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Research Paper

Adenosine receptors regulate susceptibility to noise-induced neural injury in the mouse cochlea and hearing loss

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ABSTRACT

Our previous studies have shown that the stimulation of A_1 adenosine receptors in the inner ear can mitigate the loss of sensory hair cells and hearing loss caused by exposure to traumatic noise. Here, we focus on the role of adenosine receptors (AR) in the development of noise-induced neural injury in the cochlea using A_1AR and A_{2A}AR null mice ($A_1AR^{-/-}$ and $A_{2A}AR^{-/-}$). Wildtype (WT) and AR deficient mice were exposed to octave band noise (8–16 kHz, 100 dB SPL) for 2 h to induce cochlear injury and hearing loss. Auditory thresholds and input/output functions were assessed using auditory brainstem responses (ABR) before and two weeks post-exposure. The loss of outer hair cells (OHC), afferent synapses and spiral ganglion neurons (SGN) were assessed by quantitative histology. $A_1AR^{-/-}$ mice (6–8 weeks old) displayed a high frequency hearing loss (ABR threshold shift and reduced ABR wave I and II amplitudes). This hearing loss was further aggravated by acute noise exposure and exceeded the hearing loss in the WT and $A_{2A}AR^{-/-}$ mice. All mice experienced the loss of OHC, synaptic ribbons and SGN after noise exposure, but the loss of SGN was significantly higher in $A_1AR^{-/-}$ mice than in the $A_{2A}AR^{-/-}$ and WT genotypes. The $A_{2A}AR^{-/-}$ demonstrated better preservation of OHC and afferent synapses and the minimal loss of SGN after noise exposure. The findings suggest that the loss of A_1AR expression results in an increased susceptibility to cochlear neural injury and hearing loss, whilst absence of A_{2A}AR increases cochlear resistance to acoustic trauma.

1. Introduction

Ageing and exposure to noise are major contributing factors to acquired hearing loss. Sensorineural hearing loss (SNHL) caused by acoustic overexposure is associated with cochlear injury, including the loss of sensory hair cells and primary auditory neurons in the cochlea. The classic view of SNHL is that the hair cell death is the earliest sign of degeneration in both noise- and age-related hearing loss, whilst the degeneration of primary auditory neurons occurs secondary to the loss of sensory hair cells (Bohne and Harding, 2000). This view has been challenged in the last few years from animal and human studies. Recent studies have shown that extensive loss of auditory afferent nerve synapses, rather than hair cell death, is the earliest sign of cochlear damage, and that it can occur as the primary event, even without the loss of sensory hair cells (Kujawa and Liberman, 2009, 2015; Housley et al., 2013; Fernandez et al., 2015). Cochlear neuropathy may not be obvious as a change in auditory threshold, but it leads to difficulties in speech discrimination in noisy conditions and central auditory deficits that can help explain the perceptual handicap in aging individuals with normal audiograms (Sergeyenko et al., 2013; Kujawa and Liberman, 2015; Liberman and Liberman, 2015). Cochlear neuropathy may also underlie post-noise exposure perceptual abnormalities such as tinnitus (perception of phantom sounds) and hyperacusis (intolerance to moderately intense sounds), common complaints from patients with or without threshold elevation (Bauer et al., 2007; Eggermont, 2007).

In this study, we focused on the role of adenosine receptors in the development of neural injury in the cochlea associated with

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acoustic overexposure. Adenosine is an endogenous neuromodulator whose effects are mediated by four types of G protein-coupled adenosine receptors (A_1, A_{2A}, A_{2B}, A_3). We have shown that the high affinity adenosine receptors (A_1, A_{2A}, A_3) are differentially localised in cochlear tissues with the strongest immunoexpression in sensory hair cells, supporting Deiters' cells and spiral ganglion neurons (Vlajkovic et al., 2007). A_1 and A_{2A} receptors likely have an important role in presynaptic regulation of glutamate release from the inner hair cells, consistent with their role in the CNS (Cunha, 2001, 2005). Adenosine is released from tissues in response to stress and it can promote tissue protection and repair through several modes of action. Adenosine can boost antioxidant defenses, increase oxygen supply, improve blood flow, inhibit or stimulate the release of neurotransmitters, trigger anti-inflammatory responses and promote anti-apoptotic pathways (Linden, 2005; Fredholm, 2007). Inhibitory A_1 receptors generally set the neuroprotective tone, whilst A_{2A} receptors facilitate synaptic function and may contribute to synaptotoxicity (Boison and Aronica, 2015). Neuroprotective actions of A_1 adenosine receptors in CNS disorders such as stroke, epilepsy, migraine, neurodegenerative and neuropsychiatric disorders are well documented (Boison, 2008; Jacobson, 2009; Stone et al., 2009). In contrast, A_{2A} receptor inactivation/inhibition in the CNS has been associated with neuroprotection against ischemic brain damage, traumatic brain injury, excitotoxicity and neurodegenerative diseases, such as Parkinson's and Alzheimer's (Chen et al., 2013).

We and others have previously shown that noise- and drug-induced hearing loss can be ameliorated in experimental animals by local or systemic administration of A_1 adenosine receptor agonists (Hu et al., 1997; Hight et al., 2003; Wong et al., 2010; Gunewardene et al., 2013; Vlajkovic et al., 2010, 2014; Kaur et al., 2016). However, we know little about how adenosine receptors affect injury processes in the cochlea and especially whether there is an interplay between the facilitatory and inhibitory adenosine receptor responses that may define the extent and nature of reparative processes. To identify the role of adenosine receptors in regulating the overall response of cochlear neural tissues to stress and injury, we exposed mice lacking genes for A_1 and A_{2A} receptors to acute noise and assessed functional and histological correlates of neural injury.

Both the A_1 and A_{2A} receptor gene knockout mice are viable at birth, breed normally and have only mildly impaired functionality (Fredholm et al., 2005). Importantly, studies to date suggest that there are no compensatory changes in the remaining receptors if either of these genes is deleted (Fredholm, 2010). Targeted disruption of the *A_1AR* gene results in mice with normal heart rate, blood pressure and body temperature, but with signs of increased anxiety, aggressiveness and hyperalgesia (Fredholm et al., 2005; Johansson et al., 2001). Other studies in *$A_1AR^{-/-}$* mice have demonstrated an important role of the A_1AR in modulating excitatory glutamatergic neurotransmission (Johansson et al., 2001; Fredholm et al., 2011). *$A_{2A}AR^{-/-}$* mice have normal blood pressure and heart rate (Fredholm et al., 2005), but have an increased threshold to noxious heat stimulation, are more aggressive (Fredholm et al., 2011), and show reduced ischaemic neuronal injury after occlusion of the middle cerebral artery (Chen et al., 1999). Deletion of the *$A_{2A}AR$* gene is also considered neuroprotective in several models of neurodegenerative diseases (Fredholm et al., 2005). Studies on adenosine receptor knockout mice have thus contributed to better understanding of the adenosine receptor-specific physiological processes and responses to disease (Fredholm et al., 2005, 2011). Those studies have also informed the development of therapies for many CNS disorders (Jacobson, 2009; Stone et al., 2009). Whereas CNS effects of these mutations are well defined, the effects of adenosine receptor deletions on cochlear physiology and response to stress remain to be determined. In this study, using adenosine receptor knockout mice, we characterized distinctive roles of A_1 and A_{2A} adenosine receptors in noise-induced neural injury in the cochlea.

2. Methods

2.1. Animals

The *$A_1AR^{-/-}$* and *$A_{2A}AR^{-/-}$* mouse lines (C57BL/6 background; Xiao et al., 2011) were obtained from the Legacy Research Institute (Portland, OR) and established at the University of Auckland animal facility using heterozygous inter-breeding to yield knockout and wildtype littermates. After genotyping, 6–8 week old male and female mice were assigned to the following groups: wildtype (WT, *$AR^{+/+}$*), A_1 receptor knockout (*$A_1AR^{-/-}$*) and A_{2A} receptor knockout (*$A_{2A}AR^{-/-}$*) mice. Ten mice from each genotype were exposed to acute noise, and a control group of five wildtype, five *$A_1AR^{-/-}$* and five *$A_{2A}AR^{-/-}$* mice were exposed to ambient noise (55–65 dB SPL). Control mice were used to obtain synaptic ribbon and spiral ganglion neuron counts at non-traumatic ambient sound levels. The mice were housed in standard cages in the animal housing facility (Vernon Jansen Unit) at the University of Auckland with free access to food and water. All experimental procedures in this study were approved by the University of Auckland Animal Ethics Committee.

2.2. Genotyping

The genotype of each mouse was established from ear biopsies. The ear biopsies were digested at 55 °C overnight using Direct PCR lysis reagent (Viagen Biotech, LA, CA) and Proteinase K. The genomic DNA from each sample was used as a PCR template. PCR was performed using a Gotaq green master mix (Promega, Madison, WI), diluted DNA (1:10) and PCR primers. Two sets of primers were used for detection of A_1AR deletion (W1: 5′-ACTCCATGCT-GATAACCTTC-3′; W2: 5′-GTTTCCCAAAGAAACAGACC-3′; K1: 5′-CTTCAGCACAGTTTTAGAGA-3′; K2: 5′-TTTGTGAGCCAGGGCATTGG-3′) and two for detection of A_{2A}AR deletion (P1: 5′-AGC-CAGGGGTTACATCTGTG-3′; P2: 5′-TACAGACAGCCTCGACATGTG-3′; P3: 5′-TCGGCCATTGAACAAGATGG-3′; P4: 5′-GAGCAAGGTGA-GATGACAGG-3′). The PCR program with a 35 cycle profile was performed as follows: 94 °C denaturation (30 s), 54 °C annealing (1 min) and 72 °C extension (1 min) steps using PTC-100™ Programmable Thermal Controller (MJ Research Inc., Waltham, MA, USA). PCR amplicons were separated by agarose gel electrophoresis, and visualized using SYBR safe DNA gel stain (Invitrogen). The amplicon sizes for A_1AR were 600 bp (WT) and 360 bp (*$A_1AR^{-/-}$*); for A_{2A}AR were 180 bp (WT) and 320 bp (*$A_{2A}AR^{-/-}$*).

2.3. Auditory brainstem responses

Auditory brainstem responses (ABR) are auditory evoked potentials derived from the activity of the auditory nerve and the central auditory pathways (brainstem/midbrain regions) in response to transient sound (auditory clicks or tone pips). The mice were anesthetised with a mixture of ketamine (100 mg/kg i.p.) and xylazine (20 mg/kg i.p.) and then placed onto a heating pad, to maintain body temperature at 37 °C. The ABR thresholds were measured in anaesthetised mice before and two weeks after noise exposure using computer generated (Tucker-Davis Technologies Auditory Physiology System III, Alachua, FL, USA) acoustic stimuli (tone pips). ABR were recorded by inserting three platinum electrodes subdermally at the vertex (active), mastoid region of the ear of interest (reference), and mastoid region of the opposite ear