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Changes in Purinoceptor Distribution and Intracellular Calcium Levels following Noise Exposure in the Outer Hair Cells of the Guinea Pig

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Abstract. Among the cells of the inner ear, the outer hair cells (OHCs) are the most important targets of noise-induced effects, being the most sensitive cell types. The aim of this study was to examine the effects of noise (50 Hz-20 kHz, 80 dB sound pressure level, 14 days) on intracellular calcium levels and on the expression pattern of purinoceptors in the membrane of the OHCs of the guinea pig and to measure the stiffness changes of the lateral membrane of these cells. In noise-exposed animals, the resting intracellular calcium concentration increased compared to nontreated animals and was slightly higher in the cells of the basal (219 ± 29 nM) than in the apical (181 ± 24 nM) turns of the cochlea. After application of 180 μM adenosine triphosphate, the intracellular calcium level rose by 60 ± 22 nM in cells from the apical and by 44 ± 10 nM in cells from the basal turns, significantly less than in nontreated animals. Expression of the P_{2X1}, P_{2X2}, P_{2X4}, P_{2X7}, P_{2Y1} and P_{2Y4} receptor subtypes was suppressed, while expression of the P_{2Y2} subtype did not decrease in either of the two preparations. In parallel with the increase in intracellular calcium concentration, the stiffness of the lateral wall of the OHCs was increased. Noise-induced changes in intracellular calcium homeostasis and subsequently in the calcium-dependent regulatory mechanisms may modify OHC lateral wall stiffness and may lead to reduction of the efficacy of the cochlear amplifier.

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Introduction

The influence of noise on hearing sensitivity is the focus of experiments in the field of inner ear research since many people suffer from its consequences. Sustained high-level noise causes sensorineural hearing loss due to cell damage, often leading to the death of different cell types in the inner ear (Decory, Hiel & Aran, 1991; Subramaniam et al., 1994; Henderson et al., 1994; Canlon & Fransson, 1995). Using morphological and functional examinations (distortion product otoacoustic emission [DPOAE], and auditory brain stem response [ABR]), neither hair cell damage nor change in outer hair cell (OHC) function was found by Canlon & Fransson (1995) after exposure to 81 dB sound pressure level (SPL) for 24 days. In contrast, with exposure to high-level noise (105 dB SPL for 72 h), both OHC loss and decreased amplitude of DPOAE were observed by the same authors. When using lower-intensity sound for sound conditioning before use of the traumatic noise, they found that the OHC loss and the decrease in amplitude of DPOAE were significantly lower.

In chinchillas, which were exposed to 95 dB SPL octave band noise for 15 days, the DPOAE changed; but it returned to preexposure baseline values 5 days after exposure (Subramaniam et al., 1994). Similarly, Henderson and coworkers (1994) found severe

cochlear damage in chinchillas after exposure to 119–137 dB sound.

Among the cells of the inner ear, OHCs are the most important targets of these noise-induced effects as they are the most sensitive cell type, contrary to inner hair cells or pillar cells (Ou, Bohne & Harding, 2000). High-level noise exposure causes morphological changes, such as damage to stereocilia, changes in the intracellular structures especially the mitochondria and swelling or loss of OHCs (Saunders, Dear & Schneider, 1985). These degenerative changes occur mainly in the basal 20% of the organ of Corti (Ou et al., 2000). Subsequently, these changes can lead to cell damage (Berridge, 1994; Morley, Hogan & Hakim, 1994) and alter the sensitivity of hearing.

Decory and coworkers (1991) showed the modified viability and motility of OHCs isolated from high-level noise-exposed (250 Hz, 125 dB SPL) guinea pigs. Subsequent experiments linked these changes to the Ca^{2+} entering through the surface membrane channels (Fridberger et al., 1998) and the increased intracellular calcium level. Indeed, the increased intracellular Ca^{2+} level could initiate processes that may cause stiffness changes of the lateral wall (Batta et al., 2003, 2004; Frolenkov, Mammano & Kachar, 2003) of the OHCs, and these stiffness changes may constitute an intrinsic mechanism in dynamic adaptation of the organ of Corti (Dallos et al., 1997; Batta et al., 2003, 2004). This process may be responsible for the above-mentioned motility changes. Noise exposure can also modify changes in the response to different neurotransmitters or neuromodulators, e.g., to adenosine triphosphate (ATP) (Chen, Nenov & Bobbin, 1995). In their experiments, guinea pigs were exposed to moderate-intensity narrow band noise (cut-offs at 1.1 and 2 kHz; A-scale, rms: 65 dB SPL) 24 h a day for 10–11 days. There were significant differences in the incidence and magnitudes of cation currents evoked by ATP (100 μM), with a decreased response to ATP in short and an increased response in longer OHCs.

ATP is an important neurotransmitter in the inner ear. Both main subclasses of purinergic receptors, the ionotropic P_{2X} receptors and the G protein-coupled metabotropic P_{2Y} receptors, have been described in the inner ear (Mockett et al., 1995; Parker et al., 1998; Järlebark, Housley & Thorne, 2000; Nikolic, Housley & Thorne, 2003; Szűcs et al., 2004). With respect to OHCs, Housley and coworkers (1992) examined the characteristics of the ATP-induced current, which showed the pharmacological profile of a P_{2X} purinoceptor-mediated current. Measuring the ATP-induced changes of intracellular calcium levels in OHCs, Mammano and coworkers (1999) found that the elevation of the intracellular calcium level is first detected at the apical end and then followed by a delayed rise at the basal part. This observation suggests a nonhomogenous distribution of P_2 receptors in the membrane of OHCs, which was shown by immunocytochemistry (Szűcs et al., 2004). In another set of experiments, Chen and coworkers (1995) observed that there were significant differences in the incidence and magnitudes of cation currents evoked by ATP (100 μM) in OHCs from guinea pigs exposed to chronic low-level noise.

On the other hand, the chronic release of ATP has been suggested to result in a change in the expression of purinoceptors (Maynard, Loesch & Burnstock, 1992). This mechanism was proposed to explain the altered response to an ATP analogue in the rabbit central ear artery after chronic electrical stimulation of the great auricular nerve (Maynard et al., 1992). Similar changes are suspected to happen in the OHCs of the inner ear after chronic stimulation. Indeed, chronic exposure to moderately intense sound altered the response of OHCs to the addition of ATP (Chen et al., 1995). One explanation could be that this chronic exposure to moderately intense sound modified the expression of purinoceptors in the membrane of the OHCs.

The aim of the present study was to examine the effect of noise (80 dB SPL, 14 days) on intracellular calcium levels and on the expression pattern of purinoceptors in the membrane of OHCs of the guinea pig, to establish the background of the observed functional changes of the organ of Corti by measuring the changes in stiffness of the lateral membrane of these cells. We describe an increased resting calcium concentration with a decreased response to ATP in noise-exposed animals. In parallel, expression of the P_{2X1}, P_{2X2}, P_{2X4}, P_{2X7}, P_{2Y1} and P_{2Y4} receptor subtypes was suppressed, whereas expression of the P_{2Y2} subtype did not decrease. As a result of the increased intracellular calcium level, changes in the stiffness of the lateral membrane were detected. The changes were slightly more pronounced in cells from the basal compared to the apical turns of the cochlea.

Methods

Noise Exposure

The intensity of the noise was set below the level thought to induce cellular damage based on former functional and morphological experiments. Exposure to low-level, long-term pure tone stimulus (1 kHz, 81 dB SPL, 24 days) did not reveal any significant hair cell loss or any change in the ABRs and DPOAEs in guinea pigs (Canlon et al., 1995). Guinea pigs were therefore exposed to continuous, moderate-intensity broadband noise (50 Hz-35 kHz, 80 dB SPL) 24 h a day for 2 weeks. The noise level varied < 2 dB within the space of the cage.

Cell Preparation

Young guinea pigs of either sex (150–300 g) were anesthetized by sodium pentobarbital (Nembutal; Abbott, North Chicago, IL) and

decapitated according to the approved protocol of the Animal Care Committee of the University of Debrecen, as described earlier (Szűcs et al., 2004). Briefly, the temporal bones containing the bulla were removed and the bulla was opened to expose the cochlea. Each cochlea was placed into Hank's solution containing (in mM) NaCl 142, KCL 4, $CaCl_2$ 2 and $MgCl_2$ 1. The pH was adjusted to 7.4 and the osmolarity to 310 mOsm. The dissection of the organ of Corti and the isolation and digestion (collagenase type IV, 1 mg/ml, 5 min; Sigma, St. Louis, MO) of the OHCs were carried out in this extracellular solution. For the subsequent examinations, we made two different preparations, one from the apical two turns and one from the basal turn of the cochlea.

Ca^{2+} Fluorescence Imaging

We determined intracellular calcium levels on 30 OHCs. The measurements were carried out in the extracellular solution given above. Changes in the intracellular Ca^{2+} concentration were monitored using Fura-2, as described in our earlier reports (Szappanos et al., 2004; Szűcs et al., 2004). In brief, isolated cells were placed on a coverslip and loaded with 5 μM Fura-2 AM for 20 min. Coverslips with Fura-2 AM (acetoxy methyl ester)-loaded cells were then placed on the stage of an inverted fluorescence microscope (Diaphot; Nikon, Tokyo, Japan). The excitation wavelength was altered between 340 and 380 nm by a microcomputer-controlled dual-wavelength monochromator (Deltascan; Photon Technology International, New Brunswick, NJ), whereas the emission was monitored at 510 nm using a photomultiplier at 10 Hz acquisition rate of the ratios. Intracellular [Ca^{2+}] was calculated from the ratio of measured fluorescence intensities as described (Grynkiewicz et al., 1985).

The measuring bath was constantly perfused with normal 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) Tyrode's solution (in mM, 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 11.8 HEPES-NaOH, 1 g/l glucose, pH 7.4) at a 2 ml/min rate (Econo Pump; Bio-Rad, Budapest, Hungary). Test solutions were directly applied to the cells through a perfusion capillary tube (Perfusion Pencil™; AutoMate Scientific, San Francisco, CA) with an internal diameter of 250 μm at a 1.5 μl/s rate, using a local perfusion system (Valve Bank™ 8 version 2.0, AutoMate Scientific).

Immuncytochemistry

About 50 giunea pigs were used to perform the immunocytochemistry reaction. After isolation, the cells were fixed with paraformaldehyde for 20 min. The cells were then incubated in a blocking solution of phosphate-buffered saline (PBS) supplemented with bovine serum albumin (1% BSA and 0.5% Triton X-100, Sigma) for 30 min. Subsequently, the cells were incubated with polyclonal antibody (1:300; Alomone Labs, Jerusalem, Israel) against the carboxy terminus of the purinergic receptors for 90 min, as described earlier (Szűcs et al., 2004). The cells were washed three times with PBS. The secondary antibody (labeled with fluorescein isothiocyanate) was diluted 1:1,000 in PBS. The cells were washed again three times in PBS and then incubated with propidium iodide (1:1,000) to visualize the nuclei. After washing the specimens three times, they were covered with Vectashield (Vector Laboratories, Burlingame, CA). The slides were then observed under a fluorescent microscope. To avoid making mistakes in the immunocytochemical reactions, we performed this reaction three times and compared the three reactions to each other. No cross-reactivity between subtypes was detected, and further testing for specificity showed that staining was eliminated when the primary antibody was eliminated or added in the presence of the specific homologue-blocking peptide antigen from Alomone Labs.

Measurement of Lateral Wall Stiffness

Stiffness of the lateral wall was measured by the glass micropipette aspiration technique combined with a pseudocolor image analysis method (Batta et al., 2003). Glass micropipettes were pulled from Clark EC15TF capillaries with an inner diameter of 2.8 μm. Pipettes were filled with extracellular solution and connected to the calibrated water column controlled pressure application apparatus through a polyethylene plastic tube. The stiffness parameter (Sp) was determined in the midlateral region of OHCs and calculated from the length of the aspirated lateral wall segment into the suction pipette at the end of a 30-s period of $6H_2Ocm$ negative pressure (Oghalai et al., 1998; Batta et al., 2003). All measurements were carried out within 40 min after the death of the animal.

Data Analysis

Statistical comparisons were made using Student's *t*-test and, when appropriate, paired *t*-test at $P = 0.05$. For all experiments, the standard error of the mean (SEM) is reported.

Results

Calcium Transients in OHCs

Figure 1 shows representative calcium transients recorded in OHCs under control conditions and after moderate noise exposure. Since there was no statistical difference between the data of the apical and basal cells in the control cell population, the two groups were mixed and used together as a control group. Resting intracellular calcium concentration of the OHCs was 133 ± 12 nM ($n = 14$). After addition of 180 μM ATP for 30 s to the cells, the intracellular calcium concentration rose by 266 ± 65 nM with a latency of 5.9 ± 1.0 s (Table 1).

After noise exposure, some cells showed degenerative signs and abnormal shape; they were more sensitive to injury and did not respond to ATP. Those cells fitting the morphological criteria of a healthy cell – cylindrical shape, intact stereocilia, appearance of normal turgor and normal nucleus location – showed a significantly higher resting intracellular calcium concentration. We also found a slight difference between the cells which originated from the apical and the basal turns. The resting calcium concentration in the noise-exposed cells of the apical two turns was 181 ± 24 nM ($n = 11$), whereas in the cells of the basal turn it was even higher, 219 ± 29 nM ($n = 8$, Table 1). In line with these observations, the response of the cells to 180 μM ATP for 30 s was smaller with a longer latency time, and we again found a slight difference between the two cell populations in this respect (Fig. 1). The intracellular calcium concentration rose by only 60 ± 22 nM with a latency of 8.4 ± 2.2 s ($n = 11$) in the apical turns, whereas it rose by 44 ± 10 nM with a latency of 9.9 ± 1.1s ($n = 8$) in the basal turn (Table 1).

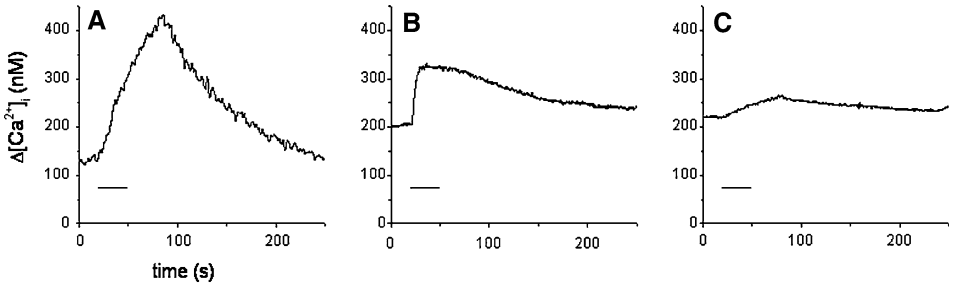

Fig. 1. ATP-induced rise in $[Ca^{2+}]_i$ in OHCs under control conditions (*A*) and after noise exposure (apical cell, *B,* and basal cell, *C*). The cells were loaded with Fura-2 AM and $[Ca^{2+}]_i$ was measured as described in Materials and Methods. Since no statistical difference was found between the data of the apical and basal cells in the control cell population, the two groups were mixed and used together as a control group. In control conditions (*A*), the representative curve shows that the intracellular calcium level rose by 278 nM with a latency of 6.3 s after addition of 180 μM ATP (30 s). In OHCs isolated after noise exposure, the rise of the intracellular calcium level was lower with a longer latency time after addition of 180 μM ATP (30 s). There was a difference between the increase in the intracellular calcium level in the OHCs of the apical (*B*) two turns and the basal (*C*) turn of the cochlea. While the amplitude of the calcium transient was 109 nM with a latency time of 8.3 s in the cell from the apical two turns, it was lower in the basal turn of the cochlea (amplitude 47 nM, latency time 10.2 s).

Table 1. Resting $[Ca^{2+}]_i$ and parameters of ATP-evoked calcium transients on OHCs Data given are mean ± SEM

	Control (n = 14)	Noise-exposed Apical (n = 11)	Basal (n = 8)
Resting $[Ca^{2+}]_i$ (nM)	133 ± 12	181 ± 24*	219 ± 29*
Amplitude (nM)	266 ± 65	60 ± 22*	44 ± 10*
Latency (s)	5.9 ± 1.0	8.4 ± 2.2	9.9 ± 1.1
Max. rate of rise (nM/s)	84 ± 14	68 ± 8*	41 ± 13*

*Significant difference ($P < 0.05$) compared to control.

Purinoreceptor Subtypes on OHCs

According to previous morphological and functional experiments, showing that the basal part of the cochlea is more susceptible to the effect of noise (Nordmann, Bohne & Harding, 2000; Ou et al., 2000; and *see above*), cells from the basal and the apical parts of the cochlea were separated in the subsequent experiments. The presence of the four P_{2X} and three P_{2Y} receptor subtypes, which were described in our previous experiments in control animals (Szűcs et al., 2004), were detectable in the membrane of the OHCs of noise-exposed guinea pigs in both the apical and the basal preparations. The expression level of P_{2X1}, P_{2X2}, P_{2X4}, P_{2X7}, P_{2Y1} and P_{2Y4} receptor subtypes was lower, while that of the P_{2Y2} receptor subtype did not change in the noise-exposed animals (Fig. 2). We did not find any significant difference in receptor expression between the cells isolated from the apical and basal parts of the cochlea.

As described in our earlier report (Szűcs et al., 2004), in the control group the cells displayed intensive labeling with P_{2X1}, P_{2X2} and P_{2X4} and only weak labeling with P_{2X7}. Antibodies against the P_{2Y} receptors revealed strong immunolabeling with all three receptors tested. In some cases, expression of the given purinoceptor was nonhomogenous (P_{2X2}, P_{2X7}, P_{2Y2}, P_{2Y4}). In contrast, expression of all purinoceptors was homogenous in the noise-exposed group.

Effect of Noise Exposure on the Stiffness of the OHC Lateral Wall from Different Cochlear Locations

As described in earlier reports, mechanical stimuli initialize the regulatory stiffness response, an intrinsic regulatory system of OHCs. The regulatory stiffness response increases OHC lateral wall stiffness, which may play a protective role against long-term noise stimulus (Batta et al., 2003, 2004).

Table 2 summarizes the Sp of the lateral wall of OHCs determined from cells isolated from noise-exposed and control animals. Data are given for both cochleobasal and cochleoapical cells. The cells had to satisfy the following morphological criteria: cylindrical shape, intact stereocilia, appearance of normal turgor and normal nucleus location. Under these experimental conditions, again no significant difference was found between the Sp of cochleobasal and cochleoapical cells of control animals. However, the influence of noise exposure on the stiffness of the lateral wall of the OHCs was not dependent on the localization of the cells along the cochlea.

Discussion

Here, we report the changes in the resting intracellular calcium level, the ATP-induced intracellular calcium transients as well as the expression pattern of several purinoreceptor subtypes due to moderate noise exposure. According to previous experiments,

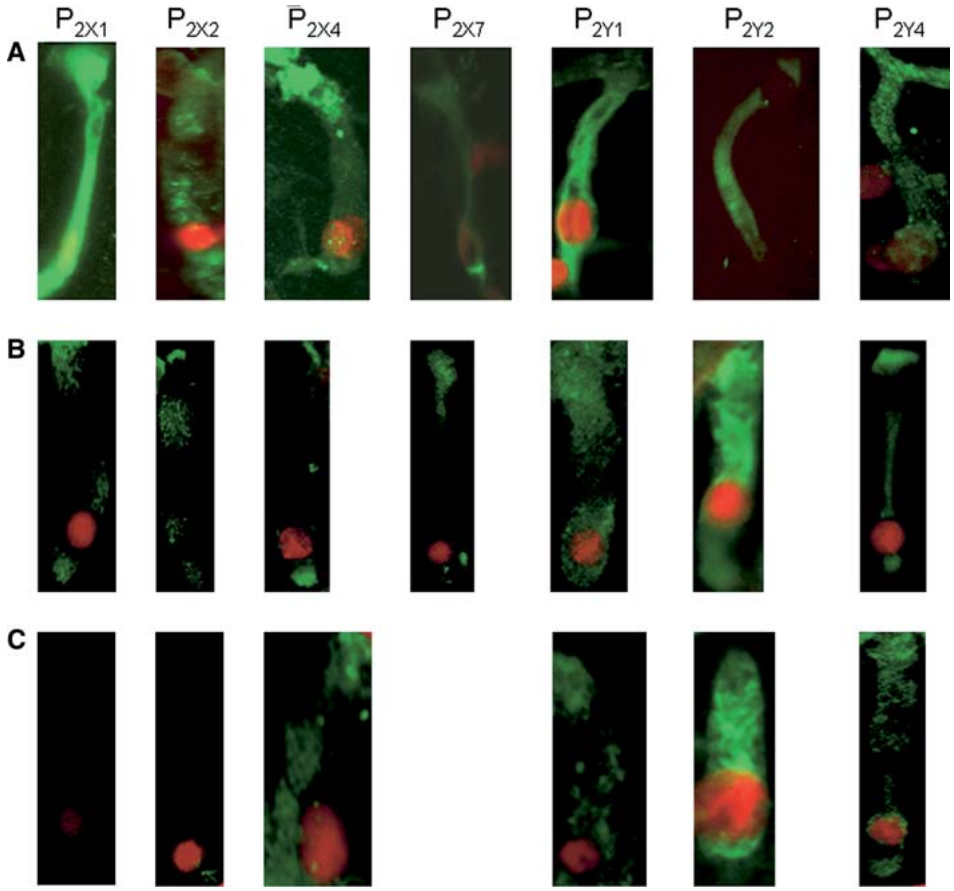

Fig. 2. Expression of purinoceptor subtypes in OHCs in control condition (*A*) and effects of noise exposure on cells isolated from the apical (*B*) and basal (*C*) parts of the cochlea. In the control group (*A*), intensive labeling was found with P_{2X1}, P_{2X2} and P_{2X4} and weak labeling was detected with P_{2X7}. Antibodies against P_{2Y} receptors revealed strong immunolabeling with all three receptors (P_{2Y1}, P_{2Y2}, P_{2Y4}) tested. For some of the purinoceptors, expression was nonhomogenous (P_{2X2}, P_{2X7}, P_{2Y2}, P_{2Y4}). In the noise-treated group (*B,* apical; *C,* basal), expression of P_{2X1}, P_{2X2}, P_{2X4}, P_{2X7}, P_{2Y1} and P_{2Y4} purinoceptors was reduced, while expression of P_{2Y2} did not decrease. The distribution of these receptors in the membrane of OHCs was homogenous after noise exposure. We did not find a difference in receptor expression between cells isolated from the apical and basal parts of the cochlea. To facilitate the comparison with control data, the intensity values in regions of interest (ROIs) defined on the cells in red-green-blue (RGB) images were also determined. The average ROI intensities on the P_{2X1}-labeled cell after noise exposure were 17 (basal cell) and 43 (apical cell), while that of the control P_{2X1}-labeled cell was 95. The ROI was 60 for P_{2Y2} labeling in control and 79 and 93 (basal and apical cells, respectively) after noise exposure.

moderate noise exposure (1 kHz, 81 dB SPL, 24 days) did not cause damage of the organ of Corti and OHC loss in guinea pigs, which was proved by morphological and functional/audiological experiments (Canlon et al., 1995). Contrary to this observation Skellet and coworkers (1996) found that in guinea pigs low-level sound exposure (65 dB SPL narrowband noise for 10–11 days) can change cochlear function.

In chinchillas exposed to 95 dB SPL octave band noise for 15 days, the DPOAEs changed, but this recovered completely to preexposure baseline values 5 days after exposure (Subramaniam et al., 1994). With the use of lower intensities (86 dB octave band noise for 24 h) in some of the chinchillas, neither a temporary threshold shift (TTS) nor changes in OHC morphology could be found. In another group of chinchillas, where a TTS could be measured after the above-mentioned treatment, the stereocilia of the OHCs were not embedded in the tectorial membrane in the basal 40% of the cochlea, reflecting the measured threshold shift (Nordmann et al., 2000).

These observations were clearly different from those on guinea pigs, where the lack of morphological changes in the organ of Corti with the use of 81 db SPL intensity sound for 24 days was reported (*see above*; Canlon et al., 1995). Therefore, the noise sensitivity seems to be different in chinchillas and guinea pigs; namely, the guinea pig is less sensitive to long-term noise exposure. This inspired us to use guinea pigs, expose them to moderate-intensity noise (80 dB SPL) for 14 days and look for possible functional changes.

Some authors explain the noise-induced functional changes with an increase in the intracellular calcium level after acoustic overstimulation (Frid-

Table 2. Effect of noise exposure on Sp of OHCs

Treatment	Apical OHC	Basal OHC
Control (n = 10)	1.27 ± 0.11	1.28 ± 0.11
Noise exposure (n = 10)	1.49 ± 0.14	1.51 ± 0.24

Data given are mean ± SEM.
P = 0.70 and 0.82 between apical and basal OHCs for control and noise-exposed animals, respectively.
P = 0.05 compared to control.

berger et al., 1998). Noise as a mechanical stimulus depolarizes the resting membrane potential. This depolarization activates voltage-sensitive calcium channels, which permit a continuous calcium influx (Sziklai, Szőny & Dallos, 2001). In our experiments, we also found an increase in the resting intracellular calcium concentration in the OHCs, which was similar to the observation of Fridberger et al. (1998), who investigated the effects of high-level (130 dB SPL) noise exposure. Based on the previous morphological and functional experiments, showing that the basal part of the cochlea is more susceptible to the effect of noise (Nordmann et al., 2000; Ou et al., 2000), we made two different preparations. Among the noise-exposed animals, not only was the measured resting intracellular calcium concentration in the OHCs higher but we also found a slight difference between the cells which originated from the apical compared to the basal turns. Namely, the calcium level was slightly higher in the cells of the basal turn (219 nM) than in the apical turns (181 nM). This higher resting intracellular concentration may explain the swelling or loss of OHCs, which could be observed mainly in the basal 20% of the organ of Corti in previous experiments (Ou et al., 2000).

Noise exposure can modify the response of OHCs to different neurotransmitters, such as ATP measured by patch-clamp experiments (Chen et al., 1995). In line with this finding, we observed that the change in the intracellular calcium level after addition of 180 μM ATP for 30 s was lower and had a longer latency time in noise-exposed animals. We also found a slight difference between cells originating from different parts of the cochlea. The intracellular calcium level rose by 60 nM in cells of the apical turns and only by 44 nM in cells of the basal turn, significantly less than the response of nonexposed animals (the amplitude of the response of control animals to 180 μM ATP was 266 nM with a latency of 5.9 s). This observation is clearly in line with the difference found in intracellular calcium levels.

The constant efferent stimulation of the OHCs and the subsequent higher level of the intracellular calcium concentration may cause downregulation of the membrane purinoreceptors, and this downregulation may be in the background of the delayed and smaller response of the cells to ATP. Using polyclonal antibodies against several subtypes of purinoceptors, we found lower intensities with P_{2X1}, P_{2X2}, P_{2X4}, P_{2X7}, P_{2Y1}, and P_{2Y4} subtypes, while the intensity of the P_{2Y2} receptor subtype did not decrease in noise-exposed compared to nontreated animals. Due to the difficulty of quantifying immunofluorescence, we could not resolve significant differences in the expression intensity between cells from the apical and basal turns of the cochlea.

It should be noted that Wang and coworkers (2003) described upregulation of P_{2X2} receptor expression in the rat cochlea following a sustained loud noise exposure (90–120 dB white noise, >6 h). The increase in expression of these ATP-gated ion channel subunits was particularly evident at the site of OHC sound transduction and may act to limit the focused amplification of sound energy. This relatively short-term response might explain why an increased resting calcium concentration was found to accompany the long-term downregulation of purinergic receptors.

An increase in the intracellular calcium concentration is assumed to cause a decrease of the stiffness of OHCs (axial and transversal) (Dallos et al., 1997; Frolenkov et al., 2003; Batta et al., 2004). The decreased OHC stiffness increases the vulnerability of the cells to mechanical insults. The regulatory stiffness response can not only compensate for the decreased stiffness due to the increased intracellular calcium level but also increase this compared to the normal level (Batta et al, 2004). In line with this expectation and with the increase of the resting intracellular calcium concentration, we found an increase of the stiffness of the OHC lateral wall. One of the possible explanations of the phenomenon is the recently described Ca^{2+}-dependent, stretch-induced regulation of the lateral wall stiffness (regulatory stiffness response), which is controlled by efferent neurotransmitters (Batta et al., 2004). This response is processed by the cytoskeleton and causes an increase in the lateral wall stiffness of OHCs (Batta et al., 2004). Loud sounds of long duration vibrate the basilar membrane with greater amplitudes, which might result in a grater stretch of the lateral wall. This may result in a permanent mechanical stimulus, which causes a persistent increase in lateral wall stiffness. An additional explanation for our results could be a metabolic modification in the cell membrane or subcortical cytoskeleton, which may also modify the stiffness of the lateral wall (Sziklai and Dallos 1997; Szőnyi et al., 1999; Sziklai et al., 2001; He, Zheng & Dallos, 2003; Batta et al., 2003, 2004).

Changes in the intracellular calcium level and the operation of stretch-gated ion channels play an important role in the process of the regulatory stiffness response. New calcium signal is needed to elicit this response. The long-term increase in the resting

intracellular calcium level causes a consecutive decrease in the amplitude of the calcium signals. This mechanism may be the reason that no significant difference was found between the Sp of cochleobasal and cochleoapical cells of control animals

In conclusion, noise-induced changes in the intracellular calcium homeostasis and subsequently in the calcium-dependent regulatory mechanisms may lead to modification of the OHC lateral wall stiffness. Changes in the micromechanical properties of the OHCs may interfere with the peripheral auditory processing, leading to a reduction of the efficacy of the cochlear amplifier.

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