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Expression of prestin mRNA in the organotypic culture of rat cochlea

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Abstract

To quantitate in absolute terms the prestin mRNA levels in the explant culture of rat cochlea, we used competitive RT-PCR with a synthetic internal cRNA standard. Prestin gene expression was found at levels of 100 fg specific mRNA/μg total RNA on postnatal day 3, which corresponds to about 300 copies per outer hair cell (OHC) and is indicative of an intermediate level of expression. Two days of culturing resulted in an increase of prestin mRNA levels and in the formation of an apical–basal gradient ($p < 0.001$). To elucidate the variations the prestin mRNA levels undergo as a result of damage to the organ of Corti, we exposed the explant cultures to ischemia and hypoxia. While total RNA was observed to remain unchanged, the numbers of OHCs and the prestin mRNA levels were found to decrease by about 20% and 35%, respectively, compared to normoxia.

In conclusion, we showed that the prestin mRNA levels during in vitro development increase and form an apical–basal gradient within 2 days in culture, similar to the postnatal in vivo development. Hypoxia and ischemia result in a decrease of the prestin mRNA level in parallel with OHC loss. The prestin mRNA level can therefore be used as marker of damage to or loss of OHCs.
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Keywords: Cochlea; Hypoxia; Ischemia; Prestin mRNA

1. Introduction

The sensitivity of hearing depends strongly on the function of special mechanosensory cells, outer hair cells (OHCs), which amplify sound-induced vibrations in the organ of Corti. This amplification is mainly the result of the protein prestin, highly expressed in OHCs and not expressed in inner hair cells (Zheng et al., 2000). The hearing threshold is elevated by 40–50 dB, when OHCs are damaged or lost (Ryan and Dallos, 1975).

Structural and functional maturation of the organ of Corti and appearance of OHC electromotility in the rat occur within the first two weeks after birth (He et al., 1994; Ludwig et al., 2001). Using antibodies specific to prestin, it was shown that prestin is highly expressed in the lateral plasma membrane of OHCs in the region in which electromotility is generated (Belyantseva et al., 2000).

Experimental studies on the quantitation of prestin mRNA in the organ of Corti under normal and damaging conditions have not been conducted. Common methods applied to study mRNA levels are dot- and Northern blots, RNAse protection assay and reverse transcription-coupled polymerase chain reaction (RT-PCR). As blot techniques usually require μg amounts of

Abbreviations: bp, base pairs; BSA, bovine serum albumin; cRNA, copy RNA; cDNA, copy DNA; DEPC, diethylpyrocarbonate; DMEM/F12, Dulbecco's modified Eagle's medium; dNTP, deoxynucleoside 5′-triphosphate; DTT, dithiothreitol; FCS, fetal calf serum; OHC, outer hair cell; PAGE, polyacrylamide electrophoresis; PCR, polymerase chain reaction; PD, postnatal day; Q-RT-PCR, quantitative reverse transcription polymerase chain reaction; TAE, tris-acetate-EDTA

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RNA they are poorly applicable in studies which have to cope with steady-state levels of few femtograms of specific mRNA. Because RT-PCR has the lowest detection limit of the three methods (O'Driscoll et al., 1993), it seems best suited for determining the minor amounts of a particular mRNA (Wang et al., 1989). Competitive PCR employing known amounts of an internal standard to be co-amplified with the endogenous target sequence is well suited for absolute quantitation. In the case of RT-PCR, the optimal internal standard is a synthetic RNA.

Considering the role prestin has to play in the hair cell function, it is important to study and understand changes in the mRNA expression of prestin induced by OHC damaging factors. Clinical and experimental studies have shown that ischemia contributes to certain forms of hearing loss (Lamm and Arnold, 1996; Orita et al., 2002; Scheibe et al., 1992, 1993, 2000; Thorne and Nuttall, 1989). Hearing disorders like sudden sensorineural hearing loss, presbyacusis and noise-induced hearing loss are suspected of being related to alterations in blood flow and ischemia (Seidman et al., 1999; Thorne and Nuttall, 1987, 1989).

To address experimentally the question of whether ischemia/hypoxia alters the prestin mRNA expression, we studied the effect of in vitro ischemia on the prestin mRNA level. Changes in the prestin mRNA level could potentially be useful as a specific marker in evaluations of OHC damage. For this study, we used an organotypic organ of Corti culture as described previously (Mazurek et al., 2003). The specimens of the organ of Corti were exposed to oxygen and glucose deprivation (= ischemia) and to oxygen deprivation (= hypoxia) in artificial perilymph for different exposure periods.

2. Materials and methods

2.1. Explant culture

We used an in vitro hypoxia model of organotypic cochlea regions from 3 to 5 day old Wistar rats. Dissection of the cochlea was performed in buffered saline glucose solution (BSG; 116 mM NaCl, 27.2 mM Na_2HPO_4, 6.1 mM KH_2PO_4, glucose 11.4 mM) under a laminar flow hood using a dissecting microscope (Stemi, SV6, Zeiss Germany). The stria vascularis, Reissner's membrane and modiolus were separated from the organ of Corti during preparation and were not considered here. The organ of Corti was separated into three parts of equal length: the basal, middle and apical segments. The mean length of one fragment was 2.4 ± 0.3 mm (mean ± SD, $n = 11$). For culturing, the segments were incubated in 4-well culture dishes (4× 1.9 cm^2, Nunc, Wiesbaden, Germany) in 500 μl Dulbecco's modified Eagle's medium/F12 Nutrient (1:1) Mixtures (DMEM/F12, Gibco, Karlsruhe, Germany) medium with 10% fetal calf serum (FCS), 10 mM hepes, 20 mM $NaHCO_3$, 5 mM L-glutamine, 10 μg/ml ciprofloxacin, 25 μg/ml insulin, 100 μg/ml transferrin, 60 μg/ml putrescine, 0.6% glucose at 37 °C (Cheng et al., 1999; Lowenheim et al., 1999). To maintain the normal architecture of the organ of Corti during culturing it is important to place and keep the segments unfolded on the bottom of the well. The organ of Corti was well preserved for up to 48 h after onset of culture without significant spreading. Hair cells were stained and counted as reported (Mazurek et al., 2003).

2.2. Ischemia

Twenty-four hours after plating, the cultures were exposed to ischemia (exposure to hypoxia in artificial perilymph without glucose) for 6 h in an incubation chamber at 37 °C (Billups-Rothenburg, Del Mar, CA, USA) as described elsewhere (Gao et al., 1999; Mazurek et al., 2003). In brief, the chamber with the plates was perfused with a calibrated gas mixture of 5% CO_2, 95% N_2 (AGA Gas GmbH, Bottrop, FRG) for 15 min. The oxygen pressure inside the culture medium was 15–20 mmHg after 10 min exposure time and reached a steady state level of 10–20 mmHg after 25 min up to the end of ischemia. At the end of the ischemia period, the artificial perilymph was replaced by its own conditioned medium and cultured for another 24 h. The explant cultures were washed and then lysed for RNA preparation. To study the dependence of prestin mRNA levels on long-term hypoxia, the cultures were exposed 24 h after plating to 38 h hypoxia in complete medium, and at the end of the hypoxia period the segments were lysed for RNA preparation.

2.3. Quantitation of prestin mRNA levels

Total RNA was isolated using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). RNA was stored at −70 °C in diethylpyrocarbonate (DEPC) water. The RNA was quantified spectrophotometrically at 260 and 280 nm, usual A_{260}/A_{280} ratios were between 1.7 and 2.1. RNA concentration was adjusted to about 100 ng/μl and the RNA stored at −70 °C until needed.

The prestin mRNA level was determined by competitive RT-PCR with an internal cRNA standard of the same length and sequence as the target mRNA except for a unique restriction site for endonuclease *Bam*H1 in the middle of the oligonucleotide. The sequence of the primers used in this study is listed in gene Accession No. NM 030840 (primer A, base pair position 1508, 5′-CAC-AGAGTCCGAGCTACACAGTC-3′; primer B, base pair position 1669, 5′-TCAGTGCGCTGCTGTA-CAAG-3′; fragment length 162 bp). The primers and the internal standard were from BioTez (Berlin, Germany).

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