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ORIGINAL ARTICLE

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Inhibition of gap junctional coupling in cochlear supporting cells by gentamicin

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Abstract Gap junctional coupling of cochlear supporting cells is thought to be responsible for spatial potassium buffering of the microenvironment of outer hair cells (OHC). OHC of the organ of Corti are considered as the target of aminoglycoside-induced ototoxicity. Due to the proposed functional relationship between OHC and cochlear supporting cells we investigated a possible involvement of the supporting Hensen cells in the ototoxic effect of the aminoglycoside gentamicin. Isolated Hensen cell pairs were superfused by gentamicin-containing bath solutions. Using the double whole-cell patch-clamp method gentamicin (10 µM) inhibited gap junctional conductance by about 90%, whereas the membrane potential of about –27 mV remained unchanged. Since the inhibitory effect was suppressed by the addition of catalase, the gentamicin mediated effect probably is due to production of free radicals. It is proposed that formation of free radicals in supporting cells inhibits gap junctional coupling whereby the spatial potassium buffer mechanism and, thus, the fine tuning of the cochlear OHC is impaired.

Key words Cochlea · Hensen cells · Gentamicin · Ototoxicity · Free radicals · Gap junctions · Double whole-cell patch-clamp

Introduction

Aminoglycoside antibiotics are in widespread clinical use. However, their nephro- and ototoxic side effects limit their application. The ototoxicity has been characterized both in vitro and in vivo as an effect on cochlear outer hair cells (OHC). Aminoglycoside-induced ototoxicity consists of reversible and irreversible phases of morphological change in the OHC (Forge 1985; Ernst et al. 1994). Although the structural damage induced by such agents in the cochlea is well documented, their specific mechanisms of action within the cochlea remain poorly understood. More recently, the production of free radicals following bioactivation of gentamicin has been demonstrated (Sha and Schacht 1999). However, it is not yet known whether gentamicin also affects the non-sensory, supporting cells (the Hensen cells) of the organ of Corti. In a recent, preliminary report, we have shown that H_2O_2 causes gap junctional uncoupling of isolated Hensen cells (Todt et al. 1999). The aim of the present study was to explore a possible effect of gentamicin on Hensen cells by analysis of gap junctional coupling. The dose dependency of gentamicin on gap junctional conductance was characterized by use of the double whole-cell patch-clamp technique. The possible involvement of free radicals was investigated by simultaneous application of catalase and gentamicin.

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Materials and methods

Pigmented, young guinea-pigs (250–400 g) with a positive Preyer's reflex were anaesthetized by a lethal intraperitoneal dose of phenobarbital (200 mg/kg). The bullae were removed and the organ of Corti dissected from the cochlea as previously described (Ernst et al. 1994). Hensen cells were isolated and digested by trypsin (500 U/ml, Sigma, Deisenhofen, Germany) in nominally calcium-free Hanks buffered saline solution (HBSS) at 22–24 °C for 30 min. The cells were settled on a cover-slip coated with Cell-Tak (Becton Dickinson) and placed in a perfusion chamber containing 200 µl HBSS. After a resting period of about 30 min, a morphologically intact Hensen cell pair was selected and the double whole-cell patch-clamp configuration established (Ngezahayo and Kolb 1994). The pipette filling solution contained (in mM): 135 K-gluconate, 5 NaCl, 5 adenosine triphosphate, Na salt (Na_2ATP), 5 ethyleneglycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 10 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 0.1 adenosine 3',5'-cyclic monophosphate (cAMP), 5 $MgCl_2$: (free [Mg^{2+}] about 1 mM), 3 $CaCl_2$ (free [Ca^{2+}] about 0.1 µM). pH was 7.2 and the osmotic pressure 295–305 mosmol/l. Gentamicin sulphate and catalase were pur-

chased from Sigma. The experiments were carried out at room temperature and data are given as mean±SEM for *n* independent cell pairs.

Results and discussion

The double whole-cell configuration was used to characterize the effect of gentamicin on gap junctional coupling of isolated Hensen cell pairs. In control experiments, a stable gap junctional conductance (G_j) (see Fig. 1A) of 55.5±16.7 nS (*n*=18) and a constant membrane potential of –27.3±6.3 mV (*n*=18) were measured for about 1 h. After application of gentamicin in concentrations of 1–100 µM, neither significant variations of the membrane potential and of the non-junctional membrane resistance, nor visible morphological changes of the cells were observed, but there was an effect on G_j. For clearer presentation one representative experiment is given in Fig. 1B. The figure shows that 1 µM gentamicin did not change the initially measured gap junctional conductance ($G_{j,0}$) significantly during the recording period of about 1 h. At 5 µM the observed time-dependent effect on gap junctional coupling varied from cell pair to cell pair. G_j either changed biphasically, as shown in Fig. 1B, or decreased slowly, as indicated by the corresponding mean time courses of three independent experiments (see Fig. 1C). Obviously, 5 µM can be considered as the threshold gentamicin concentration for a significant and reproducible reduction of gap junctional coupling. The variability probably reflects the capacity of a Hensen cell pair to overcome the gentamicin-induced uncoupling.

At higher gentamicin concentrations a monophasic change of G_j was observed. At 10 µM, G_j decreased by 20% within 7.0±2.9 min (*n*=3) and by 50% within 23.0±1.4 min (*n*=3). The degree of inhibition of G_j could not be significantly amplified by a higher gentamicin concentration of 100 µM (see Fig. 1B) which is reflected in a similar time dependent decrease of G_j by 20%(50%) within 6.5±0.5 min (10.5±2.5 min) (*n*=3) (see also Fig. 1C). In terms of gentamicin-mediated gap junctional uncoupling the data indicate that the threshold concentration of gentamicin is about 5 µM and the maximal effect on G_j is obtained already at 10 µM. It is interesting to note that this concentration corresponds to the concentration of gentamicin found in the perilymph of the guinea-pig during chronic application (cf. Tran Ban Huy et al. 1981).

Electron magnetic resonance spectrometry studies on cochlear tissue have suggested that gentamicin-induced ototoxicity is related to production of free radicals (Clerici et al. 1996). In line with this proposal we have shown recently that addition of H_2O_2 to the bath leads to gap junctional uncoupling of isolated pairs of Hensen cells (Todt et al. 1999). At H_2O_2 concentrations of ≥0.4 mM G_j decreases monophasically by about 90% of the control value within 30 min (data not shown). This time-dependent change of G_j is similar to that observed after application of 10 or 100 µM gentamicin. To test whether reduction of gap junctional coupling of Hensen cells by gentamicin in the present study is related to free radical production, gentamicin was applied in the presence of 1000 U/ml catalase in the bath. Typical recordings for the three experiments are presented in Fig. 2 for the dif-

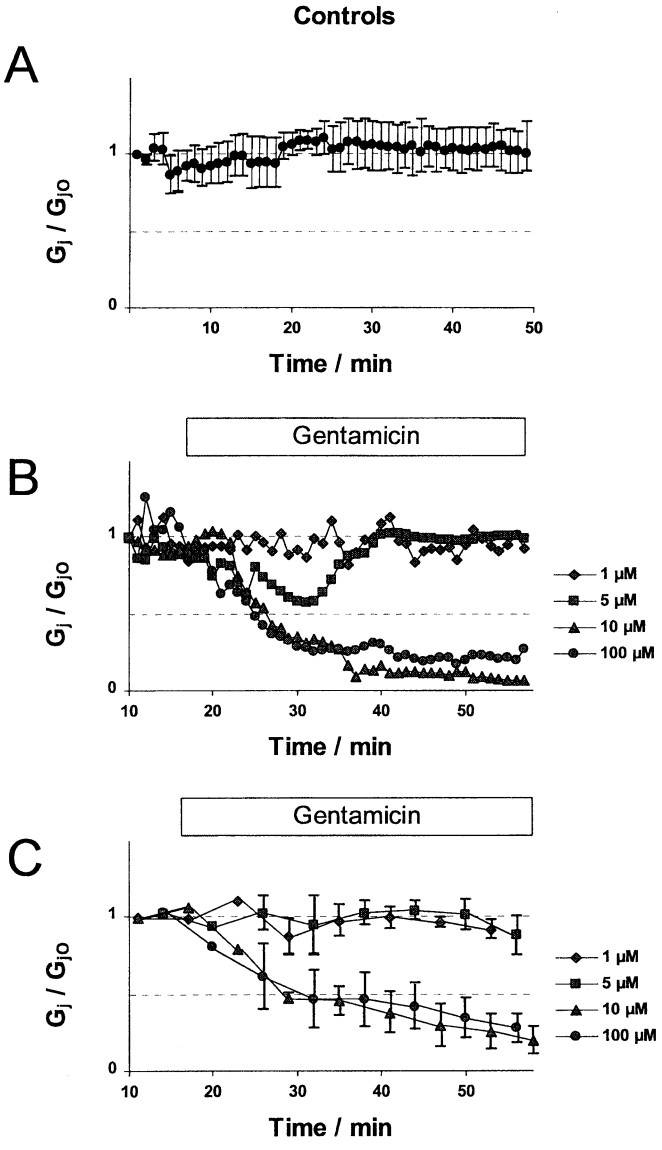

Fig. 1A–C Effect of gentamicin on the time course of gap junctional conductance (G_j) of isolated Hensen cell pairs. **A** Mean time course of G_j derived from three independent cell pairs under control conditions; **B** time course of G_j during exposure (*bar above traces*) to gentamicin (1, 5, 10 or 100 µM). Data derived from representative cell pairs are presented for each gentamicin concentration; **C** mean time course of G_j during exposure to gentamicin (1, 5, 10 or 100 µM). Three independent cell pairs were used for each data point. For clarity the data of four consecutive time points and the error bars for the data recorded up to 25 min have been omitted. G_j is shown normalized to the initial value ($G_{j,0}$, obtained as mean value 5–10 min after formation of the double whole cell configuration). Zero on the time scale indicates the start of the double whole-cell patch-clamp configuration. Cells were continuously superfused with Hanks buffered saline solution (HBSS). Gentamicin was added as indicated by the bar at 1 µM, 5 µM, 10 µM and 100 µM.

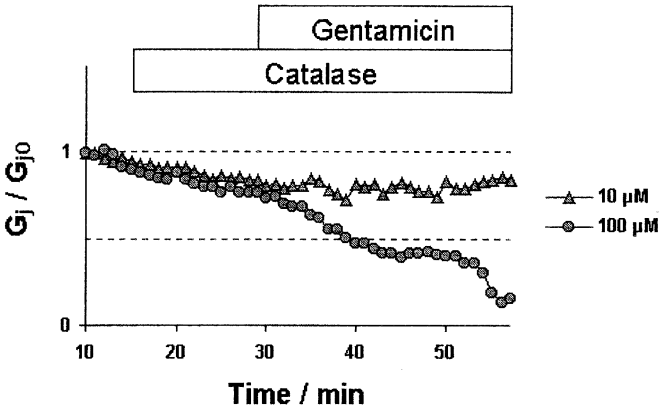

Fig. 2 Suppression of gentamicin-induced inhibition of gap junctional coupling by preincubation and simultaneous presence of catalase (1000 U/ml in HBSS). Two representative experiments are shown. Gentamicin was added at 10 µM and 100 µM as indicated. For further explanation, see legend of Fig. 1 and text

ferent concentrations of gentamicin. Comparing these traces with those in Fig. 1B it is clear that preincubation of a cell pair with catalase suppressed the gentamicin-induced decrease of G_j at 10 µM and partially suppressed that at 100 µM. In the presence of catalase, 10 µM gentamicin for 20 min yielded a value for $G_j/G_{j,0}$ of 0.84±0.16 (n=3).

The results give evidence that supporting cells could play a crucial role in gentamicin induced ototoxicity by free radical production which in turn could distort the physiological function of OHC. A direct pathophysiological effect of gentamicin on OHC can not be excluded. However, the observed inhibition of gap junctional coupling of supporting cells by gentamicin would impair the ability of spatial potassium buffering (Santos-Sacchi 1991) and, thus, change the micromechanics of cochlear OHC. A permanently varied sensitivity of OHC could be accompanied by a loss of fine tuning and a deterioration of the hearing threshold.

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