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Post exposure administration of A_1 adenosine receptor agonists attenuates noise-induced hearing loss

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ABSTRACT

Adenosine is a constitutive cell metabolite with a putative role in protection and regeneration in many tissues. This study was undertaken to determine if adenosine signalling pathways are involved in protection against noise injury. A_1 adenosine receptor expression levels were altered in the cochlea exposed to loud sound, suggesting their involvement in the development of noise injury. Adenosine and selective adenosine receptor agonists (CCPA, CGS-21680 and Cl-IB-MECA) were applied to the round window membrane of the cochlea 6 h after noise exposure. Auditory brainstem responses measured 48 h after drug administration demonstrated partial recovery of hearing thresholds (up to 20 dB) in the cochleae treated with adenosine (non-selective adenosine receptor agonist) or CCPA (selective A_1 adenosine receptor agonist). In contrast, the selective A_{2A} adenosine receptor agonist CGS-21680 and A_3 adenosine receptor agonist Cl-IB-MECA did not protect the cochlea from hearing loss. Sound-evoked cochlear potentials in control rats exposed to ambient noise were minimally altered by local administration of the adenosine receptor agonists used in the noise study. Free radical generation in the cochlea exposed to noise was reduced by administration of adenosine and CCPA. This study pinpoints A_1 adenosine receptors as attractive targets for pharmacological interventions to reduce noise-induced cochlear injury after exposure.

1. Introduction

Noise-induced hearing loss (NIHL) refers to hearing impairment caused by sustained and repeated exposure to excessive sound levels. Hearing loss from noise exposure is a leading occupational injury with up to 5% of the population at risk world-wide. NIHL is commonly attributed to occupational hazards but there is concern that it is becoming increasingly prevalent with recreational activities such as loud music from portable MP3 players. Any form of sound exposure of sufficient intensity and exposure time can lead to NIHL. Exposure to sound levels around 85 dBA leads to a temporary elevation of auditory thresholds (temporary threshold shift, TTS), which can be reversed within a few days after the exposure. However, with either sustained or more intense exposure, the change in auditory thresholds becomes permanent (permanent threshold shift, PTS). There are virtually no treatments that can ameliorate the damage to the cochlea and reduce the impact of sensorineural hearing loss. Hearing aids and cochlear implants are currently the only management options offered to hearing impaired persons, whilst pharmacological therapies for NIHL have only recently been proposed (Yamashita et al., 2005).

Noise exposure drives mitochondrial activity and free radical production, reduces cochlear blood flow, causes excitotoxic swelling of afferent nerve terminals, and induces both necrotic and apoptotic cell death in the organ of Corti (Henderson et al., 2006). Cochlear injury and the loss of auditory function from noise exposure appears to be largely due to oxidative stress and glutamate excitotoxicity (Henderson et al., 2006; Le Prell et al., 2007). This implies that compounds targeting these mechanisms could offer considerable potential as therapies for NIHL. Adenosine is an endogenous neuromodulator with the ability to boost antioxidant defences, increase oxygen supply, inhibit presynaptically the release of glutamate, trigger anti-inflammatory responses and promote angiogenesis

Abbreviations: ABR, auditory brainstem response; AP, artificial perilymph; CAP, compound action potential; NIHL, noise-induced hearing loss; NT, nitrotyrosine; RWM, round window membrane; *R*-PIA, (*R*)-N^6-phenylisopropyladenosine; SP, summating potential; SPL, sound pressure level

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(Linden, 2005; Fredholm, 2007). Adenosine release from cells under stress can thus induce cytoprotection and regeneration in a range of tissues (Linden, 2005; Fredholm, 2007). The extraordinary therapeutic potential of adenosine receptor agonists has been exploited in a variety of cardiovascular, neurological, renal, pulmonary, endocrine and inflammatory disorders including cancer (Jacobson et al., 2000; Jacobson and Gao, 2006). A considerable number of selective agonists and antagonists have been discovered in the last decade, and some of them have been evaluated clinically (Jacobson and Gao, 2006).

Adenosine receptors have been identified and localised in the mammalian cochlea (Ford et al., 1997; Vlajkovic et al., 2007). Their focal distribution in sensorineural and supporting tissues of the cochlea, leads to speculation that adenosine receptors may have a prominent role in otoprotection. Indeed, exposure to loud sound increases adenosine levels in cochlear fluids and up-regulates the expression of adenosine A_1 receptors in the chinchilla cochlea (Ramkumar et al., 2004). Previous studies have shown that adenosine receptor agonists can protect the cochlea against NIHL (Hu et al., 1997; Hight et al., 2003), cisplatin ototoxicity (Whitworth et al., 2004) and transient ischemia (Tabuchi et al., 1999). Pre-treatment of the cochlea with the broadly selective A_1 receptor agonist (*R*)-N^6-phenylisopropyladenosine (*R*-PIA) prevented noise-induced threshold shift and increased survival of the outer hair cells (Hu et al., 1997). The combination of *R*-PIA with the antioxidant glutathione monoethylester provided otoprotection against both impulse and continuous noise (Hight et al., 2003). *R*-PIA was shown to increase antioxidant enzyme activity in the cochlea and reduce lipid peroxidation (Ford et al., 1997), thus protecting cochlear tissues from oxidative stress.

Here, we investigate the potential role of adenosine receptor signalling in cochlear recovery from noise-induced injury. All compounds were administered after the cessation of noise exposure. Our results demonstrate that post-noise administration of adenosine and CCPA (selective A_1 adenosine receptor agonist) can provide partial recovery of auditory thresholds.

2. Materials and methods

2.1. Drugs

The following adenosine receptor agonists and antagonists were purchased from Sigma–Aldrich: adenosine; CCPA (2-chloro-N^6-cyclopentyladenosine), an A_1 receptor agonist; CGS-21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride hydrate), an A_{2A} receptor agonist; Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide), an A_3 receptor agonist; DPCPX (8-cyclopentyl-1,3-dipropylxanthine), an A_1 receptor antagonist; SCH-58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine), an A_{2A} receptor antagonist and MRS-1191 (3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate), an A_3 receptor antagonist. Stock solutions of these compounds were prepared in artificial perilymph solution (AP; 122 mM NaCl, 18 mM $NaHCO_3$, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4 mM D-glucose, 14 mM mannitol in 5 mM Hepes, pH 7.5). Compounds were aliquoted and stored at −80 °C.

2.2. Animals

The experiments were undertaken on male Wistar rats (8–10 weeks) with normal Preyer's reflex. Animals were supplied by the Vernon Jansen Unit (University of Auckland, New Zealand). All experimental procedures described in this study were approved by the University of Auckland Animal Ethics Committee.

2.3. Noise exposure

Rats were exposed to a broad-band noise (16 Hz–26 kHz) presented for 24 h at 90, 100, or 110 dBSPL. Noise exposures were carried out in a custom-built acoustic chamber (Shelburg Acoustics, Sydney, Australia) with internal speakers and external controls (sound generator and frequency selector). The sound levels in the cage were measured using a calibrated Rion NL-49 sound level meter to ensure minimal deviations of sound intensity across animals. The animals had free access to food and water during the exposure.

2.4. Quantitative assessment of adenosine receptor expression

The transcript levels of adenosine receptors in the noise-exposed and control cochleae were quantified by real-time RT-PCR using specific primers and TaqMan® MGB probes carrying a 5′ reporter FAM (6-carboxy fluorescein) and a 3′ non-fluorescent quencher (Applied Biosystems). The primers and probes are shown in Table 1.

A total of 32 rats were used, with 8 animals exposed to each noise level (90, 100 and 110 dBSPL) and a control group kept at ambient noise levels (around 65 dBSPL). Following noise exposure, the animals were euthanised (sodium pentobarbital, 100 mg/kg i.p.). The tympanic bulla was removed and placed in sterile 0.1 M phosphate-buffered saline (PBS, pH 7.4). The cochlea was exposed and the otic capsule removed. The membranous labyrinth and modiolus were dissected out and placed in lysis buffer (100 mM Tris–HCl, pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM DTT). Cochlear tissues were homogenized using a sterile Teflon pestle and mRNA extracted using Dynabeads Oligo $(dT)_{25}$ (Invitrogen Dynal AS, Oslo, Norway). First-strand cDNA synthesis was carried out in a 20 µl reverse transcription reaction using random hexamers and SuperScript III reverse transcriptase (Invitrogen).

Real-time PCR was performed in MicroAmp Optical 384-well reaction plates using TaqMan® Universal PCR Master Mix (Applied Biosystems), unlabelled PCR primers and FAM-labelled TaqMan MGB probes (Custom TaqMan® Gene Expression Assays). As a template, 1 µl of sample cDNA was added to a total reaction volume of 12.5 µl. The samples were tested in duplicate and data were expressed as a mean of two replicates. Negative controls without a template or reverse transcriptase were included in every PCR run. Quantitation of a house-keeping gene, glyceraldehyde-3

Table 1
Primers and probes for quantitative assessment (qRT-PCR) of adenosine receptor transcripts in the rat cochlea (GenBank accession numbers are given in parentheses).

Target	Forward	Reverse	TaqMan MGB probe 5′-FAM–NFQ-3′
A_1R (NM_017155)	ATCCTCACCCAGAGCTCCATT Position: 328–348	TGTCTTGTACCGGAGAGGGATCT Position: 414–392	TGTGGATCGATACCTC Position: 369–384
A_{2A}R (NM_053294)	GTCACCAACTTCTTTGTGGTATCG Position: 537–560	TGCTGATGGTGATAGCGAAGG Position: 621–601	CTGACATTGCAGTGGGT Position: 571–587
A_3R (NM_012896)	GGACATCTTCTACATCATCCGAAACA Position: 915–940	CGTAAAATGCACGCGTCTCT Position: 988–969	AAGCCAGTCAGATTCT Position: 952–965