Molecular and Cellular Neuroscience 48 (2011) 51–61

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Ototrauma induces sodium channel plasticity in auditory afferent neurons

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ARTICLE INFO

Article history:
Received 14 December 2010
Revised 2 June 2011
Accepted 8 June 2011
Available online 25 June 2011

Keywords:
Cochlea
Immunohistochemistry
qPCR
Spiral ganglion neurons
Voltage-gated sodium channels
Noise-induced hearing loss

ABSTRACT

Exposure to intense sound can cause damage to the delicate sensory and neuronal components of the cochlea leading to hearing loss. Such damage often causes the dendrites of the spiral ganglion neurons (SGN), the neurons that provide the afferent innervation of the hair cells, to swell and degenerate thus damaging the synapse. In models of neuropathic pain, axotomy, another form of afferent nerve damage, is accompanied by altered voltage-gated sodium channel (VGSC) expression, leading to neuronal hyperactivity. In this study, adult Wistar rats were exposed to noise which produced a mild, 20 dB hearing threshold elevation and their VGSC expression was investigated. Quantitative PCR showed decreased $Na_V1.1$ and $Na_V1.6$ mRNA expression in the SGN following noise exposure (29% and 56% decrease respectively) while $Na_V1.7$ mRNA expression increased by approximately 20% when compared to control rats. Immunohistochemistry extended these findings, revealing increased staining for $Na_V1.1$ along the SGN dendrites and $Na_V1.7$ in the cell bodies after noise. These results provide the first evidence for selective changes in VGSC expression following moderate noise-induced hearing loss and could contribute to elevated hearing thresholds and to the generation of perceptual anomalies commonly associated with cochlear damage, such as tinnitus and hyperacusis.

Introduction

The Royal National Institute for Deaf People (RNID) estimates that 12.6 million people are affected by noise-induced hearing loss (NIHL) in the UK. Many animal models of NIHL have characterised the effects of intense noise stimuli (≥120 dB SPL) presented over several hours or days on the function of the peripheral and central auditory pathways (Duan et al., 2008; Hamernik et al., 1980; Kojima et al., 2007; Salvi et al., 1979; Sullivan and Conolly, 1988). In such studies, profound or complete deafness is elicited and the changes observed in such models are loss of outer and inner hair cells (OHC and IHC, respectively), physical rupturing of cochlear structural membranes (Henderson and Hamernik, 1995; Spoendlin, 1971), and swelling and degeneration of the spiral ganglion neuron (SGN) dendrites resulting from glutamate excitoxicity (Puel, 1995; Puel et al., 1998; Spoendlin, 1971). Damage-evoked neuronal plasticity in the central auditory pathway is also observed with increased spontaneous activity in the dorsal cochlear nucleus (Kaltenbach et al., 2000; Zhang and Kaltenbach, 1998) and inferior colliculus (Salvi et al., 2000).

The pathophysiological consequences of less intense acute noise stimuli (≤115 dB SPL), where more modest hearing loss occurs, are much more relevant to human environmental exposure but have not been well characterised. In such models, little physical damage is seen and only modest structural changes in the cochlea occur, including sparse hair cell loss (Hu et al., 2009) and limited SGN degeneration (Kujawa and Liberman, 2009) although further investigation is required to fully understand the effects of such stimuli.

Voltage-gated sodium channel (VGSC) α-subunits ($Na_V1.1$–1.9) play an important role in regulating neuronal excitability. Neuronal VGSC α-subunits have been classified as either "central" ($Na_V1.1$, 1.2, 1.3, 1.6) or "peripheral" ($Na_V1.7$, 1.8, 1.9) subunits, based on their expression patterns. We previously showed $Na_V1.1$, $Na_V1.6$, $Na_V1.7$ subunits are expressed in rat SGNs (Fryatt et al., 2009), a unique VGSC phenotype that conforms to neither the conventional central or peripheral expression patterns.

In peripheral nociceptors, nerve injury and deafferentation induce profound changes in the expression and subcellular distribution of $Na_V1.8$, Na_V 1.9 (Dib-Hajj et al., 1999; Novakovic et al., 1998; Sleeper et al., 2000) and $Na_V1.3$ (Black et al., 1999; Dib-Hajj et al., 1996; Waxman et al., 1994). This strongly influences pain fibre excitability, leading to spontaneous pain, hyperalgesia and allodynia. We hypothesised that acoustic ototrauma, a stimulus known to damage the peripheral SGN processes (Puel, 1995; Puel et al., 1998; Spoendlin, 1971), has the potential to elicit similar axotomy and injury-related changes in SGN ion channel expression. We postulated that altered expression of VGSC subunits could have profound effects on SGN excitability, perhaps affecting hearing threshold and auditory perception. If changes in VGSC expression are seen following moderate NIHL, this could provide additional avenues of investigation for identifying neuronal correlates of

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doi:10.1016/j.mcn.2011.06.005

hearing loss and perceptual anomalies, such as tinnitus, as well as novel therapeutic pharmacological targets for such conditions.

Using a new model of moderate NIHL, designed to minimise physical damage to the cochlea, we investigated whether the VGSC cohort expressed in normal rat SGNs (Fryatt et al., 2009) alters following ototrauma. We demonstrate that moderate noise exposure results in elevated hearing thresholds and that this is accompanied by marked changes in endogenous VGSC channel expression at the mRNA level and altered immunohistochemical staining for $Na_V1.1$ and $Na_V1.7$. These changes represent a novel pathology that could, at least in part, contribute to auditory deficits and changes in auditory perceptions including tinnitus.

Results

Hearing thresholds before and after moderate noise exposure

In order to establish that the noise exposure protocol produced measurable changes in hearing threshold, ABR measurements were taken from all rats used in this study. Representative serial ABR waveforms measured in response to 30 kHz tone pips before and after noise exposure are shown in Fig. 1. The ABR waveform amplitudes decreased dramatically following noise exposure compared to pre-exposure levels. Additionally, the ABR waveform could not be detected at 40 dB attenuation in response to noise exposure, indicating an increase in ABR threshold at this frequency compared to the pre-exposure period.

The mean ABR thresholds and threshold elevations following noise exposure ($n=8$) are shown in Fig. 2. On completion of the noise exposure protocol, there was no detectable increase in the average hearing threshold at 12 kHz. At 16 kHz, a small increase in ABR threshold of 6 ± 3 dB ($p=0.09$) was seen following the complete noise exposure protocol. Marked elevations in ABR threshold were observed at 24 kHz and 30 kHz following both first and second noise exposures. At 24 kHz, the magnitude of the mean threshold elevations was 10 ± 4 dB ($p<0.05$) and 18 ± 4 dB ($p<0.005$) after the first and second noise exposures respectively, while the mean elevations for 30 kHz were 13 ± 3 dB ($p<0.005$) and 24 ± 3 dB ($p<0.001$) respectively.

Changes in P1 amplitudes following noise exposure

Fig. 3 shows the average P1 amplitudes from the same noise exposed animals in response to discrete tone pip stimulus steps. The P1 amplitudes in response to the 12 kHz and 16 kHz tone pips were not significantly different following the complete noise exposure protocol ($p>0.05$). However, testing the 24 kHz and 30 kHz hearing ranges of these animals revealed a significant decrease in P1 amplitude of approximately 35% following the final ABR when compared to the first ABR measurements, especially at the loudest tone pip level used ($p<0.05$-0.01).

Using the discrete stimulus attenuation steps, a simple input/output transfer function was fitted using a line of best fit for each frequency. Following the first ABR, a second order polynomial line of best fit was fitted, with the form:

$$y = lx^2 - mx + c$$

The parameters l and m in terms of x reflected the primary transfer function of the cochlea at the level of the ABR signal while the c term is related to sensitivity of the cochlea. Following the second and final ABR measurement, lines of best fit were determined using the same

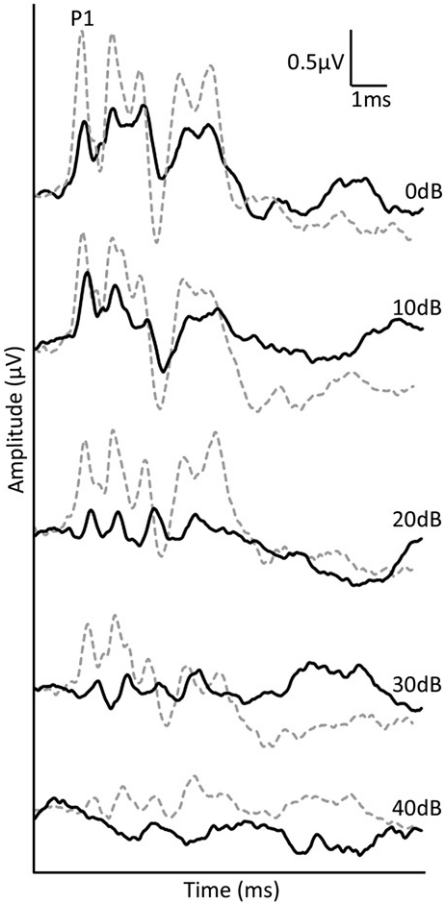

Fig. 1. Typical ABR waveforms measured from a rat before and after noise exposure. ABR waveforms in response to 30 kHz tone pips (numbers indicate the attenuation of the tone) before (dashed grey line) and after (filled black line) the complete noise exposure protocol. Following the noise exposure protocol, a decrease in waveform amplitude can be seen at all tone pip attenuations compared to the pre-noise exposure ABR waveforms while no dramatic change in waveform component latencies were observed. Additionally, the ABR waveform could not be reliably identified at 40 dB attenuation following noise exposure.

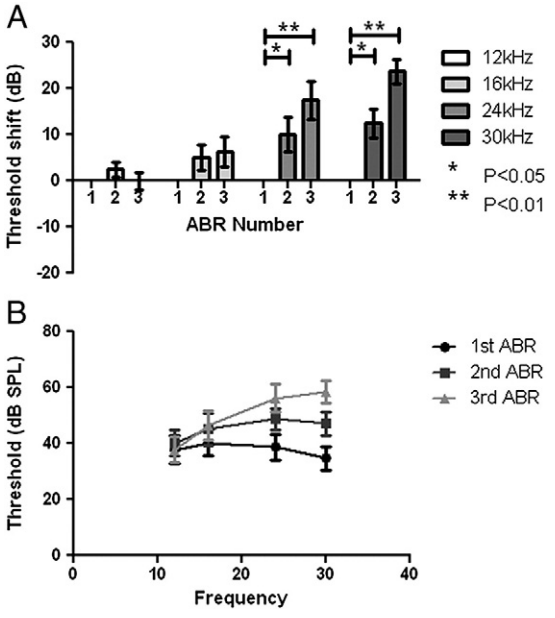

Fig. 2. Threshold elevation and hearing thresholds of noise exposed animals. Graphs of mean hearing threshold shifts (A) and hearing thresholds (B) of animals exposed to noise. (A) Hearing threshold for the 24 kHz and 30 kHz tone pips significantly increased during the second and third ABRs, (*, $p<0.05$ and **, $p<0.01$ respectively) following noise exposure. An increase in hearing threshold for 16 kHz was detected during the third ABR, after two noise exposures ($p=0.09$), while the hearing threshold for 12 kHz remained unaffected. Please note that ABR number refers to each trial as described in Fig. 9.