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Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss

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GUINEA-PIGS were exposed to a traumatic sound inducing up to 60 dB hearing loss. Beside the well described mechanical damage to outer hair cells, a total disruption of inner hair cell (IHC)–auditory nerve synapses was acutely observed within the traumatized area. To test the hypothesis that synaptic damage is due to an excessive release of glutamate by the IHCs, we examined the protective effect of the glutamate antagonist kynurenate on noise-induced hearing loss. The high degree of protection observed with kynurenate attests that dendritic damage is an important component in noise-induced hearing loss. Moreover, we demonstrate that a synaptic repair mechanism occurring within the first few days post-exposure is partly responsible for the recovery of temporary threshold shifts after an acoustic trauma. *NeuroReport* 9: 2109–2114 © 1998 Rapid Science Ltd.

Key words: Acoustic trauma; Cochlea; Excitotoxicity; Glutamate; Guinea pig; Inner hair cells; Spiral ganglion neurons; Temporary threshold shift

Introduction

Intense sound stimulation results in various structural changes leading to functional auditory impairment. Early studies have attempted to correlate the functional impairment with stereocilias of the hair cells. It is now clear that the stereocilia of the first row of the outer hair cells (OHCs) are more sensitive to trauma than those of the inner hair cells (IHCs) and those of the second and the third row of OHCs (see for review:[1]). Moreover, acoustic trauma may cause the loss of hair cells, especially OHCs. Beside these well described changes in hair cells, postsynaptic damage at the synaptic pole of IHCs has been also demonstrated. It entails a disruption of the dendrite ending of the primary auditory neurons below the IHCs, leading to synaptic uncoupling.[2–5] Recently, it has been suggested that dendrite damage might be due to excessive release of neurotransmitter from the IHCs, which is toxic (excitotoxic) to the structure and function of the primary auditory neuron.[5] This hypothesis is based upon the fact that IHCs use glutamate as a neurotransmitter (see for review [6,7]). Excess glutamate in the extracellular space below the IHCs could result in permeability changes in the postsynaptic membrane of the dendrite, leading to prolonged depolarization of ionic channel-gated postsynaptic receptors, and thus large cation influxes and passive entry of Cl^-. The resulting osmotic imbalance would cause water to move into the dendrites, increasing intracellular volume (swelling) up to the point of membrane disruption. Indeed, local application of glutamate agonists[8,9] induced a destruction of primary auditory dendrites similar to that observed after acoustic trauma.[2–5] If dendritic damage is caused by excessive release of glutamate during sound exposure, one would expect a glutamate antagonist to protect the postsynaptic target, leading to a significant reduction in functional impairment.

The aim of present experiments was to test the protective effect of a broad spectrum glutamate antagonist, kynurenate, on noise-induced dendritic damage. In a first set of experiments, we determined the vulnerability of the primary auditory dendrites to sound by exposing guinea pigs to different levels of continuous pure-tone. The threshold shifts were evaluated by recording the compound action potential (CAP) from the round window. Anatomical alteration of the dendrites as a function of acoustic input level was assessed using transmission electron microscopy. In a second set of experiments, we analysed the protective effect of the glutamate antagonist kynurenate on the noise-induced dendrite damage by comparing the effects of intense sound exposure on CAP threshold in presence and absence of kynurenate in the cochlear fluids. Because kynurenate has no effect on structures other than IHC-auditory nerve synapses,[10] this study indicates the relative involvement of synaptic and mechanical damage to hair cells after acoustic trauma.

Materials and Methods

The care and use of the animals were carried out according to the animal welfare guidelines of the "Institut de la Santé et de la Recherche Médicale" (INSERM), and under the approval of the French Ministère de l'Agriculture. Pigmented guinea pigs (250-300 g) were anaesthetized with 6% sodium pentobarbital (0.3 ml/kg, i.p.) and artificially respired. Rectal temperature was maintained at 38.5°C ± 1°C, and heart-rate monitored using EKG electrodes. Supplementary doses of sodium pentobarbital were administered as needed.

Electrode implantation and electrophysiological recordings: The right bulla was exposed postauricularly under sterile conditions, by cutting through the connective tissue and repelling the neck musculature. The bulla was opened to expose the cochlea, and a recording electrode was placed on the round window membrane. The bulla, including the recording electrode, was closed with dental cement. The round window and the reference electrode, placed in the neck muscle, were then soldered to a plug fixed on the skull.

Cochlear responses recorded from round window electrode were elicited by tone bursts of alternate polarity (1 ms rise/fall time, 8 ms duration) of varying intensity (0 to 100 dB SPL, in 5 dB steps). The rate of presentation was 10 per second. The threshold of the CAP was defined as the dB SPL needed to elicit a measurable response ($\geqslant 5\ \mu V$).

Intracochlear perfusion technique: This method has been extensively described elsewhere.[7] Briefly, the cochlea was exposed ventrolaterally and two holes (0.2 mm diameter) were gently drilled in the basal turn, one hole in the scala tympani, the other in the scala vestibuli. Artificial perilymph alone or containing 5 mM of kynurenate was perfused through the hole in the scala tympani and allowed to flow out of the cochlea via the hole made in the scala vestibuli. The rate of the perfusion was set at 2.5 µl/min. The pipette tip diameter was 0.15 mm. A ring of glue placed near its tip provided a leak proof seal between the pipette and the cochlea.

Experimental protocol: To study the vulnerability of the primary auditory dendrites to sound, 32 guinea pigs were used. A pre-exposure audiogram was obtained in each animal by averaging CAP in responses to tone pips over the frequency range of 2 to 20 kHz. Each animal was then exposed to a 6 kHz continuous tone for 15 minutes under anesthesia. The level of this pure tone ranged from 100 to 130 dB. Twenty minutes after the end of the stimulation another CAP audiogram was measured to obtained short-term threshold shift.

The protective effect of kynurenate was tested on 10 additional animals. After CAP audiogram measurement, the animals received an initial perfusion of artificial perilymph. Ten minutes after the start of a second perfusion, a continuous tone (6 kHz, 130 dB SPL) was presented for 15 minutes. During this second perfusion which lasted 35 minutes (10 min. before, 15 min. during and 10 min. after sound exposure), 5 animals received artificial perilymph alone, whilst the 5 others were perfused with artificial perilymph containing 5mM kynurenate. A last 10 min-perfusion with artificial perilymph was then performed. The CAP thresholds were evaluated after the final perfusion (i.e. 20 min. after sound exposure). The time events of the kynurenate experiment is summarized in Figure 1. Immediately after the last audiometric measurement the cochleas were classically processed for transmission electron microscopy (TEM) as already described.[9]

Long-term recovery was also tested after 130 dB SPL tone exposure in 10 animals. The CAP audiogram was measured before and 20 minutes after sound exposure in anesthetized animals and 1, 3, 10, 15 and 30 days in awake animals. For histological purpose, the cochleas were prepared for TEM 5 days (n=3) and 30 days (n=3, corresponding to the animals presented in Fig. 5) after intense sound exposure.

Results

Acute effects of acoustic trauma: These experiments were performed to determine the vulnerability of primary auditory dendrites to sound by exposing guinea pigs to different levels of continuous pure-tone. When measured 20 minutes after the exposure, - 15 min of continuous 6 kHz pure-tone-, the CAP threshold elevation was in the range of 25 dB with a 100 dB SPL exposure, and increased up to 80 dB with a 130 dB exposure. Moreover, the maximum elevation located around 10 kHz at 100dB SPL shifted towards higher frequencies with the increase of sound intensity: 16 kHz at 130 dB SPL (Fig. 2).

Examination of the traumatized cochleas revealed no dendrite damage after 100 and 110 dB exposure. After 120 and 130 dB SPL exposure, dendrite damage was systematically seen in the basal turn (3 to 5 mm from the round window). In this area, all radial dendrite endings below the IHCs were swollen (at 120 dB SPL) and disrupted (at 130 dB SPL). This resulted in an indentation of the IHC basal pole (Fig. 4A, B). In addition, classical signs of hair cell early degeneration were noticed in the first row of the outer hair cells (OHCs). All other sensory and

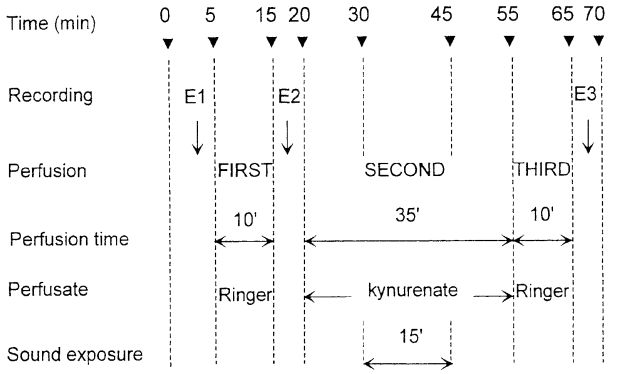

FIG. 1. Time scale of experimental protocol.

neural structures in the organ of Corti, including the supporting cells, appeared normal.

Protective effects of kynurenate on sound-induced dendrite damage: To test the hypothesis that dendrite damage observed below the IHCs was linked to an excessive release of glutamate, 5 mM of kynurenate was applied during the sound exposure (130 dB SPL). After rinsing out the drug, CAP thresholds were compared to those recorded before trauma. Our results showed that the CAP threshold shift was significantly less (about 40 dB between 8 and 16 kHz, Fig. 3) in kynurenate-treated cochleas than in controls perfused with artificial perilymph only (70 to 80 dB loss between 8 and 16 kHz, Fig. 3). TEM observations were consistent with these electrophysiological data, revealing OHC damage but no dendrite alteration below IHCs in kynurenate-treated-animals (Fig. 4).

Long-term effects of acoustic trauma: Animals exposed to 130 dB SPL pure tone were daily tested during one month. When measured one month after sound exposure, the threshold shifts were not greater than 20 dB SPL, which correspond to a partial recovery of about 60 dB. Examination of the time course showed a fast recovery of thresholds within the first week (about 50 dB recovery) followed by a slow recovery (about 10 dB) within the next two weeks to reach the 20 dB permanent threshold shift level 15 days after noise exposure (Fig. 5). TEM analysis was used to assess the morphological correlates of this functional recovery. While OHC damage was clearly observed 5 days after exposure, the pattern of innervation of IHCs looked normal, i.e., typical synaptic differentiations were observed at contacts between auditory nerve endings and IHCs (Fig. 6). When compared with control cochleas, the high density of the synaptic vesicles in the lateral efferent terminals is particularly striking, suggesting a very intense metabolic and/or functional activity of these efferents (Fig. 6).

Discussion

The protective effect of kynurenate against the acoustic trauma observed in this study confirms and extends previous results, suggesting that dendrite damage below IHCs is due to an excessive release of glutamate from the IHCs.[4,5] Moreover, the high degree of protection induced by the drug, -i.e., about 50 % reduction of noise-induced hearing loss-, attests that postsynaptic damage to the auditory dendrites

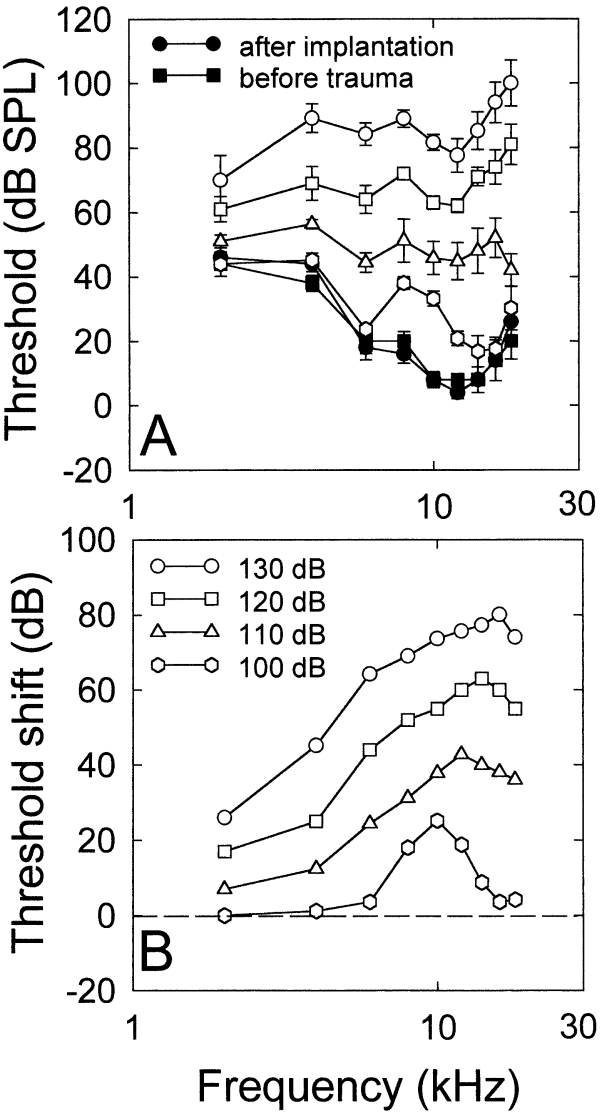

FIG. 2. CAP threshold as a function of continuous sound exposure level. (A) CAP threshold in dB SPL (mean ± SEM) just after electrode implantation (filled circle), before (filled square) and 20 minutes after continuous sound exposure at different levels. (B) Threshold shift calculated as the difference from the recording before and 20 minutes after 6kHz continuous tone exposure. Shown, are threshold shift recorded after 100 (empty hexagon), 110 (empty triangle), 120 (empty square), and 130 dB SPL (empty circle) exposure during 15 minutes.

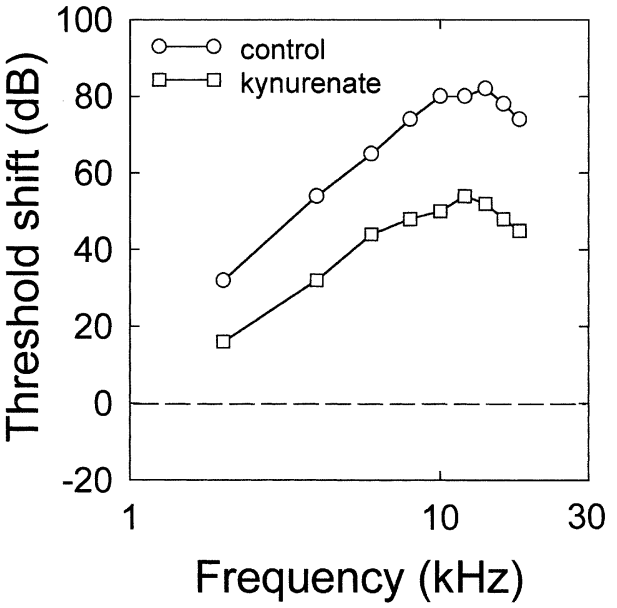

FIG. 3. Protective effect of kynurenate on noise-induced hearing loss. Shown, are data obtained after intense sound exposure during perfusion with artificial perilymph alone (n=5, empty circle) and after intense sound exposure during perfusion with artificial perilymph containing 5 mM of kynurenate (n=5, empty square). The data are expressed as means ± SEM.

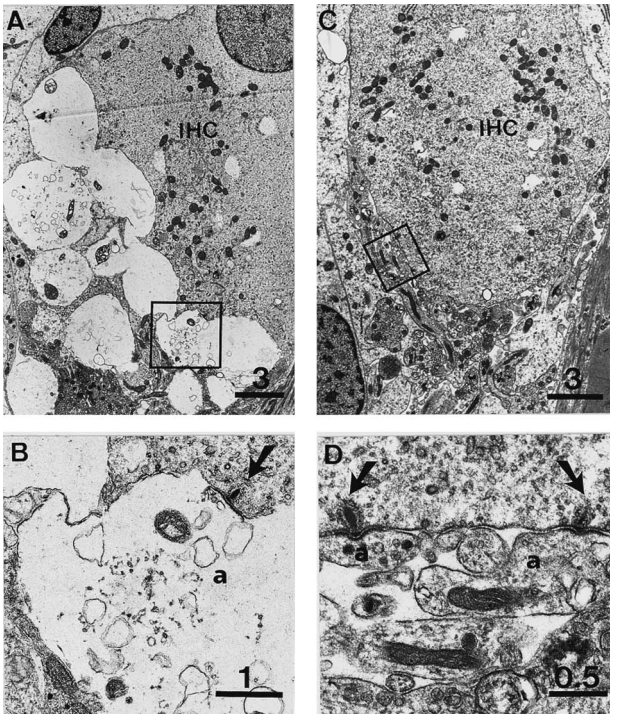

FIG. 4. Transmission electron microscopy 20 minutes after 130 dB sound exposure. (A) TEM micrograph at the base of an IHC in the traumatized area. The acoustic trauma induced a dramatic swelling of all auditory dendrites which resulted in an osmotic-induced indentation of the IHC basal pole. This massive swelling of the dendrites often results in a total disruption of postsynaptic membrane. Scale bar = 3μm. (B) Enlargement of the area framed in A. Presynaptic differentiation with a synaptic body (arrow) is seen at the IHC basal pole facing a piece of the postsynaptic membrane and remnants of the dendritic (a) cellular content. Scale bar = 1 μm. (C) Protective effect of kynurenate. No sign of dendritic damage is observed at the IHC base. Scale bar = 3 μm. (D) At higher magnification of the area framed in C, two synapses are seen, evidenced by the presence of synaptic bodies (arrows) surrounded by microvesicles. The postsynaptic afferent dendrites (a) are unaffected, due to the protective effect of kynurenate. Scale bar = 0.5 μm.

is an important component of noise-induced hearing loss. Finally, the fact that this synaptic injury was totally reversible within a week after sound exposure suggests that the synaptic repair mechanism is strongly involved in restoring function after an acoustic trauma.

Acute effects of acoustic trauma: In 1988, Puel *et al.*[11] tested the ability of kynurenate to reduce the effect of 95 dB SPL continuous sound exposure at 6 kHz for 15 minutes. Because kynurenate did not reduce the effect of this sound exposure, it was speculated that the acoustic level used was insufficient to produce dendritic damage, which would require more than 95 dB. The first set of experiments was thus designed to test the vulnerability of primary auditory dendrites by exposing guinea pigs to different levels of continuous pure-tone.

For tone exposures at 100 or 110 dB SPL, threshold elevations were lower than 40-50 dB, and no obvious abnormality at the pre- (hair cells) or postsynaptic (dendrites) levels could be observed in TEM. This suggests that, when hearing losses are not greater than 50 dB, only light mechanical and/or elec-

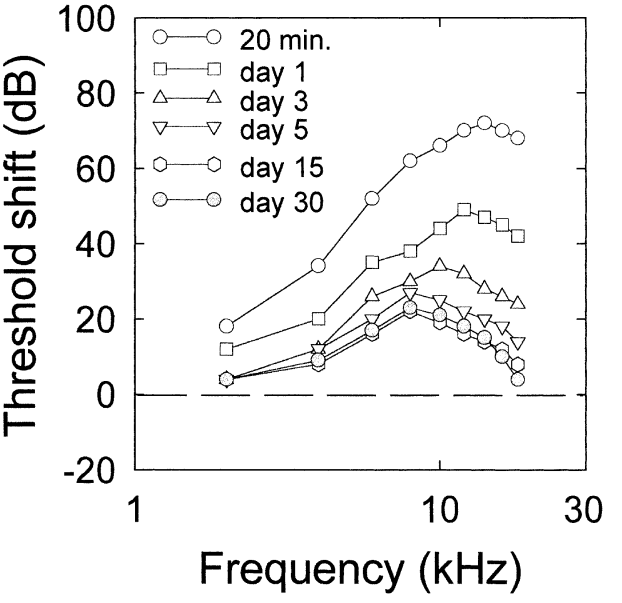

FIG. 5. Recovery of thresholds after 130 dB sound exposure. The CAP thresholds were measured 20 minutes and 1, 3, 5, 15 and 30 days after sound exposure. Note the fast CAP threshold recovery that occurred within the 5 days following acoustic exposure. A permanent threshold shift of 20 dB at 8 kHz was observed 15 days after exposure.

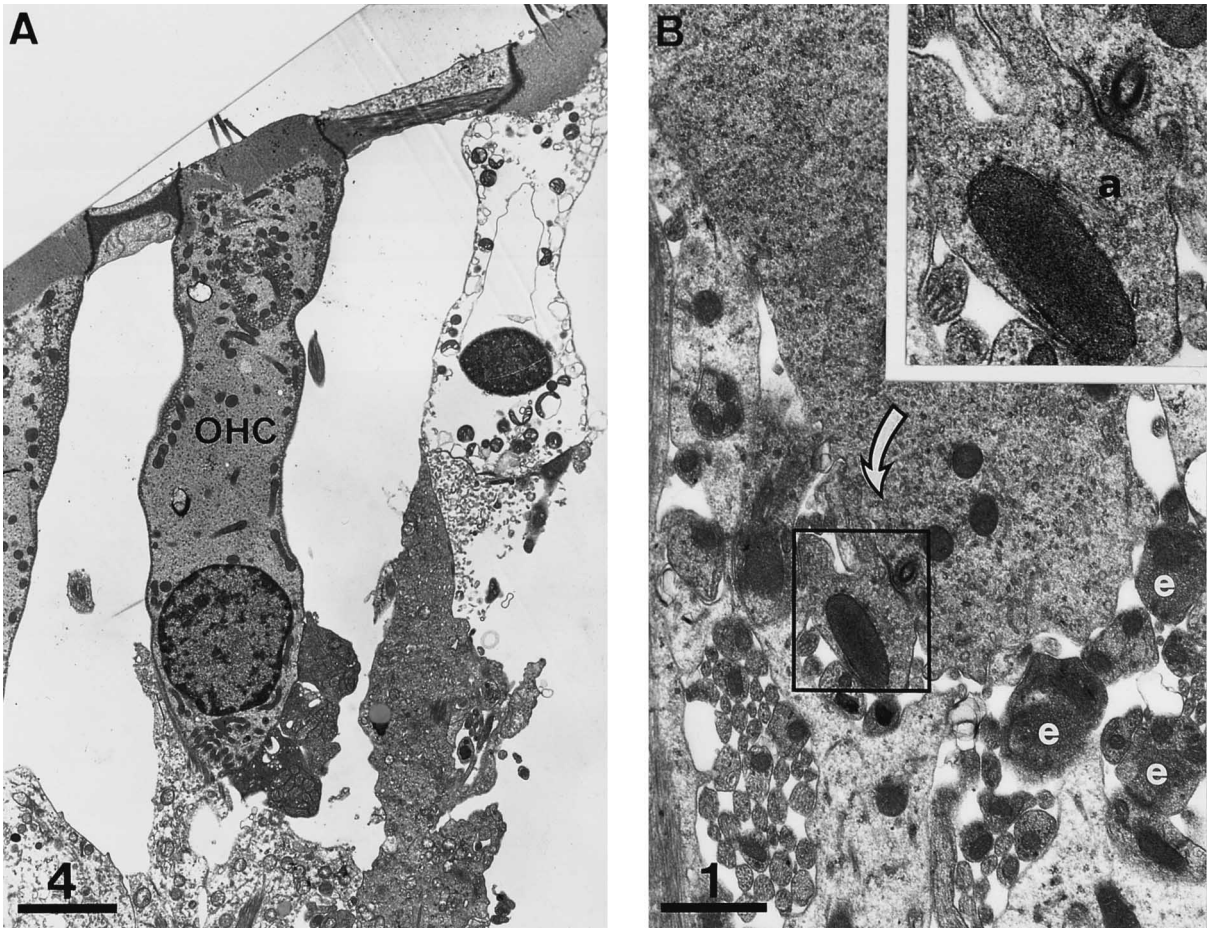

FIG. 6 Transmission electron microscopy 5 days after 130 dB sound exposure. (A) TEM micrograph of the OHCs from the traumatized area 5 days after sound exposure. Note that the first row OHC (right) is degenerating, and the second row OHC (middle) shows vacuolization and Hensen's body in the supranuclear portion. Scale bar = 4 μm. (B) TEM of the base of an IHC from the same area. As compared to Fig. 4A, a clear reorganization is observed. The auditory dendrites are again in contact with the IHC: a typical synaptic differentiation is seen (white arrow and frame). Note the dark efferent terminals (e). Scale bar = 1 μm.

trical dysfunction occurs. Indeed, Puel *et al.*[12] found that 95 dB SPL tone exposure inducing 25 dB threshold shift reduced the amplitude of the distortion product otoacoustic emissions in the same proportion, suggesting that only non-linear mechanical properties of the cochlea had been affected by the sound exposure. Increasing the sound exposure level up to 120 dB resulted in dendritic swelling and changes in OHCs from the first hair cell row in the basal portion of the cochlea. When exposure was set up to 130 dB, a dramatic increase in damage was observed, the dendrite swelling being accompanied by membrane disruption.

Protective effects of kynurenate: In order to study the involvement of pre-synaptic (hair cells) as opposed to post-synaptic (dendrites) structures in cochlear dysfunction after acoustic trauma, we prevented dendritic damage by applying the glutamate antagonist kynurenate prior to and during the 130 dB SPL sound exposure. In the tested (kynurenate) animals, hair cell damage, but no dendritic damage, were observed. Functionally, the animals showed hearing losses in the same frequency range as in controls (noise alone), but reduced by 50%. This demonstrates the major contribution of synaptic damage to noise-induced hearing loss and supports the hypothesis that excessive release of glutamate from IHCs alters the afferent dendrites and reduces their function.[5]

Long-term effects of acoustic trauma: One month after exposure, a partial recovery of the CAP-threshold was observed. The remaining threshold shift, which could be considered as permanent (PTS), after a 130 dB sound exposure was about 20 dB SPL. Histological examination of the cochleas revealed OHC damage, but the base of the IHCs appeared normal, indicating that the synaptic damage was reversible. Indeed, the reversibility of such noise-

induced damage has already been suggested by Robertson[4] who reported a recovery of dendrite swelling and N1 threshold within 48 hours after exposure to 110 dB SPL, 10 kHz for 30 minutes. In the present experiment, kynurenate reduces the traumatic effect of 130 dB exposure from 80 to 40 dB hearing loss. It can thus be speculated that at least the 40 dB of the threshold recovery observed one month after exposure could be attributed to a synaptic repair mechanism, similar to what we have described after a direct excitotoxic injury.[13] In that study, an AMPA exposure disrupted the IHC-auditory nerve synapses, but cochlear neurons regenerated their dendritic neurites and formed new functional synapses within 5 days. Nevertheless, the difference between the 40 dB threshold shift in kynurenate perfused animals and the 20 dB threshold shift measured two weeks after exposure may be accounted for by another repair mechanism, perhaps occuring in OHCs or their stereocilia.

Conclusion

This study shows that the glutamate antagonist kynurenate protects against sound-induced synaptic damage. It also indicates that the contribution of synaptic damage to noise-induced hearing loss represents about 50% of the acute threshold shift after acoustic trauma. Finally, a synaptic repair mechanism, similar to what has been observed after local application of the glutamate agonist AMPA[13], is present and may account for a large part of the functional recovery. It is tempting to propose that after trauma, the repair of the synapses predominates during a first rapid phase of the recovery (within the 5 days following sound exposure), whilst a second mechanism at OHC level is responsible for the second and slower phase of the recovery. Further experiments will thus be necessary to test this hypothesis.

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