Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 357 (2007) 14–19
BBRC
www.elsevier.com/locate/ybbrc

# Glutamate induces apoptosis in cultured spiral ganglion explants

Silke Steinbach [a,*], Jens Lutz [b]

[a] Department of Otolaryngology—Head and Neck Surgery, Technical University of Munich, Germany
[b] Department of Nephrology, Technical University of Munich, Germany

Received 28 February 2007
Available online 26 March 2007

**Abstract**

Traumatic sound exposure, aminoglycoside antibiotics, cochlea ischemia or traumatic stress leads to an excessive release of glutamate from inner hair cells into the synaptic cleft. The high glutamate concentration can cause a swelling and destruction of the dendrites of spiral ganglion neurons of type I as well as a reduction in the number of neurons. This may be a cause of hearing loss. The mechanism causing the reduction of neurons is still not known. Apoptosis, also called programmed cell death, could be involved. In this study, cultured spiral ganglion explants were incubated with glutamate in high concentrations. Neurite outgrowth was determined and additionally a new method was established for studying the morphology of single spiral ganglion neurons. For the first time it was shown that glutamate induces apoptosis of spiral ganglion neurons, which could be blocked selectively by a caspase-3 inhibitor. This could offer a new therapeutic strategy for hearing disorders.


*Keywords:* Spiral ganglion explants; Glutamate; Apoptosis; Caspase-3 inhibitor

Glutamate is considered to be the main neurotransmitter at the synapse between the inner hair cells and the dendrites of the spiral ganglion neurons of type I [1,2]. It is known that an excessive, non-physiological release of glutamate into the synaptic cleft leads to a swelling and destruction of the afferent nerve terminals [3] and reduces the number of spiral ganglion neurons of type I in the developing cochlea [4]. This may lead to hearing loss. Traumatic noise [2,5], aminoglycoside antibiotics [6,7], or cochlea ischemia [8,9] can cause such an excessive release of glutamate from inner hair cells into the synaptic cleft. Furthermore, traumatic stress caused by cochlear nerve compression is associated with a release of glutamate [10].

The detailed mechanism causing the reduction of the spiral ganglion neurons is not yet known. Apoptosis (also called programmed cell death) could contribute towards the reduction of the number of neurons. This could be an important pathogenetic mechanism involved in the pathogenesis of hearing disorders, the recognition of which could give rise to new therapeutic strategies in the future.

Achieving an inhibition of apoptosis could also be beneficial for other hearing disorders or situations accompanied by cell degeneration. There is already increasing evidence that the apoptosis of spiral ganglion neurons plays an important pathogenetic role in presbyacusis [11,12]. The survival of intact spiral ganglion cells after the degeneration of inner hair cells and before cochlear implantation is of major interest. Patients with residual hearing, who wish to have a cochlear implant, would benefit particularly from a protection of these intact spiral ganglion neurons. However, the surgery itself—especially the intraoperative insertion of the electrode—can initiate a loss of functioning auditory neurons. Finally, an induction of apoptosis by activation of caspase-3 is found in spiral ganglion cells of hydropic guinea pig cochleae [13]. Thus, apoptosis could be the explanation for the sensorineural hearing loss found in later stages of Ménière's disease.

We hypothesized that a high concentration of glutamate causes apoptosis of spiral ganglion neurons, which can be blocked by a caspase-3 inhibitor. Here we analyzed the

* Corresponding author. Fax: +49 89 4140 4853.
*E-mail address:* s.steinbach@lrz.tu-muenchen.de (S. Steinbach).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.03.098

effect of glutamate on apoptosis by incubating cultured spiral ganglion explants directly with glutamate.

## Materials and methods

*Preparation of spiral ganglion explants.* All animal experiments were approved by the local administration (Regierung Oberbayern, Munich, No. 211–25314/2002). The preparation of the spiral ganglion explants was performed as described [14–16] using gerbils (Charles River, Sulzfeld, Germany) at day 4 post-natal. After cervical transfection the heads of the gerbils were skinned, the skulls were opened midsagittally, and the brain halves were removed. The following steps were carried out under the microscope and in PBS solution. The bulla of the temporal bone was opened, the capsule of the inner ear as well as the stria vascularis and the organ of corti were removed. The spiral ganglion was identified, separated from the modiolus, cut into 3–5 pieces, and transferred to a cell culture. Post-natal day 4 gerbils were used, since the preparation of the spiral ganglion explants becomes more difficult in older gerbils as they have an increased ossification of the modiolus.

*Preparation of tissue culture wells and cell culture.* Tissue culture plates (96-well) were incubated with poly-D-lysine at 0.1 mg/ml in PBS at room temperature for 1 h. After washing with PBS, the wells were incubated with laminin at 0.01 mg/ml in PBS at 37 °C for 1 h. A basic medium was prepared of DMEM, 25 mM Hepes buffer, 1 µl/ml $N_2$-supplement, 10 µg/ml insulin, 6.15 g/l glucose, and 27.64 U/ml penicillin [15,16]. The wells were washed once with the basic medium and then the spiral ganglion explants were incubated in a culture medium at 37 °C, 5% $CO_2$, and 95% humidity for 48 h. For the culture medium 25 ng/ml NT-3 was added to the basic medium. Additionally, depending on the experimental group, 5 mM glutamate, 50 mM glutamate, or 0.015 mM caspase-3 inhibitor (Z-DEVD-FMK, Alexis, Gruenberg, Germany) was added. For each concentration 6 gerbils were prepared, i.e. 12 ears, or 48 spiral ganglion explants.

*TUNEL staining.* The spiral ganglion explants were fixed with 4% paraformaldehyde in the wells, followed by transfer under microscope control to a blotting paper. After drying they were transferred to a Petri dish. Small plastic rings were put around the explants and filled with a tissue-freezing medium. Samples were snap-frozen in liquid nitrogen and the plastic rings were removed. Five micrometer thick sections were cut with a cryostat (Leica, Wetzlar, Germany). Apoptosis was analyzed with the TUNEL assay (Boehringer, Mannheim, Germany) following the manufacturer's instructions. TUNEL positive cells had a red nucleus. Sections were counterstained with Mayer's Haemalaun. TUNEL positive cells were counted per field of view (400×) under a light microscope. The significance was tested using Weir's approach [17].

*Immunohistochemistry.* To ensure that the tissue consisted of neurons, immunohistochemistry was performed for detecting neurofilament. The spiral ganglion explants were fixed in the wells with a 1:1 mixture of acetone and methanol, washed with PBS, and incubated with a primary antibody detecting neurofilament (Boehringer, Mannheim, Germany), diluted 1:500 in PBS at 4 °C overnight. Staining was performed according to the ABC method using the Vectorstain Elite ABC kit and DAB substrate kit for peroxidase (Vector Laboratories, Burlingame, California, USA) according to the manufacturer's instructions. After photographing the neurite outgrowth of the stained spiral ganglion explant, the explant was removed from the well, dried on blotting paper, shock-frozen, and sliced as described above. After removing the tissue-freezing medium, the sections were directly analyzed under the microscope. To ensure a specific labeling of the spiral ganglion neurons near the edge of the spiral ganglion explant, explants were first shock-frozen, then sliced followed by staining for neurofilament. The spiral ganglion cell diameter was measured using Scion Image for Windows. For measuring the neurite outgrowth the five longest neurites of the explants were taken. A number of straight lines were drawn on each neurite; thus all the curves of the outgrown neurite could be followed. The neurite length was measured using Amira 3.1.1. The significance was tested using Weir's approach [17].

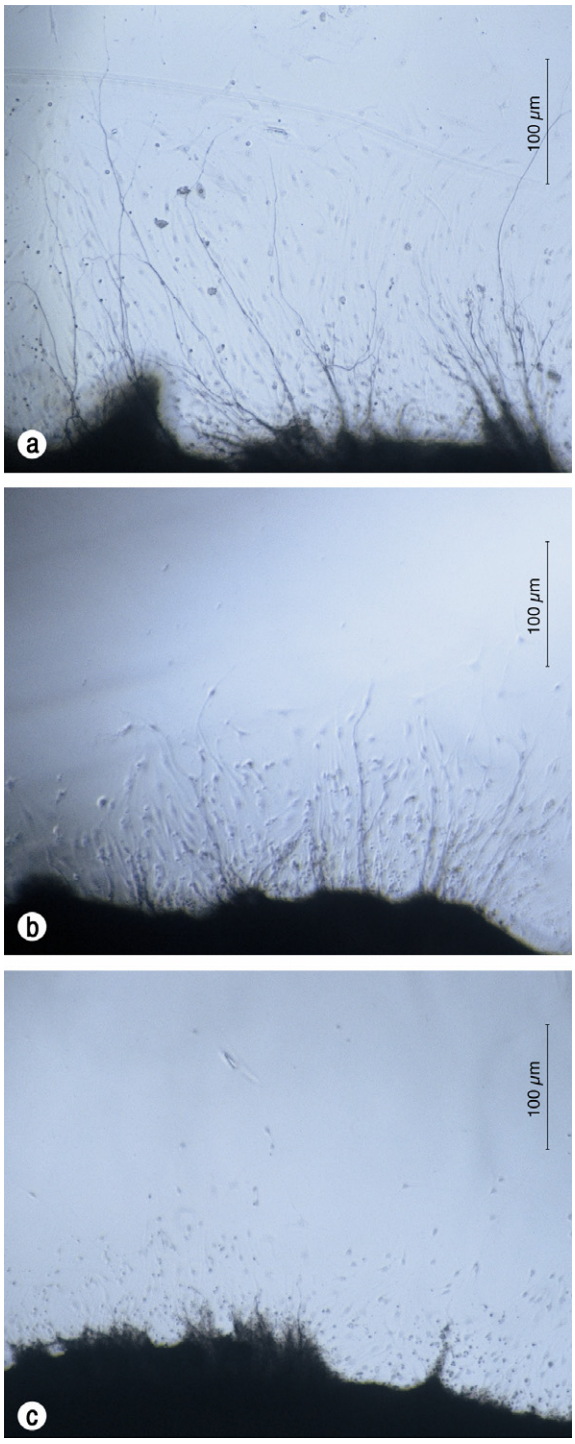


Fig. 1. Spiral ganglion explants cultured for 48 h with culture medium (a), with 5 mM glutamate (b), with 50 mM glutamate (c). Immunohistochemistry for detecting neurofilament. The higher the concentration of glutamate exposure the lesser the outgrowth of neurites.

## Results

### *Glutamate induces apoptosis in cultured spiral ganglion explants*

Fig. 1a shows a typical spiral ganglion explant incubated with culture medium for 48 h and stained by

immunohistochemistry for detecting neurofilament. The neurites extend from the spiral ganglion explant edge. Near the edge they form fascicles and further away they split into individual fibers. The neurites are adjacent to non-neuronal cells, which seem to be glial cells and fibroblasts. If the spiral ganglion explants are incubated with 5 mM glutamate for 48 h, the extension of the neurites as well as the amount of adjacent non-neuronal cells diminish (Fig. 1b). When incubated with 50 mM glutamate, there is hardly any extended neurite detectable; nevertheless, there are still some adjacent non-neuronal cells next to the spiral ganglion explant edge (Fig. 1c). If the spiral ganglion explants are incubated with 500 mM glutamate, there is no neurite and no adjacent non-neuronal cell in the area around the spiral ganglion explant (data not shown). Glutamate incubation significantly reduces the neurite extension ($p < 0.05$). After 48 h the average neurite extension without glutamate exposure is $338.9 \pm 29.4$ μm, with 5 mM glutamate exposure is $150.9 \pm 27.2$ μm and with 50 mM glutamate exposure is $30.4 \pm 3.1$ μm. Compared to spiral ganglion explants incubated without glutamate the neurite extension is reduced by 55.5% with 5 mM glutamate exposure and 91% with 50 mM glutamate exposure.

Fig. 2 shows the frozen sections of the same spiral ganglion explants that are shown in Fig. 1. Due to the thickness of the frozen sections of 5 μm the morphology of single neurons can be studied. Without glutamate incubation, small neurons (average cell diameter $11 \pm 1$ μm) and a network of neurites can be found in the spiral ganglion explant near the edge (Fig. 2a). After incubation with 5 mM glutamate some neurons swell, while others next to them retain their small size (average cell diameter $25 \pm 3$ μm) (Fig. 2b). Incubation with 50 mM glutamate produces a swelling of most of the neurons (average cell diameter $53 \pm 3$ μm) (Fig. 2c). Glutamate incubation significantly increases the spiral ganglion cell diameter ($p < 0.05$). The nucleus of the spiral ganglion neurons is unlabeled. So spiral ganglion neurons appear to have a hole in the center, which is typical for labeling of neurofilament. The location of the labeled spiral ganglion neurons near the edge of the spiral ganglion explant is identical independently if immunostaining is performed prior or after frozen sectioning.

To study the mechanism causing this swelling, a TUNEL staining was performed on frozen sections of other spiral ganglion explants. The spiral ganglion explants

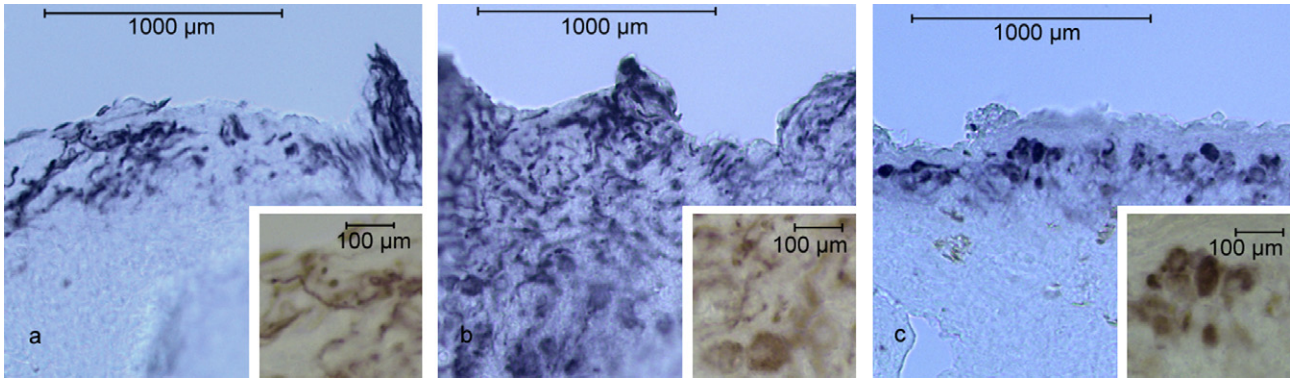


Fig. 2. Frozen sections of the same spiral ganglion explants as shown in Fig. 1, with culture medium (a), with 5 mM glutamate (b) and with 50 mM glutamate (c). Immunohistochemistry for detecting neurofilament. The higher the concentration of glutamate exposure the lesser the amount of small neurons.

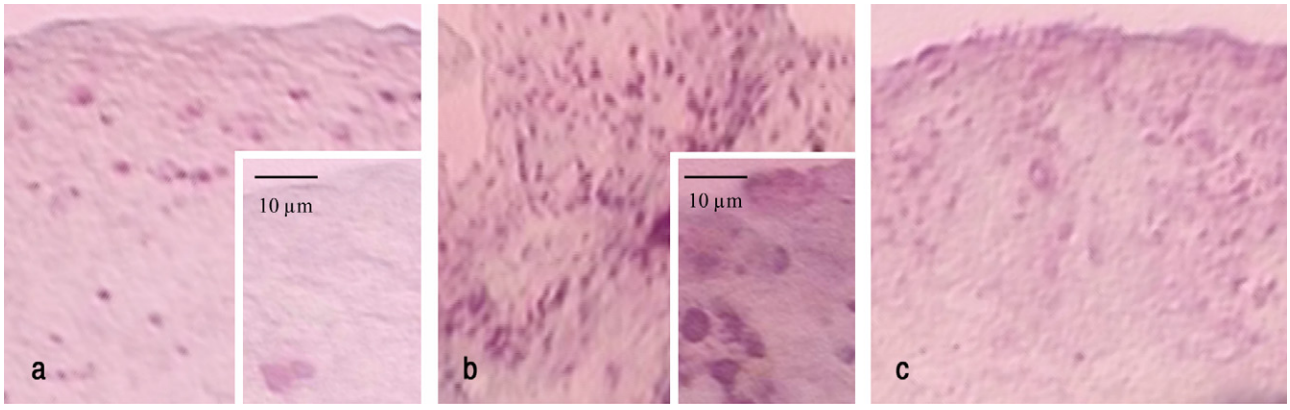


Fig. 3. TUNEL staining of frozen sections of spiral ganglion explants incubated for 48 h with culture medium (a), with 5 mM glutamate (b) and with 50 mM glutamate (c). Glutamate exposure leads to a higher number of TUNEL positive, apoptotic cells.

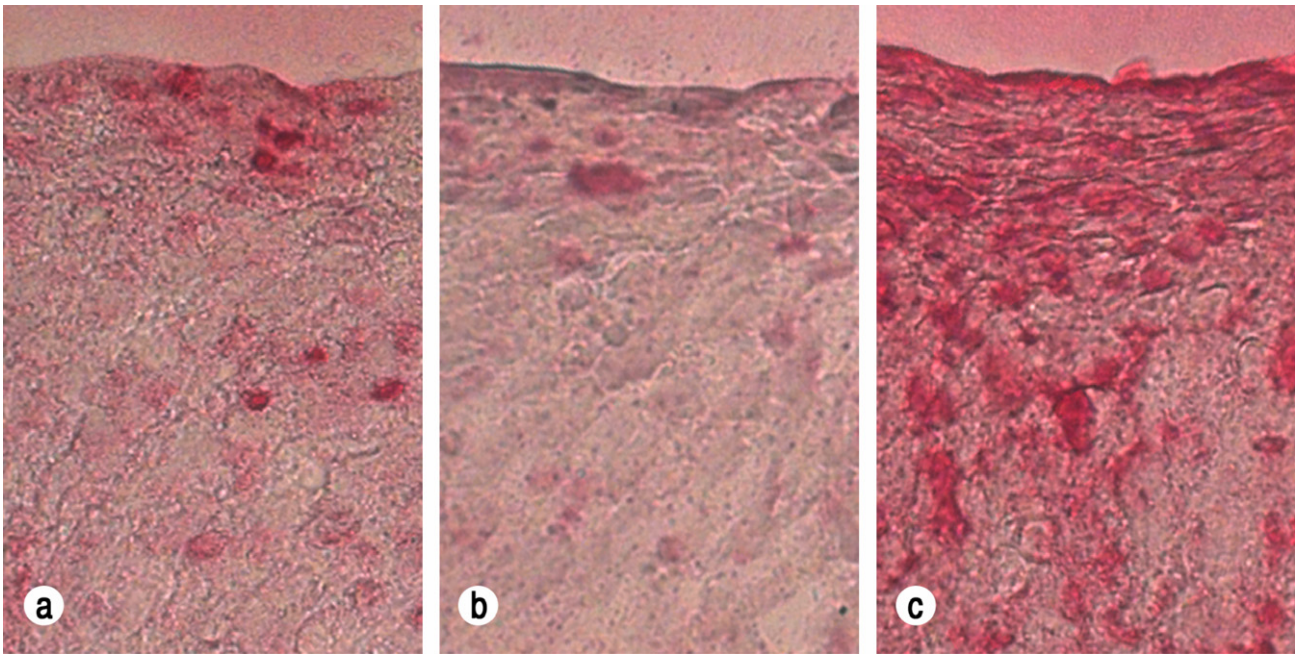


Fig. 4. TUNEL staining of frozen sections of spiral ganglion explants incubated for 48 h with a caspase-3 inhibitor (a), with a caspase-3 inhibitor and 5 mM glutamate (b), and with 5 mM glutamate (c). Apoptotic cells are significantly less with a caspase-3 inhibitor than with glutamate alone.

were incubated for 48 h with the same glutamate concentrations as described above. Glutamate incubation leads to a higher number of TUNEL positive apoptotic cells ($p < 0.05$) (Fig. 3). Without glutamate exposure an average of $2.9 \pm 0.6$ apoptotic cells per field of view can be counted, with 5 mM glutamate exposure $20.4 \pm 1.3$ apoptotic cells per field of view can be counted and with 50 mM glutamate exposure there are $20.6 \pm 1.7$ apoptotic cells per field of view.

*Glutamate-induced apoptosis can be blocked by a caspase-3 inhibitor*

Frozen sections of cultured spiral ganglion explants with TUNEL staining show few apoptotic cells after 48 h incubation with a caspase-3 inhibitor or with a caspase-3 inhibitor and 5 mM glutamate (average of apoptotic cells per field of view: $28.8 \pm 6.5$ versus $28.3 \pm 8.5$) (Fig. 4a and b), but a large number of apoptotic cells after incubation with 5 mM glutamate alone ($266.3 \pm 27.8$) (Fig. 4c). The ratio of counted apoptotic cells per field of view between spiral ganglion explants incubated with a caspase-3 inhibitor, with a caspase-3 inhibitor and 5 mM glutamate, or with 5 mM glutamate was almost 1:1:10, respectively. Spiral ganglion explants incubated with glutamate and a caspase-3 inhibitor had significantly less apoptotic cells as compared to explants incubated with glutamate alone ($p < 0.05$).

## Discussion

Glutamate exposure to spiral ganglion explants reduced the length of neurites and led to apoptosis of the spiral ganglion neurons depending on the glutamate concentration. This is the first study that directly shows that apoptosis is induced by the neurotoxic effect of high concentrations of glutamate on neurites and spiral ganglion cells.

This is in congruence with observations of Janssen et al. [4]. In his study post-natal treatment with glutamate in rats (4 g glutamate/kg/day, i.p., post-natal days 2–9) showed an elevation in high frequency thresholds on postnatal day 22, 75, and 105 as compared with the saline control group. In the morphological evaluation, sacrificed rats showed no loss of hair cells, but a dramatic and selective reduction of neurons in the basal portion of the spiral ganglion. Rats treated with glutamate on post-natal day 2–5 showed a dramatic axonal degeneration after post-natal day 5. However, the mechanism of degeneration and reduction of the spiral ganglion neurons was not further characterized.

To confirm that apoptosis is involved in the neurotoxic effect of glutamate, the spiral ganglion explants were incubated with a caspase-3 inhibitor, that blocks apoptosis. This resulted in a decreased number of apoptotic cells which was shown by the reduced number of TUNEL positive cells. Glutamate seems to be toxic for neuronal cells. Immunohistochemistry for detecting neurofilament directly showed the neurotoxic effect on spiral ganglion cells, the neurite extension was reduced and the cell diameter was increased. All of the stained spiral ganglion cells were located next to the surface of the spiral ganglion explant. Many apoptotic cells were located close to the surface where spiral ganglion cells were located.

Knowledge of the synaptic complex offers different possibilities for therapy. A more recent study by Ruel et al. [18] described an inhibition of glutamate release from inner hair cells by riluzole. Glutamate receptor antagonists such as

caroverine, MK-801, KYNA or memantine were investigated, which block the glutamate receptors on the dendrites of the spiral ganglion neurons. A local application of these glutamate receptor antagonists could be a beneficial therapeutic option. Most studies on the protective effect of glutamate receptor antagonists applied the therapeutic agent to the round window of animals prior to and shortly after noise exposure or traumatic stress of the cochlear nerve [10,19–21]. Chen et al. [22] showed a therapeutic effect of caroverine when applying 1 h after noise exposure but not when applying 24 h afterwards. However, apoptosis of the spiral ganglion neurons is a process that takes longer than reactions at the level of the synaptic complex. Therefore, a caspase-inhibitor, i.e. an apoptosis blocker, may, as a local therapeutic agent offer a longer time span after the release of high concentrations of glutamate into the synaptic cleft. Patients would profit from a longer time span after injury. Perhaps a combination of a glutamate antagonist and a caspase inhibitor would be a good solution for counteracting the short-term and long-term effects of glutamate. Other therapeutic strategies could be antioxidant agents, which in part cannot pass the blood–brain or blood–labyrinth border, as well as heat shock proteins or neurotrophic factors. The latter two agents are proteins with a low half-life.

It is known that cultured post-natal spiral ganglion cells undergo cell death after trophic factor deprivation [23–25]. Lallemend et al. [26] described TUNEL positive apoptotic spiral ganglion neurons 9 h after trophic factor deprivation, whereby the maximum number of apoptotic neurons occurred 15 h after trophic factor deprivation. Important trophic factors are the brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Both have been detected by *in situ* hybridization in the developing auditory system, whereby BDNF transcripts decrease and NT-3 mRNA is strongly expressed in the inner hair cells during all periods. NT-3 seems to be the survival factor for type I spiral ganglion neurons and BDNF for type II neurons [27]. The type I spiral ganglion neurons innervate the inner hair cells, their afferent fibers constituting 90–95% of the auditory nerve. The remaining 5–10% are type II spiral ganglion neurons that innervate the outer hair cells. In this study, NT-3, the survival factor of the spiral ganglion neurons of type I, was added in all of the experiments to prevent apoptosis by trophic factor deprivation and to show only the neurotoxic effect of glutamate.

The *in vitro* system we have developed offers new possibilities for *in vitro* research on spiral ganglion neurons. The outgrowth of the neurites from the spiral ganglion explant edge can be observed as described previously [14–16]. Moreover, the frozen sections of the same spiral ganglion explant offer the possibility of studying the morphology of single spiral ganglion neurons. Different staining can specifically characterize the single neurons.

Generally, all the studies on spiral ganglion neurons were performed in a dissociated culture [26,28,29], which automatically exposes the spiral ganglion neurons to an additional mechanical and enzymatic stress, thereby distorting experimental results particularly as regards apoptosis. This problem could be overcome by implementing our procedure of frozen sections where single spiral ganglion neurons can be observed as well.

To conclude, our results demonstrate that glutamate causes apoptosis of spiral ganglion neurons. Apoptosis can be blocked selectively by a caspase-3 inhibitor. Together with locally applied glutamate antagonists this could offer a new therapeutic strategy in hearing disorders, including tinnitus induced by an excessive release of glutamate from inner hair cells into the synaptic cleft.

## Acknowledgments

We gratefully thank Professor Dazert for showing his spiral ganglion explant preparation. The authors were funded by KKF (a hospital internal funding organization of the Faculty of Medicine of the Technical University of Munich).

## References

[1] J.-L. Puel, Chemical synaptic transmission in the cochlea, Prog. Neurobiol. 447 (1995) 449–476.
[2] N. Hakuba, K. Koga, K. Gyo, S. Usami, K. Tanaka, Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST, J. Neurosci. 20 (2000) 8750–8753.
[3] R. Pujol, J.-L. Puel, C.G. D'Aldin, M. Eybalin, Pathophysiology of the glutamatergic synapses in the cochlea, Acta Otolaryngol. (Stockh) 113 (1993) 330–334.
[4] R. Janssen, L. Schweitzer, K.F. Jensen, Glutamate neurotoxicity in the developing rat cochlea: physiological and morphological approaches, Brain Res. 552 (1991) 255–264.
[5] W. Jäger, M. Goiny, M. Herrera-Marschitz, A. Flock, T. Hökfelt, L. Brundin, Sound-evoked efflux of excitatory amino acids in the guinea-pig cochlea in vitro, Exp. Brain Res. 121 (1998) 425–432.
[6] K. Matsuda, S. Komune, T. Tono, M. Yamasaki, A. Haruta, E. Kato, A role of glutamate in drug-induced ototoxicity: in vivo microdialysis study combined with on-line enzyme fluorometric detection of glutamate in the guinea pig cochlea, Brain Res. 852 (2000) 492–495.
[7] Y. Shimizu, N. Hakuba, J. Hyodo, M. Taniguchi, K. Gyo, Kanamycin ototoxicity in glutamate transporter knockout mice, Neurosci. Lett. 380 (2005) 243–246.
[8] A. Matsubara, Y. Kawabata, Y. Takumi, S. Usami, H. Shinkawa, A. Haruta, K. Matsuda, T. Tono, Quantitative immunogold cytochemistry reveals sources of glutamate release in inner ear ischemia, Acta Otolaryngol. (Stockh) Suppl 539 (1998) 48–51.
[9] N. Hakuba, K. Gyo, N. Yanagihara, A. Mitani, K. Kataoka, Efflux of glutamate into the perilymph of the cochlea following transient ischemia in the gerbil, Neurosci. Lett. 230 (1997) 69–71.
[10] T. Sekiya, N. Shimamura, T. Hatayama, S. Suzuki, Effectiveness of preoperative administration of an *N*-methyl-D-aspartate antagonist to enhance cochlear neuron resistance to intraoperative traumatic stress: an experimental study, J. Neurosurg. 93 (2000) 90–98.
[11] S.A. Alam, T. Oshima, M. Suzuki, T. Kawase, T. Takasaka, K. Ikeda, The expression of apoptosis related proteins in the aged cochlea of Mongolian gerbils, Laryngoscope 111 (2001) 528–534.
[12] S. Usami, Y. Takumi, S. Fujita, H. Shinkawa, M. Hosokawa, Cell death in the inner ear associated with aging is apoptosis? Brain Res. 747 (1997) 147–150.

[13] D. Labbé, M. Teranishi, A. Hess, W. Bloch, O. Michel, Activation of caspase-3 is associated with oxidative stress in the hydropic guinea pig cochlea, Hear. Res. 202 (2005) 21–27.
[14] D. Brors, C. Aletsee, K. Schwager, R. Mlynski, S. Hansen, M. Schäfers, A.F. Ryan, S. Dazert, Interaction of spiral ganglion neuron processes with alloplastic materials in vitro, Hear. Res. 167 (2002) 110–121.
[15] C. Aletsee, L. Mullen, D. Kim, K. Pak, D. Brors, S. Dazert, A.F. Ryan, The disintegrin kistrin inhibits neurite extension from spiral ganglion explants cultured on laminin, Audiol. Neurootol. 6 (2001) 57–65.
[16] S. Dazert, D. Kim, L. Luo, C. Aletsee, S. Garfunkel, T. Maciag, A. Baird, A.F. Ryan, Focal delivery of fibroblast growth factor-1 by transfected cells induces spiral ganglion neurite targeting in vitro, J. Cell. Physiol. 177 (1998) 123–129.
[17] J. Bde, V. Weir, Significance of the difference between two means when the population variations may be unequal, Nature 187 (1960) 438.
[18] J. Ruel, J. Wang, R. Pujol, A. Hameg, M. Dib, J.-L. Puel, Neuroprotective effect of riluzole in acute noise-induced hearing loss, Neuroreport 16 (2005) 1087–1090.
[19] G.-D. Chen, J. Kong, K. Reinhard, L.D. Fechter, NMDA receptor blockage protects against permanent noise-induced hearing loss but not its potentiation by carbon monoxide, Hear. Res. 154 (2001) 108–115.
[20] Z. Chen, M. Ulfendahl, R. Ruan, L. Tan, M. Duan, Protection of auditory function against noise trauma with local caroverine administration in guinea pigs, Hear. Res. 197 (2004) 131–136.
[21] M.J. Khan, M.D. Seidman, W.S. Quirk, B.G. Shivapuja, Effects of kynurenic acid as a glutamate receptor antagonist in the guinea pig, Eur. Arch. Otorhinolaryngol. 257 (2000) 177–181.
[22] Z. Chen, M. Ulfendahl, R. Ruan, L. Tan, M. Duan, Acute treatment of noise trauma with local caroverine application in the guinea pig, Acta Otolaryngol. 123 (2003) 905–909.
[23] P.P. Lefebvre, T. Weber, J.M. Rigo, H. Staecker, G. Moonen, T.R. Van de Water, Peripheral and central target-derived trophic factor(s) effects on auditory neurons, Hear. Res. 58 (1992) 185–192.
[24] R. Kopke, H. Staecker, P. Lefebvre, B. Malgrange, G. Moonen, R.J. Ruben, T.R. Van de Water, Effect of neurotrophic factors on the inner ear: clinical implications, Acta Otolaryngol. (Stockh) 116 (1996) 248–252.
[25] A.G. Cheng, T. Huang, A. Stracher, A. Kim, W. Liu, B. Malgrange, P.P. Lefebvre, A. Schulman, T.R. Van de Water, Calpain inhibitors protect auditory sensory cells from hypoxia and neurotrophin-withdrawal induced apoptosis, Brain Res. 850 (1999) 234–243.
[26] F. Lallemend, P.P. Lefebvre, G. Hans, J.M. Rigo, T.R. Van de Water, G. Moonen, B. Malgrange, Substance P protects spiral ganglion neurons from apoptosis via PKC-CA2+ -MAPK/ERK pathways, J. Neurochem. 87 (2003) 508–521.
[27] H. Staecker, R. Kopke, B. Malgrange, P. Lefebvre, T.R. Van de Water, NT-3 and /or BDNF therapy prevents loss of auditory neurons following loss of hair cells, Neuroreport 7 (1996) 889–894.
[28] U. Scarpidis, D. Madnani, C. Shoemaker, C.H. Fletcher, K. Kojima, A.A. Eshraghi, H. Staecker, P. Lefebvre, B. Malgrange, T.J. Balkany, T.R. Van de Water, Arrest of apoptosis in auditory neurons: implications for sensorineural preservation in cochlear implantation, Otol. Neurotol. 24 (2003) 409–417.
[29] A.K. Lalwani, J.J. Han, C.M. Castelein, G.J. Carvalho, A.N. Mhatre, In vitro and in vivo assessment of the ability of adeno-associated virus-brain-derived neurotrophic factor to enhance spiral ganglion cell survival following ototoxic insult, Laryngoscope 112 (2002) 1325–1334.