ABSTRACT PROGRAMME

Rome, September 1-4, 2001

SUNDAY 2 SEPTEMBER, 2001
morning session

- ***Cell Signalling, Ionic Channels & Receptors***

09:00-09:10 ***Expression pattern of aquaporins in the rat inner ear (O1)***
S. Sawada, T. Takeda, S. Takeuchi, H. Kitano, A. Kakigi
Nankoku (Japan)

Water homeostasis is important for normal ear functions. Aquaporins (AQPs) in the plasma membrane facilitate water transport, which is driven by osmotic gradients across the membrane. Ten types of mammalian AQPs have been cloned to date (AQP0-9). We investigated expression pattern of AQPs using immunohistochemical method. Ten Wistar rats were used. Temporal bone was dissected followed by perfusion and fixation. Thin sections were made and incubated with specific primary antibodies (Chemicon, USA). The sections were incubated with secondary antibodies and reacted with peroxydase conjugated streptavidin and stained with DAB. AQP1 was strongly expressed in the stria vascularis, fibrocytes in the spiral ligament. The immunoreactivity of AQP4 were seen in the supporting cells (Hensen's cells) and AQP6 were in outer hair cells. The stria vascularis has a role in production of the endolymph, so it suggests AQP1 has a fuction of water transport in the stria vascularis. The outer hair cells have high motility and changes its volume rapidly. The rapid motility of outer hair cells is an important function for hearing. The results revealed AQP6 might have an role in the motility and volume change of outer hair cells. The expression of AQP4 in the supporting cells suggests that AQP4 relates to water recycling in the endolymphatic space. AQPs had unique distribution in the inner ear and we supposed it relates to the homeostais of the inner ear functions. In order to know homeostasis of the inner ear fluid and pathology like endolymphatic hydrops, further studies include its functions required.

09:10-09:20 ***Modulation of outer hair cell capacitance by chloride ions (O2)***
V. Rybalchenko, J. Santos-Sacchi
New Haven (USA)

Outer hair cell mechanical activity that derives from the cell's lateral membrane motor, prestin, likely plays a key role in auditory perception. The electrical correlate of this activity, the OHC's bell-shaped, voltage-dependent capacitance, provides information on prestin's voltage sensor, which has recently been shown to require intracellular chloride ions. (Oliver et al., in press). We have been studying this requirement in intact isolated OHCs under whole cell voltage clamp.
During the intracellular exchange of 0 mM Cl- solutions following the establishment of whole cell configuration in the extracellular presence of 140 mM Cl-, peak capacitance (pkCm) decreased to 75 +/- 10% (n=5) of its initial value and the voltage at peak capacitance (Vpkcm) shifted from -22 +/- 4 mV to 16 +/- 3 mV. Subsequent removal of extracellular Cl- , which abolished an inward chloride current monitored at zero mV, caused a further decrease in pkCm to 48 +/- 13%, and a shift in Vpkcm to 38 +/-5 mV. In some cases we observed a transient increase in pkCm following removal of extracellular Cl- in the absence of intracellular Cl-, and this was observed more readily with intracellular Cl- concentrations of 1 mM, where the rate of chloride washout through the pipette was slower.
These data indicate that nonlinear capacitance (and consequently motor activity) is rapidly modulated by the flux of chloride ions through the cell's plasma membrane, and possibly by an intracellular releasable store of chloride that is susceptible to the effects of pipette washout.

09:20-09:30 ***Chick Hair Cells Do Not Exhibit Voltage-Dependent Somatic Motility (O3)***
D.Z.Z. He, K.W. Beisel, L. Chen, D.-L. Ding, B. Fritzsch, R. Salvi
Buffalo (USA)

It is generally believed that some sort of mechanical amplification is needed to enhance the sensitivity and frequency selectivity of hearing. This amplification is presumably also responsible for the production of spontaneous and acoustic and electrically evoked otoacoustic emissions. In the mammalian ear, the basis of cochlear amplification is believed to

be the voltage-dependent somatic length change of outer hair cells (OHCs). Amphibian and reptilian ears are also sensitive and sharply tuned, but paradoxically these species lack OHCs. Nevetheless, their ears produce robust otoacoustic emissions that disappear when hair cells are destroyed. This has led to speculation that the cochlear amplifier resides in active motion of the hair-bundle. Chick ears are complex; hair cells exhibit electrical tuning, and there is a division of labor between tall and short hair cells with predominantly afferent and efferent innervation respectively. Chick ears also exhibit both otoacoustic and electrically-evoked emissions. The simplest hypothesis is that this active process is supplemented by electromechanical feedback from the short (outer) hair cells. It remains unknown, however, whether this feedback is mediated via active hair bundle motion or via somatic contractions of the short hair cells. In this study, we attempted to determine whether chick hair cells were motile by measuring their somatic length change. In addition, RT-PCR analyses were also performed to determine if there is a chick orthologue of prestin, the motor protein of OHCs. Results show that chick hair cells did not exhibit somatic motility nor did they express prestin. The lack of somatic electromotility and prestin in chick hair cells suggests that the active process might reside elsewhere, most likely in the transduction process of hair cell stereocilia.

09:30-09:40 ***Expression of HCN isoform transcript and protein in the rat cochlea (O4)***

M.J. Drescher, R.L. Barretto, J.S. Hatfield, D.G. Drescher
Detroit (USA)

Hyperpolarization-activated cation current, Ih, is thought to be attributable to the expression of hyperpolarization-activated, cyclic nucleotide-sensitive, non-selective cation (HCN) channel isoforms 1-4. Ih (also termed If) is the pacemaker current in the heart and has also been identified in excitable cells of the CNS, contributing to the maintenanceof resting membrane potential. Within the cochlea, Ih currents have been observed in spiral ganglion neurons (Mo and Davis, J. Neurophysiol. 78:3019-3027, 1997; Chen, Hear. Res. 110: 179-190, 1997). In the present investigation, RT-PCR and immunohistochemistry have been utilized todetermine expression of HCN isoform transcript and protein, respectively, in the rat cochlea. Sequencing of PCR products obtained with specific primers has indicated the existence of cDNA corresponding to HCN isoforms 1-4 in a spiral ganglion microdissected subfraction. PCR products corresponding tocDNA of HCN isoforms 1-4 were produced from an organ of Corti subfractionand HCN2 and HCN4 isoform cDNA was amplified from a lateral wall subfraction. Immunohistochemical localization studies indicate selective expression of HCN isoform protein in the cochlea. HCN1 immunoreactivity was concentrated in the spiral ganglion with most type I afferent cell bodies displaying immunostaining in all three cochlear turns. The afferent dendrites crossing the habenula perforata proceeding to the base of the inner hair cell were immunoreactive. For HCN2, the site of highest staining was the stria vascularis where immunoreactivity was concentrated in the intermediate cell region, not associated with either the surface of marginal cells facing the endolymphatic compartment or with the basal cell layer. Within the organ of Corti, HCN2 immunostaining was observed in position corresponding to the base of the outer hair cells. HCN4 immunostaining was concentrated in type III fibrocytes of the lateral wall, in the inner sulcus cells and supralimbal zone of the spiral limbus. No immunoreactivity was observed in the spiral ganglion for HCN4 and only occasional small clusters of type I afferent cell bodies were immunostained for HCN2. In conclusion, Ih current observed in the spiral ganglion neurons appears to derive primarily from the expression of cyclic nucleotide-activated HCN1, potentially regulating the firing rate and spontaneous activity of type I afferent neurons.

09:40-09:50 ***Full-length cloning of the alpha 9 nicotinic acetylcholine receptor subunit from the saccular hair cell of the rainbow trout (O5)***

D.G. Drescher, N.A. Ramakrishnan, W. Chun, X. Wang, G.E. Green, A.N. Karpenko, M.J. Drescher, S.F. Myers
Detroit (USA)

The present report describes the cloning and molecular structuraldetermination of the complete sequence of the alpha 9 nicotinicacetylcholine receptor subunit from the hair cell of the sacculus of therainbow trout, Oncorhynchus mykiss. The alpha 9 is considered to be amajor component of the cholinergic receptor expressed by hair cells ofvertebrates, with less than 39% identity to lower-numbered nicotinic alphasubunits. Narrowly distributed in tissues, the alpha 9 is likely toparticipate in mediation of the efferent signal in octavolateralis sensorysystems as either a homomeric channel or heteromeric complex ofyet-unknown stoichiometry. The native receptor may include both alpha 9and the more recently-described alpha 10 nicotinic subunit. In thecurrent study, a trout hair-cell sheet was utilized which contains thehair cell as the only intact cell type and has served as a useful modelsystem for investigations on the chemical identification of octavolateralis neurotransmitters and molecular characterization ofhair-cell receptors and channels. Since fish represent the lowest classof vertebrates, it was also of interest to examine the generality of occurrence and molecular structure of alpha 9 by comparison across thevertebrate classes. We utilized degenerate primers, RT-PCR, and RACE toelucidate the primary structure of the teleost hair-cell alpha 9 subunit.The trout saccular hair-cell alpha 9 cDNA was found to be 2,054nucleotides in length, with a coding sequence representing 572 aminoacids, including a 15 amino-acid signal peptide. The 3' untranslatedregion extended for 120 nucleotides, excluding the poly-A tail. The trouthair cell alpha 9 sequence contained typical ligand binding-site regions (A, B, C), paired cysteines common to all nicotinic alpha subunits, and membrane-spanning regions MSR I-IV. A remarkable difference was thegreatly increased

length of the MSR III-IV cytoplasmic loop - 99 aminoacids longer for trout compared to rat. Apart from this highly divergentregion, the trout hair cell alpha 9 is highly homologous with alpha 9sequences of human, rat, guinea pig, and chick. Also, a trout alpha 10sequence, previously reported from our laboratory (Green et al., Assoc.Res. Otolaryngol. Abstr. 21: 102, 1998) is presently under investigation. The current work thus extends the generality of the hair-cell cholinergic receptor to fish, the lowest of the vertebrate classes.

09:50-10:00 ***Multiple-site optical recording of mouse brainstem evoked by vestibulocochlear nerve stimulation (O6)***

M. Asako, S. Yang, T. Doi, A. Matsumoto-Ono, T. Kaneko, T. Yamashita
Kyoto (Japan)

In an attempt to detect the spatial patterning of the eighth nerve projection of the brainstem, anatomical methods such as orthograde transport of horseradish peroxidase were used. However, these methods did not provide the needed continuous information about the absolute value and time-course of varying neural excitement. Compounding this problem, the use of conventional electrophysiological methods makes it extremely difficult or impossible to detect the transmembrane voltage change because of the small size and fragility of the cells of the young brainstem. The optical imaging is very useful to investigate the mouse cochlear and vestibular nucleus in young brainstem. In this approach, we used a multiple-site optical recording system comprising a 16Å~16-element photodiode array and a voltage sensitive dye (RH-155).
We used brainstem slice preparations featuring an intact eighth nerve, and loaded depolarizing square current pulses from tungsten microelectrodes into the eighth nerve for stimulation of these preparations.
As a result, the spatiotemporal patterns of excitatory propagation were shown. These optical signals consisted of two components consisting of a spike-like fast signal and a long-lasting slow signal. All responses were abolished by tetrodotoxin. The slow signals were eliminated under a Ca (2+)-free solution. In addition, synaptic fatigue was also observed. The present study indicated the feasibility of optical recording for visually revealing the synaptic transmission in both the vestibular and cochlear nucleus.

10:00-10:10 ***Magnetic Resonance Imaging can visualise cochlear pathology in vivo (07)***

M.L. Duan, J. Qiu, B. Bjelke, A. Counter, T. Klason, A. Olofsson, E. Borg, G. Laurell
Stockholm (Sweden)

Magnetic resonance imaging (MRI) is becoming a more powerful tool in providing more information for physicians to diagnose patients who suffer from hearing loss from retro-cochlear lesions of the eight cranial nerve, cerebellopontine angle tumours and other causes. Most sensorineural hearing loss have a cochlear origin such as sudden deafness, labyrinthitis, Ménierè's disease, drug-induced hearing loss, noise-induced hearing and presbyacusis. However, the standard MRI equipment (1.5 Tesla) has obvious limitations in diagnosing cochlear pathophysiology. Even gross pathological conditions cannot be accurately identified. More recently a more powerful MRI has been developed. The three different fluid compartments in the cochlea can be visualised *in vivo* using a 4.7 Tesla MRI (Counter et al., 1999). In the present studies we develop more detail to visualise different cochlear pathological conditions *in vivo* such as genetic-induced gearing loss (German waltzing guinea pig), noise-and drug-induced hearing loss (Cisplatin and aminoglycosides). We found that MRI can visualise different cochlear pathology *in vivo* using a Gadolinium-enhanced 4.7 Tesla MRI. The new method will perhaps provide a potential means to diagnose cochlear origin inner ear disorders in the future.

10:10-10:20 ***Null mutation of the Tbx-1 gene severely disrupts the formation of the outer, middle and inner ears (O8)***

*T.R. Van De Water[1], J. Liao[2], W. Liu[3] and B. Morrow[2]
Miami, New York (USA)

The T-box family of genes all posses a common 200 amino acid DNA binding domain termed the "T-box". This T-box region is highly conserved throughout evolution with sequence homology approaching 99% in orthologous species. There are six members of the T-box family of genes (i.e. 1-6) and 2 of them have been shown to be expressed in the developing mouse inner ear complex. The T-box 1 gene (Tbx-1) is expressed in both the otic epithelium of the otocyst and in the surrounding periotic mesenchyme of the embryonic mouse inner ear as early as E9.5. A BAC transgenic mouse that contains 3 separate genes with one of these genes being Tbx-1 has been shown to have a Mondini type malformation of its inner ear and a malformation of the stapes with an associated hearing loss.

Null mutation of the Tbx-1 gene of the mouse disrupts the development of the outer, middle and inner ears. At E10.5 there are no gross differences in otocyst morphology between wild type, heterozygous and homozygous embryos. By E11.5 the otocyst has begun the formation of the complex membranous labyrinth with formation of a pars superior with its developing semicircular ducts, utriculosaccular space and endolymphatic duct and sac while the pars inferior is forming a cochlear duct as seen in the sections of both the wild type and the heterozygous embryos. In contrast, the membranous labyrinth of the E11.5 null mutant embryos has not progressed past the simple otocyst stage of

development while the anlagen of the endolymphatic duct and sac has greatly enlarged and now composes almost 1/3 of the overall volume of the developing inner ear. There is also a lack of the normal development of the tubotympanic sulcus to form the middle ear cavity in the E11.5 null mutants. The first effect of null mutation of Tbx-1 on the development of the pinna, external auditory meatus, and ossicular chain are evident in the serial sections of the head region of E13.5 embryos. In contrast to the normal patterns of development of these structures in both the wild type and heterozygous embryos, there were no detectable middle ear ossicles as well as a complete lack of either a recognizable pinna or an external auditory canal in the sections of the region of the inner ear in the null mutants.

These results show the global effect that the Tbx-1 gene has on the normal development of the outer, middle and inner ears. The Tbx-1 gene appears to be a primary organizing gene in the patterning of the ear. These results suggest that the Tbx-1 gene acts very early in the development of the outer, middle and inner ears and that it may act through downstream effects on other patterning genes such as Hmx-2, Hmx-3 and Pax-2. Studies are in progress to determine the identity of downstream target genes.

• *Nitric Oxide & Free Radicals*

11:00-11:10 ***Aging NOS II-Knock-out mice develop hearing impairment with loss of outer hair cells (O9)***
O. Michel, D. Labbe, A. Mickenhagen, M. Teranishi, M. Xiong, A. Hess, W. Bloch, K. Addicks
Cologne (Germany)

The physiological role of nitric oxide (NO) for the process of hearing is still discussed controversially. NOS II expression in the inner ear has only be shown for pathological conditions and - exceptionally - for a period in organogenesis. The following new observation concerning the hearing level in NOS II knock-out mice will add a new aspect.
A new created NOS II knock-out mouse was examined for its hearing level and inner ear morphology. In 3 groups sv129 "NOS II knock-out" mice (Group A: n=15, age 1-2 month; group B n=15, 6-8 month; group C: n=15, age 12-14 month) and in 3 control groups of sv129 wild type-mice (n=13, age 1-2 month; n=13, 6-8 month; n=5, age 12-14 month) hearing level was determined. Immediately after, cochleae removed and were processed for scanning electron microscopy (SEM).
50% of the examined NOS Il- deficient, 12-14 month mice showed in comparison to the control group (sv129 wild type) a 30-40 dB increased hearing threshold up to deafness. The electrophysiological findings could be correlated to a severe loss and a morphological irregularity of outer hair cells, as shown by scanning electron microscopy. The 2 other, younger groups with NOS Il- deficient mice and all 3 control groups showed no significant differences in regard to their hearing level and their inner ear morphology as shown by SEM.
The NOS II knock-out mice were characterized by hair cell loss 12-14 month after birth and concomitant severe hearing loss. By the hereby presented observation the role of NO in inner ear diseases gets a new facet. An explanation so far could be, that the diminished capacity of NOS II to produce NO in knock-out mice may sends cells to early apoptosis or hinders a regular defense mechanism against intrinsic or extrinsic damage.

11:10-11:20 ***Localization of soluble Guanylate Cyclase (sGC), the nitric oxide (NO) receptor, in the human cochlea (O10)***
M. Stach, B. Schneider, C. Schofer, B. Pikula, K. Frei, H. Felix
Vienna (Austria), Zurich (Switzerland)

Previous studies have shown the nitric oxide /cGMP pathway to be important to regulation of cochlear blood flow (CBF) and neuronal signaling in the mammalian cochlea. In the inner ear of rodents, studies of this system have focused mainly on the first enzyme in the pathway, nitric oxide synthase (NOS). However, localization of the NO receptor, soluble guanylate cyclase (sGC), is essential to determination of cochlear cells targeted by NO. Another aspect is the elucidation of its possible roles in function of the auditory system. Hitherto, only few data about the presence of this pathway in the human inner ear are available. Thus, the goal of this study was to identify the presence and localization of sGC, a cytosolic protein, in the human cochlea by means of immunohistochemical examination of surface preparations and cryosections of the basilar membrane and lateral wall tissue. Intensive sGC activity was detected in the inner hair cells (IHC) and adjacent nerve fibers, in pericytes surrounding small capillaries of the lateral wall tissue and in the cytoplasm of Hensen's cells. Only slight staining was observed in the outer hair cells (OHC), Deiters' cells and pillar cells in the organ of Corti. These results have to be verified by other immunohistochemical and ultrastructural methods. Our findings suggest the involvement of the NO/cGMP pathway in regulatory processes in neurotransmission, neuromodulation and cochlear blood flow in the cochlea of man.

11:20-11:30 ***Extracellular ATP-induced nitric oxide production and intracellular Ca mobilization in type I spiral ganglion cells of the guinea pig cochlea (O11)***
N. Harada, H. Cho, T. Yamashita

Osaka (Japan)

It has been suggested that nitric oxide (NO) plays a role in the auditory system. NO is synthesized by three isoforms of the enzyme nitric oxide synthase (NOS). Te neuronal isoform (nNOS) and the endothelial isoform (eNOS), which are Ca-dependent, have been detected in cochlear spiral ganglion cells of the guinea pig. However, the role of NO in auditory signal transduction pathway still remains unknown. Here we investigated the effects of extracellular ATP on NO production in acutely isolated type I spiral ganglion cells (SGCs) of the guinea pig cochlea using NO-sensitive dye DAF. SGCs were loaded with 5% BM+DAF-2 DA for 60 min. Excitation filter 485-495 nm and emission filter 510-540 nm were used respectively in the present study. Extracellular ATP induced an increase of the fluorescence intensity time-dependently. This increase was inhibited by a non-specific NOS inhibitor of L-NAME. These results suggest that ATP can induce NO production in SGCs. Using the Ca-sensitive dye Fura L-NAME and 8-Bromo-cGMP inhibited the increase of Ca induced by ATP in SGCs. ATP also could induce NO production and a Ca increase in one SGC.
The present study is the first direct evidence of NO production induced by extracellular ATP in SGC. The ATP-induced NO production was accompanied by the Ca increase. These results suggest the possibility of cross-talk between NO and Ca in SGC.

11:30-11:40 Nitric oxide modulates the cochlear inner hair cell neurotransmission (O12)
E. Oestreicher, A. Arnold, D. Felix
Munich (Germany)

Nitric oxide (NO), a free radical, is well known to be a regulator of vascular tone and mediator of neurotransmission. It has also been implicated in neuropathological conditions if released in excess. Recent immunohistochemical studies showed the localization of nitric oxide synthase in the cochlea but only few reports exists concerning the influence of NO on auditory function.
With microiontophoretic techniques and extracellular recording we investigated the action of NO on the glutamatergic neurotransmission of afferent auditory neurons of cochlear inner hair cells in guinea pigs in vivo.
Application of the unspecific NO-synthase inhibitor Ng-nitro-L-arginin methylester (L-NAME) was followed by a reduction of the firing of NMDA- and AMPA- activated neurons. The simultaneous application of the NO-donor S-nitroso-N-acetyl-penecillamine (SNAP) showed an enhancement of the spontaneous and the NMDA- /AMPA- induced firing of afferent neurons of inner hair cell.
These results demonstrate that in the mammalian cochlea NO modulates the glutamatergic neurotransmission via NMDA and AMPA receptors. This could explain in part the neuropathological effects of an excessive release of NO in the cochlea.

11:40-11:50 High concentrations of D-methionine and thiourea in the rat cochlea after round window administration (O13)
G. Laurell, M. Teixeira, O. Sterkers, D. Bagger-Sjoback, S. Eksborg, O. Lidman, E. Ferrary
Stockholm (Sweden), Paris (France)

Round window administration of drugs involves the delivery of medication directly into the inner ear via the round window, avoiding a systemic effect of the therapy. Reactive oxygen species (ROS) formation and oxidative damage are involved in some acquired inner ear disorders such as ototoxicity and noise-induced hearing loss. Antioxidants have been reported to decrease the damaging capacity activities of ROS.
This study serves to examine the transport of two tracers to the perilymphatic compartment and to cochlear tissue of the rat after round window administration. Radioactive D-methionine,a sulphur containing amino acid, and thiourea, a smaller nucleophile, were infused via a catheter to the round window niche. Samples of scala tympani perilymph from the basal turn of the cochlea were taken 17 to 254 minutes after the round window administration. Both tracers penetrated the round window readily and the highest concentration of radioactivity was found in the earliest taken samples. The perilymph-radioactivity-time curves were fitted to a one-compartment model with a half life for thiourea and D-methionine of less than one hour. The distribution of the tracers at the cellular level was analysed by autoradiography. The most intense expression was found in the lateral wall of the cochlea. We can conclude that a substantial concentration of D-methionine and thiourea can be obtained in cochlear fluids and tissues after round window administration.

11:50-12:00 Antioxidant N-L-acetylcysteine can protect cochlea from impulse noise trauma (O14)
M. Duan, J. Qiu, A. Counter, A. Olofsson, E. Borg, G. Laurell
Stockholm (Sweden)

Noise-induced hearing loss is very common in our society. Impulse noise is also a risk for human inner ear. It had been demonstrated that impulse noise such as gunfire, firework etc can cause hearing loss. It has been shown that antioxidant such as N-L-acetylcysteine (NAC) can protect the inner ear from trauma. At present we investigate if NAC can protect the cochlea from impulse noise trauma. The impulse noise level is 160 dB SPL. Guinea pig, rat and mouse were

exposed to 160 dB impulse noise from 50 to 200 times. Animals were injected with NAC (ip) at different times. We found that NAC could protect the cochlea from noise trauma. The results support that antioxidant is an effective and useful agent to protect the cochlea from noise trauma.

• *MORPHOLOGY*

12:10-12:20 ***3D TEM Modeling of Membrane Specializations between Large Ganglion Cells in the Human Spiral Ganglion (O15)***

S. Tylstedt, H. Rask-Andersen
Umea, Uppsala (Sweden)

During transcochlear procedure for a life-threatening meningioma, a human cochlea was taken out instead of its destruction during drilling. The patient had preoperatively a virtually normal hearing with full discrimination. Te specimen was freshly fixed in a 13.3% fluorocarbon-containing fixative in 2% glutaraldehyde solution and 0.05M sodium phosphate buffer, processed and sectioned for ultrastructural analysis. Consent from the patient and local ethical committee was obtained. In the apical cochlear portion 400 consecutive serial thin sections of the human spiral ganglion were analysed using TEM. Human ganglion cell bodies were mostly unmyelinated, often forming unit-like clusters surrounded by a common Schwann cell. Direct physical contact between adjacent large ganglion cells was found in so-called SC gaps and membrane specializations with three different morphological appearances could be observed. These were 1% symmetrical, 2% asymmetrical and 3% subplasmalemmal densities or condensations at or near the cell membranes. In the model, they formed groups and small areas varying in size with maximal dimensions about 3x2 microns. Structural specializations in LGCs seem unique for Man and have not so far been observed in other species. Their possible role for coding of speech is discussed.

12:20-12:30 ***Cochlear morphology and auditory function (ABR) in galectin-3 null mutant mice (O16)***

V. Couloigner, M. Bichara, F. Poirer, E. Ferrary
Paris (France)

Galectins, or galactoside-binding lectins, constitute a growing family of carbohydrate binding proteins. Among them, galectin-3 is a 30 kD protein that recognizes glycoproteins containing polylactosamines such as laminin (for review, see Hughes, Biochim Biophys Acta, 1999;1473:172-185). It has been localized in several tissues such as notochord, bone, skin, blood cells, arterial smooth muscle cells and gastrointestinal, respiratory, and renal epithelial cells. Tissue distribution of galectin-3 varies during development and as a function of cellular activation and/or pathological processes. It is required for cell-cell and cell-matrix interactions and thus plays a role in several physiological and pathological situations that involve these interactions such as embryogenesis, oncogenesis, inflammation, or infection. It is also involved in cell proliferation, RNA splicing, and is endowed with anti-apoptotic properties.
In the inner ear, the distribution and function of galectin-3 has not yet been investigated. The aim of the present work was to study the role of galectin-3 in the mouse cochlea. To this purpose, galectin-3 null mutant mice were studied both morphologically and functionally. Examination of cochlear tissues under light microscopy, as well as determination of hearing thresholds by ABR did not show any overt difference between wild-type and mutant mice. Further studies are needed to look for more subtle cochlear dysfunctions in the absence of expression of galectin-3.

12:30-12:40 ***Degeneration of cochlear neurons of mpv17-negative mice in computered-aided three-dimensional reconstruction (O17)***

A. Meyer zum Gottesberge, M. Kowalliki, M. Kassens, C. Auffenberg, H. Felix
Dusseldorf, Munster (Germany), Zurich (Switzerland)

In Mpv17-negative mice and the wild type age dependent degeneration of the SGCs occurred. Either the total number of the SGCs or density (number of cells / area in representative sections denote the patchy-like degeneration pattern seen in Mpv17-negative mouse. In order to demonstrate this degeneration pattern a new 3D reconstruction software was developed and applied. For this reconstruction the alignment-modus of the software have been used in order to match each section to the corresponding one. The area of the SGCs has been calculated by multiplication the mean cell size with the counted SGCs and then replaced by circles with exactly the same surface. For each section the calculated circles have been placed in Rosenthal´s canal and then linked with each other resulting in a complete reconstruction of the distribution of spiral ganglion cells. In the same manner the reconstruction of the Rosenthal´s canal was made and both structures were matched together. This procedure allows viewing the degeneration (survival) of the SGCs along the entire length of the cochlea. In contrast to traditional reconstruction techniques, this new computer reconstruction technique does not need internal markers, it can be applied on already serial sectioned specimens. It thus appear to be useful for 3D reconstruction of cochlear structures from animals as well as from humans (human temporal bone collections).

12:40-12:50 ***Patterns of GABA like-immunoreactivity in efferent neurons of the human cochlea (O18)***
K. Kammen-Jolly, A. Scholtz, W. Kong, M. Eybalin, A. Schrott-Fischer
Innsbruck (Austria)

Olivocochlear efferent neurons originate in the superior olivary complex of the brain stem and terminate within sensory cell regions of the organ of Corti. Components of this complex include the lateral olivocochlear bundle whose unmyelinated axons synapse with radial afferent dendrites below inner hair cells and the medial olivocochlear bundle, from which myelinated axons form a direct synaptic contact with outer hair cells. Gamma-aminobutyric acid (GABA), a major neurotransmitter of the CNS believed responsible for most fast-inhibitory transmissions, has been demonstrated with interspecies variation between mammal and primate auditory efferents. In the present study, we evaluate the immunocytochemical presence of GABA in ten human cochleae using light and electron microscopy. GABA-like immunostaining could be observed in inner spiral fibers, tunnel spiral fibers, tunnel-crossing fibers, and at efferent endings synapsing with outer hair cells. To approximate medial efferent fiber quantifications, we counted labeled terminals at the base of each outer hair cell and then compared this sum with the number of tunnel crossing fibers. We found a `branching ratio´ of 1:2 implicating a doubling in quantifiable efferent fibers at the level of the OHC. In human, the distribution of GABA-like immunoreactivity showed a consistent presence throughout all turns of the cochlea. A new method for application of immunoelectron microscopy on human cochleae using a pre-embedding technique is also presented and discussed.

SUNDAY 2 SEPTEMBER, 2001
afternoon session

• *Immunology, Endolymphatic Sac & Endolymphatic Hydrops*

14:30-14:40 ***Middle ear exotoxin and Endolymphatic sac response-an immune reaction? A morphological study of the endolymphatic sac after exposure of Pseudomonas exotoxin in the middle ear of the rat (O19)***

L. Nordang, M. Anniko, H. Rask-Andersen
Uppsala (Sweden)

The ES is presumed to be involved in immune responses in the inner ear. The ES has been shown to respond immunologically to an antigen instilled in the scala tympani. Pseudomonas Aeruginosa Exotoxin A is known to pass the round window membrane and cause cochlear functional impairment. Does the endolymphatic sac (ES) in the inner ear respond immunologically to exotoxin exposed in the middle ear?
In the present study the ES was examined morphologically after application of PaExoA in the middle ear of the rat. The ES changes were correlated with electrophysiological results using ABR measurements.
In 18 out of 32 exposed ES we found signs of inflammatory activity with raised numbers of intraluminal macrophages together with signs of epithelial alterations. There were also changes in the character and stainability of the intraluminal glycoprotein conjugates.
The ES changes may either be due to a direct exposure of toxin to the ES or possibly represent the expression of an augmented general response in the inner ear. These findings are similar to earlier description of changes in the ES after experimental labyrintitis. This is the first presentation showing altered ES activity following middle ear application of exotoxin.

14:40-14:50 ***Assessment of organ specific autoantigens in experimental autoimmune labyrinthitis by Mini Whole Gel Eluter (O20)***

S. Tomiyama
Tama, Tokyo (Japan)

Autoimmune reaction is involved in pathogenesis of Meniere's disease, bilateral progressive sensorineural hearing loss, delayed hydrops and sympathetic labyrinthitis. However, the pathogenesis of these diseases is still unknown. Therefore, experimental animal models of autoimmune labyrinthitis have been useful to investigate these diseases. We previously reported the model in bred animals that provided 100% reproducibility as well as precise immunological analysis. Single sensitization induced cellular autoimmune labyrinthitis mediated by Th1 lymphocytes. Repeated sensitization induced production of auto-antibody that was assessed by western blot and by immunohistochemistry. However, it is not enough for using crude antigen to make a close investigation. Therefore, the present study examined antigenicity of inner ear constituent fractionated by molecular gradient.
SDS soluble inner ear antigen was separated by electrophoresis in SDS-PAGE slab gel. These separated proteins are eluted in transverse direction into 14 fractionated chambers by Mini whole Gel Eluter. Mice were sensitized with these fractionated antigens after removal of SDS. Antigenicity was examined histologically.
Sensitization with fraction 5 which contained 60-70 kDa protein and with fraction 8 which contained 40-45 kDa protein demonstrated intense cellular infiltration into the inner ear as compared to sensitization with other fractionated antigen.
The study suggests that candidate of autoantigen of experimental autoimmune labyrinthitis locate in the range of 60-70 kDa and 40-45 kDa. These results supported our previous study that repeated sensitization induced production of autoantibody of 45 kDa and 66kDa.

14:50-15:00 ***Measurement of Na+, K+-ATPase activity in the Endolymphatic Sac Epithelial Cells of Guinea -Pigs (O21)***

T. Miyashita, H. Tatsumi, H. Furuta, M. Sokabe, N. Mori
Kagawa, Nagoya (Japan)

Intracellular Na+ concentration ([Na+]i) was measured in the epithelial cells isolated from intermediate portion of the endolymphatic sac (ES) of guinea-pig to examine the Na+, K+-ATPase activity in individual cells. Removal of external

K+ or application of 1 mM ouabain caused an increase in [Na+]i of ES epithelial cells. We observed two cell groups, which showed a large [Na+]i increase (from 27.9 ± 3.5 mM to 82.8 ± 10.6 mM (n = 5) and a small [Na+]i increase (from 13.2 ± 12.4 mM to 21.4 ± 11.1 mM (n = 5)), respectively. This result demonstrates that there are at least two types of cells in the intermediate portion of the endolymphatic sac according to the [Na+]i increase. The cells showing the larger [Na+]i increase may play an important role in Na+ absorption from the ES endolymph.

15:00-15:10 Vascular pathology of the endolymphatic sac in Meniere's Disease (O22)

U. Friberg, H. Rask-Andersen
Uppsala (Sweden)

Surgical excision of the endolymphatic sac in two patients with Meniere's disease undergoing labyrinthectomy showed histological evidence of occlusion of the vein of the vestibular aqueduct (VA) in its proximal region. This finding coincided with total or partial occlusion of numerous small vessels around the ES, extensive perisaccular fibrosis, flattening of residual epithelium and signs of new bone formation. If partially occluded vessels were the result of a recanalisation process was not established. Ultrastructural analysis of the thrombus-like occlusions showed foci with dense connective tissue, calcification, lipid deposits and layers of basal lamina-like material, sometimes concentrically arranged. In two additional MD patients the VVA could not be visualised and ES vessels showed no signs of occlusion. Seven control sacs taken out from patients with acoustic schwannoma had normal vasculature. Thus, at some instances, vascular occlusion in the ES and draining venous routes from the vestibule (VVA) may contribute in the pathogenesis of Meniere's disease.

15:10-15:20 Reversible changes in structure and function in the organ of Corti during hydrops (O23)

A. Flock, B. Flock
Stockholm (Sweden)

Hydrops is a condition of swelling of the membraneous labyrinth including in the cochlea a bulging of Reissner membrane into scala vestibuli. Its genesis is still not obvious but the associated clinical symptoms are quite discernable to the patient, consisting of fluctuating hearing loss, tinnitus and vertigo. We have used laser confocal microscopy to visualize the vitally-stained Reissner's membrane during the development of experimental hydrops, while at the same time recording functional changes by electrophysiological recordings. It was then noted that structural changes also occurred inside the organ of Corti. We have now explored these events at higher resolution. The scala tympani of the isolated temporal bone of the guinea pig was perfused between an opening at the base and an opening at the apex. The organ of Corti could be viewed at the apex with a 40x water immersion objective of a laser confocal microscope. The organ was stained by fluorescent probes added to the perfusate. Electrophysiological recording of the cochlear microphonic potential (CM) and the summating potential (SP) were made with an electrode inserted in the scala media at the apex. A tubing was inserted in the scala vestibuli of the basal turn. It was connected to a bath that could be elevated to produce a flow between base and the apical opening. This was accompanied by a bulging of the Reissner's membrane at the apex, mimicking hydrops. As pressure was increased stepwise to 30 cm of tank elevation, the CM was reduced and the sign of the SP turned in the negative direction. When the pressure was lowered back to 0 cm the responses returned to different degrees in different preparations, in some fully, in some partly and in some degeneration of the preparation ensued. Here we will consider reversible cases. A number of structural changes occurred: the organ tilted around a point at the habenula perforata; the surface of the organ was pushed down in parallel; the shaft of the inner pillar bent toward the tunnel of Corti; the bases of outer hair cells swing toward the tunnel; the outer hair cells shorten; the arc of Deiters cell carrying the Hensen cells swings toward the outer hair cells. When the pressure was released these events reversed.
We conclude that the organ of Corti can accomodate mechanical stress, such as imposed by increased pressure in the scala media, by adjusting its mechanical structure.

15:20-15:30 The role of anti-endothelial cell autoantibodies (AECAs) in sensorineural sudden hearing loss (O24)

F. Ottaviani, G. Cadoni, A.R. Fetoni, S. Agostino, A. De Santis, R. Manna
Rome (Italy)

Immunologic causes of sudden hearing loss (SHL) are not yet well defined although serological autoantibodies against organ specific and not organ-specific antigens of the inner ear are reported.
AECAs represent a heterogeneous group of antibodies directed against a variety of antigen determinants on endothelial cells that are detected in various inflammatory disorders. They may interfere with several endothelial cells functions and therefore may be of pathophysiological relevance.
The Authors investigated the presence of AECAs in patients affected by SHL to verify if immune-mediated vasculitis may play a role in human SHL. Forty-five consecutive patients (mean age 35 ys) affected by SHL and 20 normal subjects were included. AECA were positive in 21/45 (47%) thus significantly differing from normal population on which only 2 of 20 tested cases were positive (p=0.03). Human sera of AECA positive patients were incubated against

guinea pig cochlear sections and a positive immunoreactivity to inner ear antigens was obtained by indirect immunofluorescence assay. In conclusion, this study suggests that AECAs could be considered as markers of immune-mediated SHL.

15:30-15:40 ***Prolactinoma in some Meniere's patients: is stress involved ? (O25)***
K.C. Horner, R. Guieu, J. Magnan, A. Chays, Y. Cazals
Marseille (France)

Dizziness is a common complaint in primary care clinics and can enter the diagnostic profile of different pathologies spanning from psychiatric problems to vestibular dysfunction. Episodes of vertigo in Ménière's patients are often reported to be triggered by stress but no physiological data are available to account for the subjective link. The study involved 42 Ménière's patients hospitalized for neurectomy of the vestibular nerve for relief of incapacitating vertigo. In addition 18 patients with neurinoma of the vestibular nerve and 12 patients with facial spasm, who underwent surgery, served as controls. A blood sample was taken on the day of surgery in order to determine the level of battery of different stress hormones. The most striking observation was the presence of hyperprolactinemia (above 20 mg/l) in 14 Ménière's patients. The presence of prolactinoma was confirmed by MRI in 6 cases out of 6 investigated and the others have not yet been followed-up in this retrospective study. These observations are clearly indicative for systematic determination of prolactin levels before opting for surgery in Ménière's patients.

POSTERS (1-10)

15:50-15:55 ***Effects of direct infusion of BDNF and NT-3 on the middle-grade damaged cochleae following aminoglycoside ototoxin exposure (P01)***
H. Kuriyama, K. Takemura, M. Komeda, C. Himeno, M. Izumikawa, T. Doi, T. Yamashita
Osaka (Japan)

Recent studies show that nerve growth factor (NGF) and its family neurotrophins such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) play a crucial role during innervation of normal development and also reinnervation after cochlear insult. Current study intended to evaluate the in vivo effects of cocktail of BDNF and NT-3 on the kanamycin treated-traumatized cochlea both electrophysiologically and morphologically.
We used guinea pigs (male Hartley, 250-400g), and implanted Alzet mini-osmotic pump in the subcutaneous tissue on the back, and drugs in the pump were administrated to the scala tympani, in the flow rate of 0.5µl/hr. for 14 days. Left ears of all animals were operated and right ears were evaluated as non-operated control. In the experimental group, BDNF and NT-3 in the same concentration were filled in the pump. The control group received artificial perilymph(AP) as a placebo. After maintaining of initial infusion with BDNF and NT-3 or AP, on the experimental 14 days, animals were systemically administrated kanamycin (400mg/kg, s.c.), and 2 hours later ethacrynic acid (40mg/kg, i.v.), then the osmotic pump was switched for another 14 days infusion.
Auditory brainstem responses (ABRs) were tested before pump implantation (Day0), 3days after kanamycin administration (Day17) and the end of pump infusion (Day28).
On the day 28, the cochlea tissues were separated and proceeded to fluorescent immunohistochemistry to observe the remaining hair cell. Hair cells were counted from two randomly selected fields in each turn and calculated the surviving rate.
The ABR results of the operated side of experimental group showed the least thresholds shift due to otoxin comparing to control group and the non-operated side of experimental group. The results of hair cell counting were also indicated the highest surviving rate of outer hair cells in the operated side of experimental group.
In our study, electrophysiological evaluation as well as morphological one showed the protective effects of BDNF +NT-3 infusion on the middle-grade damaged cochleae following aminoglycoside ototoxin exposure.

15:55-16:00 ***Comparison of membrane stiffness in cochleoapical and cochleobasal outer hair cells of the guinea pig (P02)***
J. Batta, G. Panyi, I. Sziklai
Debrecen (Hungary)

Mammalian outer hair cells (OHCs) has a unique trilaminated lateral wall which presumably plays an important role in the electromechanical force coupling and shape changes. Membrane stiffness of the OHCs have already been measured by microdeformation techniques (Dallos et al., J Neurosci 17: 2212, 1997; Oghalai et al., J Neurosci 18: 48, 1998) without, however, providing with a comparative and cell-topological stiffness data in cochleoapical and cochleobasal

outer hair cells. Present study examines stiffness differences of the cell membrane along the longitudinal axis and differences between the identical membrane regions in cochleobasal and cochleoapical OHCs.
Guinea pig OHCs were prepared and incubated in Hank's solution with and without calcium. Stiffness of the lateral cell membrane was measured by a glass micropipette aspiration technique under control of phase-contrast microscope. Suction was applied to the micropipette (inner diameter of 2.83±0.25) mm by a calibrated water column. Negative pressure was applied from 4 to 10 H2O.cm (0.39 nN/mm2 -0.98 nN/mm2). The phase-contrast images of the aspirated cell membrane segments in the pipette were video-recorded, digitized and analyzed off-line. Apical (A), medial (M), near-supranuclear (S) and near-infranuclear (I) cell membrane-regions were examined. Cochleobasal and cochleoapical OHCs were isolated separetly.
A linear correlation was found between negative pressures of 4-6-8-10 H2O.cm and the length of the aspirated lateral wall independent of the cell regions. Constant application of pressure (0-120 sec) resulted in a topological difference of membrane stiffness from apex to base. No difference was found between identical membrane regions of apical and basal OHCs. The A, M, S, curves show only quantitative differences whereas I curve differs from them qualitatively. The I curve shows an exponential relation with time while curves from the supranuclear regions are complex. The latter is associated with a small active length decrease of the whole cell between 30-60 sec. Simultaneously, the length of the membrane aspirated shows a stiffness increase in the supranuclear membrane in this critical time period. Mathematical analysis of this transformation reveals a sigmoid component between 30-50 seconds. This phenomenon is not observed when OHCs were incubated in a Ca2+ -free medium which otherwise resulted in a higher stiffness of the membrane independent of location of suction point or origin of the cell in the cochlea.

16:00-16:05 Induction of apoptosis in the lipopolysaccharide (LPS)-treated vestibule of guinea pigs (P03)
K.-I.Watanabe, K. Jinnouchi, A. Hess, O. Michel, T. Yagi
Tokyo (Japan)

We immunohistochemically examined the apoptotic changes induced by endotoxin in the vestibule of guinea pigs. Lipopolysaccharide (5 mg/ml, 0.2ml), a bacterial endotoxin, was transtympanically injected into the middle ear. 48 hours after injection of lipopolysaccharide, animals were sacrificed by intracardiac perfusion of fixative. The temporal bones were then removed and immunohistochemically stained for single-stranded DNA, caspase activated deoxyribonuclease and caspase 3. Single-stranded DNA was detected after 48 hours in the dark cell area of the lipopolysaccharide group, not in the sensory epithelium. Caspase activated deoxyribonuclease was observed in the dark cell area and the sensory epithelium. Caspase 3 was also detected in the dark cell area and the sensory epithelium. During the process of apoptosis, double-stranded DNA is broken into single-stranded DNA by the action of caspases and caspase activated deoxyribonuclease. These findings suggest that apoptosis is involved in the dysfunction of the vestibule under inflammatory conditions.

16:05-16:10 Apoptotic hair cells in the vestibular epithelium lose E-cadherin-mediated cell-cell contacts (P04)
T. Kim, T. Nakagawa, T. Endo, N. Murai, J. Ito
Kyoto (Japan)

E-cadherin is known to be a major adhesion molecule in the inner ear sensory epithelia. The distribution of E-cadherin in the organ of Corti has been revealed. In addition, alteration of expression of E-cadherin in the organ of Corti following drug-induced insult has been reported. However, the expression of E-cadherin in the vestibular epithelia and its alteration after aminoglycoside intoxication is unknown. On the other hand, E-cadherin is known to be associated with regulation of apoptosis. In this study, we examined alteration of E-cadherin expression in mouse vestibular epithelia during deletion of hair cells by apoptosis.
ICR mice 10 to 20 weeks old were used. Under general anesthesia, the lateral wall of the left cochlea was exposed. A small hole was opened in the bony wall of the cochlear lateral wall. Using a microsyringe, 1 microliter of gentamicin sulfate solution (400mg/ml) was introduced to the endolymphatic space. One day after the operation, the animal was decapitated and the temporal bone was removed. The right temporal bone was used as the control. The utricle was extracted and fixed in 4% paraformaldehyde overnight. We performed immunostaining for E-Cadherin and TUNEL staining for detection of apoptotic cells. The utricle was observed by a confocal microscope.
The control specimen demonstrated a network of E-cadherin expression surrounding hair cells. TUNEL positive cells were not observed in the sensory epithelium. In gentamicin-treated utricle, TUNEL positive cells were found. The frame of E-cadherin was disrupted in the area in which TUNEL positive cells were detected.
These findings suggest that apoptotic hair cells lost cadherin-mediated cell-cell contacts.

16:10-16:15 Hypoxia-vulnerability and HIF-1 alpha activity of the organ of Corti (P05)
B.Mazurek, C.Rheinlander, E.Winter, J.Heldt, T.Kietzmann, V. Jahnke, J.Gross
Berlin (Germany)

To study the influence of hypoxia on the Corti organ, we have chosen an in vitro hypoxia-model of the cochlea of newborn rats (incubation of cochlea in Billups-Rothenburg chamber, 13-36 hours, pO2 level of the atmosphere 5-10

mm Hg). The number of inner and outer hair cells (hc) in the apical, medial and basal areas at the end of hypoxia were counted. Using this hypoxia model we determined the activation of hypoxia inducible factor 1 (HIF-1), a key transcription factor for the adaptation of cells and tissues to hypoxia. The HIF-1 activity was used as an indicator for the adaptive response of cochlea cells to hypoxia.
After 13 hours of hypoxia a reduction of the number of outer and inner hair cells could be detected: for the outer hair cells by 19,5 ± 1,9 (mean ± SEM, n= 43) and for the inner hair cells by 36,4 ± 3,3 (n=5, p<0,001). No differences between apical, medial and basal parts were found. After 36 hours of hypoxia a higher loss of inner hair cells was found (outer hair cells by 24,2 ± 2,0, n=42; inner hair cells 67,8 ± 2,7, n=36, p<0,001). HIF-1 activity was measured using a reporter gene which contains three subsequent hypoxia-response elements in its enhancer region. Since transfection of intact cochlea was not possible using standard transfection methods we prepared a single cell culture, which could be transfected easily (calcium-phosphate). Hypoxia increased HIF-1 activity differentially in dependence on the duration of hypoxia and the cochlea region. Highest activities were found in modiolus and corti organ/limbus region.
The study shows, that hypoxia induces a significant activation of HIF-1 and a decrease of inner and outer hair cells. The differential response of inner and outer hair cells can be explained by increased glycogen content and the glutamate toxicity of inner hair cells. The high activation rate of HIF-1 of modiolus and corti organ/limbus regions could contribute to the defence against hypoxia.

16:20-16:25 Proliferation is the major method of recovery in the inner ear of an amphibian, the newt (P06)

R. Taylor, A. Forge
London (UK)

In the avian auditory organ, the basilar papilla, spontaneous turnover of hair cells does not occur but damage to hair cells results in proliferation of supporting cells and recovery of the end organ. In contrast within the mammalian vestibular system it has been suggested that transdifferentiation of supporting cells takes place following ototoxic assault.
The newt, Notophthalmus viridescens, an amphibian of the order Urodeles has been used as a model to investigate regeneration within the inner ear of a vertebrate capable of regenerating many different body parts and tissues. In vitro gentamicin induced damage to the sensory epithelium results in almost complete ablation of hair cells within the saccule and the appearance of scar formations. Using both light microscopy and SEM spontaneous hair cell recovery can be observed 12 days post-treatment. TEM sections and cryosections labelled with hair cell marker suggest that non-lethally damaged hair cells do not contribute to this recovery. To investigate whether the observed recovery results from stimulation of proliferative responses intact otic capsules have been incubated with BrdU supplemented culture medium. Upregulation of proliferation commences around 6 days post gentamicin treatment with paired BrdU labelled nuclei apparent in the saccular maculae. After 19 days of incubation in BrdU following the hair cell loss there is extensive nuclear labelling and double- labelling with a hair cell marker suggests most of the new hair cells have arisen through proliferation. Assessment of recovery by non-mitotic means has been carried out using aphidicolin to block mitosis. Following gentamicin treatment and incubation with aphidicolin there is a reduction in the number of hair cells produced. Proliferation, therefore, appears to be the major mechanism of recovery within the sensory patches of the inner ear in this amphibian. This is similar to the regenerative responses found in other newt tissues.

16:25-16:30 Connexin expression during regeneration in the sensory epithelia of the inner ear (P07)

R. Nickel, A. Forge
London (UK)

Connexins (Cx) are members of a highly related protein-family which form gap junctions. Connexin composition which varies between tissues and during development, influences the physiological properties of the channels. Intercellular communication via gap junctions is known to play a role during development, cell growth and wound healing which all correlate with changes of connexin expression patterns and levels.
In the inner ear of mammals and birds, gap junctions are present between supporting cells; no connexins are expressed in hair cells. Gap junctions may regulate the transfer of signals which coordinate repair processes in the inner ear and proliferation of supporting cells.
The role of gap junctions on repair and recovery mechanisms in sensory tissues of the inner ear, is being examined by comparing connexin expression following hair cell loss in the following model systems: gentamicin-treated organotypic cultures of (1) the basilar papilla and utricle from chicken hatchlings, and (2) the organ of Corti and utricle from neonatal mice; (3) the mature organ of Corti of mice in which hair cell loss has been induced by a systemic aminoglycoside-diuretic combination. The tissues are examined by immunohistochemistry and confocal microscopy.
Cx26, Cx30,and Cx43 have been immunohistochemically localized in vivo and in vitro in the avian auditory and vestibular epithelium as well in the neonatal mouse. However the specificity of the Cx26 and Cx30 labelling is being investigated due to sequence similarities with the avian Cx31. An antibody raised to a specific peptide sequence on the carboxy terminus of the avian Cx31 is currently being characterized. Changes in the pattern of Cx43 have been found in the sensory tissue of both the basilar papilla and utricle during the first week following hair cell loss.

16:30-16:35 ***Expression of aquaporin-2 and its regulation by vasopressin in the inner ear (P08)***
T. Takeda, S. Sawada, S. Takeuchi, H. Kitano, A. Kakigi
Nankoku (Japan)

Vasopressin is known to regulate aquaporin-2 (AQP2) in kidney collecting duct and thereby control water reabsorption. We already showed V2 receptor was expressed in the inner ear and its regulation. We hypothesized vasopressin has important function for water homeostasis in the membranous labyrinth and relates to endolymphatic hydrops. To investigate roles of vasopressin in the inner ear, We studied expression of AQP2 mRNA and its regulation by vasopression in the inner ear. Wistar rats were used. Two animals (four ears) were dissected and the inner ear tissue (whole cochlea), the endolymphatic sac and kidney were removed. The mRNAs were isolated and reversetranscripted. PCR were performed using specific primer pairs for 40 cycles. Expected band by electrophoresis were shown in all three tissues and we confirmed the sequence of PCR products. To investigate the regulation of AQP2, vasopressin 20µg were administered and quantified mRNA levels using real-time PCR (LightCycler, Roche). AQP2 mRNA levels (AQP2/ß-actin ratio) in kidney had increased by 229±16%. In the inner ear and the endolymphatic sac, mRNA levels had also increased by 198±28% and 220±20% respectively. We showed the expression of AQP2 mRNA in the inner ear and the endolymphatic sac and its up-regulation by vasopressin. This results suggest AQP2 has an important role in water regulation in the inner ear similar to kidney.

16:35-16:40 ***Noise trauma in carriers of a guinea pig strain with hereditary hearing loss (P09)***
A. Skjonsberg, P. Herrlin, A.C. Johnsson, M. Ulfendhal
Stockholm (Sweden)

Noise induced hearing loss is the most common cause of auditory impairment in humans. However, great variability of the hearing loss is found among individuals exposed to the same sound levels. There are many reasons for this variability, influence of age and other risk factors etc, but also different genetic predispositions have been discussed. It is still very unclear how unexpressed hereditary hearing deficits influence the susceptibility to ototraumatic agents such as noise. The aim of this study was to investigate how the hereditary deficit in the German waltzing guinea pig affected the response to noise exposure. The German waltzing guinea pig is a new strain of animals expressing recessive hereditary inner ear degeneration (Ernstson and Ulfendahl, in prep.). In the homozygotes, which are deaf at birth, the Reissner´s membrane has collapsed upon the organ of Corti, resulting in a complete loss of scala media. The heterozygotes or carriers are symptom-free at birth but have been reported to express elevated reflex thresholds with increasing age (Ernstson & Ulfendahl, 1998).
Twenty-four animals were tested, 12 carriers of the waltzing guinea pig

16:40-16:45 ***Aquaporin-2 in Meniere's Disease (P10)***
A. K. Lalwani, I. Chiappini, G. Bolasco, S. Monini, M. Barbara, R. Filipo
San Francisco (USA), Rome (Italy)

Several studies have been carried out having as objective the localisation of mutations which may predispose to Meniere's disease (MD). Our study was focused on the observation of the aquaporin-2 (AQP2) gene. This gene has been supposed to be one of the genetic factors contributing to development of metabolic mechanisms within the inner ear. Aqp2 is one of the several different water channels, that represent critical components in the osmotic regulation. Dysfunction of these proteins is associated with fluid and electrolyte disturbance in the body, including inner ear disease. Therefore, the possibility of an AQP2 mutation could be considered in patients with MD symptoms. We report the genetic analysis of 17 patients diagnosed as MD according to the AAO-HNS guidelines criteria. After completion of a informed consent form, blood was collected from these patients and DNA extracted and stored. The AQP2 exons from patient's genomic DNA were amplified by the PCR using two different programs following the difference among the Tm of these primers (primers 1,2 and 4 Tm = 66 C, primers 3 Tm = 60 C). PCR products were analyzed. We have obtained products in 12 DNA samples. All coding exons were purified and sequensed. The sequences were analyzed to find out a mutation on AQP2 gene as a possible responsible to MD. All the exons were analyzed with care for exon 3 (position 559) and exon 4 (position 646). In fact mutations in these positions were found in patients with diabets. No AQP2 mutation was found in any of the samples analysed.

MONDAY 3 SEPTEMBER, 2001
morning session

• *Clinical Implications Of Inner Ear Research*

09:20-09:30 ***Cochlear electrical stimulation: Influence of age of implantation on Fos immunocytochemical reactions in inferior colliculi and dorsal cochlear nuclei of the rat (O26)***
W.-C. Hsu, H.-C. Wu, E. Lecain, P. Tran Ba Huy
Paris (France)

The influence of age at the time of implantation of a stimulating electrode unilaterally in the inner ear on central auditory pathways was investigated in rats deafened shortly after birth.
Immunoreactivity for *Fos* served as a functional marker of neuronal activity. Electrodes were implanted in the left cochlea of rats aged 3 weeks or 4 months. Stimulation lasted 45 min, then rats were sacrificed and tissues processed for immunocytochemistry. The younger animals showed significantly more neurons with *Fos* immunoreactivity bilaterally in the dorsal cochlear nuclei (DCN) and inferior colliculi (IC) than the older rats or control animals with normal hearing receiving the same stimulation. Activity was more prominent in the left DCN and right IC. The results show that electrical stimulation of the inner ear is more effective in younger animals in eliciting gene expression associated with development of a functional network in the auditory pathways. This suggests that deaf children should be provided with cochlear implants as early as possible.

09:30-09:40 ***Virtual Reality: Acute unilateral peripheral vestibulopathy and video-oculography (O27)***
M. Barbieri, M. Chiarlone, F. Mora, M. Bavazzano, R. Mora
Genova (Italy)

One new methodical experimental in the study of the Optocinetic Nystagmus is the one concerning the application of the virtual reality to video-oculography. With such instrumental application we want to study those ocular visual origin movements concerning the system of Smooth Pursuit from which for prolonged and repeated stimulations a two-phase movement gives rise to a direction and a slow pursuit of the aim in the opposite direction like the Vestibular Nystagmus known as Optickinetic Nystagmus. An Oculography is executed by infrareds with post elaboration of the image. It is an implementation of an algorithm of filter of the image recognition photograms. Algorithm means cleaning up from the sequence of the images not valid photograms due to the involuntary movement of the eyelids. Besides, algorithm means recognition of the sequence if the pupil is present. Mediating the position as a software of analysis and recognition of the images is possible to study visuo-graphically the nystagmus. The acquisition of such-vestibulo-ocular phenomenon is based on the position of two telecameras to infrareds, each of which is connected to an acquisition card mounted on a helmet for videogames. The patient through liquid crystals visors of the helmet observes a visual situation established by the alternation of fluent white and black bands to variable speed like Barany's drum.
Acute unilateral peripheral vestibulopathy is a severe sudden vertigo which often occurs during the night and may gradually increase over several hours and persist more than 24 hours. For a correct diagnosis it is necessary, besides careful anamnesis and otoneurologic check-up, the use of diagnostic manoeuvres and instrumental tests as galvanic test, caloric test and the examination of subjective visual vertical. We use an helmet head mounted display linked to a personal computer. This Head Mounted Display uses two visors with liquid crystals mounted on a band support very similar to that used on Clar photophore. The Head Tracker is positioned at the nape of neck. The system permits binocular vision on two screens and is comfortable even if the patient wears glasses. It is requested of observing to the patient sitting on a rigid support and with the look turned towards in front at the beginning by appropriate helmet a horizontal white line put on the centre of a black field of vision. The centre of field of vision consists of the plane of absolute horizon, around to it the line of reference goes around in accordance with movements carried out by patient. After 20 seconds the informatic system shows the width of angle expressed in degrees between the final position of line and horizontal line passing by zero. We have obtained a range of normality between 1% and 26%. The study of the subjective visual horizontal by VR has shown in 100% of cases values out of normal range.In patients with left acute Monolateral Peripheral Vestibular Dysfunction, it has got a positive final angular value as result of a head final deviation towards left, confirming data from caloric test and from the line of Maddox. These homogeneous results have been obtained also for those patients with right acute Monolateral Peripheral Vestibular Dysfunction in which the final negative angular value, as result of a head final deviation towards right, was in harmony with data of traditional methods. Using traditional methods it has been able to verify that normal subjects can single out in detail the real horizontal a contrary to patients with Monolateral Peripheral Vestibular Dysfunction who shows a visual horizontal deviation, the more important the more quickly lesion has been determined.

09:40-09:50 ***Estrogen and hearing - is there a correlation? (O28)***
E.A. Stenberg, R. Simonoska, M. Hultcrantz
Stockholm (Sweden)

Estrogen - the female sex hormone - is classically known to influence growth, differentiation and function of the reproductive tract in both females and males. In recent years it has also been established that estrogens also are important in the maintenance of the skeleton and in the cardiovascular system, where estrogens have certain protective effects. In the brain estrogens affects both the activity and the connectivity of specific neuronal populations. There are indications that estrogens may have an effect on the ear and hearing, but the relationship is not fully investigated. When comparing the hearing of elderly males and females of the normal population, there is a gender difference with a generally poorer hearing of the males than that of the females. There are also well-documented differences in auditory brainstem responses, with shorter latencies in women. Estrogen receptors exist in an a and a b ?form, and both have been shown to be present in the inner ear of normal mice and rat.
In this study immunohistochemistry was used to visualize estrogen receptor a and b in human inner ear (adult and fetal). The morphology of an estrogen receptor b knock-out mouse was also investigated. Estrogen receptor a was present in the spiral ganglion and estrogen receptor b was present in the stria vascularis in the human specimens. The possible influence of estrogen on the hearing will be discussed.

09:50-10:00 Penetrance of hearing loss for patients with Waardenburg syndrome type 1 (O29)

T.G. Markova, S.M. Megrelishvily, N.G. Zaitseva
St. Petersburgh (Russia)

Fifty years ago, P. Waardenburg confirmed that hearing impairment and pigmentary disturbance inherited together and it was not a chance coincidence. The most essential factors in being able to give an accurate estimate of chance of recurrence is accurate diagnosis, knowledge about proper manifestations and penetrance of the main symptoms. Further studies tended to emphasize variable expression of the Waardenburg gene that means that there is a wide range in the degree of hearing loss, from profound deafness to milder hearing loss to unilateral loss to normal hearing. Ten families in which there were 33 individuals affected by WS1 were examined for penetrance of sensorineural hearing loss and expressivity of the gene. According our results all affected WS1 person including those with PAX3 mutations have congenital bilateral or unilateral profound hearing loss, non-progressive. The degree of sensorineural hearing loss was not very variable in expressivity within and between families whereas all hearing affected individuals suffered from congenital profound deafness. Penetrance of sensorineural hearing loss was calculated after exclusion of the probands. A unilateral sensorineural hearing loss was present more frequently (16.7%) then bilateral (8.3%) among affected relatives. Interestingly that two probands with unilateral deafness have had history of respiratory diseases and otitis with aminoglycoside treatment while other ones with bilateral hearing loss grew up without any diseases and their mothers did not have problems during pregnancy. All those children have mutations in PAX3 gene. Hereby nor slight nor late onset have been observed. It means that susceptibility to hearing loss may arise in the early embryo or further at least in early postnatal period and appear after epigenetic influence. Nevertheless we are still unable to predict hearing loss, one of the symptoms that mostly disturb parents, because of it is not clear what accounts for the reduced penetrance of deafness.
Further studies of the role of PAX3 during embryogenesis nay reveal the mechanism by which different mutation types result in different risks for hearing and pigmentary disturbances. Also different epigenetic events during development may be the factors that determine whether a person with PAX3 mutation will be congenitally deaf or not. Alternatively, genetic background or non-random environmental factors or both may be significant.

10:00-10:10 Treatment of sudden hearing loss with recombinant tissutal plasminogen activator (O30)

A. Sovatzis, R. Mora, M. Barbieri, F. Mora
Genova (Italy)

The responsible factors of sudden hearing loss can be, as known, of different origin, but all these etiologic agents meet as one pathogenic, microthrombotic mechanism. Great interest has arisen in the past few years in the second-generation thrombolytic agents. We have decided to experiment in our patients a recombinant glycoprotein analogous tPA (tissutal plasminogen activator) able to promote the transformation of plasminogen soaked up a thrombus of fibrin into plasmin (Banyai L et al., 1983). The plasmin degrades the fibrin in small flakes that are then removed by the monocytes and macrophages scavenger system. Although plasmin can also degrade the fibrinogen, its release is localised as tPA and some forms of factor urinary plasminogen activator activate the precursor more effectively when it soaked up by a fibrin thrombus, other mechanisms are able to neutralise the plasmin freely circulating (Radcliffe & Heinze T, 1981). Rt-PA is indicated in the therapy of myocardial infarction (preinfarction, angina, venous thrombosis, pneumonic embolism and thromboembolic occlusions). The bleeding incidents are the more remarkable collateral effect of this thrombolytic therapy. A more serious side effect of the thrombolytic therapy is intracranial bleeding, the most dreaded risk of this therapy for acute myocardial infarction because of the high mortality and disability rates associated with this complication. Brain structural lesions may predispose a patient to bleeding and cerebral aneurysms should be considered as a possible contributing factor to bleeding after thrombolytic therapy (Lagares A et al., 1999). Intracerebral bleeding after combined thrombolytic and antithrombolytic therapy may be associated with cerebral amyloid angiopathy and other vascular lesions (Sloan MA et al., 1995). Such accidents lose their importance if compared to the benefits induced from therapy in terms of saved life. The contraindications to the use of rt-PA are coagulation

modifications, anticoagulant oral therapy, recent serious bleeding, history of brain haemorrhage, central nervous system damage, haemorrhagic retinopathy, gestation, childbirth nursing, severe arterial hypertension bacterial endocarditis, pericarditis, acute pancreatitis, ulcerous illness of gastro-enteric and urinary system, in the last three months, neoplasia, surgical operation, cirrhosis liver failure (Sloan MA et al., 1995).
We have studied 40 subjects who reported a sudden hearing loss at the first ENT visit. Te audiometric examination and the acoustic otoemissions confirmed the evidence of+a sensoneurinal deficit. They didn't show any modifications of the waves at the ABR. Te patients were subdivided in two groups: group A and group B. During the recovery in our clinic, the 20 patients of group A were treated with the traditional endovenous therapy. Instead the 20 patients of group B were submitted to the treatment with the rt-PA: we used a dosage of 1.5 mg of rt-pa in 100 ml of physiological saline, twice a day. Every day we submitted the patients to an audiometric examination. At the term of treatment, we have repeated the audiometric examination and the acoustic otoemisions. In the group A, we noted an improvement of the auditory threshold only in 4 patients (20%), without a significative improvement of the subjective symptomatology. In the group B, 15 patients (75%) reported not only a regression of their symptoms, but an improvement of the audiometric examination and the acoustic otoemissions also. In fact, the waves were transformed from fail to pass.
As mentioned earlier, all the etiologic factors responsible for sudden hearing loss meet as microthrombotic pathogenic mechanism. For this reason we thought to appraise the effectiveness of a medicine of fibrinolytic type (rt-PA) for the treatment of the sudden hearing loss. At the present moment there is no literature regarding the treatment of sudden hearing loss with rt-PA, although a number of studies have been done using heparin, steroids and thrombolytic drugs. Heparin improves microcirculation of the inner ear and could be a new and effective method for the treatment of sudden hearing loss (WALCH C et al., 1996) The 1st generation thrombolytic drugs, such as Urokinase and Streptokinase, in comparison to rt-PA, are known to be less effective, have a longer half-life and a higher rate of incidents (HAGEN R. et al., l991). It is our intention, stimulated by the hearing test data observed in our patients, to evaluate a new therapeutic protocol for sudden hearing loss. The choice of the rt-PA is also based on the probable thrombotic aetiology of sudden hearing loss and on the data of relative literature to other non-traditional drugs experimented in the therapy of the sudden hearing loss.
Human studies have to follow and be done parallel to studies on the effects of the drug on the auditory cells in vitro and the evaluation of its effects on animals. The dosage administered should be notably inferior to that normally used for acute myocardial infarction cases, thus avoiding collateral effects.

10:10-10:20 ***A new therapy for tinnitus (O31)***
M. Barbieri, T.J. Yoo, G. Cordone, A. Salami, B. Jankowska, F. Mora, A. Sovatzis, R. Mora
Genova (Italy), Memphis (USA)

In our study we have used a recombinant glycoprotein analogous tPA (tissutal plasminogen activator) able to promote the transformation of plasminogen soaked up a thrombus of fibrin into plasmin. The contraindications to the use of rt-PA are: coagulation modifications; anticoagulant oral therapy; recent serious bleeding; history of brain haemorrhage; central nervous system damage; haemorrhagic retinopathy; gestation; childbirth; nursing; severe arterial hypertension; bacterial endocarditis; pericarditis; acute pancreatitis; ulcerous illness of gastro-enteric and urinary system in the last three months; neoplasia; surgical+operation; cirrhosis liver failure.In our ENT department, we have introduced in our experimental study, 60 patients of age included between 25 and 70 years, and both sexes. Te subjects were complaining at the first examination about a tinnitus risen for at least 15 days. Te patients were submitted to an audiometric examination, otoacoustic emissions, ABR and eventually to an encephalic RM. Every subject was evaluated the grade of his disease by a subjective scale of evaluation: the range of evaluation was from 0 to 4. In particular: no disease; occasional diseaese; little constant disease; big diseaese; unbearable diseaese. We have randomly subdivided the patients in two groups: the subjects belonging to group A have been submitted to the traditional pharmacological therapy (vasodilatators, steroids and vitamins). Instead, the patients belonging to the group B have been treated for 15 days with rt-PA. We used a dose of 3 mg of rt-PA in 100 ml of physiological saline, twice a day. During the hospitalisation the patients were further submitted to audiometric tests and OAE. At the end of the treatment all the patients were again submitted to the audiometric examination, otoacoustic emissions and the subjective score scale.In the group A we have only found an improvement of the subjective symptomatology. In fact the score from 3% became 2%. We didn't see any change of the audiometric examination neither the otoacoustic emissions. In all the patients of group B we have seen an improvement of the audiometric threshold of about 10 dB and in the 40%, the otoacoustic emissions from fail became pass. The symptomatology improved from 3% scores to 1%.

• *Development & Regeneration*

11:00-11:10 ***Inner ear abnormalities in Igf-1 mouse mutants (O32)***
I. Varela-Nieto, G. Camarero, M. Angeles Villar, J. Contreras
Madrid (Spain)

Insulin-like growth factor IGF modulates inner ear cell proliferation, differentiation and survival in culture. Its function in human hearing was first evidenced by a report of a boy with a homozygous deletion of the Igf-1 gene who showed severe sensorineural deafness (Woods et al. 1996). To better understand the in vivo role of IGF1 during inner ear differentiation and maturation, we analyzed cochleae of Igf 1 gene knockout mice at postnatal days (P5, 8 and 20) using stereological methods and immunohistochemistry. Mutant mice showed reduction in size of the cochlea and cochlear ganglion, although less severe than whole body dwarfism. An immature tectorial membrane and a significant decrease in the number and size of auditory neurons were evident at P20. IGF-1-deficient cochlear neurons showed increased caspase-3-mediated apoptosis along with aberrant expression of the early neural markers nestin vimentin and Islet. Cochlear ganglion and fibers innervating the sensory cells of the organ of Corti, presented increased expression of vimentin, while levels of neurofilament and myelin were decreased in P20 mouse mutants. In addition, an abnormal synaptophysin staining of the somata of cochlear ganglion neurons and sensory hair cells suggested the persistence of an immature pattern of synapses distribution in the organ of Corti of these animals. These results demonstrate that lack of IGF 1 in mice severely affects postnatal survival, growth, differentiation and maturation of the cochlear ganglion cells and causes abnormal innervation of the sensory cells in the organ of Corti.

11:10-11:20 ***Targeted disruption of mammalian hairy and Enhancer of split homologues 1 and 5 (Hes1 and Hes5) leads to upregulation of a bHLH factor (Math1) and formation of supernumerary inner ear hair cells (O33)***

F. De Ribaupierre, R. Kageyama, F. Guillemot, A. Zine
Lausanne (Switzerland), Kyoto (Japan)

The bHLH genes Hes1 and Hes5 (mammalian hairy and Enhancer-of-split homologues) can influence cell fate determination by acting as negative regulators to inhibit the action of bHLH positive regulators during the CNS development. It has been shown that a bHLH transcription factor Math1, mammalian homologue of Drosophila atonal is a positive regulator of inner ear hair cell differentiation (Bermingham et al., 1999; Zheng and Gao, 2000). We show by using RT-PCR analysis that Hes1, Hes5 and Math1 are expressed in the developing mouse cochleae. In situ hybridization revealed a widespread expression of Hes1 in the greater epithelial ridge (GER) and in lesser epithelial ridge (LER) regions. Hes5 is predominantly expressed in the LER, supporting cells and in a narrow band of cells within the GER. Examination of cochleae from Hes1-/- mice showed a significant increase in the number of IHCs, while cochleae from Hes5-/- mice showed a significant increase in the number of OHCs. In the vestibule, targeted deletion of Hes1 and to a lesser extent Hes5 lead to formation of supernumerary hair cells in the saccule and utricle. These defects in the patterning of hair cells were more severe in Hes1-/-;Hes5+/- and Hes1+/-;Hes5-/- backgrounds. The supernumerary hair cells in the mutant mice showed an upregulation of Math1. These data indicate that Hes1 and Hes5 participate together for the control of inner ear hair cell production, likely through the negative regulation of Math1.

11:20-11:30 ***Hensen's cells acquire specific hair cell markers in vitro, strengthening their role as precursors of supernumerary OHCs (O34)***

P.P. Lefebvre, B. Malgrange, M. Thiry, T.R. Van de Water, L. Nguyen, G. Moonen
Liege (Belgium)

During development, the normal production of hair cells is complete by embryonic day 16. When E19 organ of Corti explants are cultured, supernumerary OHCs are produced, without any additional cell proliferation. In this study, we identify the cells within the cochlea that retain the capacity to differentiate into OHCs. Quantitative analysis of cell types present demonstrates that, when the number of OHCs increases in organ of Corti explants: 1) the total number of cells remains constant; 2) the number of Deiters' cells increases; 3) the number of tectal cells decreases, and 4) the number of Hensen's cells decreases.

Myosin VIIa has been reported to initiate its expression in HCs of E14 mouse otocysts and corresponding to rat E16.5 inner ears. In E19 rat organ of Corti explants, either at time of excision or after 5DIV myosin VIIa immunolabel, was highly specific to HCs including the supernumerary OHCs that differentiate in the explants. The other markers, i.e. Jag2, Math1 and actin, have been shown to be expressed in inner ear HCs as early as E17 in mice (i.e. E20 in the rat inner ear). Double immunolabelling for myosinVIIA / Jag2 , myosinVIIA / Math1 and myosin VIIA/ phalloidine of E19 rat organ of Corti at time of excision revealed that myosin VIIa was present in all auditory HCs. A weak expression for Jag2 was observed and restricted to HCs, while Math1 did not immunostain the sensory epithelium . All HCs, including supernumerary OHCs, were strongly immunopositive for presence of Jag2 and Math1 in the E19, 5DIV explants. In these 5DIV explants, the Jag2 and Math1 antibodies also immunostained both tectal cells and Hensen's cells. Math1 immunopositivity is detected earlier than Jag2 immunolabelling in these cells, respectively after 2 and 5DIV respectively. Math 1 immunoreactivity decreased but was still present in Hensen's cells after 5 DIV. Tectal cells and Hensen's cells never immunostained for presence of myosin VIIA. Staining of actin by FITC-phalloidin does not clearly identify OHCs in E19 explants at time of excision. When phalloidin labelling of actin was performed on E19 explants after 5 DIV, both the normal configuration of HCs and all rows of supernumerary OHCs were stained, with the exception of the most distal row of supernumerary OHCs, suggesting that the last row of supernumerary OHCs

comprises the most immature of these newly differentiated cells.The results of this study suggest that Hensen's cells retain the capacity to differentiate into tectal cells, which differentiate into OHCs.

11:30-11:40 Antimasking effect of sound-evoked efferent activity on the aged guinea pig (O35)

G. Attanasio, M. Barbara, R. Filipo
Rome (Italy)

One of the most complaints of aged individuals with presbyacusis is difficulty understanding speech, even in quiet. The decline of speech understanding has been attributed to peripheral and central degenerative changes that affect abilities in frequency analysis, temporal processing, and cognitive processing. The purpose of this study is to describe the antimasking effects of sound evoked efferent activity on the CAP of guinea pigs and to compare the strenght of these effects as a function of age.
All the animals were implanted with permanent electrode on the round window and the CAP amplitudes were recorded in quiet and with simultaneous masking noise. To half animals the crossed olivo-cochlear bundle (COCB) was severed at level of the floor of the forth ventricle, while to the remaining guinea pigs a vestibular neurectomy was performed in order to interrupt the entire OCB. After three weeks resting the contralateral sound enhancement of the CAP amplitudes obtained from adult guinea pigs were compared to those from guinea pigs over 3 years old. The results of the present study suggest that the antimasking effect can be explained on the basis of activation of the one-third of total MOC neurons projecting from ipsilateral olivary complex. The antimasking effect seems to disappeare with aging, even with no change in distribution of the efferent fibers along the organ of Corti.

11:40-11:50 Expression and localization of prestin and the sugar-transporter Glut-5 during development of electromotility in cochlear outer hair cells (O36)

I.A. Belyantseva, H.J. Adler, G.I. Frolenkov, B. Kachar
Bethesda (USA)

Electromotility, i.e. the ability of cochlear outer hair cells (OHCs) to contract and elongate at acoustic frequencies, is presumed to depend on the voltage-driven conformational changes of "motor" proteins present in the OHC lateral plasma membrane. Recently, two membrane proteins have been proposed as candidates for the OHC motor. A sugar transporter, GLUT-5, was proposed based on its localization in the OHCs and on the observation that sugar transport alters the voltage sensitivity of the OHC motor mechanism. Another candidate, "prestin", was identified from a subtracted OHC cDNA library and shown to impart voltage driven shape changes to transfected cultured cells. We used antibodies specific for these two proteins to show that they are highly expressed in the lateral membrane of OHCs. We also compared the postnatal expression patterns of these proteins with the development of electromotility in OHCs of the apical turn of the rat organ of Corti. The patch-clamp recording of transient charge movement associated with electromotility indicates that half of the maximal expression of the motor protein occurs at postnatal day 9. Prestin incorporation in the plasma membrane begins from postnatal day 0 and increases progressively in a time course coinciding with that of electromotility. GLUT-5 is not incorporated into the lateral plasma membrane of apical OHCs until postnatal day 15. Our results suggest that while GLUT-5 may be involved in the control of electromotility, prestin is likely to be a fundamental component of the OHC membrane motor mechanism.

11:50-12:00 Cortical processing of vestibular sensation and its habituation: a PET study (O37)

Y. Naito, I. Tateya, S. Hirano, K. Funabiki, M. Inoue, J. Ito
Kyoto (Japan)

The purpose of this study is to analyze the central mechanisms of vestibular habituation by using functional brain imaging. We applied hot orcold air irrigation to the external auditory canal of 12 normal subjects by an air-caloric stimulator, and monitored eye movement using infrared CCD video system. Habituation of vestibulo-ocular reflex induced by repeated caloric stimulation was monitored, and changes in regional cerebral blood flow (rCBF) were measured by a PET (positron emission tomography) scanner. rCBF data were analyzed by SPM (Statistical Parametric Mapping) to identify the regions that are activated or deactivated significantly during vestibular stimulation and habituation. The average slow phase eye velocity (SPV) during caloric stimulation were; 7.4 degree/sec for the 1st irrigation, 6.3 degree/sec for the 2nd irrigation, 4.5 degree/sec for the 3rd irrigation and 4.3 degree/sec for the4th irrigation. The SPV decreased along with the repetition of caloric stimulation, and the SPV during the 3rd and the 4th irrigation was significantly lower than that during the first irrigation. We compared rCBFbetween the 1st and the 4th measurement. The regions that exhibited significantly lower rCBF during the 4th caloric stimulation, which were thought to be deactivated along with vestibular habituation, were the bilateral parieto-insular regions, the right precuneus (BA7), the motor area(BA4), the supplementary motor area (BA6), the left cingulate gyrus (BA24) ,the right inferior temporal gyrus (BA20) and the left cerebellar hemisphere.On the other hand, the regions that exhibited higher rCBF at the 4th caloric stimulation were the left anterior cingulate gyrus (BA24, 32), the right anterior cingulate gyrus (BA32), the right cingulate gyrus (BA29) and the right parahippocampal gyrus and the bilateral visual cortices (BA17,

18). Since the activation of the anterior cingulate gyrus and the visual cortex were associated with decrease in caloric response, it was suggested that these areas might be related to vestibular habituation.

12:00-12:10 ***Effects of an augmented acoustic environment on age-related reductions in distortion product otoacoustic emissions for three mouse strains (O38)***

G.K. Martin, C. Candreia, B.L. Lonsbury-Martin
Miami (USA)

Willott and Turner (1999) recently reported that an augmented acoustic environment (AAE) delayed age-related hearing loss (AHL) in C57BL/6J (C57)and DBA/2J (DBA) mice as measured behaviorally with either acoustic startleor prepulse inhibition, or electrophysiologically with the auditorybrainstem response (ABR). The present study in C57, DBA, and WB/ReJ (WB)mice determined if the effects of an AAE could be observed at the outer haircell (OHC) level by measuring distortion-product otoacoustic emissions (DPOAEs). WBs were purposely derived from parental stock that showed anatypical AHL pattern in which one ear displayed normal DPOAEs, whereas thecontralateral ear exhibited the characteristic age-related decrements inemissions. Beginning at 25 d of age, mice from all three strains wereexposed for 12 h nightly to a 70-dB SPL broadband AAE (200-ms pulses at2/s), centered at approximately 10 kHz. Age-matched control mice werehoused under similar conditions, but without the AAE. 2f1-f2 DPOAEs wererecorded monthly in the form of DP-grams, i.e., DPOAE levels as a functionof primary tone frequency, at the three primary-tone levels of L1=L2=55, 65,75 dB SPL, and at geometricñmean (GM) frequencies ranging from 5.6-48.5kHz(f2=6.3-54.2 kHz), in 0.1-oct steps. In the C57 mice, ABRs were also recorded at 6, 8, 12, 16, 24, and 32 kHz to confirm the presence of an AAE effect. For DBAs, due to the rapid onset of AHL, DPOAEs were measured onlyonce after 1 mo of AAE exposure. In these mice, no effects of the AAE weredetected. However, in the C57s, by 4 mo of age, clear differences betweenexperimental and control DPOAEs were observed. That is, control C57s showedaverage DPOAEs that were at noise-floor levels between GM frequencies from25-48.5 kHz, while the AAE ears exhibited moderately reduced DPOAEs over arestricted range of GM frequencies between 25-35 kHz. These differencespersisted until the final measurement age of 6 mo. Additionally, after 1 moof AAE exposure, the WBs also showed a tendency for the AAE to delay the AHLtypically exhibited by this strain, although this effect was notstatistically significant. These findings, in general, indicate thatexposure to an AAE can retard the loss of OHC function associated with AHLin some mouse strains.

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MONDAY 3 SEPTEMBER, 2001
afternoon session

- ***APOPTOSIS AND CELL DEATH***

14:15-14:25 ***Apoptosis---An important death pathway for noise induced cochlear lesion (O39)***
T.M. Nicotera, D. Henderson, B.H. Hu
Buffalo (USA)

One of the major consequences of exposure to intense noise is a loss of hair cells (HCs) in cochleas. Here we report involvement of apoptotic mechanisms in the progression of HC death in the chinchilla cochlea. Chinchillas were exposed to a 4 kHz narrow band noise at 105 dB SPL for 1 hour and sacrificed at various times after the exposure. Three questions regarding the death pathway of HCs are addressed. First, what is the driving force for the continued HC death after exposure to a traumatic noise? Morphological examination of propidium iodide-stained HC nuclei from the cochleas examined at three time intervals after the exposure (30 minutes, 6 and 48 hours) revealed nuclear changes consistent with both apoptosis and necrosis. However, the apoptotic indicators in the HCs predominated the post-exposure cochlear lesion. Examination of the OHC morphology clearly indicates that apoptosis is the primary cell death mechanism responsible for the progression of the cochlear lesion after noise exposure in the chinchilla cochleas. Secondly, what signaling pathways are activated that result in apoptosis? The activation of three distinct members of the caspase family was detected with carboxyfluorescein labeled fluoromethyl ketone peptide inhibitor of caspase. Caspase-3, a major executor of apoptosis, was activated in apoptotic HCs, but not in necrotic cells. Two of the upstream caspases, caspase-8 and caspase-9, were also activated in the apoptotic cells. Since caspase-8 and caspase-9 represent two different upstream signaling pathways, activation of both caspases suggests existence of the multiple pathways regulating apoptotic processes. Finally, which cellular structures are affected during early phases of OHC apoptosis? Activated caspase has been found to cleave many intracellular molecules. In this study, we examined the cleavage of F-actin, an important cytoskeletal protein, in the cochlear OHCs after noise exposure. The organs of Corti were double-stained using FITC-labeled phalloidin for F-actin and propidium iodide for OHC nuclei. Confocal microscopic examinations showed that the fluorescence associated with F-actin was decreased in the OHCs possessing condensed nuclei, but remained unchanged in the OHCs with swollen nuclei. The change in F-actin labeling occurred coordinately with the changes in the nuclear morphology of the apoptotic cells, which was prevented by administration of caspase-3 inhibitor (Z-DEVD-FMK). However, the treatment did not affect nuclear breakdown. These results indicate that F-actin cleavage is an important early cellular event in apoptotic development in OHCs following exposure to traumatic noise.

14:25-14:35 ***A mouse experimental model for apoptosis of inner ear hair cells (O40)***
T. Nakagawa, N. Murai, T. Endo, T. Kim, N. Yamamoto, I. Tateya, J. Ito
Kyoto (Japan)

Apoptosis of inner ear hair cells has been investigated by use of guinea pigs and rats as experimental animals. However, a mouse model for apoptosis of hair cells is suitable for molecular analyses. Thus, we developed a mouse experimental model for apoptosis of hair cells.
ICR mice were used as experimental animals. Under general anesthesia, a retorauricular incision was made, and the tympanic bulla was exposed. A small hole was made in the tympanic bulla for exposure of the round window or cochlear basal turn. We used two methods for application of gentamicin into the inner ear, placement of gelfoam soaked with gentamicin solution on the round window membrane and direct injection of gentamicin solution after cochleostomy. The animals were sacrificed on the first, third and fifth postoperative days. The temporal bones were collected and were fixed with PFA. Cochleae and utricle were prepared for whole-mount staining, ampullar cristae were made into frozen sections. These specimens were stained by the TUNEL method and DAPI for evaluation of the occurrence of apoptosis.
Our surgical procedure required only 3-5 min for exposure of the cochlea. In animals applied through the round window membrane, apoptosis in hair cells was most frequently seen 5 day after treatment, especially in cochlear basal turn. In specimens treated with direct injection, most of apoptotic hair cells appeared 1 day after treatment. Ampullar cristae showed most severe degeneration.

This mouse model for hair cell apoptosis is useful for molecular analyses of the process of apoptosis and consequent regeneration.

14:35-14:45 ***The Pathway of Apoptosis in Mouse Hair Cells Induced by Aminoglycoside Toxicity (O41)***
N. Murai, T. Nakagawa, T. Endo, T. Kim, J. Ito
Kyoto (Japan)

Since Forge et al reported that apoptosis is involved in the mechanism of inner ear hair cell damages following administration of aminoglycosides, apoptosis of inner ear hair cells has received wide-spread attention in the field of inner ear biology. Although many of the researchers so far have used guinea pigs or rats as materials, it is now of great interest to utilize genetically manipulated mice mutant in, for example, genes related with cellular differentiation. Here, in order to establish an aminoglycoside ototoxicity model which can be applied to molecular researches utilizing genetically manipulated mice, we have investigated the pathway of apoptosis following local administration of aminoglycosides into the mouse inner ear.
ICR mice 10 to 20 weeks old with normal Preyer's reflex were used. Under general anesthesia, lateral wall of cochlea was exposed. After cochleostomy, 1 microliter of gentamycin sulfate solution (400 mg/ml) was microinjected into the endolymphatic space. One day after the operation, the animal was decapitated under deep general anesthesia, and temporal bone was removed. Crista ampullaris of the lateral semicircular canal was extracted and was fixed with 4% paraformaldehyde, and frozen sections were prepared for immunohistochemistry. Between apoptotic cells and normal hair cells, differences of the intracellular distribution of cytochrome-C and Bax, which are substances related to apoptosis cascade, was investigated. DAPI staining was performed to detect apoptotic cells.
In normal hair cells, cytochrome-C showed distribution similar to that of mitochondria, while Bax was faintly expressed in whole cytoplasm. On the other hand, in apoptotic hair cells, cytochrome-C was expressed in whole cytoplasm, while Bax showed a cluster-like distribution.
It has been reported that Bax, a bcl-2 family member, is localized to cytosol and that it translocates to mitochondria following such assaults causing cell damages as ischemia to cerebral neurons. The translocation of Bax is considered to activate terminal caspases by increasing permeability of mitochondrial membrane and resulting in releasing cytochrome-C through the membrane. Thus, Bax is known as a potent proapoptotic molecule. In the present study, apoptosis induced by aminoglycoside ototoxicity was related to the translocation of Bax and release of cytochrome-C from mitochondria. These results suggest that Bax mediates the pathway of mitochondrial apoptosis and that it plays an important role in aminoglycoside ototoxicity.

14:45-14:55 ***Over expression of bcl-2 in organ of Corti explants and cell cultures containing auditory neurons modulates oxidative stress induced sensory cell death (O42)***
H. Staecker, W. Liu, X. Wei, B. Malgrange, P. Lefebvre, T. Van De Water
Baltimore (USA), New York (USA), Liege (Belgium)

Apoptosis triggered by oxidative stress has been shown to play a significant role in several cochlear pathologies including ageing, sound trauma and ototoxicity. *Bcl-2* plays a key role in controlling the induction of apoptosis by protecting the mitochondrial viability. Using gene delivery techniques we have blocked the initiation of apoptosis by cisplatin and neomycin by overexpressing the anti-apoptotic protein *bcl-2* in *P3* and adult organ of Corti cultures. Effects of gene therapy mediated delivery of *bcl-2* was also assessed in dissociated *P3* auditory neuron cultures treated with cisplatin. Outcomes of transduction were measured by determining total hair cell and auditory neuron survival levels in cultures pretreated by transduction with either adenovirus *bcl-2* (*AV bcl-2*) or herpes virus *bcl-2* (*HSV bcl-2*) and challenging both control and transduced cultures with either cisplatin or neomycin. Overall over expression of *bcl-2* resulted in prevention of a significant degree of ototoxicity induced cell death prevented the initiation of neurotrophin withdrawal related neuronal death and prevented loss of the mitochondrial potential ψ.

POSTERS (11-26)

15:00-15:05 ***Spontaneous regeneration of hair cells after gentamicin insult in long time cultured postnatal mammalian rat utricular macula explants (P11)***
D. Berggren, M. Werner, W. Liu, D.A. Frenz, T.R. Van De Water
New York (USA), Umea (Sweden)

In the normal postnatal mammalian vestibular sensory epithelium renewal of hair cells is a very rare event but following ototoxic damage there is a hair cell renewal response with partial restoration of the vestibular sensory epithelium. There

is an ongoing debate about the actual mechanism that underlies the regeneration response of vestibular sensory epithelium after exposure to ototoxic drugs. It has been suggested that replacement hair cells can arise directly from non-mitotic transdifferentiation of support cells into new hair cells. In contrast, other studies have shown that a substantial number of hair cells can survive the gentamicin insult even when their stereocilia are lost indicating self-repair of damaged hair cells as an important contributor to mammalian vestibular hair cell renewal and recovery. We have developed an in vitro method for long-term culture of 4-day-old rat utricular maculae which differs from earlier described methods. This method is suitable for immunohistochemical studies as well as for LM-EM morphological studies. We will present our hair cell renewal results from long-time (4 weeks) cultured utricles that following exposure to genatmicin have been allowed to recover either with or without an antimitotic drug(aphidicolin). Whole mount utricles were immunostained with phalloidin-FITC to visualize stereociliary bundles. Loss of hair cell bundles was nearly complete three days after exposure to genatmicin. Renewal of hair cell bundles was abundant in cultures recovering in normal medium but also it did occur in the presence of the antimitotic drug. Our present conclusion is that the spontaneous renewal of hair cells after gentamicin insult to the utricular maculae includes mitotic events as well as processes without mitotic activity.

15:05-15:10 p27kip1 plays a role in degeneration of mouse vestibule after aminoglycoside treatment (P12)

T. Endo, T. Nakagawa, N. Murai, T. Kim, J. Ito
Kyoto (Japan)

P27 is known to play a certain role in regulation of cell proliferation in the inner ear during development.Recently, involvement of p27kip1 in degenerative process of chicken basilar papillae was reported.In the present study, we examined the expression of p27kip1 in the healthy normal vestibular epithelia and those damaged by aminoglycosides.
ICR mice 10 to 20 weeks old with normal Preyer's reflex were used. Undergeneral anesthesia, the lateral wall of the left cochlea was exposed. A small hole was opened in the bony wall of the cochlear lateral wall. Using a microsyringe, 1 microliter of gentamicin sulfate solution (400mg/ml) was introduced to the endolymphatic space. One day after the operation, the animal was decapitated and the temporal bone was removed.The right temporal bone was used as the control. Crista ampullaris of the lateral semicircular canal was extracted and fixated in 4% paraformaldehyde and frozen sections were prepared for immunohistochemistry. We performed immunostaining for p27kip1 and DAPI staining for detection of apoptotic cells.
In healthy matured vestibular epithelia p27Kip1 expression was found in supproting cell nuclei,not in hair cell nuclei.In damaged vestibular epithelia. apoptosis nuclei were observed in the hair cell layer. Some of supporting cells lacked p27Kip1 expression.In addition a few hair cells showed p27Kip1 expression.
These findings indicate that p27Kip1 plays a certain role in the degenerative process of vestibular epithelium following the aminoglycside treatment.

15:10-15:15 Local application of sodium thiosulfate prevents cisplatin-induced hearing loss (P13)

A. R. Fetoni, J. Wang, M. Guitoon, J. Galli, J.-L. Puel
Montpellier (France), Rome (Italy)

Cisplatin is extensively used in the cancer chemotherapy. In addition to its antitumour properties, this compound induced deafness and tinnitus. When given intraperitoneally sodium thiosulfate (STS) blocks both the ototoxicity and the antitumoral properties of cisplatin. Here, we develop a strategy to rescue hearing from cisplatin by using a local application of STS.
Cisplatin was administered (2 mg/kg, I.P.) for 5 days. Audiograms was performed from electrode implanted on the round window. The control animal received local a pplication of artificial perilymph via an osmotic minipump implanted in the right ear. The experimental animal received local application of STS (10mM). In all animals, the left ear served as to control. At the end of experiments, the animals were sacrificed, and the cochleas proceeded for morphological evaluation.
Cisplatin induced a high-frequency hearing loss of about 60 dB, due to a massive loss of outer hair cells in the cochlear basal turn. The control animals sustained a bilateral high-frequency hearing loss. In the experimental group, the ear treated with STS retained entirely normal hearing thresholds and no significant hair cell loss.
This suggests that local therapy could be a powerful strategy to protect the auditory function from cisplatin chemotherapy.

15:15-15:20 Extracellular ATP-induced nitric oxide production and intracellular Ca++ mobilization in type I spiral ganglion cells of the guinea pig cochlea (P14)

N. Harada, H. Cho, T. Yamashita
Kansai (Japan)

It has been suggested that nitric oxide (NO) plays a role in the auditory system. NO is synthesized by three isoforms of the enzyme nitric oxide synthase (NOS). The neuronal isoform (nNOS) and the endothelial isoform (eNOS), which are Ca++ dependent, have been detected in cochlear spiral ganglion cells. of the guinea-pig. However, the role of NO in

auditory signal transduction pathway still remain unknown. Here we investigated the effects of extracellular ATP on NO production in acutely isolated type I spiral ganglion cells (SGCs) of the guinea pig cochlea using NO-sensitive dye DAF-2. SGCs were loaded with BM DAF-2 DA for 60 min. Excitation filter and emission filter 540 nm were used respectively in the present study.
Extracellular ATP induced an increase of the fluorescence intensity time-dependently. This increase eas inhibited by a non specific NOS inhibitor of L-NAME. These results suggest that ATP can induce NO production in SGCs. Using the Ca++ sensitive dye FURA (L-NAME and Bromo cGMP) inhibited the increase of Ca++ induced by ATP in SGCs. ATP could also induce NO production and a Ca++ increase in one SGC. The present study is the first direct evidence of NO production induced by extracellular ATP in SGC. The ATP-induced NO production was accompanied by the Ca++ increase. These results suggest the possibility of cross-talk between NO and Ca++ in SGC.

15:20-15:25 Therapeutic efficacy of magnesium in the acoustic trauma (P15)
H. Haupt, F. Scheibe, L. Cherny
Berlin (Germany)

Using auditory brainstem response (ABR) audiometry, we have recently demonstrated in the guinea pig that post-exposure magnesium (Mg) treatment has the potential of significantly reducing impulse noise induced hearing loss. In the present study which relied on our standard therapy model, ABRs were compared with distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAPs) to obtain more information on the therapeutic efficacy of Mg in the acoustic trauma in terms of the extent and location of damage. Hair cell stereocilia were examined by scanning electron microscopy. The experiments were performed on anesthetized guinea pigs exposed to a selected 167 dB peak level (Leq,1s 127 dB) impulse noise series (1/s) for 4 min. After the exposure, the animals were injected either a dose of 0.29 mmol MgSO4 s.c./100g for 3 days or saline as a placebo. The Mg group received drinking water with an additive of 39 mmol MgCl2/l for one week and the placebo group tap water (0.43 mmol Mg/l) alone.
One week after the noise exposure, the hearing functions measured were significantly less affected in the Mg-treated animals than in the placebo group. Mg was found to be most effective on those frequencies at which the evoked potentials were most seriously impaired. The differences between the two experimental groups were most pronounced in the ABR permanent threshold shift (PTS) which amounted to 15-26 dB over the frequency range tested (0.5-32 kHz). The DPOAE measurements (f2 = 1.5-16 kHz) revealed differences between the two groups amounting to 7-19 dB in the PTS and to 2-13 dB in the reduction of amplitudes (L1/L2 = 70/60 dB), with the highest differences observed within the lower frequency range. The differences in the CAP threshold shifts which were evaluated by comparison of the ipsi- and contralateral ears (0.5-32 kHz) were 5-17 dB, and there was significance in the frequency range of up to 8 kHz. The morphological findings also revealed an Mg-related reduced susceptibility of hair cell stereocilia to impulse noise exposure. The CAP threshold shifts reflected the morphological damage most obviously. The relevant underlying protective mechanisms of Mg in acoustic trauma are discussed.

15:25-15:30 Age-related hearing loss after gentamicin treatment in patients with Meniere's disease (P16)
M. Hillerdal, U. Friberg, A. Svedberg
Uppsala (Sweden)

Forty-six patients with severe Meniere's disease were treated with gentamycin intratympanally. The mean age at onset of disease was 51 years (range 25-75) and at treatment 60 years (range 36-81). 22 patients were under the age of 60 and 24 patients were over the age of 60 at the time of treatment. As controls served 125 conservatively treated patients with Meniere's disease. Their mean age at onset of disease was 47 (range 16-76) and at a fictitious date of treatment 53 (range 29-79), with 82 patients > 60 years and 43 < 60 years. Gentamicin was injected intratympanally once daily 30 mg, during 2-3 consecutive days. Seven patients required 2 additional injections after a mean time of 6 months (2-23 months) and in 2 of these another 1-2 injections were given after 12 and 27 months. Evaluation of the treatment results was carried out according to the AAO-HNS guidelines. Student t-test was used for the statistical analysis.
After treatment, 12 patients (26%) had a loss > 10 dB in the speech frequency PTA, 32 patients (70 %) had no change and 2 patients (4%) had a hearing improvement. The mean hearing loss was 8 dB from 62 dB pre-treatment to 70 dB posttreatment. In the control group, 29 patients had a decreased hearing level (23%), 82 (66%) remained unchanged and in 14 (11%) hearing was improved. The mean hearing loss in the control group was 4 dB from 41 to 45 dB. The hearing loss in both groups was significant. Five patients (11%) in the gentamicin group lost more than 30 dB after treatment (range 37-64 dB) compared to 3 patients (25%, range 30-42 dB) in the control group. Four patients became deaf in the gentamicin group but none among the controls.
Hearing loss after gentamicin related to age
Patients older than 60 years had a significant hearing loss posttreatment, while younger patients had a hearing looss which was not significant compared to controls. Four of the five patients who had losses greater than 30 dB after genatmicin treatment were in the age-group > 60 years. Of the 3 controls who lost more than 30 dB, 2 were younger and 1 older than 60 years.
An increased sensitivity to the ototoxic effects of gentamicin was noted with increasing age in Meniere patients. This should be considered when treating older Meniere patients with gentamicin intratympanally.

15:30-15:35 ***Inducible nitric oxide synthase (iNOS) inhibitor prevents kanamycin-induced ototoxicity on the guinea-pig cochlea (P17)***

M. Izumikawa, M. Koneda, N. Harada, C. Himeno, M. Yagi, T. Doi, H. Kuriyama, T. Yamashita
Osaka (Japan)

We investigated the production of nitric oxide (NO) in the cochlea on the ototoxicity after kanamycin administration using the new fluorescence indicator, DAF-2DA (4,5-diaminofluorescein diacetate), for direct detection of NO. We also determined whether iNOS inhibitor, ONO 1714, administered chronically and directly into the left scala tympani via an osmotic pump, would lead to decrease NO production in the cochlea after aminoglycoside-induced cochlear insult. We observed the dramatic increase of NO production after kanamycin treatment compared to normal ABR control animals. Direct infusion of the iNOS inhibitor could suppress kanamycin-induced NO production. On ABR, the ears treated with the iNOS inhibitor showed significantly smaller threshold shifts than the contralateral ears at all frequencies. Histologically, the ears treated with the iNOS inhibitor showed significantly less outer hair cell loss than the contralateral ears.
Te present sudy shows the first direct evidence of NO production induced by kanamycin ototoxicity. Our results also suggest that iNOS is supposed to be concerned with the NO production following kanamycin treatment and the iNOS inhibitor can protect the cochlear function and morphology. These results indicate that NO must have a direct role on kanamycin-induced ototoxicity.

15:40-15:45 ***Self-defense against aminoglycoside ototoxicity in the guinea-pig (P18)***

J.A.A. Oliveira, D.M. Canedo, M. Rossato, M.H. De Andrade
Ribeiro-Preto (Brazil)

Amikacine is a semisynthetic aminoglycoside. It acts against most of the microbial species. Amikacin limitation of the therapeutic application is the ototoxicity which promotes permanent lesions in the cochlear system. Aminoglycoside antibiotics have ototoxic potential. The target cells are preferentially the outer hair cells in the cochlear basal turns. Aminoglycoside antibiotics can quelate iron forming a complex with oxidative properties and promotes the formation of free radicals responsible for production of lesions in the hair cells. The objective of the present investigation was to detrmine whether the use of aminoglycoside amikacin at small doses may lead to the occurrence of some types of resistance to or protection against ototoxicity of the drug by analizing lesions to the organ of Corti bu scanning electron microscopy. The sudy was conducted on 31 guinea-pigs that were divided into 4 groups. Amikacon was administered intramuscular.
Histological studies were performed by scanning electron microscopy (Jeol-JMS. Three cochleae were discarded.
Group A and B showed normal organs of Corti; group C presented total cellular damage to the outer hair cells in the two more basal turns and in the first and second rows of apical turns. Group D demonstrated marked otoprotection of the outer hair cells in two more basal turns in comparison of group C. Otoprotection in turn 1 was 56% of the cochlea and in turn 2 was 51% of the cochlea. Results were statistically significant. Based on the results of this research we may then propose that the hair cells were able to develop a mechanism of otoprotection during the application of the non-ototoxic stimulus which later permitted resistance to the damaging dose.

15:45-15:50 ***Selective aspects of human pathology in high-tone hearing loss of the aging inner ear (P19)***

A.W. Scholtz, K. Kammen-Jolly, R. Glueckert, B. Hussl, H. Rask-Andersen, A. Schrott-Fischer
Innsbruck (Austria), Uppsala (Sweden)

It is believed that as individual's age increases, the thresholds for high tone frequency sound may concordantly increase and coactively result in a progressive hearing loss described as presbyacusis. Based on correlation between audiometric measures of aged patients and histologic findings garnered from post-mortem examinations, four types of presbyacusis have been characterized: sensori-neural, neural, strial and conductive (Schuknecht and Gacek, 1993). Otopathologic changes to the inner ear as a direct function of ag, however, remain controversial. The focus of this investigation involves the pathological impact on remaining sensory structures in patients having sensori-neural degeneration. The currect study presents 7 human temporal bones extracted from patients aged 53 to 67 years with high tone hearing loss and with no known history of extraordinary environmental events involving head or noise trauma, acoustic overstimulation, or ototoxicity. In previously published findings of these specimens, all but one temporal bone failed to demonstrate a meaningful correlation between audiometric measurements and loss of functional hair cell populations with secondary retrograde degeneration of nerve fibres. In all pathological specimens, the greatest incidence of degeneration was seen at the cuticular plate. Conclusively, our findings present three implications in the aging human cochlea: first, audiometric measures that represent a high-tone hearing loss may take various forms with respect to ultrastructural patterns of degeneration and surviving structures; secondly, the incidence of lipofuscin and lysosome granules does not correlate with the degree of hearing loss; theirdly, as shown only in guinea-pigs (Anniko, 1998), high tone hearing loss can be associated with deformation of the cuticular plate.

15:50-15:55 Optical imaging of glycinergic inhibition in the vestibular and cochlear nuclei (P20)

T. Doi, S.-M. Yang, M. Asako, T. Kaneko, S. Jing, A. Matsumoto-Ono, T. Yamashita
Osaka (Japan)

Glycine is considered to be a major inhibitory neurotransmitter in the cochlear nucleus (CN) and vestibular nucleus (VN). Using multiple-site optical recording technique coupled with voltage-sensitive dye, the spatiotemporal activity in neuron populations was observed in both the cochlear and vestibular nucleus in the newborn mouse brainstem after electrically stimulating the cut end of the vestibulocochlear nerve. In both the CN and VN, the optical responses were obviously enhanced by bath-applied strychnine (50 µM), which is a glycine receptor antagonist. An excitatory enhancement occurred in the central areas of the responses in CN and VN as well as at the edges of the response areas. Moreover, the enhancement of the central areas of the responses was clearly stronger than that at the edges of responses in both the CN and VN. Optical imaging enables us to visualize the spatiotemporal extent of the inhibitory receptive field after the application of strychnine. Our present data thus provides further evidence that glycine is an inhibitory neurotransmitter in the CN and VN in the newborn mouse brainstem.

15:55-16:00 Combined early effects of corticosterone CHALLENGE and endotoxin INFECTION on auditory sensitivity in male rats (P21)

P. Siaud, D. Maurel, M. Lucciano, Y. Cazals
Marseille (France)

The immune and endocrine corticotropic system are strongly reactive in the early phase of an integrated defense response triggered by an infectious aggression. The present study investigated whether this condition could affect the functioning of the cochlea. The Experiments were performed on conscious free-moving normal male rats. Some rats were adrenalectomized (ADX) and supplemented or not with corticosterone (Ci). The endotoxin lipopolysaccharide (LPS) was injected through an indwelling cannula in the right carotid. Auditory nerve acoustic sensitivity was measured through compound action potential (CAP) audiograms recorded from an electrode chronically implanted close to the auditory nerve. Animals were ramdomly divided in six groups: control (C), C + LPS, ADX, ADX + LPS, ADX + Ci and ADX + Ci + LPS. A first experiment examined C, ADX and ADX + Ci groups. In a second experiment on control animals, an LPS dose/effect relation was explored using LPS doses of 1, 25 and 250 µg/kg. In a third experiment combinations of ADX and ADX + Ci together with a 25 µg/kg LPS injection were investigated. Adrenalectomy was found to induce some improvement in cochlear acoustic sensitivity in control cochleas which was partially reversed by corticosterone supplementation. Endotoxin-challenge induced significant losses of auditory sensitivity, of up to about 20 dB, for the two higher LPS doses. Combination of adrenalectomy and endotoxin-challenge (at the dose of 25µg/kg) provoked a drastic loss in cochlear acoustic sensitivity which did not occur with corticosterone supplementation. These results indicate that corticosterone influences cochlear function in both a basal condition and in an inflammatory process. Effects of LPS on cochlear function most certainly act through generalized overshoot in inflammatory processes, and in this pathological condition corticosterone protected the cochlea.

16:00-16:05 Characteristics and aetiology of tinnitus. A study in 123 patients (P22)

C. Nicolas-Puel, R. Lloyd Faulconbridge, M. Guitton, J.-L. Puel, M. Mondain, A. Uziel
Montpellier (France)

Tinnitus is generally classified into objective or subjective. However, this classification is of little use in clinical practice because the incidence of objective tinnitus is extremely low. Moreover, because this definition exists, there is little stimulus to further evaluate clinical characteristics of tinnitus. There are many descriptive studies of tinnitus but few which are quantitative and so the aim of this study was to highlight the clinical characteristics of tinnitus and to attempt a quantitative assessment in relation to any underlying aetiologies. A study was undertaken on a population of 123 patients attending a tinnitus clinic between 1998 and 2000. A questionnaire allowed detailed evaluation of the characteristics of tinnitus, including such variables as the circumstances in which the tinnitus was first noticed and evaluation of its intensity and frequency. A full neurootological examination was performed with the aim of diagnosing an aetiology. Types of tinnitus were correlated with the aetiology of the underlying deafness. 81% of tinnitus patients had an endocochlear deafness and of these acoustic trauma, endolymphatic hydrops and presbyacusis were the commonest diagnoses, 25%, 32% and 23%, respectively. From the history, 100% of the patients with noise trauma and 91% with presbyacusis described their tinnitus as a stable, high-pitched whistle. Those patients with active Meniere's disease or Menier-like syndrome described a fluctuant low-pitched buzzing tinnitus. Analysis of those patients with a stable high-pitched tinnitus associated with a high-frequency hearing loss shows a statistically significant correlation between the elevation of the audiometric threshold and the loudness of the tinnitus. In addition, to better characterising tinnitus, this clinically-based approach should provide significant progress in understanding the fundamental mechanisms of tinnitus and in the evaluation of any treatment.

16:05-16:10 Short-term effects of simulated microgravity on auditory sensitivity in the rat in relation to intracerebroventricular pressure (P23)

D.L. Mauriel, P. Siaud, M. Lucciano and Y. Cazals
Marseille (France)

Physiological effects of microgravity can be well simulated using the hindlimb supension model in the rat. In previous experiments (Maurel et al. 1996) we showed a transient increase in intracerebroventricular pressure (Picv) from 10 to 60 minutes after suspension. Other experiments in the guinea pig have shown that increasing pressure in the inner ear induces transient losses of cochlear sensitivity (Horner and Cazals 1991). In the present study cochlear nerve acoustic sensitivity was examined after hindlimb suspension in conscious free-moving rats chronically implanted with an electrode close to the auditory nerve. Compound action potential (CAP) audiograms were taken daily during three weeks without suspension for control, and at 10, 30, 60 minutes and 24 hours after suspension. After suspension CAP audiograms showed a 10-25 dB loss of sensitivity from 0.5 to 4 kHz and above 32 kHz whereas around 8 kHz no change was observed. The results appear to divide rats in two groups, one group showing a greater loss being significant at 10 minutes and maximum between 30-60 minutes after suspension, after 24 hours the loss was reduced without complete return to normal however. These observed losses in cochlear acoustic sensitivity appear concomitant with previously documented variations of Picv which was found to increase 2.8-fold during the first 30 minutes, 2-fold during the next 30 minutes and return to normal after 24 hours. Rats of the other group presented smaller non significant losses and at the highest frequencies only. The variability between the two groups seems related to a better absolute sensitivity in pre-test audiograms in rats of the first group. Deleterious effects of increased intracochlear pressure have often been speculated whereas few evidences are available (e.g. Horner and Cazals 1991, Buki et al 2000, Flock and Flock 2000). Overall this model provides new experimental evidence relating increased pressure with moderate cochlear sensitivity losses affecting differently various frequencies. Simple mechanical alteration seems insufficient to explain frequency specificity and variability between individuals. In this experimental model, physiological stress responses affecting the corticotropic axis are known to occur, as diurnal alterations in corticosterone and ACTH rhythm and plasma levels (Assenmacher et al 1995) which certainly also play a significant role.

16:10-16:15 Release of TGF BETA 1 in a neurinoma cell Line (P24)

M.G. Valente, M. Bizzarri, F. Ronchetti, D. Bernardeschi, M. Finocchiaro, M. Barbara, R. Filipo
Rome (Italy)

TGF beta 1 can play a crucial role in the growth regulation of acustic neuroma. The release of the TGF beta 1 in a standardized serum free colture was investigated on a human neurinoma cell line.
A cell line was derived from a human acustic neuroma. The analysis of the TGF beta 1 released from the line was performed on the medium of serum free cultures by the ELISA method. The TGF beta 1 release was investigated at 24, 48, 72 and 96 hrs of incubation .
The data demonstrated:
a) a statistical significant increase ($p<0.001$) of TGF beta 1 release at 24 hrs of incubation as compared to negative control with medium alone being 0,142 + 0.01 and 0,005+0.001 U.A. respectively
b) a statistical significant increase ($p<0.001$) of TGF beta 1 release at 24 hrs of incubation as compared to release at 48, 72, 96 hrs
c) the release at 48, 72 and 96 hrs although demonstrating a costant decrease of TGF beta 1 showed a significant difference ($p<0.001$) as compared to negative control.
The data clearly demonstrate that neurinoma cell releases a high quantity of TGF beta 1 and the kinetic of TGF beta 1 release shows the highest value after 24 hrs and decreasing values later.
This studies on the kinetic of release of TGF beta 1 could be useful for a better understanding of tumor growth and for more efficient therapies planning.

16:15-16:20 Kinetics of the potassium induced cell shape changes in the OHCs of the guinea-pig (P25)

Z. Farkas, I. Sziklai
Debrecen (Hungary)

The phenomenon of slow shortening in the outer hair cells (OHCs) induced by potassium is well-known. The mechanism of this slow motile response is, however, still uncertain. Osmotic changes, increase of [Ca++]i , role of the sodium-calcium exchanger are reported to be involved in the process.
Present study examines time-related alterations of the motile response in different concentrations of potassium in the presence or absence of calcium.
Guinea pig OHCs from the cochlear apex were isolated by enzymatic treatment followed by mechanical triturization in Hank's solution. Potassium rich Hank's solution was released from a glass micropipette (ID 25 microm) in a distance of about 150 microm. Simultaneously an outlet pipette removed the potassium solution from the chamber. The cells were examined under an inverted Zeiss microscope (Axiovert 100) and video recorded. Cell lenght measurements were performed off-line. The potassium concentrations were 12.5, 25, 50 mM and osmotically balanced to 300 mosm.

Calcium-free solutions were prepared by substituting the calcium with magnesium and adding 5mM EGTA. Cytochalasin B was used in 50mM concentration.
The lowest concentration of potassium (12.5 mM) induced a rapid cell shortening in the first 30 second (n=9) which is than followed by a *plateau* during the whole observation period (10 min). The curve does not differ from those obtained in a calcium-free incubation medium (n=7). Both 25 (n=10) and 50mM (n=10) potassium evoked a different motile response kinetics. The fast shortening of the first 30 second observed with the 12.5 mM k+ was replaced by a slow monotonic cell lenght decrease. Incubation in calcium free medium resulted in a same response curve to 25 mM k+ (n=10) as to 12.5 mM k+. Conversly the 50 mM K+ induced shortening kinetics did not change in calcium free solution (n=10). Cytochalasin B decreased the overall response of cell shortening to 25 mM K+ (n=10) but did not change the response pattern as to fast or saturated part of the curves irrespective to calcium presence or absence (n=10).
The results argue for a K+ concentration-dependent mechanism which counteracts the fast cell concentration in the first 30 seconds of application.

16:20-16:25 ***Reaction of the cells of the stria vascularis on aminoglycoside antibiotics (P26)***
S. Ilijskaya
St. Petersburgh (Russia)

The ototoxicity of aminoglycoside antibiotics on cochlear hair cells loss is a widely known, however, the details of its influence on cells of stria vascularis (SV) not yet evident. The purpose of this study was to examine the morphological and functional changes of the cells of the SV after administration of the kanamycin. Experiments were carried out on the pigmented guinea pigs that were injected intramuscularly by kanamycin with the dosage of 400 mg/kg during 7 (I group of animals) and 14 days (II group), the third group of animals was injected during 14 days and was investigated after 30 days past later. The fourth group was a control. The marginal cells (MC) of SV are considered to be responsible for producing the endocochlear potential and for secreting the endolymphatic fluid. In the first experimental group of animals in MC the quantity of vacuoles was increased. Apparently, the rising of secretory activity is the compensate reaction. The most pathological changes are found in the marginal and intermedial cells of the SV of the second group of the animals. Some of marginal cells were destructed. The quantity of secretory vacuoles in MC was sure less, than one's in the control group. In the SV of the third group of animals were not discovered the destroyed MC but number of secretory vacuoles were less then in norm. So, aminodlycoside antibiotic entails the ultrastructural disturbance of the cells of SV and inhibits the secretory activity of MC.

• JUNIOR "SPOENDLIN" AWARD LECTURE

17:00-17:15 ***The importance of class D L-Type Ca^{2+} channels in the mouse inner ear (JuAw)***
R. Glueckert
Innsbruck (Austria)

Based on electrophysiologic and pharmacologic properties, calcuim channel activity in hair cells of the cochlea have been characterized as predominantly voltage-gated, L-type Ca+2 channels. The LTCCs (Voltage-gated, L-type calcuim channels) evidenced in the inner ear contain a pore forming a1D subunit (D-LTCC) found widely expressed in neurons and neuroendocrine cells of mammal. D-LTCCs recently evidenced in hair cells of the chick basilar papilla suggest this isoform may play a participatory role in the afferent synaptic transmission of the cochlea. As depolarization opens voltage-activated Ca2+ channels, incoming Ca2+ions open Ca2+ activated K+- channels which results in a hyperpolarized hair cell and a consequent release of afferent neurotransmission. Platzer (2000) reported that Ca2+ currents which couple sound-evoked depolarization with neurotransmission in cochlear inner hair cells, are primarily mediated by D-LTCCs. The aim of the present study was to investigate the patterns of degeneration during prenatal and neonatal development in the a1D KO mouse cochlea. Forty a1D KO mice cochleae ranging from ages 3 days to 1year were processed by the block surface method. Eleven wild type mice were included as a control. Measurements of click-evoked auditoy brainstem responses confirmed a profound deafness in all neonatal KO mice. Histological findings using light and electron microscopy revealed that at 15 days of age, degeneration can be seen in both inner and outer hair cells, at synaptic contacts, and along afferent nerve fibers while efferent nerve fibers remained intact. Interestingly, as the population of afferent nerve fibers were found progressively diminished by degenerative processes, efferent nerve fibers appeared to compensate by forming direct synaptic contact with inner hair cells. At five weeks of age, a notable degeneration of spiral ganglion cells could be seen and by 1 year, nearly all spiral ganglion cells and sensory cells of the organ of Corti were absent. The functional role of LTCCs in the cochlea are further discussed.

TUESDAY 4 SEPTEMBER, 2001
morning session

- ***Electrophysiology Of Cochlea And Auditory Pathways***

09:10-09:20 ***Some novel aspects about the expression and function of BDNF in the auditory system (O43)***
M. Knipper, J. Tan, A. Limberger, L. Minichielle, R. Klein, T. Schimmang, H.P. Zenner, U. Zimmermann
Tubingen, Heidelberg, Hamburg (Germany)

It has been shown that during early developmental periods BDNF plays a crucial role for vestibular neurons, while NT-3 maintains survival of cochlear neurons (Pirvola et al., 1992; Ernfors et al., 1995; Fritzsch et al., 1995). Less is known about the role of BDNF during later periods. We recently observed an altered expression pattern of BDNF to correlate with rearrangement of fibers in the cochlea prior to hearing function (Wiechers et al. 1999). We noted that a BDNF/trkB/Shc adapterprotein activated Ras/MAPK signalling pathway appears to guide parts of late vestibular innervation patterning as the Calyx formation around type I hair cells. In contrast formation and maintenance of distinct synapses in the cochlea prior to function as e.g. the afferent type II synapse at outer hair cells results from a trkB/ Shc adapterprotein independent BDNF activity (Minichiello et al., submitted). TrkB/trkC/ Shc mutants or trkB /Shc minus mutants present an animal model for studying some aspects of the loss of BDNF activity for auditory function. To get more insight in the functional role of BDNF in the mature cochlea we cloned different BDNF exons I-IV using primers specific for alternate 5` exons and rat genomic DNA as template, and amplified transcripts using PCR. In situ hybridization analysis revealed a differential expression pattern of distinct BDNF splice variants along the auditory tract. The results are discussed in the context of development and maintenance of normal auditory function.

09:20-09:30 ***Early changes in frequency and amplitude of spontaneous otoacoustic emissions (SOAEs) following the onset of contralateral broadband acoustic stimulation (O44)***
J. Smurzynski, G. Lisowska, R. Probst
Basel (Switzerland)

It has been previously reported that SOAEs typically increase in frequency and decrease in amplitude in the presence of contralateral broadband noise. However, methods applied to determine these findings have only used time windows of several seconds in duration. This does not allow the detailed time-course of SOAE changes following the onset of contralateral stimulation (CS) to be measured. The purpose of this study was to evaluate early changes in SOAEs occurring after the onset of a broadband CS.
Measurements were made of 17 ears with strong multiple SOAEs in 9 subjects. Spontaneous OAEs were measured using an ER10A microphone and a Tucker-Davis system, which also controlled the timing of the CS. An ER3 insert earphone was used to deliver Gaussian noise contralaterally at 65 dB SPL. In a subset of subjects, additional measurements were made for the CS at 55 and 45 dB SPL. Data were sampled for 1.23 s with the onset of the recording window at 409 ms prior to the abrupt onset of CS. For each ear, a set of 50 waveform samples was stored for off-line analysis. Averaged spectra of the microphone signal recorded without CS (the first 409-ms interval) and with CS (the last 409-ms interval) were computed with a frequency resolution of 2.4 Hz.
For the vast majority of SOAEs examined, CS produced an increase of SOAE frequency (by up to 20 Hz) and a decrease of SOAE level. In many cases, the SOAEs were suppressed into the noise floor and in some instances, the decrement of the SOAE level exceeded 20 dB which is a much larger reduction than previously reported for longer time windows placed later after the CS onset. The magnitude of the CS effects on the frequency and level shifts of SOAEs diminished with a decrease of the CS level. However, even for the CS of 45 dB SPL, a substantial reduction of the SOAE levels was observed. In a few ears with SOAEs closely related in frequency, a suppression of one SOAE by the CS resulted in an enhancement of another SOAE within the same frequency region.
The results indicate that CS alters SOAEs with a very short latency relative to the onset of the CS. One potential mechanism may be attributed to the stapedius muscle reflex at levels below its clinically measured threshold. The

results may also be explained by assuming that efferent activity evoked by the CS changes the membrane properties of the outer hair cells. Thus, changes in SOAE frequency introduced by CS represent changes in the tuning of the SOAE generator.

09:30-09:40 ***Rapid adaptation of DPOAEs in humans: The effects of binaural and contralateral stimulus conditions (O45)***
D.W. Smith, R.L. Miller
Durham (USA)

Distortion product otoacoustic emissions (DPOAEs) at 2F1-F2 decrease in amplitude, by up to 10 dB, following signal onset. This rapid adaptation, with a time constant of <100 ms, has been shown in cats to be due to the normal function of the medial olivocochlear efferent system (Liberman et al., 1996). A recent study (Kim et al., 2001) has shown a similar adaptation of DPOAEs in humans, suggesting this measure could also serve as a measure of efferent physiological function in man. The present study measured the 2F1-F2 emission in a group of human listeners to characterize the changes in rapid adaptation under binaural and contralateral stimulus conditions. Both ears in each subject were initially assessed by measuring dpgrams. The amplitude of the 2F1-F2 distortion product was measured in 1/7th octave steps from 800 Hz to 8.0 kHz. In the present studies, the F2/F1 was 1.21, with F1 amplitude 70 dB SPL and F2 65 dB SPL. Primary tone duration was 12 s. The ear with the largest emissions in each subject was chosen for detailed study and the F1 yielding the largest distortion product amplitude was employed. DPOAEs elicited by primary tones presented ipsilaterally were compared in the same subjects with emissions elicited by binaurally-presented primaries. The ipsilateral-only emissions were also compared with the same measure taken in the presence of low-level, contralateral broadband noise. When presented, the contralateral noise was 5 s in duration, beginning 4 s after the onset of the primaries and was presented at levels of 60 and 70 dB SPL. Rapid adaptation was demonstrable in 15 of 16 subjects studied and, consistent with the recent report in humans from Kim and colleagues (2001), the magnitude of the rapid adaptation observed varied across subjects, ranging from approximately 0.4 to 2.5 dB. In general, the magnitude of the rapid adaption increased when the primaries were presented simultaneously to both ears, as compared with emissions measured to the ipsilateral only primaries. As expected, contralateral noise further suppressed the DPOAE, in a manner that was intensity dependent, with a time constant similar to that of the initial rapid adaptation observed at the onset of the primaries.

09:40-09:50 ***The behavior of evoked otoacoustic emissions during and after postural changes (O46)***
E. De Kleine, H.P. Wit, P. Avan, P. van Dijk
Groningen , Maastricht (The Netherland), Clermont-Ferrand (France)

Click-evoked and stimulus frequency otoacoustic emissions (CEOAEs and SFOAEs, respectively) were studied in humans during and after postural changes. The subjects were tilted from upright to a recumbent position (head down 30 degrees) and upright again. Due to the downward posture change, CEOAEs showed a phase increase (80 degrees at 1 kHz) and a level decrease (0.5 at 1 kHz), especially for frequency components below 2 kHz. For SFOAEs, the typical ripple pattern showed a positive shift along the frequency axis, which can be interpreted as a phase shift of the inner ear component of the microphone signal (90 degrees at 1 kHz). This also occurred mainly for frequencies below 2 kHz. The altered posture is thought to cause an increase of the intracranial pressure, and consequently of the intracochlear fluid pressure, which results in an increased stiffness of the stapes system. Computations from a middle-ear model in which the stiffness of the stapes system was altered, are well in agreement with our observations.

09:50-10:00 ***Otoacoustic emissions, cochlear mechanics and hearing impairment (O47)***
A. Moleti, R. Sisto, M. Lucertini
Rome (Italy)

Twenty years after their discovery, otoacoustic emissions (OAEs) are now a powerful experimental tool for probing the inner ear function. The resonant nonlinear nature of the cochlear response and the discovery of self-sustained spontaneous emissions (SOAEs) led many authors, already in the 80's (Bialek and Wit, 1984, etc.), to propose cochlear models based on limit-cycle nonlinear oscillators. In the simplest formulation of these models the effective oscillators are directly interfaced to the oval window, for all frequencies (embedded oscillator models). The propagation of the acoustic signal along the basilar membrane is described by transmission line equations in more complex full cochlear models (Talmadge et al., 1998). The understanding of the cochlear mechanisms, and of how they are reflected in the features of the OAE recordings, is important also from a clinical viewpoint. A correct schematization of the cochlear mechanics is necessary indeed to select the measurable OAE parameters which are more directly and univocally connected to hearing functionality. Pass-fail tests, based on the reproducibility of the evoked signal, are already widely used for neonatal screening purposes. Another possible OAE application is the early detection of hearing loss. Such an application requires a higher degree of insight into the cochlear mechanisms. We have developed a new embedded oscillator model (Sisto and Moleti, 1999), whose analytical behaviour is in better agreement with the OAE phenomenology than the commonly used Van der Pol oscillator models. We have recently found a significant

correlation between hearing loss and the presence of long-lasting OAEs (Sisto et al., 2001). We have also studied the correlation of OAE latency with hearing loss, finding significantly longer latencies at frequencies immediately below the impaired range. In a new study we have measured a set of OAE parameters, whose correlation with audiometric hearing loss had been previously demonstrated, in normal ears of bilaterally normal subjects and in normal ears of monolaterally impaired subjects. Preliminary results show a significant difference between these two populations. This is an indication that these OAE parameters could be useful for the early detection of exposure effects that are not audiometrically detectable yet.

10:00-10:10 Electrocochleography in auditory neuropathy (O48)

E. Arslan, R. Santarelli, V. Magnavita
Padova (Italy)

Auditory neuropathy is a disorder identified by the absence or the severe impairment of auditory brainstem responses (ABRs) with the preservation of otoacoustic emissions. It is generally accepted that the lesion should be localized at the level of the inner hair cells, the auditory nerve fibers or the synapse in between. The presence of otoacoustic emissions indicates that the outer hair cells are spared and possibly they are functional.
We performed an audiologic evaluation in 5 patients showing distortion product otoacoustic emissions and absent ABRs. Since these findings could be attributed both to a selective damage involving the peripheral afferent component and/or to a de-synchronization of brainstem neural generators, patients underwent the recording of transtympanic electrocochleography (ECochG). The results were:
1) One of the two children (aged 1) with hyperbilirubinemia at birth showed only the cochlear microphonic in the electrocochleographic recording; the second child (aged 4) had also a high amplitude summating potential;
2) The third patient (aged 17) had a severe impairment of speech discrimination out of proportion of the auditory threshold. The ECochG performed in this subject consisted in the summating potential followed by a neural activity highly desynchronized which was identifiable at stimulation intensities lower than the hearing threshold;;
3) Of the two patients with only a moderately elevated PA threshold in eectrocochleography, the first child (aged 5) showed a moderate hearing loss at low frequencies and a severe impairment in speech discrimination associated with bilateral optic nerve atrophy. A compound action potential with normal amplitude and latency was identifiable in the ECochG recording till to a stimulation intensity corresponding to the PTA threshold. The second child had a profound hearing loss at the behavioral evaluation in spite of the peripheral threshold estimated by EcochG recordings.
These results suggest that electrocochleography can be useful in the assessment of the auditory neuropathy since only the CAP detection in ECochG recordings is a reliable estimate of the auditory peripheral function in the presence of a de-synchronized ABR.

10:10-10:20 Quantification of audiogram fine-structure as a function of hearing threshold (O49)

J.W. Horst, H.P. Wit and F.A.J. Albers
Groningen (The Netherlands)

Many normal ears exhibit a characteristic fine-structure in their audiograms related to oto-acoustic emissions. The presence or absence of oto-acoustic emissions (OAEs), and by implication of audiogram fine-structure, may be regarded as an indication of the state of cochlear functioning.
We investigated the amount of fine-structure in patients with Meniere's disease. In Meniere's disease a large variation of hearing thresholds between patients and also between ears of the same patient can be found. Both ears (often an affected and an unaffected ear) were investigated.
Data are presented from 47 ears of 25 patients with hearing thresholds varying from about 0 to 70 dB HL.
We measured the fine-structure in the frequency range from 500 to 3500 Hz; this agrees with a part of the basilar membrane from 9 to 23 mm from the apex. The fine-structure was characterized by means of the number of peaks Np and the average peak height Hp.
We found a negative correlation between hearing loss and strength of fine-structure, i.e. the higher the thresholds the smaller Np as well as Hp.
Also Np and Hp were correlated, i.e. the more peaks the higher the average peak height.
The accumulated peak height Sp=Np.Hp showed a strong dependence on the hearing loss. In cases of strong fine-structure Sp reached values above 200 dB.

10:20-10:30 Evaluation of anesthesia effects in a rat animal model using high and medium stimulus intensity otoacoustic emission protocols (O50)

S. Hatzopoulos, J. Petruccelli, and A. Martini
Ferrara (Italy), Worcester (USA)

Recent studies (Harel et al,1997) have suggested that ketamine compounds increase the amplitude level of the TEOAE and DPOAE responses in a gerbil animal model. Since the major application of the OAEs is the detection of

sensorineural hearing losses, expressed by alterations of the OAE responses, it is important to define the time window in which the anesthesia effect on the OAEs is not present.
The anesthesia effect on the OAE recordings was evaluated in 3 groups of 64 Sprague -Dawley rats (mean weight 225 ± 20 gr). Thirty due animals were tested with TEOAEs, 27 animals with DPOAEs and 5 animals with both OAE types.The anaesthetic (equal volumes of ketamine mixed with xylazine or atropine and saline solution) was administered in two consecutive phases. In phase one, the animal received an intra-peritoneal dose (1 ml / kg of body weight) and upon the first signs of muscular relaxation (phase two) a second half-volume dose was administered subcutaneously**.** The TEOAEs were recorded according to a nonlinear protocol and were evoked by a 63.5 dB SPL click stimulus. The DPOAEs (cubic distortion products) were evoked by two different asymmetrical protocols with primaries set at 60-50 and 50-40 dB SPL. The OAE responses were recorded in 10 min intervals, starting 5 min after the first dose and ending at 60 min post treatment.
An analysis of the data of both OAE types with a repeated measures model indicated the following: (1) There is a decrease of the TEOAE response level, the TEOAE correlation value and the S/N ratios in the tested frequencies 12-14 min after the administration of the anesthesia, but these differences were not distinguishable from random variation (not statistically significant). (2) The DPOAE responses evoked by a 50-40 protocol showed the highest S/N decrease, but the time differences were also not significant.
These findings from the rat model contrast the data available in the literature from a gerbil animal model and suggest that the previous data were treated, erroneously, by statistical methods which did not consider time as a variable.

11:00-11:10 Activation of G-proteins participates in ototoxic hair cell damage (O51)
A.F. Ryan, K. Pak, A. Sahakian, A. Battaglia
La Jolla (USA)

GTP-binding (G-) proteins play a major role in signaling pathways that lead from the cell membrane to the nucleus, and participate in many other intracellular processes. We evaluated the role of G-proteins in ototoxicity in vitro. The pan-G-protein inhibitor GDPßS was found to inhibit the ototoxicity of gentamicin and cisplatin. The size of G-proteins activated by ototoxins was determined using a radiolabeled azide GTP analog that can be linked to activated G-proteins by UV light, followed by gel electrophoresis. Exposure to gentamicin and cisplatin activated a small (21 kDa) G-protein. The azide analog then was used to isolate the G-protein, which was analyzed by trypsin digestion followed by matrix-assisted laser desorption and time-of-flight spectroscopy. The peptide fragment spectrum was matched to the predicted trypsin fragment profile of the EMBL protein database. The match with the highest probability was H-Ras. A specific Ras inhibitor, FTI-277, was found to inhibit both gentamicin and cisplatin ototoxicity. An inhibitor of Mek, a MAP kinase kinase that often links Ras to the Erk MAP kinases, did not inhibit ototoxicity but in fact enhanced hair cell damage. The results suggest that ototoxicity involves activation of a Ras-dependent but via Mek/Erk independent signal transduction pathway.

11:10-11:20 Recovery of cisplatin ototoxicity in guinea pigs in relation to cisplatin dose (O52)
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Utrecht (The Netherland)

The objective of the present study was to further characterize cochlear recovery after cisplatin damage.We equipped albino guinea pigs with permanent round window electrodes. Cisplatin was injected intraperitoneally on a daily basis at either 1.5 or 2.0 mg/kg/day. Treatment was stopped when the criterion of >40 dB loss in the compound action potential iso-response level at 8 kHz had occurred. Either shortly or long after this stop, the endocochlear potential (EP) was measured and all animals were sacrificed for histology. With a cisplatin dose of 2.0 mg/kg/day, the time needed to reach the criterion hearing loss varied from 5 to 11 days. With 1.5 mg/kg/day this period lasted longer, the cumulative dose being the first order predictor. The cochlear potentials gradually recovered in the first two weeks after treatment. At the lower frequencies, recovery was often complete. At the higher frequencies, complete recovery was never seen. EP was depressed when measured just after treatment but had normal values long after. Basal outer hair cell (OHC) loss was found for both the short and the long post-treatment period. Thus, loss and recovery of cochlear potentials can for a large part be explained by loss and recovery of the EP. Recovery is limited by permanent OHC loss.

11:20-11:30 The effects of combined exposure to noise and ethyl benzene on hearing in rats (O53)
N.L.M. Cappaert, S.F.L. Klis, H. Muijser, G.F. Smoorenburg
Utrecht (The Netherlands)

The effects on hearing of simultaneous exposure to the ototoxic organic solvent ethyl benzene and broad-band noise were evaluated in rats. The effects of three ethyl benzene concentrations (0, 300 or 400 ppm) and three noise levels (95 or 105 dB SPL or background noise at 65 dB SPL) and all their combinations were investigated after 5 days exposure at 8 hours/day. Distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAPs) were affected after 105 dB noise alone, and after 105 dB noise in combination with ethyl benzene (300 and 400 ppm). However, the amount of loss for these combinations did not exceed the loss for 105 dB noise alone. Outer hair cell (OHC) loss after

exposure to 300 ppm ethyl benzene was located in the third row of OHCs. At 400 ppm, the loss spread out to the second and first row of OHCs. Noise alone hardly affected the OHC counts except for minor loss in the first row of OHCs after 105 dB SPL. Noise at 105 dB in combination with ethyl benzene at 300 and 400 ppm, however, showed OHC loss greater than the sum of the losses induced by noise and ethyl benzene alone.

11:30-11:40 ***Gentamicin-induced changes of expression of PKC isoforms in an immortalized embryonary auditory cell line (O54)***

I. Lanzoni, M. Previati, D. Bindini, C. Vitali, A. Martini, C. Parmeggiani, S. Capitani and L. Bertolaso
Ferrara (Italy)

To investigate the biochemical mechanisms underlying the gentamicin cytotoxic side effects we used an immortalized cell line (OC-k3) from the organ of Corti of transgenic mice H-2Kb-tsA58 (Kalinec et al., 1996). This mouse carries a conditionally expressed, temperature-sensitive immortalizing gene that perpetuates cell division. The OC-k3 cells express simultaneously specific auditory sensory cell markers (such as myosin VIIa and acethylcoline receptor α-9), the supporting marker connexin 26, the specific IHC marker OCP2 and the neuroepithelial precursor nestin. These data add support to the hypothesis of a common progenitor for hair cells and supporting cells in the organ of Corti of rodents. This cell line was used to investigate the biochemical pathways involved in cell death induced by aminoglycosides treatment, i.e. streptomicin, kanamicin and gentamicin. In our model gentamicin induced apoptotic cell death at a concentration of 50 μM, which had previously shown in organ culture to selectively kill hair cell but not ganglion cell. Cell death induced by gentamicin displayed the morphological changes associated to apoptosis. In addition, after gentamicin treatment the intracellular reactive oxygen species (ROS) increased reaching a maximum levels to 1h, followed by increase of GSH efflux and a contemporaneous decrease of intracellular GSH levels. In addition, PKC isoform expression level was investigated. We detected five isoforms, PKC α, βI, βII, γ and ζ. Among these, PKC α was progressively down-regulated without resynthesis, while PKC ζ increased and translocated to the nucleus, during gentamicin treatment. BDNF (brain-derived neurotrophic factor) was able to reverse the apoptotic effect of the aminoglycoside, and to suppress intracellular GSH variations such as the changes of PKC α and ζ expression, suggesting that the depletion of GSH and the concomitant increase of ROS could be related to the long-term modulation of PKC and that these events could mediate the cytotoxic effect of gentamicin.

11:40-11:50 ***Role of iNOS in Cisplatinum-ototoxicity (O55)***

M.-A. Teranishi, D. Labbe, W. Bloch, K.-I. Watanabe, O. Michel
Cologne (Germany)

Cisplatin (CDDP), an antitumor drug commonly used in head and neck tumours, has dose-limiting side effects such as ototoxicity and nephrotoxicity. Recent reports demonstrate that the expression of iNOS is enhanced in the cochleae of animals treated with CDDP and CDDP-ototoxicity is suppressed by a combined treatment of a NOS inhibitor, L-NAME. In the present study, we investigated the roles of iNOS in CDDP-ototoxicity using iNOS knock-out mice. Materials/Methods: Mice (controls and iNOS knock-out mice) were injected with CDDP (15 mg/kg) intraperitoneally. Before the experiment and four days after the injection, ABR measurements were performed. Immunohistochemistry were carried out to check the expressions of NOS isoforms.
Results: In the control animals, ABR thresholds were distinctly elevated and the expression of iNOS in the cochleae was enhanced and body weight was remarkably decreased. Those toxic effects induced by CDDP were suppressed in iNOS knock-out mice.
Conclusion: A suppression of iNOS provides a protective effect on CDDP-ototoxicity, possibly via the decreased production of peroxynitrite.

11:50-12:00 ***Cisplatin-dependent cytotoxicity in Organ of Corti-derived immortalized cells (O56)***

C. Vitali, L. Bertolaso, D. Bindini, I. Lanzoni, A. Martini, C. Parmeggiani, S. Capitani, M. Previati
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Cisplatin is an anticancer drug currently used for treatment of a wide range of tumours. This drug shows two major undesired side effects, nephro- and oto-toxicity, whose mechanisms of action are only partially understood. In particular, elucidation of the cellular mechanisms underlying ototoxicity is impaired by the small size of tissue and the short lifetime span in culture of cells freshly prepared from inner ear. To overcome these difficulties we used a cell line (OC-k3) developed from the Organ of Corti of transgenic mice, which expresses the large T antigen of SV40 and, in presence of γ-interferon, is driven to proliferate indefinitely. This cell line is positive for neuroepithelial precursor nestin, for specific auditory cell markers as myosin VIIa and acetylcholine α-9 and for IHC specific marker OCP2; on the other hand, it lacks of glial and neuronal markers.
In our cell model, cytotoxicity occurred after 24-48 hour by cisplatin incubation at concentration from 13 to 200 μM. When treatment was carried out for the whole time explored, cell death occurred by necrosis at all the tested

concentrations; otherwise, when cisplatin was left in the culture medium for some time (2-4-6-8 hour), we observed at 48 hour that cell death was time- and concentration dependent. In addition, the adherent cells showed, by DAPI staining and electron microscope examination, some morphological hallmarks of apoptosis, as nuclear fragmentation in presence of membrane and mitochondrial integrity. No DNA ladder was detected, indicating absence of exonucleasic activity.

Treatment by 13 μM cisplatin led to ROS increase and diminution of intracellular GSH. Furthermore, fluorescence of cell loaded by Rhodamine 123 diminished, indicating a probable transient compromission of mitochondrial $\Delta\psi$, that was reversed by presence of reducing factors as n-acetylcisteine, GSH and vitamin C. Rotenone, an inhibitor of mitochondrial complex I, behaved as cytotoxic and increased ROS, which further increased during cisplatin and rotenone coincubation. Several substances reduced cisplatin-induced apoptosis, as suramin and PD98059, indicating the involvement of specific transduction pathways in cisplatin toxicity, and in particular of ERK cascade.

As a whole, these data shows that cisplatin can trigger apoptosis time and concentration dependent, while excesses of these factors lead to cell necrosis. Complex pathways could be involved in inducing apoptosis, including ERK recruiting and production of ROS, probably by complex I inhibition. Depletion of intracellular GSH could more widely affect the redox balance, contributing to drive the cell toward programmed cell death.

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