Oral presentations IEB Debrecen 2004

AUDIOLOGY

O1: MINOCYCLINE AND MDL28170 REDUCE GENTAMICIN OTOTOXICITY

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Gentamicin (GM) side effects on cochlear structures and functions are well known, but not the intracellular molecular mechanisms that lead to aminoglycoside ototoxicity. Hair cell death occurs by apoptosis, with the activation of enzyme cascades such as caspase or calpain proteases, and with the release of cytochrome c. To test this hypothesis we used as potential otoprotector minocycline, a second-generation tetracycline antibiotic wich inhibits caspases and release of cytochrome c, and MDL28170, a dipeptide able to act as a selective calpain inhibitor.
The organs of Corti of postnatal day 3 (P3) rat were dissected and kept in culture. After in vitro treatment tissues were fixed and labeled with fluorescent falloidin, and hair cells were counted by a fluorescent microscope. Cochlear organotypic cultures were treated with GM alone or in combination with different doses of minocycline, and MDL28170. Treatment with GM induced a dose-dependent loss of outer hair cells (OHC) and inner hair cells (IHC).
Addition of minocycline to the GM-treated cultures greatly reduced the amount of GM induced hair cell damage. The greatest protection was achieved with 100 μM of minocycline. Application of minocycline alone had no adverse effects on hair cell survival. A MDL 28170 dose-effect curve was obtained: lower concentrations resulted not toxic. In cultures treated with highest concentration of GM, MDL28170 showed a dose-dependent protective effect.
These data can support a role of calpains cytochrome c in GM induced hair cell apoptosis.

O2: SODIUM ENOXAPARIN FOR TREATMENT OF IMMUNE-MEDIATED SENSORINEURAL HEARING LOSS (IMSNHL)

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Immune-mediated sensorineural hearing loss (IMSNHL) is characterised by rapidly progressive hearing loss that has not confirmed etiology: treatment guidelines are controversial but include corticosteroids, cytotoxic agents and

plasmapheresis. Actually, the literature does not report others therapeutic protocols for IMSNHL treatment with sodium enoxaparin or other kinds of unfractionated heparin. The aim of the study was to assess the efficacy of sodium enoxaparin in the treatment of IMSNHL.
Sixty patients, who had suffered from IMSNHL, have been analysed. These were randomly assigned to two groups: in the first group enoxaparin was administered subcutaneously at a dose of 2000 I.U. twice a day for 10 days while the second group (control) received a placebo (0.2 millilitres of physiological solution) with the same way of administration. Every day all patients underwent the following instrumental examinations: liminar tonal audiometry; otoacoustic emission; otoacoustic products of distortion. Blood tests where performed at the beginning and at the end of the treatment. Specifically excluded were patients with abnormal known coagulation.
On discharge, the 86% of patients treated with enoxaparin presented a subjective abatement of tinnitus and the 72% of the patients an hearing improved. No patient experienced side effects from this treatment.
We have tested sodium enoxaparin in these patients affected by IMSNHL and a high number of patients shown a marked improvement of their symptoms. This study suggests further studies to confirm these data but this kind of therapy appears to give encouraging results in the treatment and diagnosis of IMSNHL.

O3: THE EFFECT OF LONG TERM NOISE EXPOSURE ON BEHAVIORAL AUDIOGRAMS AND TRANSIENT OTOACOUSTIC EMISSIONS

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Firing noise is characterized by a rapid pressure change and sharp peak of sound pressure level in the range of 160-190 dB SPL. In the current research, we examined ears vulnerability to exposure of small-arm firing noise in a population of combat soldiers who were initially exposed to this type of noise during their basic training. Measurements included Transient Evoked Otoacoustic Emissions (TEOAE) and standard audiograms. Measurements were initially performed prior the soldiers’ basic training and were repeated several times during the experimental period. None of the ears developed a significant hearing loss during the 2 years of the experimental period. We define slight hearing loss (SHL) if a threshold shift of a more than 10 dB was obtained in at least one of the tested frequencies. About 57% of the ears developed SHL after 2 years of noise exposure. TEOAE was analyzed in wide-band frequency range and narrow-band of 500 Hz around different center frequencies. Wide-band TEOAE level (Em) decreased as a function of time. The most significant decrease was obtained in the low frequency range following the first exposure. Increase in TEOAE levels was obtained in the high frequency range following a short rest period. About 67% of the tested ears that prior to the noise exposure had medium wide-band

TEOAE level (2 < Em <8 dB) developed SHL. On the other hand, among the ears whose were either very low (Em < 2 dB) or very high (Em > 8 dB), only less than 38.5% of them developed SHL. We suggest a prediction for ears vulnerability to noise exposure on basis of their TEOAE prior to the noise exposure.

O4: AN EXPLANATION FOR THE SYMPTOMS OF MENIERE'S DISEASE

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There can be a higher threshold of hearing without any dysfunction or loss of hair cells. If the stimulus energy does not reach the inner hair cells (IHC) there will not be a sensation of hearing. This can be considered to be a pathophysical problem and can potentially explain the early symptoms associated with endolymphatic hydrops. The concept of energy flow may provide a reasonable explanation for the initial low-frequency and fluctuating hearing loss that occurs with hydrops. With the increased pressure of endolymphatic hydrops, the BM will be distended and there will be changes in its stiffness and compliance. The compliance change causes the location where the sound passes through the BM to be more basal. Thus, with hydrops, low frequency sounds may never reach the apical receptors. This may be considered to be a pathophysical loss that occurs with no physiological injury. Thus, the fluctuating threshold shifts are a product of a physical change in the path of the cochlear sound. Thus, anything that results in a loss of endolymph with a restoration of normal pressure may affect a return to normal hearing by returning the area of compliance to its original location. The secondary hearing loss that starts with the higher frequencies may have an ionic basis. The repeated rupturing of Reissner's membrane with the admixing of perilymph and endolymph causes a lowering of the potassium concentration in the endolymph. Electroreception by the IHC apparently requires a high concentration of endolymphatic potassium. The basal IHC may be most sensitive to a lowering of the potassium concentration.

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AUDIOLOGY, PHYSIOLOGY

O5: A SISTEMATIC IDENTIFICATION OF NEW GENES INVOLVED IN NON-SYNDROMIC DEAFNESS

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Non syndromic hearing loss is the most common form of genetic deafness and is a very heterogeneous trait with more than 100 loci. This extreme genetic heterogeneity suggests that there are many different processes within the inner ear that, once altered, can cause hearing loss.
A mutational screening of a series of 10 candidates genes has been performed on 500 hearing impaired unrelated patients from many countries. All candidates genes have been selected on the basis of their expression in the organ of Corti, to their possible role in the Machanism of Transduction of the signal, to the existence of animals models and Loci candidates. The analysis of mutations has been straight led by DHPLC and Sequencing.
This strategy proved to be exstremely useful and effective since permitted us to find causative mutations in the gene MYO1A in the year 2003, while the ivolvement of MYH14 gene in causing hearing loss as consequence mutations was demonstrated by our group in the year 2004 . This last gene is a non-muscle myosin, exspressed in cochlea, seems to be strictly connected with trunsduction mechanism of auditive signal. On the contrary, using the same systematic approach , we were unable to find causative mutations in the following candidate genes : CX45, BARHL1 , TIMM13b, PRESTIN , PMCA2.
Our systematic approach for the screening of candidate genes for hearing loss, based on high-throughput DNA molecular analysis using DHPLC coupled with direct sequencing, has proven to be highly satisfactory since allowed us to reach in relatively short time positive results in terms of rapid progression in identification of genes involved in deafness and/or hearing loss.

O6: BK AND CAV1.3 ION CHANNELS IN THE COCHLEA

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Transmitter release in IHCs is dependent on Ca2+ influx through voltage-activated Ca2+ channels, which have been specified as Cav1.3 (or a1D). The

molecular basis of the IHC L-type Ca2+ currents was investigated using a mouse model lacking Cav1.3 subunits. Hair cell degeneration in Cav1.3-/- mice was noted starting in OHCs in the apical cochlear turns around P14 and involving IHCs later on (Platzer Thet laarlg.e, 200 conductance voltage- and Ca2+-activated potassium (BK) channel has also been suggested to play an important role in the signal transduction process of cochlear inner hair cells (IHCs), contributing to phase locked receptor potentials up to high sound frequencies (Kros et al., 1998). Analyzing the hearing function and cochlear phenotype of BK channel a-knockout mice (BKa-/-) we recently could demonstrate hair cell degeneration starting in OHCs in the basal /midbasal cochlear turns around the 8 weeks postnatally (Rüttiger et al., 2004). We here demonstrate the expression pattern of Cav1.3- and BK-mRNA and protein using whole mount in situ hybridisation and immunohistochemistry and compare the known phenotypes of both Cav1.3-/- and BK-/- knockout mice. The results are discussed in the context of a possible differential role of the ion channels in outer hair cells along the tonotopic axis.

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O7: METABOLIC ROLE MAY BE MORE INVOLVED IN HEARING LOSS INDUCED BY IMPULSE NOISE EXPOSURE

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Impulse noise-induced hearing loss (ININHL) induced by gunshots, firework and industrial noise is common in our society. Underlying mechanism is considered to cause more mechanical damage to hearing organ. However, there is no direct evidence to convince the expectation. The aim of the present study is to test that metabolic role in impulse noise exposure may be more involved.

We measured auditory brainstem response (ABR) thresholds in the animals exposed to impulse noise at 160 dB SPL peak value. The confocal microscope was applied to detect changes of hair cells in exposed animals. We also analyse the ion concentration of perilymph. In addition, the animals were injected glutamate receptor antagonist caroverine to if it could protect the cochlea against impulse noise trauma.

We found that the animals exposed to impulse noise were significantly protected by caroverine. Hair cell change was found to correlate to post-impulse noise exposure time and ion concentration of the perilymph were not significantly changed due to impulse noise exposure.

Metabolic role is more involved in impulse noise exposure at 160 dB SPL peak value and pharmacological therapy is possible to treat/protect impulse noise trauma.

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GENETICS

O8: CISPLATIN-INDUCED GENE-TRANSCRIPTION IN ORGAN OF CORTI-DERIVED CELLS

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Cisplatin (CDDP) is an anticancer drug used in treatment of a wide range of tumors. However it shows side effects as nephro- and ototoxicity, whose cell and molecular mechanisms are only partially understood. To elucidate the cellular mechanisms specifically underlying CDDP ototoxicity, we used a cell line (OC-k3) developed from the organ of Corti of transgenic mice. We investigated the gene expression profiles originated by CDDP. We compared these data to expression profiles obtained from public data banks or from CDDP-treated tumor cell lines (HL60) and we searched for a confirmation of translation by WB.
Immunohistochemistry, protein phosphorylation and mass analysis, ROS evaluation and DNA microarray was used.
In our cell model, we observed that CDDP induced a time- and concentration dependent cell death, that seems to occur by apoptosis. As detected by microarray analysis in the 3 to 12 hours interval of treatment, CDDP strongly enhanced gene transcription as a whole. In particular, we observed a time dependent variation of the mRNA,of several enzymes involved in signal transduction and ROS generation. These findings could explain the ability of suramin, an inhibitor of growth factor and P2 receptors, and butylated hydroxytoluene, a lipophilic radical scavenger, to partially protect OC-k3 cell from cisplatin toxicity.
These data indicate that binding to DNA can not be the main cause of cytotoxicity in non-cancer cells. On the contrary, activation of intracellular signalling pathways, ROS production, and the related alteration of the redox balance can have a key role in CDDP-triggered apoptosis.

O9: ROLE OF INSULIN-LIKE GROWTH FACTOR I (IGF-I) IN VESTIBULAR DEVELOPMENT

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The development of the otocyst into a mature inner ear is controlled by extrinsic and intrinsic factors. The insulin-like growth factor (IGF) system plays an important role in the development of the nervous system being fundamental system for its differentiation, functionality and maintenance. The IGF system is formed by three factors (Insulin, IGF-I and IGF-II), three cellular receptors (IGF receptors type 1 and 2 and insulin receptor) plus six binding proteins that control IGFs activity. IGF-I is expressed in the developing otic epithelia and cochleovestibular ganglia of different species being essential for the normal development and function of the vertebrate inner ear (Varela-Nieto et al., 2004). IGF-I deficiency in mouse severely affects postnatal survival, differentiation and maturation of the cochlear ganglion (Camarero et al., 2001; and 2002). In humans the lack of IGF-I is associated with sensorineural deafness (Bonapace et al., 2003).
Here we present the study of the vestibular system in Igf-1 -/- deficient mice. We have studied the expression of IGF system members at postnatal day five (P5) and P20 in the vestibular organ. Our results indicate that the vestibular ganglion and the sensoriephitelium express insulin, IGF-I, IGF-II and their receptors. The vestibular phenotype of the Igf-1-/- mouse shows a severe disorganization in the neurosensorial ephitelia with abnormal hair cell morphology. The vestibular ganglion presents a delayed neuronal maturation, altered myelination in neurons, and a reduced size of the ganglion at P20. Some alterations in neuroephitelium ending nerves have also been found. IGF-I deficiency, therefore,
causes alterations in the vestibular organ, playing IGF-I an important role in its postnatal development and maturation.

O10: FUNCTIONAL ANALYSIS OF CONNEXINS

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Connexins comprise a family of vertebrate trasmembrane proteins that assemble intracellularly to form oligomeric channels clustered at gap junctions. They are involved in several human disorders: in particular, mutations in five members of the connexin gene family, including CX26, CX31 and CX30 have been shown to underlie distinct genetic forms of deafness. Our aim is to characterize the connexins' complex and find novel functional interactors of these proteins. To perform this analysis we cloned the wt cDNAs of CX26, CX30, CX31, CX43, CX45 and some mutated cDNAs like Cx26 35delG and W44S, Cx30 T5M, Cx31 D66del, in two expression vectors: the first one codifies for a fusion protein CX-GFP, the other for an HA-CX protein. After the transfection of the clones in gap junction-deficient HeLa cells, the GFP allows us to confirm the right localization of the proteins, while using the antibody that recognize the HA tag we can

characterize the connexins' complex through immunoprecipitation, western blot and mass spectrometry. At the same time we are able to use the RNA extracted from cells transiently transfected with our clones to perform microarrays using our chip made of 1000 genes cochlea-expressed. The fusion proteins go to the plasma membrane and form functional connexons, as we observed in an immunoflorescence assay performed with the confocal microscopy technique, so we are testing the conditions for the isolation and the characterization of the immunocomplex. Furthermore, we are preparing the high-quality RNA for the expression profiling assay. These different approaches are important to better understand connexins' function and we hope that they will allow us to identify new candidate genes for non-syndromic deafness.

O11: TRANSCRIPTION PROFILING OF INNER EARS FROM POU4F3 DREIDEL MICE IDENTIFIES GFI1 AS A TARGET OF THE POU4F3 DEAFNESS GENE

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Pou4f3 (Brn3.1, Brn3c) is a class IV POU domain transcription factor that has a central function in the development of all hair cells in the human and mouse inner ear sensory epithelia. A mutation of POU4F3 underlies human autosomal dominant non-syndromic progressive hearing loss DFNA15. Through a comparison of inner ear gene expression profiles of E16.5 wild type and Pou4f3 mutant deaf mice using a high density oligonucleotide microarray, we identified the gene encoding growth factor independence 1 (Gfi1) as a likely in vivo target gene regulated by Pou4f3. To validate this result, we performed semi-quantitative RT-PCR and in situ hybridizations for Gfi1 on wild type and Pou4f3 mutant mice. Our results demonstrate that a deficiency of Pou4f3 leads to a statistically significant reduction in Gfi1 expression levels and that the dynamics of Gfi1 mRNA abundance closely follow the pattern of expression for Pou4f3. To examine the role of Gfi1 in the pathogenesis of Pou4f3-related deafness, we performed comparative analyses of the embryonic inner ears of Pou4f3 and Gfi1 mouse mutants using immunohistochemistry and scanning electron microscopy. The loss of Gfi1 results in outer hair cell degeneration, which appears comparable to that observed in Pou4f3 mutants. These results identify Gfi1 as the first downstream target of a hair cell-specific transcription factor and suggest that outer hair cell degeneration in Pou4f3 mutants is largely or entirely a result of the loss of expression of Gfi1.

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PATHOLOGY

O12: ISCHEMIA-INDUCED APOPTOSIS AND NECROSIS RATES IN EXPLANT CULTURES OF THE RAT ORGAN OF CORTI

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Hypoxia and ischemia are considered to be important pathogenetic factors in bringing about hearing loss via hair cell death. To develop new preventive and therapeutic approaches, it is important to determine the role of the main pathways, apoptosis and necrosis, in cell death.
We used an in vitro model of the 3-5 days old newborn rat cochlea. The organotypic cultures were divided into their apical, middle and basal parts which were exposed to mild (3 h) or severe (4 h) ischemia in glucose-free artificial perilymph in a Billups chamber, pO2 10-20 mm Hg. The cultures were stained 24 h after onset of ischemia using propidium iodide (PI; staining of necrotic cells), in situ DNA End Labeling Assay (ApopTag ISOL Ligation Kit, Intergen; staining of apoptotic cells) and tetramethyl rhodamine isothiocyanate labelled phalloidin (Sigma; staining of inner and outer hair cells, IHCs, OHCs). The number of stained cells per 100 µm organ of Corti was counted.
Ischemia induced a decrease of the number of both IHCs and OHCs. The hair cell loss was more severe for the IHCs than it was for the OHCs. The rate of apoptotic and necrotic hair cell death increased in parallel to the duration of ischemia (e.g., in the middle part, 4 cells/100 µm after 3 h, 8 cells/100 µm after 4 h). The ratio of PI/ISOL stained cells in the cochlear fragments is in the range of one, indicating an almost 1:1 proportion of apoptosis and necrosis.
Ischemia triggers death of hair cells in the organ of Corti via apoptosis and necrosis. Mild and severe ischemia revealed an almost identical share of the apoptosis and necrosis rates in the apical, middle and basal cochlear parts.

O13: INTERACTION OF FREE CHOLESTEROL WITH THE INNER EAR MEMBRANES

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Several studies suggest a contribution of hypercholesteremia to the development of sensorineural hearing loss. Cholesterol is a prominent component of the

mammalian plasma membrane. An enhanced incorporation of free cholesterol may alterate the function of the membrane and influences its stiffness and finally induces cell death. Loss of function of the peroxisomal Mpv17 protein in Mpv17-/- mice causes focal segmental glomerulosclerosis and sensorineural deafness associated with increased cholesterol level in serum. In early stages of inner ear degeneration severe malformation of the cellular and plasma membrane have been observed. They include a formation of membrane whirls in the stria vascularis and a collapse of the lateral wall of the outer hair cells (OHCs) which appear floppy and wrinkly indicating alterations of membrane stiffness. A histochemical technique that allows visualization and mapping of free cholesterol in the inner ear tissue at light- and electronmicroscopical level was applied. The reaction products were found mainly localized at membranes and mitochondria especially in structures contributing to the ion transport, e.g. stria vascularis. In the organ of Corti an incorporation of free cholesterol into the lateral wall of the OHCs leads to floppy and folded appearence of the cells. Furthermore, a separation of the layer of the laterall wall of the outer hair cells was evident indicating a posssible toxic effect of enhanced free cholesterol and an interaction with the micromechanics of the outer hair cells.

O14: CHARACTERISATION OF RECOVERY AFTER CISPLATIN OTOTOXICITY: ENDOCOCHLEAR POTENTIALS AND CISPLATIN CONCENTRATIONS

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Previous work has shown that within the first 2 weeks after an ototoxic insult caused by cisplatin treatment, substantial recovery of acoustically evoked cochlear potentials occurs (Klis et al., 2002, Hear. Res. 164, 138-146). This recovery appeared to originate with recovery of the endocochlear potential (EP). Directly after the insult, the EP had decreased to typically 50 mV and it had returned to normal (~80 mV) 4 weeks after. In the present work we proceed to characterise the recovery process by refining the time resolution of these measurements. Albino guinea pigs, equipped with permanent round-window electrodes, were treated daily with an i.p. injection of 1.5 mg/kg cisplatin until the compound action potential threshold (3 μV criterion) shifted by 40 dB or more. We continued electrocochleography for 0, 2, 3, 5 or 7 days after which the EP was measured in a terminal experiment. In addition to the EP measurement, the cisplatin concentration was determined in blood and perilymph with atomic absorption spectrometry (AAS). Results thus far show parallel recovery in the EP and the electrocochleographic thresholds with possibly the exception of the first couple of days. This suggests that another factor may be responsible for initial recovery, e.g., a pharmacokinetic factor. However, AAS measurements of the Pt

concentration in blood and perilymph did not support this hypothesis. Pt concentrations were around 100 µg/L in perilymph in the animals sacrificed at day 0 and dropped below the detection limit afterwards. In blood, the Pt concentration dropped gradually, but slowly from around 1300 µg/L at day 0 to 400 µg/L after 7 days.

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INNERVATION

O15: ALTERED BASILAR ARTERY PERMEABILITY IN RESPONSE TO COCHLEAR APPLIED CAPSAICIN: A PRIMARY SENSORY INNERVATION CONNECTING HEADACHE AND INNER EAR

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Trigeminal neurogenic inflammation is one explanation for the development of vascular headaches. The migraine-related inner ear symptoms of phonopobia, tinnitus, fluctuation in hearing perception, and increased noise sensitivity provide indirect evidence for a connection to basilar artery migraine. The aim of this study was to determine if a physiological basis for neurogenic inflammation exists between cochlear and the basilar artery. Capsaicin induced neurogenic inflammation induced plasma extravasation in the basilar artery, as observed by colloidal silver leakage with darkfield microscopy and Evans Blue extravasation by laser-scanning confocal microscopy. The capsaicin application caused a dose and time dependent permeability increase both in the basilar artery and the anterior inferior cerebellar artery (AICA). The most marked extravasation occurred with 0.01% capsaicin, but the effect was also significant with 0.001%, 0.1%, and 1% capsaicin. Sixty minutes following capsaicin Evans Blue extravasation occurred the internal elastic membrane and external elastic membrane of basilar artery and AICA. The colormetric determination of cohlear Evans Blue extravasation also showed significant quantitative differences between the treated, contralateral and control groups. These results characterize a functional connection between the cochlea and vertebro-basilar system through the capsaicin sensitive primary sensory neurons. We propose that vertigo, tinnitus, and hearing deficits associated with migraine arise by excitation of the trigeminal ganglion and plasma extravasation. Cochlear dysfunction may also can trigger basilar and cluster headache.

O16: INTERACTION BETWEEN INNER EAR HAIR CELLS AND GRAFTED ES CELL-DERIVED NEURONS IN VITRO

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Embryonic stem (ES) cells are the basis of cellular therapy in a range of organs. ES cells are the candidates as transplants for replacement of various types of inner ear cells. Recently, the methods for neural induction of ES cells have been established. This study examined the interaction between neural differentiated ES cells and inner ear hair cells using organ-culture systems.
We used the co-culture of ES cells with PA6 cells, mouse skull bone marrow cells, as a method for neural induction of ES cells. ES cells genetically labeled with GFP were used in the present study. Colonies that formed on the PA6 layer during the 6 days of culture were collected, and prepared as cell suspensions. Mouse cochlea or utricle sensory epithelia obtained from P3 mice were co-cultured with ES cell suspensions for 7 days. Some of the sensory epithelia were pre-treated with aminoglycosides. After the culture period, the sensory epithelia and ES cells were fixed with 4% PFA, and provided for histological analysis. Immunostaining for myosin VIIa and TuJ1 was performed to demonstrate locations of hair cells and neural tissues. The formation of synaptic connections was estimated by immunostaining for synaptophysin. Histological analyses revealed massive elongation of neurites from ES cells. The majority of ES cells located around cultured sensory epithelia, while some ES cells were found closely to sensory epithelia. Some neurites derived from ES cells were adjacent to sensory hair cells. Neurites from ES cells adjacent to hair cells frequently expressed synaptophysin.
These findings indicate the potential of ES cell-derived neural cell transplants for the restoration of the nervous system in the inner ear.

O17: NEURONAL CHAIN FROM THE AUDITORY CORTEX TO THE HAIR CELLS IN THE COCHLEA: DIRECT EVIDENCE BY TRANSNEURONAL LABELING

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Nuclei of the ascending and descending auditory pathways are interconnected through a series of commissural and ipsilateral connections. Each stage of the descending auditory pathway could serve as descending part of regional feedback loops. We used the pseudorabies virus to label the descending auditory system. Following primary infection of neurons, the pseudorabies virus is transported retrogradely in the axon, invades and destructs the cell body and infects other contacting neurons transsynaptically. Seventeen adult male guinea pigs were utilized. A suspension of the virus was slowly injected into the cochlea.

Animals were sacrificed following different survival times. Brains were transcardially fixed and removed from all animals. Virus-infected cells were visualized by immunoperoxidase staining of cryosections. After different survival times, labeled cells were detected in auditory brainstem nuclei, in the auditory cortex and in several monoaminergic regions. On the basis of different survival times, three stages of infection were distinguished. Stage 1 (25 hours) was characterized by infection of the olivocochlear cells in the superior olive bilaterally, while stage 2 (37-72 hours) was identified by labeling in the inferior colliculus and in other auditory and non-auditory structures of the brainstem. Following longer survival times (stage 3, 90-108 hours) infected cells were additionally seen in the medial geniculate body and auditory cortex bilaterally. The present work indicates that the descending auditory system is not only a loosely interconnected chain of feedback loops, but also a descending chain of neurons which is able to conduct information from the auditory cortex to the hair cells in the cochlea.

O18: DISTRIBUTION OF GLUR2 RECEPTORS IN SPIRAL GANGLION NEURONS

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Murine type I and type II spiral ganglion neurons show distinctive firing properties that vary with cochlear location (Reid, Flores-Otero, & Davis, J.Neurosci. 24, 2004). To determine whether apex/base distinctions are also present at synapses, we have begun to examine the distribution patterns of AMPA receptors which mediate neuronal responses to neurotransmitter released from hair cells. Because of the central role that calcium plays in neuronal modulation, signaling, and regulatory events, we have focused on GluR2, an α-subunit that has a regulatory effect on Ca2+ permeation through the AMPA receptor pore. Immunocytochemistry was utilized to visualize GluR2 α-subunits in type I and type II spiral ganglion neurons. We observed that anti-GluR2 staining was significantly enriched in type II spiral ganglion neurons isolated from the apex and base of the cochlea (luminance = 35.5 ± 1.2, n=81 and 45.0 ± 4.4, n=30, respectively) compared to their type I apical and basal counterparts (17.6 ± 0.5, n=142 and 27.0 ± 1.2, n=62, respectively; $P < 0.01$). We also noted enhanced GluR2 antibody labeling in basal type I and II neurons compared to type I and type II neurons isolated from the apex ($P<0.01$ for each comparison). These data clarify earlier reports by others of higher levels of GluR2 mRNA expression in small spiral ganglion neurons. In conclusion, our data indicate that GluR2 α-subunit density varies with characteristic frequency for both the type I and type II spiral ganglion neurons. Future experiments will evaluate other AMPA receptor subunits known to be expressed in the spiral ganglion and to determine the distribution patterns of these receptors in adult animals.

O19: THE PARTICIPATION OF THE TIP39-CONTAINING NEURONS IN THE AUDIOGENIC STRESS RESPONSE

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Recently a new endogenous ligand of the parathormone-II receptor has been discovered: the tuberoinfundibular peptide of 39 residues (TIP39). TIP39 is localized within some circumscribed neuronal cell groups in rat. Among these areas some are known to be auditory-responsive. Materials and methods:In the present study, double immunostaining for TIP39 and the gene c-fos was performed, following a stressful noise stimulation, to investigate the participation of the TIP39-containing neurons in the audiogenic stress response. The expression of the gene c-fos is a reliable marker for neuronal activation. Results:Among else the subparafascicular (SPF), parvicellular SPF (SPFPC), posterior intralaminar (PIL) thalamic nuclei in the midbrain and the medial paralemniscal nucleus (PL)in the pons showed c-fos expression. Several neurons in these nuclei synthesize TIP39, as proven by the immunohistochemistry. A large number of Fos-immunoreactive cells were present in the parvicellular subdivision of the hypothalamic paraventricular nucleus. This part of the nucleus is fairly well innervated by very fine, varicose TIP39-positive fibers. A relatively low (24%) percentage of TIP39-positive neurons in the SPF were double stained with Fos in response to loud noise. 18-21% of the Fos-positive neurons in the acoustic thalamus (SPFPC-PIL) stained for TIP39. However, Fos immunoreactivity was induced in 50% of the TIP39-immunoreactive cells in the SPFPC. The vast majority (70%) of the medial PL neurons were double-stained, four out of five TIP39-ir neurons responded to loud noise with c-fos expression.Discussion:Based on these results, we may suppose that, TIP39 may participate in the neuronal procession of the audiogenic stress.

Oral presentations IEB Debrecen 2004

PHARMACOLOGY

O20: CHEMICALS THAT CAUSE OXIDATIVE STRESS PROMOTE NOISE INDUCED HEARING LOSS

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We have studied a series of chemical contaminants present in both work and ambient environments for their ability to potentiate noise-induced hearing loss. These chemicals have in common the potential to impair intrinsic antioxidant pathways. We have hypothesized that disrupting antioxidant pathways renders the cochlea more vulnerable to reactive oxygen species that are produced even by moderate noise exposures. Rats were exposed to acrylonitrile (50mg/kg for 5 days) alone, noise alone (4 hours at 95 and 97dB OBN for 5 days), both acrylonitrile + noise, and to no treatment. DPOAEs obtained 1 day and 4 weeks following the last treatment were compared to each subjects pre-exposure DPgram. CAP thresholds were also measured 4 weeks following treatment. The data to be presented show that a high production chemical, acrylonitrile, which depletes glutathione and generates cyanide can produce significant auditory impairment and loss of outer hair cells even though the noise exposure alone and the chemical exposure alone have no permanent effects on hearing and do not produce loss of outer hair cells. Only rats receiving combined treatment to acrylonitrile + noise show disruption in DPOAE amplitude, loss of threshold sensitivity, and loss of outer hair cells. Parallel studies demonstrated that rats which received PBN, a spin-trap agent that forms adducts with reactive oxygen species, were protected from the combined exposure to acrylonitrile + noise, suggesting that oxidative stress was responsible for the potentiation of noise effects by this chemical agent.

Supported in part by NIH grant DC05503 and NIOSH grant OH03481.

O21: PROTECTION STRATEGIES FOR NIHL USING N-L-ACETYLCYSTEINE (L-NAC) . DATA FROM THE ACOUSTICAL PERIPHERY

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Noise induced Hearing Loss (NIHL) is known to cause mechanical and

metabolical alterations to the Organ of Corti. Data in the literature (Kopke et al, 2000; Ohinata et al, 2003) have suggested that the administration of L-NAC protects partially against oxidative stress. Recently Janssen et al, 2003; and Gorga et al 2003, have provided evidence that mild sensorineural impairment can be well monitored by DPOAEs and that in pharmacological treatments DPOAE levels are very well correlated with ABR threshold estimates (Hatzopoulos et al, 2002). Recently Duan et al (2004) using LNAC for protection against impulsive noise, suggested that the efficacy of L-NAC in the rat is dose dependent. We have tested the L-NAC dose dependant protection in the Sprague Dawley rat model, monitoring cochlear function with TEOAEs, DPOAEs (Fratio= 1.18 , 60-50, 50-40, 40-30 asymmetrical protocols) and ABR . The animals were exposed to continuous 8 kHz noise of 115 dB for 4 hours and were treated with 250, 300 and 350 mg/Kg of L-NAC, 30 min prior to the noise exposure and twice daily after the exposure. Data were collected post 24, and 168h. Regression analyses and two-way ANOVAs were used to analyze the data. Both the electrophysiological and acoustical measurements have indicated that not all L-NAC dosages provide protection (at 168 h). The best results were obtained from the lower L-NAC dosage and have indicated that even in this dose-regime the protection effect (i.e. integrity of the OHC function) is partial, resulting in a 55-60% performance in comparison to the pre-exposure data . The reasons for which higher L-NAC dosages lower protection against NIHL needs to be further investigated , if L-NAC is intended to be administered to human clinical trials.

O22: COMPARTMENTALIZATION ESTABLISHED BY CLAUDIN-11-BASED TIGHT JUNCTIONS IN STRIA VASCULARIS IS REQUIRED FOR HEARING THROUGH GENERATION OF THE ENDOCOCHLEAR POTENTIAL

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Claudins are cell adhesion molecules working at tight junctions (TJs), which are directly involved in the compartmentalization in multicellular organisms. The cochlea includes a very peculiar compartment filled with endolymph. This compartment is characterized by high K^+ concentration (~150 mM) and positive endocochlear potential (EP) (~90 mV), both of which are indispensable conditions for cochlear hair cells to transduce acoustic stimuli to electric signals. These conditions are thought to be generated by stria vascularis adjacent to the endolymph compartment. Stria vascularis itself constitutes an isolated

compartment delineated by two epithelial barriers, marginal cells and basal cells. Since TJs of basal cells are primarily composed of claudin-11, claudin-11-deficient (*Cld11*$^{-/-}$) mice were generated with an expectation that in these mice the compartmentalization in stria vascularis is affected. Auditory brainstem response measurements revealed that *Cld11*$^{-/-}$ mice were suffered from deafness. In the *Cld11*$^{-/-}$ cochlea, no obvious gross morphological malformations were detected, but freeze-fracture replica electron microscopy showed that TJs disappeared from basal cells of stria vascularis. In good agreement, tracer experiments showed that the basal cell barrier was destroyed without affecting the marginal cell barrier. Importantly, in the *Cld11*$^{-/-}$ cochlea, the K^+ concentration was maintained around the normal level (~150 mM), whereas EP was suppressed down to ~30 mV. These findings indicated that the establishment of the stria vascularis compartment, especially the basal cell barrier, is indispensable for the hearing ability through generation/maintenance of EP, but not of high K^+ concentration of endolymph.

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FLUID HOMEOSTASIS

O23: A COMPARISON OF DEHYDRATIC EFFECTS OF V2-ANTAGONIST (OPC-31260) ON THE ENDOLYMPHATIC SPACE

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We have already reported that OPC-31260 (OPC) application to the scala tympani reduced endolymphatic hydrops. In the present study, we investigated whether or not the same effect is also obtained by a systemic administration or a local infusion via the round window (RW application), that are more suited for clinical use.
Three experiments were performed. In Experiment 1, the increase ratios of the cross-sectional area of the scala media
(IRs-S) were quantitatively assessed in guinea pigs with experimentally-induced endolymphatic hydrops, and IRs-S were compared among three groups of no OPC-application, systemic application (100 mg / kg / 8 hour, 3 times, oral) and RW application (OPC (1 mg/ animal) mixed with xanthan gum). In Experiment 2, plasma vasopressin (p-VP) concentrations were measured in non-operated guinea pigs with the systemic application of OPC. In Experiment 3, EP was measured in non-operated guinea pigs with the RW application of OPC. EM findings of the stria vascularis and the hair cells were also studied.
Systemic and RW applications of OPC significantly reduced the endolymphatic hydrops. Folding of the distended Reissner's membrane was also noted in experimentally-induced hydropic ears. But, systemic application resulted in the distension of the Reissner's membrane in non-operated ear with any change of the endolymphatic volume. A systemic application produced a high p-VP. A RW application of OPC produced no electrophysiological and morphological change in the inner ear.
A drug delivery via the round window is the most suitable for a clinical use of OPC for the medical decompression of endolymphatic hydrops.

O24: IMMUNOLOCALIZATION OF VASOPRESSIN V2 RECEPTOR AND AQUAPORIN-2 IN THE RAT INNER EAR

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Fluid homeostasis of the inner ear is essential for normal cochlear and vestibular functions. Vasopressin V2 receptor (V2R) and Aquaporin-2 (AQP2) might play an important role, since V2R and AQP2 mRNA is revealed in the cochlea and endolymphatic sac (ES). However, localization of V2R and AQP2 is still obscure. The aim of this study is to investigate the immunolocalization of V2R and AQP2 in the inner ear.
Fluorescent immunohistochemistry of V2R and AQP2 was applied to the rat cochlea and ES using antibodies of specific to V2R and AQP2 antigen. Immunostain of frozen sections were observed with confocal laser microscopy.
Strong immunoreactivity of both AQP2 and V2R was obtained in the stria vascularis and ES. The immunoreactivity was specific to both antigens, since pre-absorption control showed negative staining in both cochlea and ES.
In the inner ear, V2R-AQP2 system seems to play an important role in fluid homeostasis since both V2R and AQP2 were expressed in the stria vascularis and ES which relate the production and absorption of endolymph.

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MORPHOLOGY

O25: DEVELOPMENTAL ANALYSIS OF SEMICIRCULAR CANAL AND OTOCONIA IN HEAD TOSSING MICE

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In the previous study, we analyzed the ENU mutant mice that show autosomal recessive hereditary pattern and abnormal behavior of head tossing and circling movement. It starts to show the circling and head tossing behavior since the age of 3 weeks. In present study, we observed the abnormal development of membranous inner ear using paint injection. Apoptotic changes of embryo are also examined using immunocytochemistry. From E12 to E15, both superior semicircular canal (SSCC) and lateral canal (LSCC) normally develop into the ring shape from cystic structure as central parts come in contact each other. But the posterior canal (PSCC) does not develop and stay just as it is in the form of a cyst. By E18, PSCC could not be seen and the common crus show a thick stout like shape. TUNEL staining of cryosection in E13 ENU mouse reveals active apoptosis around membranous inner ear. In order to analyze the change of otolith shape and size according to the age, we performed SEM study on both macula sacculi and macula utriculi at different ages. The shape and size of otolith are highly variable regardless of its age and we could not find a specific pattern of those. The sizes of otolith is about 4.5 ~ 160 μm in utricle and 6.5 ~ 98 μm in saccule. Linkage analysis and genetic mapping are now currently undergoing for cloning of disease-related gene and this will provide useful information about human disorder of hearing and balance function.

O26: ESTROGEN RECEPTORS IN THE INNER EAR DURING DIFFERENT STAGES OF PREGNANCY

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Older women, up to menopause, in the normal population tend to develop less severe hearing loss compared to males. In Turner's Syndrome (loss of one X

chromosome, 45,X) affecting 1:2000 newborn girls, estrogen deficiency due to streak ovaries is the dominant problem. This results in a short stature, failure to enter puberty spontaneously and infertility. Ear and hearing problems are common among these patients and affects outer, middle and inner ear. The middle-aged women frequently complain of a rapid onset of social hearing problems, due to a premature aging of the ear (presbyacusis). Can estrogen have an impact on hearing?
Thirty-four rats, in 5 different groups, in different time periods of pregnancy have been investigated in order to study the effect of estrogen receptors and estrogen on the inner ear.
Rats were sacrificed after blood samples showing estrogen levels at different stages of pregnancy and studied
immunohistochemically for estrogen receptors which were quantified.
Estrogen receptors are present in the inner ear of the rat, and differ during pregnancy.
Estrogen receptors are present in the inner ear during pregnancy and are up and down regulated depending on the stage of pregnancy, proposing that estrogen may affect hearing.

O27: THE MECHANISM OF IN VITRO BLEB FORMATION FROM INNER HAIR CELLS

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Hair cells (HC) are noted to have a high level of vesicular based membrane cycling in the apical cuticular plate area. We observed that blebs form on the apical membrane of inner hair cells (IHCs) in vitro and studied their exocytotc origin.
After organ of Corti dissection, fluorescent Annexin V was used to label phosphatidylserine on the apical membrane of HCs. A number a reagents were applied in the bath to affect cell physiology. Results. A single bleb always emerged at the vestigial kinocilium location and grew to a maximum diameter of ~10 μm over 1-2 hours. No polymerized actin was observed in the lumen of blebs. Since cell death can result in blebs, tests of apoptosis and necrosis were made and gave negative results. Lowering intracellular turgor pressure did not prevent blebbing. IHC bleb formation was correlated with elevated concentration of intracellular Na+. Blocking apical Na+ influx or by replacement of bath Na+ with NMDG prevented Na+ loading and bleb formation. Bleb formation was also blocked by depletion of intracellular ATP, blocking cAMP or inhibition of vesicular transport with Ly 294002 or BFA. Conclusions. The mechanism of blebbing is not associated with cellular apoptosis and necrosis and not the result of excess intracellular pressure. Blebs are due to a disruption of apical plasma membrane recycling that is linked to the Na+ loading in vitro.

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MICROMECHANICS

O28: REGULATORY STIFFNESS RESPONSE OF THE OHC LATERAL WALL IS REGULATED BY ACETYLCHOLINE AND GABA

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A force feedback within the cochlear partition is provided by the OHC electromotility,called cochlear amplifier.Its dynamic adjustement to actual requirements is regulated by several mechanisms,including the efferent innervation.The efficacy of the mechanical feedback is dependent on the global stiffness of the cells against the motor proteins work.The stiffness of cochlear outer hair cells consists of a'dynamic'tiffness, which is associated to the conformational changes of the prestin molecules,and a'static'stiffness which is processed by the cytosceleton.The effect of ACh and GABA on the micropipette aspiration evoked stiffness changes(including of a regulatory stiffness response)of the OHC lateral wall were examined. We found a Ca2+dependent, stretch induced regulation of the'static'stiffness(regulatory stiffness response)and a stretch
induced slow cell contraction.Both of the regulatory stiffness response and the stretch induced slow cell contraction are controlled by the efferent neurotransmitters.A cochleobasally biased ACh response and a cochleoapically biased GABA response was found. A steady-state loud sound stimulus generates large vibrations of the basilar membrane and concomitantly stretches the lateral wall of the OHCs which evokes greater stretch-induced cell shortening.The efferent neurotransmitters increased the stretch induced lateral wall stiffness response and cell shortening. These changes can interfere with cochlear micromechanics and can decrease the effectivity of the electromotile force feedback. These are in agreement with in situ measurements of Friedberger et al.(1998)on the effects of acoustic overstimulation in the guinea pig.

O29: MECHANOELECTRICAL TRANADUCTION OF ADULT INNER AND OUTHER HAIR CELLS

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Sensory receptor cells of the mammalian cochlea are morphologically and functionally dichotomized. Inner hair cells (IHC) transmit auditory information to the brain, while outer hair cells (OHC) amplify the mechanical signal, which is then transduced by IHCs. Amplification by OHCs is likely mediated by their somatic motility in a mechanical feedback process. OHC motility in vivo is thought to be driven by the cell's receptor potential. The first steps towards the generation of the receptor potential are the deflection of the stereociliary bundle, and the subsequent flow of transducer current through the mechanosensitive transducer channels located at their tips. Quantitative relations between transducer currents and basilar membrane displacements are lacking, as well as their variation along the cochlear length. To address these matters, we simultaneously recorded OHC (or IHC) transducer currents (or receptor potentials) and basilar membrane motion in an excised and bisected cochlea, the hemicochlea. This preparation permits recordings from adult OHCs and IHCs at various cochlear locations while the basilar membrane is mechanically stimulated. Furthermore, the stereocilia are deflected by the same means of stimulation as in vivo. Here we show that asymmetrical transducer currents and receptor potentials of OHCs are significantly larger than previously thought, they possess highly restricted dynamic range, and strongly depend on cochlear location. The transducer currents and receptor potentials were also recorded from the IHCs and compared with those of the OHCs.

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Distorsion Product Otoacoustic Emission (DPOAE) is a widely used differential diagnostic method for diagnosing inner-ear disorders. It provides us information on the condition of the outer hearing cells (OHCs). Otoacoustic emission only occurs when the OHCs are functioning normally. Changes in thresholds of DPOAE curves can provide us important information on the activity of the OHCs. The inner ear shows non-linear properties if the OHCs are functioning normally. If OHCs are injured and thus don't function properly, the system stops showing non-linear properties.
If we have a system with periodic excitation and with the addition of white noise the signal-to-noise ratio (SNR) on the output increases at least for small noise intensities, we call this phenomenon stochastic resonance (SR). Our goal was to elucidate how white noise influences the intensity of DPOAE. If there is emission then that specific ear surely has non-linear behaviour, which in turn is the basic property needed for SR.

O31: DETAILED DPOAE LEVEL AND PHASE MAPS IN NORMAL AND NOISE-DAMAGED RABBITS EARS.

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Knight and Kemp (JASA 107:457, 2000) described a method in which they derived detailed plots of DPOAE level and phase as a function of f2/f1 ratio and DPOAE frequency. They identified two predominate DPOAE phase patterns. Horizontal phase banding was viewed as evidence for “wave-fixed” DPOAE generation while vertical phase banding was ascribed to “place-fixed” DPOAEs. Shera & Guinan (JASA 105:782, 1999) attributed to these two modes to “distortion” and “reflection” mechanisms. We examined detailed DPOAE level and phase plots in normal and noise-damaged rabbit ears. It was hypothesized that noise damage would disrupt outer hair cell organization and alter the normal distribution of phase patterns. The f1 and f2 primary tones for eliciting DPOAEs were generated by a DSP board mounted in a Macintosh personal computer and presented over ER-2 speakers. Ear-canal sound pressure, measured with an ER-10A microphone assembly, was sampled and synchronously averaged (n= 4) by the DSP. DPOAEs were measured in response to primary-tone sweeps at constant ratios in .025 increments, from .025 to 1.5, with DPOAE frequency steps of approximately 44 Hz from 1.4 to 4.4 kHz. DPOAE level was directly plotted while the phase was corrected for primary-tone phase variation and unwrapped before plotting. One ear of 8 rabbits was exposed to a 110-dB SPL OBN centered at 10 kHz for 2 h. The other unexposed ear served as a control. Rabbits were tested at 3 stimulus levels (55/45, 60/55, 65/65 dB SPL). In unexposed ears the 2f1-f2 DPOAE showed phase patterns characteristic of “wave-fixed” DPOAEs will little evidence for “place-fixed” generation. In contrast, the 2f2-f1 DPOAE behaved like a “place-fixed” emission. Following noise exposure, the 2f1-f2 DPOAE exhibited a substantial number of vertical phase bands consistent with an increase in the amount DPOAE generated by a reflection mechanism.

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IEB Debrecen 2004 : POSTER SESSION I.

GENETICS

P1: POSSIBLE ROLES OF TGF-BETA IN INNER EAR DEVELOPMENT IN MICE

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The otic vesicle (otocyst), the primordium of the inner ear, is formed by thickening and invagination of the otic placode originating from the surface ectoderm. The epithelium of the otic vesicle undergoes complex morphogenetic movement to form the cochlea and semicircular canals. In addition, a fraction of neuroblasts, which arise from the otic vesicle, delaminate and join neural crest-derived glial cells to form the cochleovestibular ganglion (CVG). In E10.5 mouse embryos, transforming growth factor-beta (TGF-beta) was found to expressed in the otic epithelium and its localization overlapped partially with that of NeuroD which is a marker of delaminating neuroblasts. TGF-beta type I and type II receptors were also detected in the otic epithelium. In order to examine the roles of TGF-beta in the differentiation of the otic vesicle, we treated the otic vesicles of E10.5 mouse embryos with TGF-beta in vitro. It was found that TGF-beta promotes enlargement of the CVG. Our findings suggest that TGF-beta contributes to the development of the inner ear in mouse embryos.

P2: HEARING STUDY IN APOLIPOPROTEIN E GENE DEFICIENT MICE

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The relationship between the hyperlipidemia and sensorineural hearing loss remains obscure. In this study, the cochlear histology, hearing changes and their relationships with hyperlipidemia, atherosclerosis and endothelial dysfunction in ApoE knock-out (ApoE-KO) mice were examined. Ten-week old ApoE-KO mice, fed either atherosclerotic (1.25% cholesterol) or normal diet, were used. Wild type mice (C57BL/6J) were served as normal controls. Fourteen weeks later, marked hyperlipidemia, atherosclerosis, endothelial dysfunction and hearing impairment, especially in high frequency, were developed in ApoE-KO mice, as compared with C57BL/6J mice ($P<0.001$), and were exacerbated by

atherosclerotic diet. A high positive correlation between hearing loss and the extent of atherosclerosis and plasma total cholesterol levels was found. Hearing loss, was detected in all ApoE-KO mice. Hair cell loss mainly at the base turn, the thickening of vascular intima in spiral modiolar artery (SMA) in cochlea were also found, and these histological changes were exacerbated by atherosclerotic diet. Furthermore, eNOS in aortic wall and cochlea were distinctly reduced in ApoE-KO mice. These results demonstrate that the hyperlipidemia and atherosclerosis can induce the alterations of the cochlear morphology and may contribute to hearing loss.

P3: MOLECULAR ANALYSIS OF NOISE-INDUCED HEARING LOSS

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Noise-induced hearing loss (NIHL) is a major health problem. Exposure to mild noise causes a temporary threshold shift (TTS), whereas intense noise can damage the cochlea, leading to a permanent threshold shift (PTS). The long-term goals of our research program are to elucidate the molecular basis of NIHL in mammals. We exposed rats for 90 min to broad band noise (2-20 kHz) ranging from 98 dB to 120 dB to produce TTS and PTS. RNA from whole cochlea was examined for changes in gene expression at two different times following the noise exposure. Using cDNA arrays, we examined global changes in gene expression immediately after noise in the rat cochlea and showed that six immediate-early genes (IEGs) were induced 15 min following the 120 dB noise exposure (PTS). We extended these results by using quantitative RT-PCR analysis to examine the induction of these IEGs in the rat cochlea 2.5 hr after the noise, using three noise intensities (103 dB, 113 dB, and 120 dB). We observed even greater induction of each of these IEGS at this later time. Using oligonucleotide arrays (Affymetrix GeneChips) to profile gene expression in cochlear RNA from rats exposed to these three noise intensities, we identified approximately 1000 genes whose mRNA levels exhibited a 2-fold change vs non-exposed control rats, at one or more noise intensities. We are currently analyzing these gene expression profiles to identify genes that are involved in protective pathways, versus those that will activate cell death pathways. These genes
and pathways will provide targets for future interventions to enhance the protective pathways and block cell death pathways following noise overstimulation.

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P4: ROLE OF HSF1 AND HEAT SHOCK PROTEINS IN THE RODENT

COCHLEA

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Hearing loss is a major health problem for both the young and old. Excessive noise can cause either transient or permanent hearing loss. Approaches for protecting the cochlea from noise damage throughout life would be highly desirable. We have focused on the classic stress-inducible protective pathway regulated by the Hsf1 transcription factor. Hsf1 is activated by many different stresses, leading to induction of genes for heat shock proteins (Hsps), the downstream target genes of Hsf1. To examine the role of the Hsf1 pathway in the cochlea, we used mouse genetic models that lack Hsf1. We exposed wild-type and Hsf1-null mice to whole body hyperthermic stress to identify the Hsps controlled by Hsf1. Heat shock was accomplished by raising the body temperature to 3.3oC above basal temperature for 15 min, followed by a 30 min recovery period at room temperature. Levels of mRNA for eight Hsps were measured by quantitative RT-PCR. Five of the eight Hsp genes we examined showed significant induction following heat stress in wild-type, but there was no induction of these genes in Hsf1 null mice. In wild-type CBA mice, induction of Hsp27, Hsp70.1, and Hsp70.3 by heat stress decreased with age (between 12 – 22 months). We also examined the physiological response to noise overstimulation in Hsf1 null mice and their normal littermates. Exposing wild-type mice to a mild noise produced only a temporary threshold shift (TTS), whereas the same noise exposure caused some permanent threshold shift (PTS) in Hsf1-null mice. These studies suggest that Hsf1-controlled gene expression plays a protective role in the cochlea following noise overstimulation and that this protective response decreases with age.

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P5: DFNA5: THE IDENTIFICATION OF A FIRST HEARING IMPAIRMENT EXON?

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Three mutations have been described in the DFNA5 gene in 3 families with autosomal dominant non-syndromic hearing impairment. Although different at the genomic DNA level, these mutations all lead

to skipping of exon 8 at mRNA level. In all 3 families the hearing loss is progressive, sensorineural and starting in the high frequencies. Despite extensive computational analysis, no putative function for DFNA5 could be deduced.
DFNA5-/- mice were generated through deletion of exon 8 by targeted recombination, simultaneously mimicking the human mutation. To test the hearing, frequency-specific ABR (Auditory-evoked Brainstem Response) measurements were performed. Transfection experiments in mammalian cell lines (HEK293T; COS-1) using GFP-tagged wild-type and mutant DFNA5 were performed and cell death was analysed using flow cytometry and fluorescence microscopy.
ABR tests could not demonstrate significant differences between DFNA5-/- mice and their wild-type littermates at different ages in 2 genetic backgrounds. After transfections with mutant DFNA5-GFP, cell death approximately doubled when compared with transfections with wild-type DFNA5-GFP.
The fact that DFNA5-/- mice have normal hearing, in combination with the fact that only mutations leading to exon 8 skipping have been described for human hearing loss, led to the hypothesis that DFNA5 associated hearing impairment is caused by an unusual mechanism where only skipping of one exon leads to disease. We hypothesize that this represents a specific gain-of-function mutation, with the truncated protein exerting a deleterious new function. This hypothesis was supported by transfection experiments as transfection with mutant DFNA5-GFP significantly increased cell death.

P6: GDNF, ARTEMIN, PERSEPHIN AND THEIR RECEPTORS GFRA1, GFRA3 AND C-RET MRNAS ARE UPREGULATED IN RAT MODIOLUS FOLLOWING NEOMYCIN-INDUCED

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Hair cell loss following drug- and noise-induced trauma induces apoptosis in spiral ganglion cells (SGC). It has been shown that nerve growth factors, especially members of the transforming growth factor b (TGF-b) superfamily, play key roles for the protection of SGC and enhance the functional excitability of the auditory nerve in response to stress. The aim of the present study was to determine gene expression patterns of the glial cell line derived-neurotrophic factor (GDNF) family members and their corresponding receptors in the modiolus and inferior colliculus of deafened rats. Adult rats were deafened by local inner ear-injection of 10% neomycin and sacrificed after 26 days. The gene expression of GDNF, persephin, artemin and neurturin, their glial cell line derived-neurotrophic factor receptors (GFR)a1, GFRa2, GFRa3 and c-ret (rearranged during transforming) was determined by semiquantitative RT-PCR using GAPDH expression as an internal standard. Significant downregulation of persephin gene

expression as well as slight, but no significant changes in transcript levels of artemin, GDNF and c-ret was detected in IC following deafening. In contrast artemin, GDNF, GFRa1, GFRa3 and c-ret RNA expression were significantly upregulated in modiolus of deafened rats. These data indicate endogenous survival effects of artemin and GDNF as well as their receptors GFRa1, GFRa3 and c-ret on SGC over a certain period following deafening.

P7: CLINICAL CHARACTERIZATION OF HEARING LOSS CAUSED BY THE A1555G MUTATION IN THE MITOCONDRIAL 12S rRNA GENE

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Hearing loss is a common sensory disorder affecting 1 in 1000 newborns. A genetic origin is accounted in at least 50% of the cases. Mutations in the mitochondrial DNA have been found associated with syndromic and nonsyndromic forms of deafness. In particular, the A1555G mutation in the 12S rRNA gene has been associated with aminoglycoside-induced and adult onset nonsyndromic deafness. The resulting phenotype varies considerably, ranging from severe, to moderate progressive hearing loss and completely normal hearing. The final phenotype is thought to be due to the interaction between genetic and environmental factors. To determine the phenotypic and audiologic alterations of A1555G carriers we have performed a clinical study of patients affected of nonsyndromic hearing loss and their relatives.
The cases who tested positive for the A1555G mutation and their families were included in the study. Clinical information
such as the severity and age of onset of hearing impairment, the exposure to ototoxic substances and other medical diagnoses has been recorded. The audiological evaluation consisted in otoscopy, pure-tone audiometry, impedanciometry, acoustic reflexes and otoacoustic emissions.
The A1555G mutation was identified as the cause of deafness in 21% of individuals with other affected relatives. Our
results indicate that the affection is bilateral and endocochlear, with acuphens as an additional symptom in half of the cases. The A1555G mutation causes alterations in the cochlear physiology of all carriers. This gives further evidences of the important role played by nuclear factors, which determine the clinical manifestation of the A1555G in terms of age of onset and severity of hearing loss.

INNERVATION

P8: THE HUMAN SPIRAL GANGLION: NEW INSIGHTS IN

ULTRASTRUCTURE, SURVIVAL RATE AND IMPLICATIONS FOR COCHLEAR IMPLANTS

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Our knowledge about the ultrastructure of the human spiral ganglion is still limited. More information about its morphology, organization and spiral ganglion cell (SGC) survival rate in deaf people may give us a better understanding regarding mode and site of electrical stimulation of the neural elements in patients with cochlear implants. Scanning (SEM) and Transmission (TEM) Electron Microscopy of temporal bones from normal hearing and a long term deaf demonstrate the differences in organization of neuronal structures and gives indications about spiral ganglion cell survival rates in humans. Specimens were obtained at surgery for large life-threatening petro-clival meningioma. Excellently preserved human tissue could be obtained after decalcification and observation in a field emission Scanning Electron Microscope. New ultrastructural information may have great clinical implications as to the understanding of degeneration pattern of the neural components of the human cochlea as well as for cochlear implantation since duration of deafness is one of the most crucial factors for the success of a cochlear implant.

The authors are supported by the European Community Project QLG3-CT-2002-01463 Austrian Science Foundation FWF project P15948-B05 and the National Bank Foundation Österreichische Nationalbank 8745, the Swedish Scientific Council (VR proj no. 03908) Hörselskadades riksförbund (hrf) and Stiftelsen Tysta Skolan.

P9: EFFECTS OF NEUROTROPHIC FACTORS AND ELECTRICAL STIMULATION IN DEAFENED GUINEA PIG

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A primary cause of deafness is the damage of receptor cells in the inner ear. Clinically it has been demonstrated that effective functionality can be provided by electrical stimulation of the auditory nerve. Subsequent to sensory cell loss there is a secondary degeneration of afferent nerve fibers. The effects of neurotrophic factors (NTFs) were tested in chemically deafened guinea pigs mimicking the clinical situation in humans. NTFs may be important for the survival of neurons.

Studies were performed in guinea pigs deafened with kanamycin (450mg/kg) SQ 2 hours prior to ethacrynic acid (60mg/kg). Baseline aABR confirmed deafness. NTFs were applied locally via mirocannula-osmotic pump to the scala tympani. Another group of deafened guinea pigs were implanted with a single ball electrode inserted approximately 3 mm into scala tympani via round window. Control groups included normal hearing animals and animals that survived 3 days to 7 weeks post deafening. Efferent nerve fibers were labeled with synaptophysin, afferent with parvalbumin. Findings on the characteristics of maintained or possible regrown afferent and efferent fibers as well as spiral ganglion neurons using
transmission electron, light and confocal microscopy are discussed. Quantification of afferent nerve fibers in the osseous spiral lamina near the habenula perforata and spiral ganglion cells throughout the whole cochlear turns in the different groups are presented.

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MICROMECHANICS

P10: CORRELATION BETWEEN SLOW MOTILITY, FAST MOTILITY AND LATERAL WALL STIFFNESS IN THE OUTER HAIR CELLS OF THE GUINEA-PIG

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Outer hair cells (OHCs) regulate cochlear sensitivity to sound by two types of motor mechanisms: slow and fast motility. Slow length changes can modify geometry of the organ of Corti, consequently regulate cochlear micromechanics. Fast motility is assumed to increase the input sensitivity of inner hair cells at low sound intensites. No experimental evidence exists upon the correlation between different stages of slow motile shortening, magnitude of electromotile response and stiffness of the lateral wall of OHCs.
Slow motile length changes of isolated, apical turn OHCs were induced by a perfusion of saline (flow rate: 0.6 µl/min) as a mechanical challenge (n=4) or 12.5 mM KCl solution for 90 s as a chemical challenge (n=4) with and without ocadaic acid (n=4), a phosphatase inhibitor. Fast motility was evoked by square pulses from ±35mV to ±240mV during the slow shortening and recovery period. Cell movements were recorded by an optoelectronic system. Stiffness of lateral wall was mesured by a micropipette aspiration technique (n=5).
Saline perfusion caused a reversible shortening of 766 nm as well as K+ (1448 nm). Slow shortening increased lateral wall stiffness by 22% (from 1.25 to 1.52 nN/µm), decreased electromotility both to small voltage (from 200 to 65 nm) and

maximum applied voltage (from 729 to 245 nm), significantly. Ocadaic acid blocked slow shortening, increased lateral wall stiffness (to 1.5 nN/μm), decreased magnitude of fast motility both to ±35mV (98 nm) and to ±240mV (437 nm).
Slow motile shortening increases stiffness of the lateral wall wich decreases, simultaneously, the magnitude of fast motility of OHCs. Lateral wall stiffness is increased in the absence of slow shortening and resulted in a fast motile performance decrease when ocadaic acid is applied.

AUDIOLOGY

P11: THE CHANGE OF DISTORTION PRODUCT OTOACOUSTIC EMISSIONS UNDER DIFFERENT CAUSES OF INNER EAR DAMAGE

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To investigate the manifestations of DPOAEs under different causes of inner ear damage.
DPOAE,conventional pure tone audiometry(PTA) and expanded high frequency audiometry(EHFA) were examined in
adults with normal hearing,noise exposure workers and gynecologic cancer patients with first time cisplatin administration. DPOAE amplitudes,conventional pure tone hearing thresholds and expanded high frequency hearing thresholds were analysed.
In differet insult conditions,some conventional DPOAE amplitudes were decreased,earlier than the changes of conventional pure tone hearing thresholds in corresponding. Expanded high frequency DPOAE amplitudes were decreased,but the changed frequencies were varied.
Conventional DPOAE was more sensitive than conventional PTA and expanded high frequency DPOAE. Conventional DPOAE was more sensitive than conventional PTA and expanded high frequency DPOAE. Conventional DPOAE was a potential implement to early diagnose and detect noise-induced hearing loss and ototoxicity of cisplatin.

P12: cGMP DIRECTLY MODIFIES PRESTIN'S FUNCTION

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The protein prestin plays a crucial role in mediating outer hair cell (OHC)

electromotility, which is thought to provide the local active mechanical amplification of the cochlear response to sound [Zheng et al., Nature 405, 149-155, 2000]. It has been shown that the efferent neurotransmitter acetylcholine (ACh) can modify the electromotile response of OHC [Sziklai et al. 1996] by triggering the cGMP/PKG cascade (Szönyi et al. 1999). Previously, we were able to demonstrate that prestin is one of the targets of this phosphorylation process. Two potential cGMP-depended protein kinase phosphorylation sites were identified. Phosphorylation of these sites increases charge displacement as measured with nonlinear capacitance (NLC). To study the phosphorylation process itself, we used the inside-out patch-clamp method, where the "naked" prestin molecule can be measured without interference from other cytosceletal proteins or other factors. Prestin cDNA was transfected into a human kidney cell line. As a signature of prestin's electrically evoked charge movement, NLC functions were measured in protein-expressing cells. Prestin's NLC function increased up to 3-fold after cGMP was applied to the intracellular side of the membrane. This effect was abolished after the two PKG targeted sites had been mutated. These data suggest the possibility that cGMP has a direct effect on prestin since the bath solution lacked the phosphorylation agent, ATP.

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P13: HIRESOLUTION BIONIC EAR: COMPARISON OF BENEFIT BETWEEN HIRES AND CONVENTIONAL SOUND PROCESSING

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Since the inception of cochlear implants, speech perception performance in cochlear-implant users has improved dramatically. The increase in benefit is directly related to improvement in cochlear-implant technology and in the capability for processing and transmitting more sound information to the auditory system. The electronics platform of the HiResolution Bionic Ear provides a wide input dynamic range (82 dB), high-fidelity input amplitude resolution (12-bit analog-to-digital converter), and enhanced frequency resolution (70,000 samples/sec). The Bionic Ear has a digital processing capability of 100 mips, which allows representation of fine temporal structure out to 2800 Hz and an overall stimulation rate of 90,000 pulses per second delivered to up to 16 sites in the cochlea. Comparison of benefit in adults who have used both conventional sound processing and HiResolution (HiRes) sound processing implemented in the Bionic Ear show that speech perception is better and sound quality is preferred when using HiRes. Moreover, comparison of auditory skill development in very young children (implanted at 12-18 months of age) using conventional or HiRes sound processing indicate that children using HiRes master basic auditory skills at faster rates than do children using previous sound-processing algorithms. These data indicate that both adults and children

continue to benefit from advances in microprocessor and digital-signal-processing technology applied to date. HiRes has the capability to implement other potentially advantageous algorithms, including current steering, electrode "tuning," and hair-cell-model-based stimulation. Patients implanted with the CII or HiRes 90K devices will have access to those new advancements without requiring additional surgery.

P14: ACOUSTIC STIMULATION PROMOTES THE FRAGMENTATION OF DNA IN THE COCHLEA OF GUINEA PIGS

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A loud acoustic stimulation is known to cause the inner ear disturbance. We examined the apoptotic changes induced by a loud acoustic stimulation in the cochlea of guinea pigs.
The loud speaker was placed beside the right ear. Stimuli were pure tone and the frequency was 2kHz. The sound pressure level was 125dB. Animals were divided into four groups; 1)control (n=6), 2)the acoustic stimulation (2 hours of noise exposure; n=6), 3)the acoustic stimulation (5 hours of noise exposure; n=6) and 4)the acoustic stimulation (20 hours of noise exposure; n=6). All animals were sacrificed after 7 days. The temporal bones were fixed via cardiac infusion of fixative and immunohistochemically stained using a specific antibody for single-stranded DNA. The auditory brain stem responses (ABR) were measured before and 0, 1 and 7 days after the acoustic stimulation.
The temporal bones in the control and 2 hours stimulation group did not show any fragments of DNA. Fragments of DNA were detected in the 5 and 20 hours stimulation groups. Fragmented DNA was detected in the organ of Corti and the lateral wall of the 5 hours stimulation group and only in the organ of Corti of the 20 hours stimulation group. The threshold shift of ABR was elevated in all acoustic stimulation group immediately and 1 day after the acoustic stimulation. In 2 hours stimulation group, the threshold shift recovered to the pre-stimulation level, whereas 5 and 20 hours stimulation group, the threshold shift did not recovered to the pre-stimulation level.
During the process of apoptosis, double-stranded DNA is broken into single-stranded DNA. Our findings suggest that apoptosis is involved in the pathogenesis of noise induced inner ear damage.

P15: THE AMPLITUDE MODULATION FOLLOWING RESPONSE (AMFR) AS A TOOL TO ASSESS HEARING IN NEONATES

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The AMFR, (a.k.a. auditory steady state response, ASSR) is rapidly becoming a viable audiometric tool because it can assess hearing in a frequency specific way. Its main application is in assessment of hearing in infants and neonates. However, few studies have been done in this age group. The purpose here was to determine optimal stimulation and analytical procedures for evoking and detecting the AMFR in this population. Babies were not sedated and usually asleep. The AMFR was recorded from a surface electrode placed on the forehead and referenced to linked mastoid electrodes. Sounds were delivered to both ears and the scalp potentials were averaged over several modulation periods. A magnitude squared coherence algorithm was used to detect the AMFR in the averaged signals . We found that a half-wave rectified sinusoidal envelope was more effective than a sinusoidal envelope. Low modulation frequencies (<35 Hz) were the least effective in evoking the AMFR, whereas the most effective modulation frequencies covered a higher and wider range (~40 to 90 Hz) independent of the carrier frequencies (500 to 4000 Hz). Modulating band-pass noise with a half-wave rectified sinusoid was an efficient way to evoke the AMFR and could be detected near hearing thresholds. Thus, the use of selected noise bands may be effective stimuli for evoking the AMFR in infant screening.

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P16: A MUTATION IN THE MYOSIN VIIA MOTOR DOMAIN CAUSES AUTOSOMAL DOMINANT HEARING LOSS (DFNA11)

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Hereditary hearing loss is the most common sensorineural deficit, affecting millions of people world wide. We identified a novel missense mutation, in an Italian family with dominant sensorineural non-syndromic hearing loss, in the unconventional Myosin VIIA gene, which is reported to be responsible for both non-syndromic deafness (DFNB2 and DFNA11) and for a syndromic form of deafness, Usher type IB.
Family individuals were analyzed by audiometrical tests. We performed a genome wide linkage and a mutational analysis by directed sequencing. Moreover, we performed a molecular modelling analysis, building models for wild

type and mutant Myosin VIIA motor domain complexed with ADP and Mg.
The genome-wide scan found linkage to locus DFNA11. Sequencing of the MYO7A gene in the linked region identified a new missense mutation
resulting in a change (alanine/valine) in the motor domain of the Myosin VIIA. This change is absent in not affected individuals and in 100 control individuals. The missense mutation found in this family is in a highly conserved amino sequence at the N terminal domain. Molecular modelling shows that an unfavorable steric interaction arises between a gamma methyl and a carbonyl oxygen.
Analyzing an Italian family with a neurosensorial dominant form of hearing impairment we identified a new point mutation in the coding region of the MYO7A gene, that imply the change of an alanine, for a valine, another hydrophobic aminoacid. This mutation is located in the motor domain, but in a protein region, until now, never implicated in altered protein function. A modelled protein structure of Myosin VIIA motor domain provides evidence for a significant functional effect of this missense mutation.

P17: PREOPERATIVE GLUCOSE METABOLISM CORRELATES WITH SPEECH PERCEPTION AFTER COCHLEAR IMPLANTATION

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Outcome of cochlear implantation (CI) shows a large variation especially in prelingual deaf group. In the previous study of 5~7 year-old patients who showed the most variable outcome of CI, the group with poor postoperative speech perception had greater metabolism in ventral part of the cerebral cortex, including bilateral fusiform gyri (BA 20) and right inferior occipital cortex (BA 18). We were interested in examining if the metabolism of the same regions during preoperative period would predict the post CI speech perception ability in all pediatric deaf patients, aged from 1.5 to 12 yrs old. Materials and Methods: 18F-FDG positron emission tomography (PET) images were performed preoperatively in congenital deaf children (N = 36, mean age = 6.5 yrs old). The speech perception was measured with the Korean CID (Central Institute for the Deaf) test without visual cues at around 2 years after CI. Covariate analysis was performed between the count of FDG-uptake and K-CID score after removing age factor as a nuisance variable, using SPM99. Results: Left fusiform gyrus (BA 20) showed significant negative correlation with K-CID ($p<0.005$, uncorrected, extent threshold=25). A smaller, but positive correlation was found in bilateral parietal lobe including bilateral precuneus (BA 7) and right inferior parietal lobe (BA 40). Conclusions: The greater metabolism in the ventral pathway of the visual cortex, especially in the left fusiform during the preoperative years, was correlated with lower speech perception ability after CI. This finding consisting

with the previous result indicated that the left fusiform hypermetabolism might interfere with the acquisition of appropriate speech perception ability after cochlear implantation in the pediatric deaf patients.

P18: COMPARISON BETWEEN TREATED AND NON-TREATED PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS

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(Idiopathic) Sudden Sensorineural Hearing Loss (ISSHL) is a disease which etiology is not known. Different hypotheses about the etiol therefore been developed, resulting in several treatment policies:

- Corticosteroids/ autoimmune disorders,
- Antiviral treatment/ viral diseases (herpes virus),
- Hemodilutive therapy/ rheological disturbances, and
- Surgery/ cochlear fistulas between inner ear and middle ear.

The low incidence, the rate of spontaneous recovery and the possibility of more than one cause make the efficacy of the treatment impossible to evaluate at any clinic since each physician only sees a few patients with ISSHL per year.

The developing Swedish national database for ISSHL gathers data from patients at a number of clinics to provide a basis for studies to investigate the effect of different treatments on outcome. A questionnaire is requested for each patient covering background, current diseases, examinations, treatment and audiogram at the beginning of ISSHL and after tree months.

The poster will describe the first 200 patients that were reported to the database over a period of 18 months and relate the treatment provided to the outcome and to the appearance of the initial audiogram.

IEB Debrecen 2004 : POSTER SESSION II.

PATHOLOGY

P19: ELECTRON MICROSCOPIC STUDY OF ELECTROMAGNETIC FIELDS ON THE NEUROEPITHELIAL STRUCTURES OF THE INNER EAR OF RATS

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This work was dedicated to study the effects of long term exposure to time varying extremely low frequencyn electromagnetic fields on the neuroepithelial structures of the inner ear of rats.
This work was conducted on 15 male adult albino rats: 5 rats were sham exposed and served as normal controls and 10 rats were exposed to time varying extremely low frequency [50 hz] and 10-mT flux density for one hour for 30 days.
By the end of the experiment the animals were anaesthetised, perfused with the proper fixative then decapitated. The inner ears were perfused and temporal bones obtained. The specimens were put in a decalcifying agent for 5 days. The specimens were prepared for both histological and scanning Electron microscopical study.
Histological changes in all experimental (study) group specimens were observed. The cochlea showed variable degrees of affection ranging from cytoplasmic vacuolation of some supporting cells to complete destruction of the organ of Corti. Stria vascularis showed atrophied lining cells. Spiral ganglionic cells appeared swollen with karyolitic nuclei. The vestibular neuroepithelial structures revealed milder response in the form of cytoplasmic vacuolation of both hair cells and supporting cells.
Results of our study indicate that long term exposure to 10-mT extremely low frequency time varying electromagmetic fields caused degenerative changes in the neuroepithelial structures of rat's inner ear.

P20: THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN A EXPERIMENTAL MODEL OF NOISE INDUCED HEARING LOSS

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Recent studies suggest that alterations in physiological cochlear blood flow play an important role in noise-induced hearing loss. However, direct evidence of blood flow disturbance in the cochlea is still debated. Recent investigations have identified a number of microvascular alterations during noise exposure. These alterations seem to be sufficient to induce localized periods of stasis, alterations in vascular permeability and local ischemia. In this study, we used a model of noise exposed guinea pig and evaluated whether this is associated with an overexpression of vascular endothelial growth factor (VEGF). VEGF is a highly specific mitogen for vascular endothelial cells. It induces endothelial cell proliferation, promotes cell migration, and inhibits apoptosis. In vivo VEGF induces angiogenesis as well as permeabilization of blood vessels, and plays a central role in the regulation of vasculogenesis. Animals were exposed for 40 minutes to 120 dB SPL of continuous pure tone of 6 kHz. Auditory function was monitored by measuring thresholds of compound action potential (CAPs). We analyzed the expression patter of vascular endothelial growth factor (VEGF) and its receptors (Flt-1 and Flk-1) in the inner ear performing an immunohistochemical study and a Western Blot analysis. We report morphological and quantitative data about the expression of these crucial angiogenic molecules in the cochlea of noise exposed animals. In this animal model, the cochlear VEGF expression increase after noise in the stria vascularis. Our findings provide new evidence of possible relationships between VEGF and noise, suggesting that vascular abnormalities might play a role in noise associated hearing loss, with potentially important clinical implications.

Research supported by MIUR

P21: THE ROLE OF MEASLES VIRUS IN THE PATHOGENESIS OF OTOSCLEROSIS

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Etiology of otosclerosis is still unknown. Persistent measles virus (MV) infection of the otic capsule is assumed to be one of the etiologic factors in otosclerosis. Chronic viral antigen expression on the surface of infected cells can induce a secondary autoimmune reaction against the otic capsule. Conceptually, TNF-alpha and RANK-ligand overexpression can lead to osteoclast activation

and lytic bone resorption in the otosclerotic foci. Nucleic acid (mRNA, vRNA, DNA) was extracted from pulverized, frozen stapes footplate samples of otosclerotic patients. MV RNA was amplified by RT-PCR. An Edmonston- and a Schwartz-type MV served as a positive control and cortical bone fragments, stapes superstructures, incus and malleolar samples served as negative controls. TNF-alpha mRNA was amplified also by RT-PCR. A phytohaemagglutinin (PHA-M/P) treated mononuclear and non-adherent cell line served as a positive control. Metabolic activity of osteoclasts in the otosclerotic foci was estimated by cytochemistry (GAPA-score) and anti-human CD51 immunofluorescence assay.Among 115 otosclerotic patients, 70 stapes footplate samples contained measles MV RNA. MV RNA was not detected in other bone specimens of the patients. Etiologic role of MV in the pathogenesis of otosclerosis should be considered. The 45 negative samples may be genetically determined otosclerotic cases or stapes fixations due to other etiology.

P22: TIME COURSE OF ELECTROCOCHLEOGRAPHIC MEASURES AFTER CO-ADMINISTRATION OF KANAMYCIN AND FUROSEMIDE

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In animal models of deafness as used in cochlear-implant related studies often co-administration of kanamycin and a loop diuretic is applied to produce functional loss of cochlear hair cells. However, the extent to which hair cells remain functional after the deafening procedure, thus the actual extent of deafness, varies (Nourski et al. Hear Res 187:131-133, 2004). Hence, auditory-nerve responses to electrical stimuli in deafened animals might not only reflect the auditory-nerve function itself but also the condition of remaining hair cells. We investigated the remaining cochlear function in a guinea-pig deafness model over a course of weeks. Guinea pigs (n=20) were implanted with a round-window electrode. The animals were treated once with a co-administration of kanamycin (400 mg/kg, sc) and furosemide (100 mg/kg, iv). Compound action potentials (CAPs) and cochlear microphonics (CMs) were recorded before and at various times after treatment (immediate, 1, 2, 4, 7 days, 2- 8 weeks). Acoustical stimuli were tone pips of various frequencies (2 – 16 kHz) and broadband clicks. Immediately after treatment CAP thresholds shifted around 60 dB in all animals. In about 50 % of the animals the thresholds continued to increase until a 80-100 dB shift was reached after 2 days, which in most cases remained stable. However, in the other animals, the CAPs recovered after 1 day, but deteriorated again after 2 days. Deterioration progressed until 4-7 days after treatment. The final threshold shifts were smaller and more variable as a function of time than in the group which did not show transient recovery. We suggest assessment of the treatment effect after 1 day. In case of recovery a second treatment might

be considered to reach an adequate deafness.

P23: AGE-RELATED HEARING LOSS

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Age-related hearing loss is the most common cause of adult auditory deficiency affecting modern societies. By the analysis of mouse mutants, we could recently show that a lack of the neurotrophin BDNF or its receptor TrkB leads to specific cochlear innervation defects and hearing loss during postnatal development (Schimmang et al., 2003). Phenotypic correlations between these mutants and aged animals with hearing loss lead us investigate the expression of BDNF during the process of aging. BDNF expression was drastically reduced in cochlear neurons of aged animals suggesting a neuronal basis for the observed hearing loss. To characterize this neuronal defect we examined the expression of voltage-dependent potassium channels, Kv1.1 and Kv3.1, which have been shown to be modulated by BDNF in cochlear neurons in vitro (Adamson et al., 2002). Expression of Kv channels was found to be reduced in aged hard hearing animals. The relevance of this finding was further underlined by the analysis of BDNF and TrkB mutant mice, which showed a complete absence of Kv channel expression. Our results suggest that BDNF is a key player for maintenance of neuronal function in the adult inner ear and that age-related hearing loss develops due to a lack of BDNF signaling which negatively influences neuronal activities reflected by a downregulation of Kv channels.

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P24: LOCAL AND SYSTEMIC APPLICATION OF SALICYLATE HAVE DIFFERENTIAL EFFECTS ON BDNF EXPRESSION IN THE COCHLEA

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Brain-derived neurotrophic factor (BDNF) plays a crucial role in short- and long-term synaptic plasticity changes. In this study, the regulatory effect of neuronal activity on BDNF expression in the auditory system was investigated. Salicylate, known to change neuronal activity in the auditory system, was applied either

locally to the round window niche or systemically. Expression of BDNF-transcripts from BDNF promoter III and IV were determined by means of in situ hybridisation and RT-PCR assays. An upregulation of c-Fos and BDNF exon III and IV was observed in the cochlear neurones after the systemic and local application of salicylate, indicating an altered excitability of these neurones. BDNF
transcripts from both promoters were also found in the auditory cortex where a differential effect of locally and systemically applied salicylate was observed. Data suggest that BDNF is an effector of auditory activity which responds differentially to altered neuronal activity arising either from the cochlea or the auditory cortex.

P25: ELECTROPHYSIOLOGIC MEASUREMENT OF SULFADIMETHOXINE-INDUCED OTOTOXICITY IN JAUNDICED GUNN RATS

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Accumulation of unconjugated bilirubin, especially in newborns, in the central auditory pathway and basal ganglia causes neurological damage and deafness. Effect of unconjugated bilirubin on the brain is known well through auditory brainstem responses (ABRs). However, effect of unconjugated bilirubin on the cochlea is not defined completely. The purpose of this study is to evaluate the bilirubin ototoxicity using ABR and DPOAE in jaundiced Gunn rats before and after sulfadimethoxine injection. Experiments were conducted on three littermate P19(postnatal 19 days) homozygous(jj) and one heterozygous Gunn rats, five littermate P21 jj and one littermate nj Gunn rats. P21 jj and nj Gunn rats were re-tested in three weeks in the same condition. ABRs were measured with auditory stimuli of 100-microsecond pulse width click and DPOAEs were measured at 8kHz, 16kHz and 22kHz. Response amplitudes of DPOAEs before 1 mg/g sulfadimethoxine injection were compared with them after the injection. Thresholds of ABR were increased in P19 and P21 groups starting within the first day after injection and became normalized at 10 days after injection. The interwave intervals I – III and I – V were increased and normalized. Even though sulfadimethoxine injection induced significant ABR abnormalities in P19 and P21 groups, no DPOAE abnormalities were found in these groups. Unconjugated bilirubin induced pathologic changes at or higher than the brainstem level with intact cochlear function. ABR abnormalities were only observed in P19 and P21 groups because the level of bilirubin in blood is higher and the central auditory pathway is more immature than PND 42 group. We also found the possibility of spontaneous reversibility of the hyperbilirubinemia-related ototoxicity.

FLUID HOMEOSTASIS

P26: A TRABECULAR PERIMODIOLAR MESHWORK IN THE HUMAN COCHLEA – ITS POSSIBLE SIGNIFICANCE FOR PERILYMPH TURNOVER

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We performed high resolution scanning electron microscopy of immediately fixed human cochleae. Tissue was obtained during skull base surgery for life-threatening petro-clival meningioma after consent had been obtained from the local medical ethical committee and the patient. SEM analysis of the endosteal relief revealed a discontinuous trabecular meshwork situated perimodiolarly in the scala tympani and vestibuli in the first and second turn. It consisted of a bony and fibrous meshwork of collagen and reticular fibres incompletely covered with a thin duplicature of the mesothelial cell sheath of the endosteal surface. There is an intimate relationship to the arterial (scala vestibuli) and venous (scala tympani) vascular system. Its structure shows striking similarities to the trabecular meshwork of the eye and its connections to Schlemm's canal draining the eye fluid. We propose that this perimodiolar meshwork, not described earlier, may play a role both for secretion and drainage of perilymph within the human cochlea.

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P27: DYE DISTRIBUTION IN A COCHLEA MODEL USING DIFFERENT TYPES OF MODIFIED COCHLEAR IMPLANT ELECTRODES

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Cochlear implants (CI) present the treatment of choice in most cases of inherited or post-lingually acquired deafness. However, there is still great variability for the individual success of each patient. The number of spiral ganglion cells (SGC) is considered to be among the factors defining the effectiveness of cochlear implants. Reports on animal studies show the protective effects of neurotrophic

factors (NF) on SGC's. A device for local inner ear treatment is therefore of great interest. We modified a Contour™ electrode, as this device already contains an inbuilt lumen, and tested it for the purpose of drug delivery to a plastic cochlea model. This cochlea model consisted of a plastic canal, representing two turns of the human cochlea. It was used to visualize the distribution of a dye (methylene blue) inside the modelled scala tympani. Three different electrode prototypes with openings at varying locations were used to release the dye: a) at the tip, b) at the tip and the side of the electrode, c) only at the side of the electrode (6 mm from the tip). A mechanical pump was used to drive the system at pump rates of 100 µl/h, 10 µl/h, and 1 µl/h. Applying the higher pump rates results showed that the dye was distributed more rapidly using the prototype with the opening at the tip than only having an outlet at the side of the array. Testing the device carrying two delivery outlets resulted in a faster dye distribution along the "active"; part of the electrode array as with the prototype having only one opening at the tip. The dye concentration changes along the whole cochlea model have been assessed using analySISÒ software (Soft Imaging System), allowing
to quantify the dye distribution in a spatial and time dependent manner.

PHARMACOLOGY

P28: ARGON PROTECTS HAIR CELLS IN RAT ORGAN OF CORTI EXPLANTS EXPOSED TO OTOTOXIC SUBSTANCES

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Recently, we have demonstrated the protective effect of the noble gas argon on hypoxia-exposed hair cells in rat organ of Corti explants. To further characterize the effects of argon, we tested its ability to protect the hair cells against the toxicity of the platinum-based anticancer drug cisplatin and of the aminoglycoside antibiotic gentamycin.
Organotypic cultures of the newborn rat cochlea, 3-5 days old, were divided into their apical, middle and basal parts
which were exposed to increasing concentrations of either cisplatin (7.5, 15, 25 µM) or gentamycin (5, 15, 40 µM). Unexposed cultures served as controls. The explants were cultured in a perilymph-like solution under oxygen-nitrogen atmosphere (21% O2, 5% CO2, 74% N2) or under oxygen-argon atmosphere (21% O2, 5% CO2, 74% Ar) for 48 h. The cultures were phalloidin-labelled and the number of inner and outer hair cells (IHCs, OHCs) per 100 µm was counted.
Cisplatin (25 µM) induced a mean loss of about 25% for the IHCs and of some 30% for the OHCs. In the argon group, the mean IHC loss was reduced by about 10% (n.s.) and the OHC loss by about 20% ($p < 0.05$). Gentamycin (40 µM) induced a mean IHC loss of about 25%, but the loss was completely prevented from occurring by the argon atmosphere. The middle and basal parts suffered the most severe OHC loss (of 80%) which argon reduced by about 25%.
The ototoxicity of gentamycin and cisplatin has been related to the production of reactive oxygen species. Therefore, we assume that the protective effect of argon is mediated by the inhibitory effect of free radical-forming processes.

P29: PROTECTIVE EFFECTS OF HUMAN RECOMBINANT ERYTHROPOIETIN ON HAIR CELL LOSS IN RAT EXPLANT CULTURES

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The aim of the study was to evaluate the effect of recombinant human erythropoietin (rhEPO) on ischemia-induced hair cell loss in an organotypic

cochlea culture. The results were compared with those obtained using IGF-1 (insulin like growth factor-1) and EGF (epithelial growth factor).
Organotypic cultures of the newborn rat cochlea, 3-5 days old, were divided into their apical, middle and basal parts and
exposed to ischemia (3.5 h) in glucose-free artificial perilymph in a Billups chamber, pO2 10-20 mm Hg without (controls) or with rhEPO (5 ng/ml,
Roche), rhIGF-1 (50 ng/ml, R&D) or rhEGF (200 ng/ml, R&D). 24 h after onset of ischemia, the cultures were stained using propidium iodide (PI; staining of necrotic cells), in situ DNA End Labeling Assay (ApopTag ISOL Ligation Kit, Intergen; apoptotic cells) and tetramethyl rhodamine isothiocyanate labelled phalloidin (Sigma; inner and outer hair cells), and the number of stained cells per 100 µm was counted.
Ischemia increased the numbers of PI and ISOL stained cells in the middle and basal parts of the cochlea (by 5 cells/100 µm). rhEPO and rhIGF-1 significantly attenuated the loss of hair cells via protection of apoptosis as well as necrosis (rhEPO by 20%, IGF-1 by 30%). EGF had no effect on the ischemia induced hair cell loss.
rhEpo attenuates the ischemia induced hair cell loss by reducing apoptosis and necrosis. rhEpo has been in clinical use for more than a decade and found to be well tolerated. Now, clinical studies have to clarify whether rhEPO could be an effective therapy for the prevention of hearing loss via a hair cell protective mechanism.

P30: ELEVATION OF SUPEROXIDE DISMUTASE INCREASES ACOUSTIC TRAUMA FROM NOISE EXPOSURE

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The generation of superoxide has been implicated as a cause of cochlear damage from excessive noise. Cu/Zn superoxide dismutase (SOD1) will generally protect against superoxide-mediated tissue injury, but protection by this enzyme against noise trauma is controversial. The aim of the present study was to elucidate effects of increasing SOD1 on noise-induced hearing loss.
We compared ABR threshold shifts between C57BL/6 mice administered lecithinized SOD1 (PC-SOD) and those applied
physiolosical saline, and between C57BL/6 mice overexpressing SOD1 and littermates. The activity of SOD and catalase was assessed in cochlear specimens obtained from animals treated with PC-SOD and those with saline. To examine the expression of hydroxynonenal (HNE), a product of lipid peroxidation

by hydroxyl radical, immunostain for HNE was performed in cochlear specimens in both experimental groups.Results:Noise exposure caused significantly higher threshold shifts in PC-SOD-treated animals than physiological saline-treated ones. Cochlear tissues of PC-SOD-treated animals exhibited significant elevation of the levels in the SOD activity, but not in the catalase activity, in comparison with saline-treated ones. Intense immunostaining for HNE was seen in the cochleae of PC-SOD-treated animals after noise exposure, while staining in the cochleae of saline-treated ones was barely detectable. Transgenic mice overexpressing SOD1 suffered a significantly higher ABR threshold shift than non-transgenic littermates from noise exposure. These findings indicate that increasing SOD1 enhances auditory dysfunction following noise exposure.Conclusions:Our data point to the complexity of ROS homeostasis in the cochlea and measures of protection for auditory function.

P31: ROLE OF P53 IN NOISE-INDUCED HEARING LOSS: PROTECTION WITH PIFITHRIN AND CH65

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Recent studies suggest that p53 may play an important role in initiating apoptosis in cochlear hair cells. Zheng et al. (2003) observed that Pifithrin-alpha(PTF), a p53 inhibitor, protects against cisplatin-induced hair cell loss in organotypic cochlear and vestibular cultures. Carney et al. (2004), has demonstrated protection against noise-induced hearing loss (NIHL) using CH65, a putative inhibitor of pp60c-src protein tyrosine kinase. The goal of the present study was to determine the role of p53 in apoptotic signaling following noise exposure and whether p53 expression could be blocked using CH65 or PTF. The chinchillas were used. One ear received a 30 µl drop of CH65, the other control ear is treated with DMSO. Chinchillas were exposed to 75-pairs of impulse noise at 155 dB pSPL. Auditory thresholds were measured with ABR responses, then the animal was anesthestized and the unfixed cochleas were stained with propidium iodide (PI) and p53 immunolabeling. Additional animals were assessed at 4 and 24 hs after noise. Both apoptosis and necrosis coexist in the cochlear lesion after noise exposure. Impulse noise caused an upregulation of phospho-p53 serine 30 min after the expression of p53 increased in hair cells survival 24 hours after noise exposure. Treatment of the RW before exposure with CH65 or PFT decreased the apoptotic and necrotic cells and suppressed the p53 immunolabeling. These data suggest that p53 activation occurs in response to noise via changes in cellular attachment and/or increases in
oxidative stress and confirm the pivotal role of p53 in the cell death pathway. Suppression of p53 expression with CH65 and PFT constitute a potential protective strategy against NIHL.

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PHYSIOLOGY

P32: EXPRESSION OF CO-FACTORS OF THYROID HORMONE RECEPTORS IN THE COCHLEA

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Recent data showed that thyroid hormone (TH) regulates expression of KCNQ4 and prestin differentially via distinct TH receptors (TR) in outer hair cells: prestin expression is enhanced by TH (Weber et al., 2002, PNAS 99), while in the case of KCNQ4 expression TH is needed to overcome a repressive activity of TRalpha1 aporeceptors (Winter et al., 2004, submitted). Since these completely different mechanisms of regulation occur in the same cell, a thrilling and open question is how and on which level this difference is realized. TRs are known to act in combination and association with different co-factors, which can either be co-repressors or co-activators. Based on the observed repression of the KCNQ4 gene by TRalpha1 aporeceptors, we analysed the expression patterns of two known co-repressors: SMRT and NcoR in the inner ear. Appropriate genes were cloned and riboprobes for in situ hybridisation were generated to analyse the expression patterns. In parallel RT-PCR was performed on isolated outer hair cells as well as the cochlea at different developmental stages. Data will be presented and discussed in the context of a presumptive role of these co-repressors during hair cell differentiation.

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P33: LOCAL APPLICATION OF SALICYLATE INDUCES ALTERATION OF BEHAVIOR IN AN ANIMAL MODEL FOR PHANTOM AUDITORY EXPERIENCE

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Behavioral conditioning studies on rats have been proven to be a valid animal model for the evaluation of acute and chronic phantom auditory experience (tinnitus). We recently developed an animal model for short-term acute induced phantom auditory sensations in rats on the basis of systemic application of salicylate (Rüttiger et al., 2003). To elucidate the cell specific consequences of phantom auditory experiences in rat we now analysed the change in expression

of activity dependent genes in cochlear spiral ganglion neurons and auditory cortical tissues after application of salicylate locally to the round window of the cochlea and systemic injections. The changes of gene expression were similar in the peripheral auditory system (cochlea) for local and systemic application of salicylate. However, the changes of gene expression differed for local and systemic application in auditory cortical tissues (Tan et al., 2004, Hadjab et al., 2004). The question arises whether local application of salicylate - similar to systemic application of salicylate - might induce phantom auditory experience (tinnitus) in the behavioral model. We here introduce first results dealing with this question and discuss the data in the context of the advantages and the constraints of the various application methods for the examination of phantom auditory perception.

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P34: CHARACTERIZATION OF POTASSIUM CURRENTS AND MORPHOLOGY OF GUINEA PIG DEITERS CELLS

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The role of the OHCs in the amplifier mechanism of the cochlea is mediated by the fast motility of the OHCs (Dallos et al.,1995,1997; Sziklai et al.,1996). The Deiters' cells are in close contact with the OHCs (Laffon et al.,1996). The contribution of the Deiters' cell in the active mechanism of the cochlea is still not clear. Methods: Young guinea pigs were anesthetized by Nembutal, the temporal bones and the cochleae were exposed. After dissection, the organ of Corti was digested (Collagenase type IV). Currents were recorded using the standard whole cell configuration. Results:During depolarization for +50 mV from a holding potential of -70 mV, an outward current could be detected. The biphasic inactivation process may represent expression of two simultaneously activated current components. Using specific K+ channel blockers, we found, that Margatoxin (MgTx)and Pandius Imperator scorpion toxin (PI1) block equally both two components of the outward current, while Charybdotoxin (ChTx) blocks the two current components by different Kd values. We found morphological diversity among the shapes of Deiters' cells. Those cells, which attached to shorter OHCs, had bigger cell bodies whereas others attached to longer OHCs and had smaller cell bodies. The cells could be separated into two morphological groups. Each group of cells shows the same kinetics behavior in the current activation and inactivation, but the magnitude of the current components are different. Conclusion: The two different groups of Deiters' cells, which have different cell

shapes and different expression of potassium channels may contribute to the active mechanism of the cochlea in a different degree.

P35: TWO DIFFERENT DESTINATIONS OF ENDOCYTOSED PLASMA MEMBRANE IN GUINEA-PIG OUTER HAIR CELLS

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Although hair cells are known to exhibit rapid endocytic activity around the cuticular plate, destinations of trapped membrane are unknown. Rapid endocytosis and intracellular traffic in guinea-pig cochlear outer hair cells (OHCs) were investigated using the membrane probe FM1-43. OHCs were mechanically isolated from the adult guinea-pig cochlea. FM1-43 (10 µM) was applied locally to OHCs by pressure injection through a grass capillary. FM1-43 application caused the apical surface membrane to fluorescence immediately. After terminating application, fluorescence began to decrease exponentially back to its initial value (τ = 58 ± 8 s; N=3). Fluorescence of the infracuticular zone, containing Hensen's body, also increased; about 30 s after terminating the application, fluorescence began to decrease, exponentially with τ = 97 ± 9 s (N=3), but remained at an elevated intensity. In contrast, fluorescence intensity at the basal pole continued to increase after termination of application. It is evident that trapped vesicles at the apical pole were transported to the basal pole. It has been suggested that endocytosed vesicles transport to subsurface cisternae (SSC), which consist of several layers of endoplasmic reticulum (ER) in the lateral wall. Therefore, DiOC6 (0.5 µg/ml, 1-min incubation time, wash out) was used to double-stain ER. This resulted in staining of the entire plasma membrane. After terminating the application, FM1-43 fluorescence intensity was reduced at the apical surface. However, in the lateral wall, fluorescence continued to increase and reached a plateau. FM1-43 was clearly co-localized with DiOC6. In conclusion, trapped vesicles at the apical pole were transported to two different locations; namely to the basal pole and to the SSC.

P36: SK2 IN THE COCHLEA

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Hair cells are the targets of olivocochlear fibers that carry efferent inhibitory feedback from the brain. In particular, IHCs initially are contacted by olivocochlear efferent fibers that make contacts with IHCs before targeting OHCs (Simmons et al., 1996). In the adult system IHCs are innervated mainly by afferent fibers, having few if any remaining efferent contacts (Liberman et al., 1990). In contrast, adult OHCs are the principal targets of cholinergic olivocochlear efferents (Guinan, 1996). The efferent feedback to IHCs and OHCs is predominantly provided by the release of acetylcholine (ACh), that acts on AChR a 9/a 10 subunits. In OHCs and IHCs calcium influx through the receptor complex is presumed to activate nearby calcium-dependent SK2 channels, leading to inhibitory postsynaptic currents(Oliver et al., 2000; Elgoyhen et al., 1994, 2001; Glowatzki et al., 2000). Accordingly, we note the localization of SK2 channel protein during the early (IHC), respectively late (OHC) postnatal period. Aiming to identify transciptional regulators for SK2 we could identify thyroid hormone (TH) as one of the presumptive modulators. While under hypothyroid conditions the SK2 protein in OHCs is completely absent most interestingly SK2 expression persists in IHCs. Using in situ hybridisation, immunohistochemistry, reportergene study, RT-PCR and EMSA we start to elucidate the obvious differential regulatory role of TH on SK2 ion channels in IHCs and OHCs.

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MORPHOLOGY

P37: POSSIBLE ROLES OF TGF-BETA IN INNER EAR DEVELOPMENT IN MICE

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The otic vesicle (otocyst), the primordium of the inner ear, is formed by thickening and invagination of the otic placode originating from the surface ectoderm. The epithelium of the otic vesicle undergoes complex morphogenetic movement to form the cochlea and semicircular canals. In addition, a fraction of neuroblasts, which arise from the otic vesicle, delaminate and join neural crest-derived glial cells to form the cochleovestibular ganglion (CVG). In E10.5 mouse embryos, transforming growth factor-beta (TGF-beta) was found to expressed in the otic epithelium and its localization overlapped partially with that of NeuroD which is a marker of delaminating neuroblasts. TGF-beta typeI and typeII receptors were also detected in the otic epithelium. In order to examine the roles of TGF-beta in the differentiation of the otic vesicle, we treated the otic vesicles of E10.5 mouse embryos with TGF-beta in vitro. It was found that TGF-beta promotes enlargement of the CVG. Our findings suggest that TGF-beta contributes to the development of the inner ear in mouse embryos.

P38: INVESTIGATION OF THE DEL35G MUTATION OF THE GENE GJB2 AMONGST COCHLEAR IMPLANTEDPATIENTS AND THEIR RELATIVES IN SZEGED, HUNGARY

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The mutation of GJB2 gene, which codes the connexin26 channel protein subunit, is the most frequent cause of nonsyndromic hearing loss. Our aim was to screen our own population of cochlear implanted patients and their relatives, searching for the del35G mutation of the GJB2 gene.
During our work we collected blood samples from our population, prepared genomic DNA, and carried out 2 AS-PCR reactions on each DNA sample. Thus we could examine whether the given subject is heterozygous, homozygous, or does not carry the del35G mutation at all.

Where it was possible, we collected blood samples from blood relations, and investigated their del35G mutation status, so that we could do pedigree analysis as well.

P39: ELECTRICAL COMPOUND ACTION POTENTIAL MEASUREMENTS IN COCHLEAR IMPLANT USERS

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The threshold levels in cochlear implant patients are well correlated to electrically evoked brainstem responses (EABR). The electrically evoked compound action potentials wich are closely related to the EABR, would also show a similar correlation with behavioral threshold. Determining threshold levels requires subjective responses to a series of sophisticated psychophysical percepts. It is often difficult for cochlear implant patients. However, the neural response telemetry (NRT) system makes the measurement of compound action potential threshold possible.

NRT examinations were performed in 27 cochlear implant users with Nucleus 24 channels cochlear implants. We used MP1 stimulation and MP2 recoding mode. Five electrodes (3, 5, 10, 15, 20) were measured in all patients. The starting current level was 10 μA below the threshold level and it was increased up to the comfort level by 5 μA steps. Our goal was to look for correlation between behavioral subjective thresholds and compound action potentials.

The action potentials could be elicited in 26 patients in all measured electrodes. The NRT threshold values were highly correlated with electrical threshold levels obtained through subjective responses. Above NRT threshold neural responses increased linearly. Near the comfort level this linearity changed in several cases. Our results suggest that the electrically elicited neural responses may yield very important information for device fitting in patients with cochlear implants.

P40: NT-3 ENHANCES THE NEURONAL DIFFERENTIATION OF GRAFTED NEURAL STEM CELLS IN AN OTOTOXIN-DAMAGED MOUSE COCHLEAR EXPLANT

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Most types of acquired hearing loss arise from damage to, or loss of, cochlear hair cells and associated spiral ganglion neurons. Numerous studies have

indicated that neural stem cells (NSC) are multipotent cells that have retained the capacity to differentiate into a variety of cell types depending on their response to local environmental cues. The initial objective of our research is to assess the ability of NSC to integrate into injured cochlear epithelium and to replace damaged hair cells and/or associated neurons.To this end, we isolated NSC from the spinal cord of embryonic GFP transgenic mice and grafted them into organotypic cultures of ototoxin-damaged postnatal day-3 mouse cochleae. The expression of GFP, hair cell, and neuronal differentiation markers were evaluated 4 days post-grafting.
Our data indicate that a supplementation of the culture medium with neurotrophin-3 (NT-3) enhanced NCS expression of neuronal markers (tubulin, MAP2) at the expense of astrocystic differentiation marker (GFAP). In contrast, NT-3 supplementation did not enhance the expression any of the hair cell differentiation markers (Math1, Myosin 7a). There was a significant difference in the neuronal differentiation in the NT-3 supplemented, ototoxin-damaged cochlear explants as compared to the level obtained in the NT-3 non-supplemented, damaged, explants. In the NT-3 supplemented ototoxin-damaged explants, a large number of the grafted NSC were found to differentiate into neurons that also
generated peripheral processes.These findings suggest that combining NSC grafting with an adequate level of neurotrophin support can create a useful strategy to repair and/or replace lost or damaged auditory neurons in a traumatized cochlea.

P41: FUNCTIONAL AND MORPHOLOGICAL EXAMINATIONS OF A MOUSE MODEL CREATED BY A CONDITIONAL KNOCKOUT OF GJB2 GENE

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Hereditary deafness affects about 1 in 2,000 children and mutations in the GJB2 gene are the major cause in various ethnic groups. GJB2 encodes connexin26, a putative channel component in cochlear gap junction. However, the pathogenesis of hearing loss caused by the GJB2 mutations remains obscure. The generation of a mouse model to study the function of connexin26 during hearing has been hampered by the fact that gjb2 knockout mice are embryonic lethal. We generated targeted disruption of Gjb2 using Cre recombinase controlled by P0. Targeted disruption of Gjb2 caused profound deafness from birth but has never

reach maturation (ARO, 2004). Apparent degeneration of the organ of Corti was recognized, together with presumably secondary reduction of numbers of spiral ganglion cells. In order to further evaluate the detailed pathogenesis of deafness, we assessed functional and morphological examinations in a mouse model of gjb2 conditional knockout.
Auditory brainstem response (ABR) derived from tone bursts in KO mice resulted in elevation of threshold by 60 to 80 dB in all frequencies (8, 12, 16, 20 kHz).
Immunohistochemistry demonstrated expression of connexin 26 in the organ of Corti in the littermates, while almost no expression was identified in that of KO mice. In littermates, strong expression of beta-catenin, ZO-1 and F-actin was observed in pillar cells, while pillar cells, especially outer pillar cells, of KO mice exhibited weak or faint expression of these molecules. These findings suggest that dysfunction of gap junctions in the organ of Corti affects other cell-cell junctions resulting in degeneration of the organ of Corti.

P42: NF-kappaB IS A SURVIVAL FACTOR FOR IMMATURE AUDITORY HAIR CELLS

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Death of auditory hair cells (HC) in the inner ear as a consequence of aging, disease, acoustic trauma, or exposure to ototoxins results in irreversible hearing loss. However, little is known about the molecular mechanisms that underlie protection of these valuable cochlear elements. Here we examined the role of NF-kappaB, a ubiquitous transcription factor that plays a major role in the regulation of many apoptosis- and stress-related genes, in mediating HC survival. We analyzed organ of Corti (OC) explants of 5-day old rat pups, and found evidence of a constitutively active form of NF-kappaB that localizes predominantly to the HCs. Selective inhibition of NF-kappaB through use of a cell-permeable inhibitory peptide caused transcriptional down-regulation of gadd45beta, an anti-apoptotic NF-kappaB target, activation of caspase-3 and cell death by apoptosis. Application of the ototoxic drug gentamicin did not affect intracellular distribution of NF-kappaB, suggesting that gentamicin toxicity is not mediated through interference with the NF-kappaB pathway. In contrast to p5 animals, immunohistochemical analysis showed differential staining patterns for NF-kappaB in the cochleae of adult animals and no evidence of RelA (p65) in the adult OC. Taken together, our data suggest an important function for NF-kappaB in promoting survival of immature auditory hair cells.