axis by inhibiting the expression of type 2 deiodinase (Dio2) in the ventrobasal ependymal cell layer of the third ventricle (EC). To examine whether the photoperiodic response of Dio2 expression involves a circadian clock system, we investigated the expression of two clock genes (Per1 and Bmal1) in the EC of male Syrian hamsters kept under short- or long- day conditions (SD-hamsters and LD-hamsters, respectively). Per1 mRNA levels varied rhythmically peaking at ZT15 in both LD- and SD-hamsters. Maximal values were twofold higher in LD- than SD-hamsters. PER1 protein levels also fluctuated and peaked at ZT21; they were higher in LD- than in SD-hamsters. Expression of Bmal1 was rhythmic peaking at ZT03 in both LD- and SD-hamsters, but the amplitude was not affected by the photoperiod. To evaluate the functional relationship between expression of Per1 and Dio2, we examined mice with targeted deletion of Per1 and found that Dio2 expression was not induced under long-day conditions. Thus, rhythmic clock gene expression in the EC is an important mechanism for the photoperiodic regulation of gonads in mammals. Additional investigations showed an unaltered temporal expression of Per1, but an upregulation of Dio2 expression in mice lacking the Mel1a-receptor. All data suggest that at least two independent mechanisms are involved in the photoperiodic control of Dio2 expression: one depends on melatonin and the other involves rhythmic expression of Per1.

Category: Lecture

Titel:2-arachidonoylglycerol elicits neuroprotective effects via its action on abnormal-cannabidiol-sensitive receptors on microglial cells

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Abstract:

Endocannabinoids like 2-arachidonoylglycerol (2-AG) exert neuroprotective effects after brain injuries. According to current concepts these neuroprotective effects are due to interactions between 2-AG and cannabinoid (CB)₁ receptors on neurons. Moreover, 2-AG modulates migration and proliferation of microglial cells, the intrinsic immune cells of the central nervous system which are rapidly activated after brain lesion. This effect is mediated via CB₂- and abnormal-cannabidiol (abn-CBD)-sensitive receptors. Here, we investigated whether the abn-CBD-sensitive receptor on microglial cells contributes to 2-AG-mediated neuroprotection in organotypic hippocampal slice cultures (OHSCs) after excitotoxic lesion induced by NMDA (50µM) application for 4h. This lesion caused neuronal damage and accumulation of microglial cells within the granule cell layer. To analyze the role of abn-CBD-sensitive receptors for neuroprotection and microglial cell accumulation two specific agonists of the abn-CBD-sensitive receptor, abn-CBD or 2-AG and two specific antagonists, O-1918 and CBD, were applied to the lesioned OHSC. Propidium iodide (PI) labeling was used as a marker of degenerating neurons and isolectin B4 (IB4) as marker of microglial cells. Application of both, abn-CBD and 2-AG to lesioned OHSC significantly decreased the number of IB4+ microglial cells and PI+ neurons in the dentate gyrus. Application of O-1918 and CBD reverted these effects. When microglial cells were depleted by preincubation of OHSC with the bisphosphonate clodronate (100µg/ml) for 5 days prior to excitotoxic lesion, 2-AG and abn-CBD lost their neuroprotective effects. We therefore propose that the endocannabinoid 2-AG exerts its neuroprotective effects via activation of abn-CBD-sensitive receptors on microglial cells.

Category: Lecture

Titel: Studies on the pathomechanism of neurodegeneration in peroxisomal biogenesis disorders

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Abstract:

Purpose: To elucidate the pathogenesis of neurodegeneration in peroxisomal biogenesis disorders.

Methods: Neuronal death was evaluated by nuclear staining with Hoechst 33342 and immunostaining of active caspase-3 in primary cortical cultures from wild-type, heterozygous and homozygous PEX11beta knockout (KO)-mice. The redox state of cortical neurons was determined using the redox-sensitive dye dihydroethidine to detect the cellular level of reactive oxygen species (ROS) and by measuring the protein level of different antioxidant enzymes. The contribution of peroxisome proliferator-activated receptor alpha (PPARalpha) and NO-synthases (NOS) activation to neurodegeneration in PEX11beta-deficiency was evaluated using specific agonist/antagonist of the respective signalling molecules.

Results: In primary neuronal cultures prepared from the medial neocortex of heterozygous and homozygous PEX11beta-KO fetuses (E19) we observed increased neuronal death accompanied by reduced neurite outgrowth and network formation. In neurons from homozygous knockout mice the abundance of peroxisomes was reduced by half. The cellular ROS level was higher in neurons from heterozygous and homozygous PEX11beta-KO mice compared to those from wild-type littermates. The protein levels of catalase and SOD1 were only slightly different between all three genotypes. The SOD2 level was up-regulated in neurons from heterozygous mice – those from PEX11beta-KO mice could not increase SOD2. Treatment with either tocopherol, the PPARalpha-antagonist MK886 or the inducible NOS inhibitor 1400W was able to inhibit neuronal death. PPARalpha activation led to cell death only in wild-type cultures.

Conclusions: Our results suggest that neurodegeneration in peroxisomal biogenesis disorders involves oxidative stress, activation of PPARalpha and of inducible NOS.

Category: Poster