Published in final edited form as:

J Neurosci Res. 2010 September ; 88(12): 2598–2609. doi:10.1002/jnr.22421.

The Role of Adenosine Kinase in Cochlear Development and Response to Noise

Srdjan M. Vlajkovic^{1,*}, Cindy X. Guo¹, Nuwan Dharmawardana¹, Ann Chi Yan Wong¹, Detlev Boison⁴, Gary D. Housley^{1,3}, and Peter R. Thorne^{1,2}

¹ Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand ² Section of Audiology, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand ³ Department of Physiology and Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, Sydney, Australia ⁴ RS Dow Neurobiology Laboratories, Legacy Research, Portland, USA

Abstract

Adenosine signalling has an important role in cochlear protection from oxidative stress. In most tissues, intracellular adenosine kinase (ADK) is the primary route of adenosine metabolism and the key regulator of intracellular and extracellular adenosine levels. The present study provides the first evidence for ADK distribution in the adult and developing rat cochlea. In the adult cochlea ADK was localised to the nuclear or perinuclear region of spiral ganglion neurones, lateral wall tissues and epithelial cells lining scala media. In the developing cochlea, ADK was strongly expressed in multiple cell types at birth, and reached its peak level of expression at postnatal day 21 (P21). Ontogenetic changes in ADK expression were evident in the spiral ganglion, organ of Corti and stria vascularis. In the spiral ganglion, ADK showed a shift from predominantly satellite cell immunolabelling at P1 to neuronal expression from P14 onwards. In contrast to the role of ADK in various aspects of cochlear development, ADK contribution to the cochlear response to noise stress was less obvious. Transcript and protein levels of ADK were unaltered in the cochlea exposed to broadband noise (90–110dB SPL, 24 hours) and the selective inhibition of ADK in the cochlea with ABT-702 failed to restore hearing thresholds after exposure to traumatic noise. This study indicates that ADK is involved in purine salvage pathways for nucleotide synthesis in the adult cochlea, but its role in the regulation of adenosine signalling under physiological and pathological conditions is yet to be established.

Keywords

adenosine kinase; cochlea; development; noise; ABT-702

INTRODUCTION

Adenosine is a constitutive cell metabolite released from most cells mainly via specific bi-directional transporters or as a result of cell damage (Fredholm et al., 2001). Another potential source of adenosine is the activity of ectonucleotidases that breakdown extracellular ATP and cAMP to adenosine (Zimmermann, 2001). Extracellularly, adenosine is a signaling molecule for P1 (adenosine) receptors that modulate a variety of physiological

*Corresponding Author: Dr SM Vlajkovic, Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand, Ph 64 9 3737599; Fax 64 9 3737499; s.vlajkovic@auckland.ac.nz.

responses in mammalian tissues (Fredholm et al., 2005). Released adenosine is inactivated by adenosine deaminase, or removed from the extracellular space by nucleoside transporters (Baldwin et al., 1999). Following adenosine uptake into the cells, adenosine is phosphorylated to AMP by adenosine kinase (ADK) or degraded to inosine by intracellular adenosine deaminase (Fredholm et al., 2001).

The expression and distribution of adenosine receptors and nucleoside transporters in the rat cochlea have been previously characterised (Vlajkovic et al., 2007; Khan et al., 2007). High-affinity adenosine receptors (A_1 , A_{2A} , A_3) are differentially expressed in the organ of Corti, spiral ganglion neurones, lateral wall tissues and cochlear blood vessels (Vlajkovic et al., 2007). Adenosine stimulates cochlear blood flow (Muñoz et al., 1999), but has no effect on cochlear resting and sound-evoked electrical potentials (Muñoz et al., 1995; Ford et al., 1997a). Similarly, sound-evoked cochlear potentials in rats exposed to ambient noise are minimally altered by local administration of selective A_1 , A_{2A} or A_3 receptor agonists (Wong et al., 2010). However, loud sound (Ramkumar et al., 2004; Wong et al., 2010) and ototoxic drugs such as cisplatin (Ford et al., 1997b) induce up-regulation of A_1 adenosine receptors in the cochlea, suggesting a role of these receptors in the cochlear response to oxidative stress. Indeed, other studies have shown that the local application of *R*-phenylisopropyladenosine (*R*-PIA), an A_1 adenosine receptor agonist, onto the round window membrane can prevent cochlear injury from continuous and impulse noise (Hu et al., 1997; Hight et al., 2003). In addition, *R*-PIA and 2-chloro-N⁶-cyclopentyladenosine (CCPA) reduce cisplatin-induced auditory threshold shifts (Whithworth et al., 2004), most likely by promoting the antioxidant defence system (Ford et al., 1997a). More recent studies (Wong et al., 2010; Vlajkovic et al., 2010) have demonstrated that adenosine and the selective A_1 adenosine receptor agonists CCPA and adenosine amine congener (ADAC) ameliorate cochlear injury and reverse hearing loss after exposure to traumatic noise. Further studies are thus warranted to determine the full potential of adenosine signalling in mitigating cochlear pathologies based on oxidative stress.

ADK is the primary route for adenosine metabolism and the principal regulator of intracellular and extracellular adenosine concentrations in the brain (Boison, 2006). Experimental evidence suggests that this enzyme may have a pivotal role in the brain response to injury (Boison, 2006, 2008). The rapid elevation of adenosine levels following ischemia, traumatic brain injury or status epilepticus suggest that the adenosine system shifts rapidly into a protective mode under these conditions (Boison, 2006). Transient down-regulation of ADK after acute brain injury protects the brain from further seizures and cell death, whilst up-regulation of ADK is associated with epileptogenesis and neuronal loss (Gouder et al., 2004; Li et al., 2008). Regulation of adenosine levels by ADK may also have a key role in the susceptibility of the brain to ischemic injury induced by middle cerebral artery occlusion (Pignataro et al., 2007, 2008). ADK is therefore emerging as an extraordinary therapeutic target in seizures and stroke, and this background of neuroprotective action represents a rationale to investigate a role of ADK in cochlear protection from environmental challenges such as loud sound.

In this study, we investigated the expression and developmental regulation of ADK expression in the rat cochlea as a foundation for the noise studies. Whilst the differential regulation of ADK in the early postnatal and adult cochlea suggested a dual role of the enzyme in cochlear development, functional studies could not link ADK to noise-induced cochlear injury.

MATERIALS AND METHODS

Animals

The experiments were carried out on adult male Wistar rats (8–10 weeks) with normal Preyer's reflex and early postnatal (P1–P21) rats. 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.

Cochlear mRNA Isolation and Screening for Adenosine Kinase Transcript

Animals were euthanised for tissue collection using sodium pentobarbital (100 mg/kg i.p.). The tympanic bulla was removed and placed in sterile 0.1M phosphate buffered saline (PBS; pH 7.4). The cochlea was exposed and the otic capsule removed. The membranous labyrinth and modiolus were homogenised in lysis buffer (100 mM Tris-HCl, pH 8.0; 500 mM LiCl; 10 mM EDTA, pH 8.0; 1% LiDS; 5 mM DTT) using a sterile Teflon pestle. Polyadenylated RNA was extracted using Dynabeads mRNA DIRECT (DynaL A.S., Oslo, Norway). First-strand cDNA synthesis was carried out in a 20- μ l reverse transcription (RT) reaction using random primers (Invitrogen, Groningen, The Netherlands), dNTPs (Amersham Biosciences, Piscataway, NJ, USA) and Superscript III reverse transcriptase (Invitrogen). The complementary DNA was amplified by PCR using rat-specific primers for adenosine kinase (GenBank accession number NM_012895). The forward primer sequence was 5'-TGG CTT CTT TCT CAG CGT CT-3' (position: 564–583) and the reverse 5'-ACT CCA CAG CCT GAG TTG CT-3' (position: 1127–1108). Control experiments omitted reverse transcriptase from the first strand cDNA reaction mixture. PCR with a 40 cycle profile was performed as follows: 94°C denaturation (1 min), 60°C annealing (1.5 min), 72°C extension (2 min) steps using PTC-100™ Programmable Thermal Controller (MJ Research Inc., Waltham, MA). The PCR product was purified using the Pure Link™ PCR purification kit (Invitrogen) and the identity of the amplicon was confirmed by DNA sequencing.

Western Blot Analysis

For Western blotting, rat cochleae were extracted, decapsulated in chilled (4°C) 0.1M PBS, and homogenized with a micropestle. Cochlear proteins (80 μ g) were dissolved in non-reducing Laemmli's sample buffer (1% SDS, 20% glycerol, 0.1% bromophenol blue, 125 mM Tris, pH 6.8), separated by SDS-PAGE on 10% polyacrylamide gel, and electrophoretically transferred to a PVDF membrane (Roche Diagnostics, Auckland, New Zealand). Identically processed rat lung and liver tissues (80 μ g) were used as positive controls. After blocking with 5% skim milk and 2% normal goat serum in TBS-T (20 mM Tris pH 7.2, 137 mM NaCl, 0.1% Tween 20), the membrane was probed for 2 hours with a polyclonal rabbit antibody (1:4000) raised against recombinant mouse ADK. The specificity of this antibody was previously characterised in tissues of *Adk*^{+/+} and *Adk*^{-/-} mice (Gouder et al., 2004). The blotted membrane was incubated for 1 hour with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (dilution 1:8000) before the bands were visualized by chemiluminescence (ECL™ Western blotting analysis system, Amersham Biosciences, Piscataway, NJ, USA).

ADK Immunohistochemistry

High resolution imaging of ADK immunostaining in cochlear tissues was provided by laser scanning confocal microscopy. Rats were euthanized with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 4% paraformaldehyde (PFA) in a 0.1 M phosphate buffer. Rat cochleae were removed and fixed overnight in 4% PFA. P14, P21 and adult cochleae were decalcified in 5% EDTA solution for 7 days, whilst P1 and P7 cochleae were processed without decalcification. After overnight cryoprotection in 30% sucrose, they were

rinsed in 0.1 M phosphate-buffer (PB, pH 7.4), snap-frozen in isopentane at -80°C and cryosectioned at $30\text{ }\mu\text{m}$. The sections were placed in 24-well plates (Nalge Nunc Int., Rochester, NY, USA) in sterile 0.1 M PBS. The tissues were permeabilised with 1% Triton X-100 for 1 hr, and non-specific binding sites were blocked with 5% BSA and 5% normal goat serum (Vector Laboratories, Burlingame, CA, USA). Primary ADK antibody (dilution 1:500) was applied overnight at 4°C . In control experiments, the primary antibody was omitted. The sections were then incubated with the secondary antibody (Alexa 488 goat anti-rabbit IgG, dilution 1:400; Molecular Probes, Eugene, OR, USA) for 2 hr at room temperature. The sections were rinsed several times in PBS, mounted in Citifluor (Citifluor Ltd, London, UK) and screened for ADK labelling using a confocal microscope (TCS SP2, Leica Leisertechnik GmbH, Heidelberg, Germany) with 488 nm excitation and 520nm bandpass emission via Scanware software (Leica). A series of 6–10 optical sections were collected for each specimen, and image analysis was performed on an optical section from the centre of the stack. At least four cochleae obtained from different animals were analyzed for each age group.

Noise Exposure

For gene expression analysis, adult Wistar rats were exposed to a broadband noise presented for 24 hours at 90, 100, or 110 dBSPL. For the ADK inhibition study, adult rats were exposed to 8–12 kHz band-limited noise presented for 2 hours at 110 dBSPL and the cochleae were harvested 1 hour or 72 hours after noise exposure. 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), with animals placed in cages. The sound levels in the chamber at the level of the cages were measured using a calibrated Rion NL-49 sound level meter to ensure minimal deviations of sound intensity. The animals had free access to food and water during noise exposure.

Quantitative Assessment of ADK Expression in the Noise-exposed Cochlea

The transcript levels of ADK in the noise-exposed and control rat cochleae were quantified by real-time RT-PCR using specific primers and TaqMan® MGB probes carrying a 5' reporter FAM (6-carboxyfluorescein) and a 3' non-fluorescent quencher (Applied Biosystems, Foster City, CA, USA). The forward primer sequence was 5'-CACCCAAGGGAGAGATGACACTATA-3' (position: 852–876), the reverse primer was 5'-TGTC AACGATCTCTTCCTGGTTTTG-3' (position: 943–919) and the TaqMan MGB probe was 5'-FAM-ATGTC ACTGCTTTCCC-NFQ-3' (position: 893–908). A total of 64 rats were used in this study: 8 animals exposed to each noise level (90, 100 and 110 dBSPL) and a control group kept at ambient noise levels (~60 dBSPL); there were two recovery times (1 hour and 72 hours). 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 PBS. Cochlear mRNA was extracted using Dynabeads and first-strand cDNA synthesis carried out as described previously.

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, β -actin, was performed for all samples as an endogenous reference. β -actin specific primers and VIC-labelled probes are proprietary to Invitrogen. Amplification and fluorescence detection were carried out using the 7900HT Fast Real-Time PCR System (Applied Biosystems). The thermal cycling protocol included 2

min at 50°C, 10 min at 95°C, and a 40 cycle profile: 15 sec at 95°C and 1 min at 60°C. The data were analysed using the Sequence Detector v2.3 software (Applied Biosystems). Gene expression levels of ADK were normalised to the reference β -actin gene expression. The relative gene expression (fold change) of ADK was calculated using the $2^{-\Delta\Delta C_T}$ method (Livak & Schmittgen, 2001). Fold change greater than ± 2 was considered significant.

Treatment with ABT-702

A selective ADK inhibitor ABT-702 (4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine) (McGaraughty et al., 2005) was obtained from Sigma-Aldrich. ABT-702 (5 mg) was dissolved in 0.25 mL of DMSO (20 mg/mL) and then in 9.75 mL of distilled (MilliQ) water to prepare a 0.5 mg/mL stock solution. This solution was aliquoted and stored at -20°C for later use. The ABT-702 injection dose (1.5 mg/kg/day) was given intraperitoneally 6 hours after the cessation of noise exposure (8–12 kHz; 110 dB SPL; 2 hours), followed by 4 consecutive injections at 24 hour intervals. The same volume of vehicle solution was given to control animals.

Auditory Brainstem Responses

ABR represents the activity of the auditory nerve and the central auditory pathways (brainstem/mid-brain regions) responding to the sound. ABRs were obtained by placing fine platinum electrodes subdermally at the mastoid region of the ear of interest (active electrode), scalp vertex (reference) and mastoid region of the opposite ear (ground electrode). A series of auditory clicks (10 μsec square wave, alternative polarity) or pure tone pips (4 – 28 kHz; 5ms, 1.5ms rise-fall time) were presented with decreasing intensity (5 dB steps) using a Tucker-Davis Technologies auditory physiology workstation (Alachua, FL, USA).

All ABR measurements were performed in a sound attenuating chamber (Shelburg Acoustics, Sydney, Australia). Rats were anaesthetised with the mixture of Ketamine (75 mg/kg) and Xylazine (10 mg/kg) intraperitoneally, and then placed onto a heating pad, to maintain body temperature at 37°C . Responses were averaged at each sound level (1024 repeats with stimulus polarity alternated), and an artefact rejection was set to exclude responses when the peak-to-peak amplitude exceeded 15 μV . The ABR threshold was defined as the lowest intensity (to the nearest 5 dB) at which wave I–V response could be visually detected above the noise floor.

ABRs were recorded before noise exposure and at intervals (30 minutes, 7 days and 14 days) after noise exposure.

Statistical Analysis

Results are presented as the mean $\pm$ S.E.M. The comparison of ABR thresholds at each frequency was performed using a Student's unpaired t-test assuming unequal variances. The α level was set at $P = 0.05$.

RESULTS

Adenosine Kinase Expression in the Cochlea

The ADK transcript was detected in the rat cochlea by RT-PCR (Fig. 1A). Generated PCR product corresponded to the predicted size of DNA fragment. There was no reaction product in control PCR reactions where the RT step was omitted (-RT). The identity of the amplicon was confirmed by DNA sequencing.

The Characterisation of Adenosine Kinase Antibody in Rat Tissues

The specificity of the ADK antibody in rat tissues was established by Western blot analysis of tissue extracts from rat lung, liver and cochlea. All bands were detected in the size range of 44–46 kDa (Fig. 1B), consistent with the molecular size of ADK in mouse tissues (Gouder et al., 2004). The bands obtained with rat liver and lung tissues were stronger than the cochlea, in line with abundant ADK expression in mouse liver and lung (Gouder et al., 2004). Two closely related bands, 44 kDa and 46 kDa respectively, were present in the extracts of rat liver and lung, representing alternatively spliced isoforms previously described in human (McNally et al., 1997) and mouse tissues (Gouder et al., 2004). Only the long nuclear isoform (~46 kDa), but not the short (cytoplasmic) isoform, was expressed in the adult rat cochlea (Fig. 1B).

Adenosine Kinase Distribution in the Adult and Developing Rat Cochlea

In the adult cochlea, nuclear ADK immunofluorescence labelling was evident in neural, epithelial and connective tissues (Fig. 2A). This includes fibrocytes in the spiral ligament and marginal cells of the stria vascularis (Fig. 2C), spiral ganglion neurones and satellite cells (Fig. 2D), endothelial cells of the modiolar blood vessels (Fig. 2E), sensory outer hair cells in the organ of Corti (Fig. 2F) and non-sensory epithelial cells lining scala media, including outer sulcus cells (Fig. 2C) and inner sulcus cells (Fig. 2F). Immunolabelling was absent when the primary antibody was omitted (Fig. 2B).

In the developing cochlea at P1, ADK was expressed in the spiral ganglion, spiral limbus, greater epithelial ridge, organ of Corti and spiral ligament (Fig. 3A). Strong immunostaining in the organ of Corti (sensory inner and outer hair cells, supporting Deiters' and Claudius cells) extended to the outer sulcus cells (Fig. 3A1). In the spiral ganglion, both neurones and satellite cells were labelled, but stronger immunolabelling was observed in satellite cells (Fig. 3A2). High resolution images clearly demonstrate predominantly satellite cell labelling in the spiral ganglion at P1 (Fig. 3C1), compared to the predominantly neuronal labelling at P14 (Fig. 3C2). At P7, ADK expression was transiently down-regulated in all regions of the cochlea (Figs. 3B, B1, B2). At P14, ADK immunostaining was again strong in the spiral ganglion, interdental cells of the spiral limbus, and inner and outer sulcus cells, and also appeared for the first time in the stria vascularis (Fig. 4A). Immunolabelling of the supporting Claudius cells, outer sulcus cells and their root processes extending to the spiral ligament is shown with higher resolution in Fig. 4A1. The marginal cells in the stria vascularis were strongly immunostained at P14 (Fig. 4A2), as well as the Deiters' and Hensen's cells in the organ of Corti (Fig. 4A3). At this age, neuronal immunolabelling was predominant in the spiral ganglion (Fig. 4A4), as indicated earlier. At P21, the strongest ADK immunolabelling was observed in the spiral ligament, stria vascularis, organ of Corti, spiral limbus and spiral ganglion (Fig. 4B). Fig. 4B1 shows ADK expression in the spiral ganglion neurones, and Fig. 4B2 in the organ of Corti (inner hair cells and supporting Deiters' cells) and inner sulcus cells. At P21, ADK immunolabelling extended to all three cell layers (basal, intermediate and marginal) of the stria vascularis (Fig. 4B3). The lateral wall immunofluorescence signal included both nuclear and cytoplasmic labelling, specifically the stria vascularis, root processes of the outer sulcus cells, and Type 3 fibrocytes in the spiral ligament (Fig. 4B4). No staining was observed when the primary antibody was omitted and Fig. 4C represents a negative control for all age groups.

Adenosine Kinase Expression in the Noise-exposed Cochlea

ADK transcript levels in noise exposed rat cochleae were quantified using real-time RT-PCR (Fig. 5A). The results show that ADK expression was not altered at noise levels that induce either temporary (90 and 100 dB SPL) or permanent (110 dB SPL) hearing loss. There was no change in ADK expression immediately after noise exposure or 72 hours after

exposure. ADK protein levels in the noise-exposed cochleae analysed by semi-quantitative immunohistochemistry reconciled with gene expression levels (data not shown).

ABT-702 does not confer Protection from Noise-induced Cochlear Injury

In this study, rats exposed to traumatic noise (8–12 kHz, 2 hours at 110dB SPL) were treated with ABT-702, a selective ADK inhibitor, for 5 consecutive days after the cessation of noise exposure. ABR thresholds were measured prior to noise exposure (baseline), post-exposure and at intervals after ABT-702 treatment. Fig. 5B shows threshold shifts for auditory clicks and pure tones (4, 16 and 28 kHz) representing different frequencies of the audible spectrum in rats. Threshold shifts ranged from 30 dB to 65 dB for auditory clicks and pure tones immediately after noise (Fig. 5B), but subsided significantly after 7 days. ABR thresholds measured 7 and 14 days after noise exposure were comparable in animals treated with ABT-702 and the vehicle solution. Other modalities of ABT-702 treatment were also investigated, such as increasing drug concentration to 5 mg/kg and delivering as a single intraperitoneal injection 6 hours after exposure; or drug application onto the round window membrane of the cochlea using Gelfoam technique (Wong et al., 2010). None of these treatments mitigated noise-induced hearing loss (data not shown).

DISCUSSION

The present study provides the first comprehensive analysis of the expression and distribution of adenosine kinase (ADK) in the mammalian inner ear. Primarily the long (nuclear) ADK isoform was expressed in the rat cochlea, whilst from Western blot data, both long and short (cytoplasmic) isoforms were strongly expressed in control rat tissues (liver and lung). In the adult cochlea, nuclear or perinuclear ADK labelling was predominant in tissues involved in sensory transduction (sensory hair cells), auditory neurotransmission (spiral ganglion neurones), and the maintenance of electrochemical homeostasis of cochlear fluids (marginal cells of the stria vascularis). ADK immunolabelling was also present in the non-sensory epithelium in scala media (inner and outer sulcus cells, supporting cells in the organ of Corti), satellite cells in the spiral ganglion and modiolar blood vessels. The distribution of ADK is consistent with the extensive distribution of high-affinity A₁, A_{2A} and A₃ adenosine receptors in the adult rat cochlea (Vlajkovic et al., 2007), suggesting that ADK could be the principal regulator of adenosine (P1 receptor) signalling in the cochlea. Another source of adenosine is the activity of ectonucleotidases that hydrolyse extracellular ATP to adenosine in cochlear fluid spaces (Vlajkovic et al., 1996, 1999, 2002, 2006; O'Keeffe et al., 2010a).

Whilst the early postnatal expression of P2 receptors and ectonucleotidases provides evidence for a role of purinergic P2 receptor signalling in cochlear development (Nikolic et al., 2001, 2003; Huang et al., 2005, 2010; Greenwood et al., 2007; O'Keeffe et al., 2010b), the role of adenosine (P1) signalling in cochlear ontogenesis is largely unknown. Here, we present immunohistochemical evidence of differential ADK spatiotemporal expression in the developing rat cochlea. Changes in the expression levels as well as tissue distribution of ADK across age in the developing cochlea imply a dual role of ADK. Up-regulation of ADK promotes purine recycling for ATP and nucleic acid synthesis, whilst down-regulation can enhance ambient adenosine levels and potentially provide molecular cues for the development of sensorineural tissues.

ADK is extensively expressed throughout the rat cochlea at birth, which is associated with cell proliferation during early stages of development and is consistent with its putative role in cell proliferation in the central nervous system (Studer et al., 2006). After transient down-regulation at P7 in most regions of the cochlea, ADK expression again becomes very

strongly expressed after the onset of hearing (P14) in the neural and supporting tissues involved in ionic and metabolic maintenance.

The principal changes in ADK expression during development were observed in the spiral ganglion, stria vascularis and the organ of Corti. In the spiral ganglion, a shift in expression was observed from predominantly satellite cell ADK immunolabelling at P1 to neuronal staining from P14 onwards. This period is very interesting from the developmental point of view, as the rat cochlea matures rapidly within the first two postnatal weeks, including consolidation of the Type I and Type II spiral ganglion afferent innervation of the inner and outer hair cells respectively (Hafidi et al., 1993; Huang et al., 2007). The satellite cells are glial cells wrapped around somata of the spiral ganglion neurons and they are present in the rat cochlea at birth (Romand and Romand, 1985). Like astrocytes, satellite cells are interconnected by gap junctions and may influence embryonic development of sensory neurones (Hanani, 2005). As high intracellular ADK activity reduces the extracellular adenosine concentration, strong ADK activity in satellite cells in the first week of cochlear development suggests low tonic activation of adenosine receptors on spiral ganglion neurones. At P7, down-regulation of ADK in satellite cells coincides with myelination of the spiral ganglion neurones in the rat cochlea (Romand and Romand, 1985). Increased adenosine levels support myelination in the CNS (Stevens et al., 2002; Studer et al., 2006), hence temporary ADK down-regulation in satellite cells and neurones around P7 may promote adenosine release and subsequently myelination of the Type I spiral ganglion neurones. At P14, neuronal expression of ADK becomes predominant, and this pattern continues to the adulthood. Strong neuronal ADK expression supports neuronal development by impeding myelination (Wu and Boison, 2007) and promoting adenosine recycling for nucleic acid synthesis (Studer et al., 2006). It is interesting to note that in the mouse brain ADK expression shifts from neurones to astrocytes at the same stage (around P14) of development (Studer et al., 2006). This shift in opposite direction can reflect both organ (brain vs cochlea) and species (mouse vs rat) differences in ADK expression.

ADK expression in the marginal cells of the stria vascularis was evident at P14, and transiently extended across all three cell layers (basal, intermediate and marginal cells) of the stria at P21, only to be expressed in marginal cells in the adult cochlea. The stria vascularis is responsible for K^+ secretion into the endolymph and the generation of the endocochlear potential, the main driving force for sensory transduction (Wangemann, 2006). Since none of the three main types of adenosine receptors (A_1 , A_{2A} , A_3) is expressed in the stria vascularis (Vlajkovic et al., 2007), a role for ADK in this tissue is likely in adenosine recycling for ATP synthesis. The marginal cells of the stria vascularis are known as the principal storage site for ATP (White et al., 1995; Muñoz et al., 2001), and released ATP at that location inhibits K^+ secretion via $P2Y_4$ receptors, thus reducing the endocochlear potential and hearing sensitivity (Sage and Marcus, 2002; Hur et al., 2007). These cells have high metabolic rate (Wangemann, 2006) and ATP synthesis may also be required for energy metabolism.

ADK is differentially expressed in the sensory inner and outer hair cells and supporting Deiters', Hensen's and Claudius cells in the organ of Corti of the developing cochlea (Table 1). Whilst strong ADK expression in these cells at birth is likely required for nucleic acid synthesis, at later stages of cochlear development it may be required for ATP synthesis as the organ of Corti is the site of rich purinergic signalling network which includes ATP release, $P2X$ and $P2Y$ receptors and ectonucleotidases (Housley et al., 2006, 2009). The absence of ADK in the inner hair cells and Deiters' cells in the adult cochlea suggests reduced adenosine removal from the local pericellular spaces and enhanced adenosine signalling via adenosine receptors abundantly expressed in these cells (Vlajkovic et al.,

2007). Stimulation of A₁ receptors may be crucial for the survival of these cells under stress (Vlajkovic et al., 2010; Wong et al., 2010).

ADK thus might be essential for fine tuning of the adenosine signalling system during cochlear development, and may also have a role in cochlear remodelling after injury. Down-regulation or inhibition of ADK has strong neuroprotective role in experimental models of epilepsy and stroke (Boison, 2006), which provides a rationale for the therapeutic targeting of ADK to mitigate cochlear injury.

Transcript levels of ADK were not altered immediately after exposure to sound pressure levels that induce either temporary or permanent threshold shift. Longer survival time after noise exposure (72 hr) also did not affect ADK transcript levels, suggesting that the enzyme may have a limited role in cochlear response to noise stress. In comparison, ADK expression levels show dynamic changes in the brain following seizures (Gouder et al., 2004; Fedele et al., 2005) or focal ischemia induced by transient middle cerebral artery occlusion (Pignataro et al., 2008).

Previous studies have shown that pharmacological inhibition of ADK is effective therapeutically with an improved side-effect profile compared with A₁ receptor agonists (Kowaluk and Jarvis, 2000; Jarvis et al., 2002; Gouder et al., 2004). ABT-702 is a novel generation of ADK inhibitors (McGaraughty et al., 2005) lacking the off-target effects of classical ADK inhibitors such as 5'-amino-5'-deoxyadenosine or 5'-deoxy-5-iodotubercidin. ABT-702 was shown to readily cross the blood brain barrier and brain levels of ABT-702 are approximately one third of plasma levels (Suzuki et al., 2001). This orally active drug has an exceptional potency (ED₅₀ = 0.7 µmol/kg) in thermal hyperalgesia (Jarvis et al, 2000; Lee et al., 2001) and neuropathic pain (Suzuki et al., 2001).

ABT-702 was used in our study as a potential adenosine augmentation therapy to ameliorate noise-induced cochlear injury and hearing loss. However, noise-induced threshold shifts in rats were unaffected by ABT-702. Increasing drug concentration and changing the delivery route (direct application onto the round window membrane) did not alter the lack of effect. It is not clear from this study whether ABT-702 was unable to cross the blood-perilymph barrier after systemic administration or the round window membrane after local administration, as there was no attempt to determine drug or adenosine concentrations in cochlear fluids. One should also consider a possibility that ABT-702 cannot inhibit the nuclear isoform of ADK predominantly expressed in the cochlea. In comparison, the cytoplasmic form of ADK predominantly expressed in the brain is readily inhibited with 5-iodotubercidin (Gouder et al., 2004), with subsequent increase in ambient adenosine levels.

The present study thus provides the first evidence for ADK expression and tissue distribution in the adult cochlea, and offers a glimpse into the role of ADK in the development of highly specialised cochlear tissues. Further studies, however, are required to clarify the role of ADK in cochlear response to noise injury.

Acknowledgments

This study was supported by the NZ Lottery Grants Board, Royal National Institute for Deaf People (RNID, UK), Deafness Research Foundation (NZ), and grant NS061844 from the National Institutes of Health (NIH, USA).

Grant information: This study was supported by the NZ Lottery Grants Board, Royal National Institute for Deaf People (RNID, UK), Deafness Research Foundation (NZ), and grant NS061844 from the National Institutes of Health (NIH, USA)

References

- Baldwin SA, Mackey JR, Cass CE, Young JD. Nucleoside transporters: molecular biology and implications for therapeutic development. *Mol Med Today* 1999;5:216–224. [PubMed: 10322314]
- Boison D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol Sci* 2006;27(12):652–658. [PubMed: 17056128]
- Boison D. Adenosine as a neuromodulator in neurological diseases. *Curr Opin Pharmacol* 2008;8(1):2–7. [PubMed: 17942368]
- Fedele DE, Gouder N, Güttinger M, Gabernet L, Scheurer L, Rüllicke T, Crestani F, Boison D. Astroglia in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation. *Brain* 2005;128 (Pt 10):2383–2395. [PubMed: 15930047]
- Ford MS, Maggirwar SB, Rybak LP, Whitworth C, Ramkumar V. Expression and function of adenosine receptors in the chinchilla cochlea. *Hear Res* 1997a;105:130–140. [PubMed: 9083810]
- Ford MS, Nie Z, Whitworth C, Rybak LP, Ramkumar V. Up-regulation of adenosine receptors in the cochlea by cisplatin. *Hear Res* 1997b;111(1–2):143–152. [PubMed: 9307320]
- Fredholm BB, Ijzerman AP, Jacobson KA, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001;53:527–552. [PubMed: 11734617]
- Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. *Int Rev Neurobiol* 2005;63:191–270. [PubMed: 15797469]
- Gouder N, Scheurer L, Fritschy JM, Boison D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J Neurosci* 2004;24(3):692–701. [PubMed: 14736855]
- Greenwood D, Jagger DJ, Huang LC, Hoya N, Thorne PR, Wildman SS, King BF, Pak K, Ryan AF, Housley GD. P2X receptor signaling inhibits BDNF-mediated spiral ganglion neuron development in the neonatal rat cochlea. *Development* 2007;134(7):1407–1417. [PubMed: 17329369]
- Hafidi A, Despres G, Romand R. Ontogenesis of type II spiral ganglion neurons during development: peripherin immunohistochemistry. *Int J Dev Neurosci* 1993;11:507–512. [PubMed: 8237466]
- Hanani M. Satellite glial cells in sensory ganglia: from form to function. *Brain Res Brain Res Rev* 2005;48(3):457–476. [PubMed: 15914252]
- Hight NG, Sandra LM, Henderson D, Robert F, Burkard F, Nicotera T. Noise-induced hearing loss in chinchillas pre-treated with glutathione monoethylester and R-PIA. *Hear Res* 2003;179:21–32. [PubMed: 12742235]
- Housley GD, Marcotti W, Navaratnam D, Yamoah E. Hair cells – beyond the transducer. *J Memb Biol* 2006;209(2–3):89–118.
- Housley GD, Bringmann A, Reichenbach A. Purinergic signaling in special senses. *Trends Neurosci* 2009;32(3):128–141. [PubMed: 19232752]
- Hu BH, Zheng XY, McFadden SL, Kopke RD, Henderson D. R-phenylisopropyladenosine attenuates noise-induced hearing loss in the chinchilla. *Hear Res* 1997;113:198–206. [PubMed: 9387999]
- Huang LC, Greenwood D, Thorne PR, Housley GD. Developmental regulation of neuron-specific P2X3 receptor expression in the rat cochlea. *J Comp Neurol* 2005;484(2):133–143. [PubMed: 15736235]
- Huang LC, Thorne PR, Housley GD, Montgomery JM. Spatiotemporal definition of neurite outgrowth, refinement and retraction in the developing mouse cochlea. *Development* 2007;134(16):2925–2933. [PubMed: 17626062]
- Huang, LC.; Thorne, PR.; Vlajkovic, SM.; Housley, GD. Differential expression of P2Y receptors in the rat cochlea during development. 2010. (submitted)
- Hur DG, Lee JH, Oh SH, Kim YH, Lee JH, Shin DH, Chang SO, Kim CS. KCNQ1/KCNE1 K⁺ channel and P2Y4 receptor are co-expressed from the time of birth in the apical membrane of rat strial marginal cells. *Acta Otolaryngol Suppl* 2007;(558):30–35. [PubMed: 17882567]
- Jarvis MF, Yu H, Kohlhaas K, Alexander K, Lee CH, Jiang M, Bhagwat SS, Williams M, Kowaluk EA. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2, 3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties. I. In vitro characterization and acute antinociceptive effects in the mouse. *J Pharmacol Exp Ther* 2000;295(3):1156–1164. [PubMed: 11082453]

- Jarvis MF, Mikusa J, Chu KL, Wismer CT, Honore P, Kowaluk EA, McGaraughty S. Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats. *Pharmacol Biochem Behav* 2002;73(3):573–581. [PubMed: 12151032]
- Khan AF, Thorne PR, Muñoz DJB, Wang CJH, Housley GD, Vlajkovic SM. Nucleoside transporter expression and adenosine uptake in the rat cochlea. *Neuroreport* 2007;18(3):235–259. [PubMed: 17314663]
- Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. *Expert Opin Investig Drugs* 2000;9(3):551–64.
- Lee CH, Jiang M, Cowart M, Gfesser G, Perner R, Kim KH, Gu YG, Williams M, Jarvis MF, Kowaluk EA, Stewart AO, Bhagwat SS. Discovery of 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-d]pyrimidine, an orally active, non-nucleoside adenosine kinase inhibitor. *J Med Chem* 2001;44(13):2133–2138. [PubMed: 11405650]
- Li T, Ren G, Lusardi T, Wilz A, Lan JQ, Iwasato T, Itohara S, Simon RP, Boison D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J Clin Inv* 2008;118:571–582.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 2001;25:402–408. [PubMed: 11846609]
- McGaraughty S, Cowart M, Jarvis MF, Berman RF. Anticonvulsant and antinociceptive actions of novel adenosine kinase inhibitors. *Curr Top Med Chem* 2005;5(1):43–58. [PubMed: 15638777]
- McNally T, Helfrich RJ, Cowart M, Dorwin SA, Meuth JL, Idler KB, Klute KA, Simmer RL, Kowaluk EA, Halbert DN. Cloning and expression of the adenosine kinase gene from rat and human tissues. *Biochem Biophys Res Commun* 1997;231(3):645–650. [PubMed: 9070863]
- Muñoz DJ, Thorne PR, Housley GD, Billett TE, Battersby JM. Extracellular adenosine 5'-triphosphate (ATP) in the endolymphatic compartment influences cochlear function. *Hear Res* 1995;90(1–2):106–118. [PubMed: 8974987]
- Muñoz DJB, McFie C, Thorne PR. Modulation of cochlear blood flow by extracellular purines. *Hear Res* 1999;127:55–61. [PubMed: 9925016]
- Muñoz DJB, Kendrick IS, Rassam M, Thorne PR. Vesicular storage of adenosine triphosphate in the guinea-pig cochlear lateral wall and concentrations of ATP in the endolymph during sound exposure and hypoxia. *Acta Otolaryngol* 2001;121(1):10–15. [PubMed: 11270486]
- Nikolic P, Housley GD, Luo L, Ryan AF, Thorne PR. Transient expression of P2X(1) receptor subunits of ATP-gated ion channels in the developing rat cochlea. *Brain Res Dev Brain Res* 2001;126(2):173–182.
- Nikolic P, Housley GD, Thorne PR. Expression of the P2X7 receptor subunit of the adenosine 5'-triphosphate-gated ion channel in the developing and adult rat cochlea. *Audiol Neurotol* 2003;8(1):28–37. [PubMed: 12566690]
- O'Keeffe, MG.; Thorne, PR.; Housley, GD.; Robson, SC.; Vlajkovic, SM. The role of NTPDase5 and 6 in regulation of P2Y receptor signalling in the cochlea. 2010a. (submitted)
- O'Keeffe MG, Thorne PR, Housley GD, Robson SC, Vlajkovic SM. Developmentally regulated expression of ectonucleotidases NTPDase5 and NTPDase6 and UDP-preferring P2Y receptors in the rat cochlea. *Histochem Cell Biol*. 2010b (in press).
- Pignataro G, Simon RP, Boison D. Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J Cereb Blood Flow Metab* 2007;27(1):1–5. [PubMed: 16685255]
- Pignataro G, Maysami S, Studer FE, Wilz A, Simon RP, Boison D. Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. *J Cereb Blood Flow Metab* 2008;28(1):17–23. [PubMed: 17457365]
- Ramkumar V, Whitworth CA, Pingle SC, Hughes LF, Rybak LP. Noise induces A1 adenosine receptor expression in the chinchilla cochlea. *Hear Res* 2004;188(1–2):47–56. [PubMed: 14759570]
- Romand R, Romand MR. Qualitative and quantitative observations of spiral ganglion development in the rat. *Hear Res* 1985;18(2):111–120. [PubMed: 4044415]
- Sage CL, Marcus DC. Immunolocalization of P2Y4 and P2Y2 purinergic receptors in strial marginal cells and vestibular dark cells. *J Membr Biol* 2002;185(2):103–115. [PubMed: 11891569]

- Stevens B, Porta S, Haak LL, Gallo V, Fields RD. Adenosine: a neuron-glia transmitter promoting myelination in the CNS in response to action potentials. *Neuron* 2002;36(5):855–868. [PubMed: 12467589]
- Studer FE, Fedele DE, Marowsky A, Schwerdel C, Wernli K, Vogt K, Fritschy JM, Boison D. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neurosci* 2006;142(1):125–137.
- Suzuki R, Stanfa LC, Kowaluk EA, Williams M, Jarvis MF, Dickenson AH. The effect of ABT-702, a novel adenosine kinase inhibitor, on the responses of spinal neurones following carrageenan inflammation and peripheral nerve injury. *Br J Pharmacol* 2001;132(7):1615–1623. [PubMed: 11264257]
- Vlajkovic SM, Thorne PR, Muñoz DJB, Housley GD. Ectonucleotidase activity in the perilymphatic compartment of the guinea pig cochlea. *Hear Res* 1996;99:31–37. [PubMed: 8970811]
- Vlajkovic SM, Housley GD, Greenwood D, Thorne PR. Evidence for alternative splicing of ecto-ATPase associated with termination of purinergic transmission. *Mol Brain Res* 1999;73:85–92. [PubMed: 10581401]
- Vlajkovic SM, Thorne PR, Sévigny J, Robson SC, Housley GD. NTPDase1 and NTPDase2 immunolocalization in mouse cochlea: implications for regulation of P2 receptor signalling. *J Histochem Cytochem* 2002;50:1435–1442. [PubMed: 12417608]
- Vlajkovic SM, Vinayagamoorthy A, Thorne PR, Robson SC, Wang CJH, Housley GD. Noise-induced up-regulation of NTPDase3 expression in the rat cochlea: implications for auditory transmission and cochlear protection. *Brain Res* 2006;1104:55–63. [PubMed: 16815340]
- Vlajkovic SM, Abi S, Wang CJ, Housley GD, Thorne PR. Differential distribution of adenosine receptors in rat cochlea. *Cell Tissue Res* 2007;328:461–471. [PubMed: 17285327]
- Vlajkovic SM, Lee KH, Wong A, Guo CX, Gupta R, Housley GD, Thorne PR. Adenosine amine congener mitigates noise-induced cochlear injury. 2010. (submitted)
- Wangemann P. Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. *J Physiol* 2006;576(1):11–21. [PubMed: 16857713]
- White PN, Thorne PR, Housley GD, Mockett B, Billett TE, Burnstock G. Quinacrine staining of marginal cells in the stria vascularis of the guinea-pig cochlea: a possible source of extracellular ATP? *Hear Res* 1995;90(1–2):97–105. [PubMed: 8975010]
- Whitworth CA, Ramkumar V, Jones B, Tsukasaki N, Rybak LP. Protection against cisplatin ototoxicity by adenosine agonists. *Biochem Pharmacol* 2004;67:1801–1807. [PubMed: 15081879]
- Wong A, Guo CX, Gupta R, Housley GD, Thorne PR, Vlajkovic SM. A₁ adenosine receptor agonists attenuate noise-induced hearing loss. *Hear Res* 2010;260:81–88. [PubMed: 19995597]
- Wu NL, Boison D. Adenosine kinase expression modulates expression of myelin proteolipid protein. *Open Neurosci J* 2007;1:15–19.
- Zimmermann H. Ectonucleotidases: Some recent developments and a note on nomenclature. *Drug Dev Res* 2001;52:44–56.

Fig. 1.

Adenosine kinase (ADK) expression in the rat cochlea. **A:** RT-PCR generated amplicons (shown in duplicate) are of expected size for ADK at 564 bp (SYBR Safe™ agarose gel) compared with a 1 Kb DNA ladder. No bands were observed in control lane (-RT, reverse transcriptase omitted). DNA sequencing verified the identity of the amplified cDNA. **B:** Immunoblots with rat liver and lung protein extracts show two closely related bands at 44–46 kDa. Rat cochlea shows a single band at 46 kDa corresponding to the long (nuclear) ADK isoform.

Fig. 2.

Adenosine kinase distribution in the adult rat cochlea. **A:** Low resolution image of the adult rat cochlea stained with the ADK antibody. Immunostaining is present in the spiral ligament (sl), stria vascularis (sv), organ of Corti (oc), interstitial cells (idc) of the spiral limbus (lim) and spiral ganglion neurones (sgn), but is absent from the tectorial membrane (tm). **B:** No immunolabelling was detected when the primary antibody was omitted. **C:** Immunolabelling of the spiral ligament (sl) and outer sulcus cells (osc). **D:** Strong nuclear immunolabelling in spiral ganglion neurones (sgn) and weaker staining in satellite cells (sc). **E:** ADK immunolabelling of the modiolar blood vessels (mbv). **F:** Immunostaining in the outer hair cells (ohc) and inner sulcus cells (isc). Scale bars, 50 μ m.

Fig. 3. Adenosine kinase distribution in the developing rat cochlea (postnatal days 1 and 7). **A:** ADK is expressed in the spiral ganglion (sg), spiral limbus (sl), greater epithelial ridge (GER), organ of Corti (oc) and spiral ligament (sl) at postnatal day 1 (P1). **A1:** High resolution image of the ADK immunolabelling in the organ of Corti at P1. **A2:** In the spiral ganglion, ADK immunoreactivity in satellite cells (sc) was stronger than in neurones. **B, B1, B2:** At P7, ADK immunostaining is reduced in all cochlear regions compared to P1. **C1, C2:** Differential staining of spiral ganglion neurones (sgn) and satellite cells (sc) at P1 and P14 respectively. Scale bars, 50 μ m (A, B); 20 μ m (A1, A2, B1, B2). Abbreviations: cc, Claudius cells; dc, Deiters' cells; ihc, inner hair cells; ohc, outer hair cells; osc, outer sulcus cells.

Fig. 4.

Adenosine kinase distribution in the developing rat cochlea (postnatal days 14 and 21). **A:** At P14, strong immunostaining was observed in the spiral ganglion (sg), interdental cells (idc) of the spiral limbus (lim), inner sulcus cells (isc), organ of Corti (oc), outer sulcus cells (osc), spiral ligament (sl) and stria vascularis (sv). **A1:** Detail of immunostaining in Claudius cells (cc) and outer sulcus cells (osc) at P14. **A2:** Immunostaining of marginal cells (mc) in the stria vascularis (sv) is evident for the first time at P14. **A3:** Labelling of Deiters' cells(dc) and Hensen's cells (hc) in the organ of Corti (oc) at P14. **A4:** In contrast to P1, predominant neuronal ADK immunolabelling was observed in the spiral ganglion (sg) at P14. **B:** At P21, the ADK immunostaining was comparable to P14. **B1:** Staining in the spiral ganglion (sg). **B2:** High resolution images of immunolabelled Deiters' cells (dc) and inner hair cells (ihc) in the organ of Corti (oc) and inner sulcus cells (isc). **B3:** At P21, ADK extended to all three cell layers of the stria vascularis (marginal, intermediate and basal). **B4:** Details of immunostaining in the outer sulcus cells (osc) and their root processes in the spiral ligament (sl) and Type 3 fibrocytes at P21. **C:** No immunofluorescence was observed in the absence of the primary antibody. Scale bars, 50 μ m (A, B, C); 20 μ m (A1–4, B1–4).

Fig. 5.

A: Transcript levels of adenosine kinase in the noise-exposed rat cochleae assessed by qRT-PCR 1 hour and 72 hours after the cessation of noise exposure. Control cochleae were obtained from rats exposed to ambient noise (~60 dB SPL). Data presented as mean $\pm$ SEM ($n = 8$). Fold changes in either direction greater than 2 were considered significant. **B:** Threshold shifts (ABR) in rats exposed to 8–12 kHz band noise for 2 hours at 110 dB SPL. ABR were measured in response to auditory clicks and pure tones before noise exposure (baseline), and at intervals (30 minutes, 7 days and 14 days) after noise exposure. Treatment with ABT-702 or vehicle solution commenced 6 hours after the cessation of noise exposure. Data are expressed as means $\pm$ SEM ($n = 8$).

Table 1

The overview of adenosine kinase expression in the developing rat cochlea

Tissue	Cell type	P1	P7	P14	P21	Adult
Epithelial	greater epithelial ridge / inner sulcus	++	+	++	++	++
	outer sulcus cells	++	++	++	++	++
Sensory cells	inner hair cells	++	-	-	+	-
	outer hair cells	++	+	-	+	++
Supporting cells	Deiters' cells	++	-	++	++	-
	Hensen's cells	++	-	++	++	-
	Claudian cells	++	++	++	++	++
Spiral ganglion	neurons	+	+	++	++	++
	satellite cells	+++	+	+	+	+
Lateral wall	spiral ligament	++	++	++	++	++
	stria vascularis	+	+	++	++	++
Spiral limbus	interdental cells	++	-	++	++	+

Intensity of immunostaining was scored as follows: (-)not detectable; (+) weak staining; (++) moderate staining; (+++) strong staining.