Cochlear Adaptation and Implications for Noise-Induced Hearing Loss

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ABSTRACT

Noise-induced hearing loss stems from over-exposure of sensory and neural tissues to sound resulting in their injury. There is accumulating evidence that the cochlea also adapts to moderate levels of background noise, most likely for it to sustain sensitivity to important incoming sounds, and that these adaptive mechanisms confer some protection on the cochlear tissues to excessive sound exposure. We describe here a molecular mechanism involving an ATP ($P2X_2$) receptor in cells lining the cochlear endolymphatic space, which enables long-term adaptation to chronic noise exposure but also may explain aspects of Temporary Threshold Shift (TTS) and sensitivity to Permanent Threshold Shift (PTS) (Housley et al., 2013). Comparing *P2rx2*-null and wild type mice we have shown that activation of the $P2X_2$ receptor by ATP secreted into the endolymph in response to moderately intense sound leads to a TTS that recovers within 24hrs. Additionally, *P2rx2*-null mice exhibit enhanced susceptibility to PTS with higher noise levels (>95dBSPL) indicating that this adaptation confers some protection to noise exposure. A mutation in the human *P2rx2* gene leads to the dominantly inherited, progressive sensorineural hearing loss, DFNA41, which is exacerbated by noise exposure (Yan et al., 2013). These studies imply that aspects of TTS may reflect ATP receptor-dependent adaptation rather than cochlear injury and that loss of this adaptation increases sensitivity to noise damage. Adaptation mechanisms in the cochlea need to be taken into account when considering the damaging effects of noise and may explain individual differences in susceptibility to Noise-Induced Hearing Loss.

INTRODUCTION

Noise-induced hearing loss (NIHL) is a significant health problem affecting a large number of people world-wide (Sliwinska-Kowalska and Davis 2012). The search for treatments and preventative strategies is essential to reducing or eliminating the effect of noise on hearing and the health burden from this condition.

Exposure to excessive sound leads to temporary and/or permanent functional changes in the auditory system, which are manifest as either a temporary or permanent threshold shift (TTS and PTS) (Wong et al., 2013). Although the pathological basis of TTS is not well defined, PTS can be correlated to permanent loss or degeneration of the sensory cells, synaptic structures and auditory nerve in the cochlea (Wong et al., 2013). These temporary and permanent changes in function can be correlated, to some extent, to the noise exposure parameters and are considered as quantifiable indicators of the extent of noise injury in the inner ear.

However, there is considerable variability in the individual response to both the temporary and permanent effects of noise exposure, suggesting inherent differences in susceptibility to the damaging effects of noise. The mechanisms underlying these differences are not well established, but we consider here that some may relate to variations in the ability of the cochlea to normally adapt to its environment including background noise exposure. There are many cochlear processes that contribute to its adaptation to physiological levels of noise, predominately to enable detection of important acoustic signals in increasing levels of background noise. These include those mediated by the medial olivocochlear efferents regulating outer hair cell reverse transduction and the lateral olivocochlear efferents, which may protect the primary afferent nerve terminals innervating inner hair cells from excessive stimulation (Maison et al., 2013). Down-regulation of the expression of AMPA receptors in the inner hair cell primary afferent synapse reduces the steady-state response of the cochlea to noise (Chen et al., 2007). These processes occur over relatively short time scales and whilst most likely these mechanisms are to improve signal detection in noise, there is evidence that they also provide neuroprotection to moderate level noise exposure.

Our recent studies (Housley et al., 2013, Yan et al., 2013), described here, provide evidence for a novel molecular process that enables a more sustained adaptation to background noise than the short-term processes described above. This is based on molecular signalling pathways in the cochlea mediated by extracellular adenosine tri-phosphate (ATP) and ATP receptors on cochlear sensory and supporting cells (Housley et al., 1998, 1999, King et al 1998). Importantly, with more sustained exposure (eg 30minutes) this adaptive response in animals is manifest as a temporary shift in auditory thresholds (ie TTS) with similar characteristics and time course to the noise-induced TTS in humans, suggesting that some components of TTS are due to the cochlea adapting to noise exposure, rather than an injury response (Housley et al., 2013). The lack of this adaptive mechanism leads to enhanced susceptibility to PTS and age-related hearing loss in animals and humans (Yan et al., 2013).

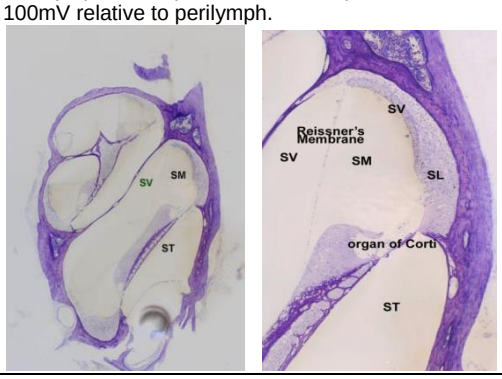
ROLE OF PURINERGIC SIGNALLING IN COCHLEAR ADAPTATION AND TTS

ATP signalling is involved in short- and long-term cochlear processes including development, regulation of vascular tone, and homeostasis of cochlear fluid electrochemistry. There are two types of ATP (purinergic, P2) receptor, the ATP-gated ion channels (P2X receptor subunits 1-7) and the G-protein coupled receptors (P2Y receptors). Activation of the P2X receptors by extracellular ATP leads to an increased non-selective cation conductance across the cell membrane, whereas the P2Y receptor activation leads to intracellular Ca^2 signalling. Many of the different sub-types of these large families of receptors are expressed in different parts of the adult and developing cochlea and mediate the various physiological responses in cochlear tissues (Huang et al., 2010).

Activation of one particular type of P2X receptor, the $P2X_2$ receptor, by ATP leads to a reduction in the endocochlear potential (EP) and dampens the hair cell's responsiveness to sound (Thorne et al., 2004). $P2X_2$ is highly expressed by sensory and supporting cells in tissues lining the endolymphatic space or Scala Media of the cochlea (Fig.1). These include the apical surfaces of the sensory hair cells of the organ of Corti, and the epithelial cells of Reissner's membrane (King et al., 1998). A characteristic of the cochlear endolymph, the positive EP (~+80 to +100 mV) is generated by the stria vascularis and maintained by resistances of the tissues lining

the endolymphatic compartment, including the Reissner's membrane (Fig 1). Together with the negative intracellular resting potential of the hair cells, the EP provides the driving force necessary for sound transduction. Experimentally, both EP and the hair cell membrane potential are reduced by the introduction of ATP into the cochlear endolymph and activation of the $P2X_2$ subunits (Thorne et al., 2004, Telang et al., 2010). The opening of these ion channels reduces the compartment resistance, thus increasing current flow across Reissner's membrane and the hair cell apical membrane, and effectively reducing the driving force for sound transduction and hair cell sensitivity (Fig 2). A consequence of this is a reduction in auditory thresholds. Continued noise exposure in animals leads to a sustained release of ATP into the endolymphatic compartment (Munoz et al., 2001), suggesting that similar events are occurring naturally during noise exposure and could lead to longer lasting effects.

Figure 1: Cross-section of mouse cochlea showing fluid chambers, Scala Media (SM) (endolymph), and Scala Vestibuli and Scala Tympani (SV, ST) (perilymph). The organ of Corti with the sensory cells and the lateral wall with the spiral ligament (SL). Endolymph in SM is separated from the perilymph by Reissner's membrane. The endolymph has a positive electrical potential of ~80-100mV relative to perilymph.

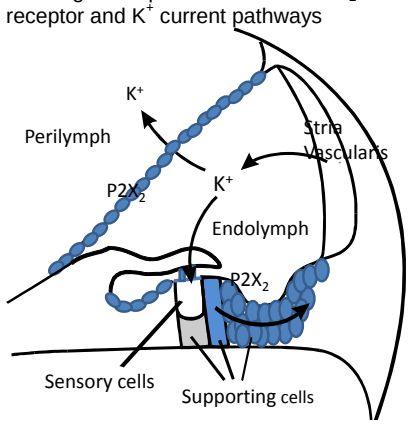


To investigate this, we evaluated the responses to noise in mice lacking the $P2X_2$ receptor (*P2rx2*$^{(-/-)}$) (Housley et al., 2013). Interestingly, these animals show little if any noise-induced TTS, as evaluated by auditory brainstem response (ABR) audiometry, with prolonged moderately intense noise exposure (30 min; 85 dBSPL; 8–16 kHz band-pass noise). As reported, the TTS in the wild-type mice (expressing $P2X_2$) measured by a click stimulus was 10.6 ± 1.4 dB (click stimulus, $p < 0.001$), or 15.6 ± 4.8 dB at 16kHz ($p = 0.012$), compared with only 2.1 ± 0.9 dB ($p=0.045$) or 1.4 ± 2.6 dB ($p=0.596$) respectively in *P2rx2* knockout mice (*P2rx2*$^{(-/-)}$). This effect on ABR thresholds was replicated in the Distortion-Product OtoAcoustic Emissions (DPOAE) measurements which showed a similar loss of threshold in the wild type mice, but which was absent in the *P2rx2*$^{(-/-)}$ mice. As the DPOAE measures the influence of the outer hair cell-mediated active process in the cochlea, responsible for fine tuning and frequency selectively, these data suggest that the effect of ATP can be linked to a suppression of the cochlear micromechanical responses.

Thus, the abolition of the $P2X_2$-mediated signalling pathway completely eliminates noise-induced low-level TTS. We have further shown that the time course for development of the $P2X_2$ -mediated TTS was approximately 20 minutes and that the recovery had a time constant of 12.3 hours and recovered fully within 24 hours. These characteristics of the TTS in wild-type mice are very similar to the characteristics of TTS from prolonged moderately-intense exposure in humans, where the auditory thresholds can take up to 16 hours to fully recover (Patuzzi, 1998). These data therefore support the view that at least some component of TTS induced by prolonged noise exposure is due to an ATP-mediated shunt of the transduction current away from the hair cells. This is the first identification of a molecular pathway that causes noise-induced TTS.

We have speculated that $P2X_2$ receptor activation, which we assume occurs as a result of noise-induced ATP release in the cochlea (Housley et al., 2013), reflects a physiological adaptation to noise rather than an injury response. In support of this the fact that thresholds are preserved with moderately-high level noise exposure in the mice without *P2rx2* expression indicates that it is unlikely that any tissue damage has occurred at that level of noise exposure. Thus the underlying cause of the TTS in the wild-type mice is a $P2X_2$ receptor-mediated effect on the transduction process in the hair cells. We have proposed that this adaptation enables the cochlea to maintain sensitivity to physiological signals in background noise. The initiating step for this would be the activation of ATP-gated ion channels and increased ionic fluxes across Reissner's membrane and the apical pole of the hair cell. However, this mechanism alone cannot explain the enduring loss of hair cell function, as the effect is prolonged substantially beyond the duration of the noise exposure whilst released ATP is rapidly removed by the extracellular ATP-hydrolysing enzymes (ectonucleotidases) (Vlajkovic et al., 1998). This indicates that the effect involves more sustained yet reversible cellular mechanisms which have yet to be identified. These mechanisms likely involve the outer hair cells, given the sustained and corresponding changes to the DPOAE and cochlear micromechanics that are observed.

Figure 2: Schematic of the cochlear partition showing the expression sites of $P2X_2$ receptor and K^+ current pathways



ROLE OF COCHLEAR ADAPTATION AND DEVELOPMENT OF PTS AND PRESBYACUSIS IN MICE

We speculated that if the ATP-mediated process causes a dampening of the hair cell response, it may also be otoprotective against high level noise exposure that causes permanent damage. Indeed, higher levels of noise exposure (2 hours; 100 dBSPL; 8 – 16 kHz band-pass noise) caused substantially greater PTS, as assessed by ABR, in the transgenic *P2rx2*$^{(-/-)}$ mice (32dB greater than the wild type mice at 16kHz) (Housley et al., 2013). Histological analysis of the cochlear tissue from the animals with PTS revealed no difference in the extent of sensory cell loss between wild type and the transgenic mice, but a significant atrophy of the spiral ganglion neurons was observed in the noise-exposed *P2rx2*$^{(-/-)}$ mice, as well as a reduction in the number of synaptic ribbons at the primary afferent synapse. These structural changes indicate the PTS is derived from neural rather than hair cell injury. This, we propose, arises from glutamate excitotoxicity at the afferent boutons from the sustained release of glutamate from the inner hair cells (Puel et al., 1998). This could occur in the *P2rx2* null mice if normally the ATP-mediated adaptation protects the cochlear hair cells from overstimulation and excessive glutamate release.

COCHLEAR ADAPTATION AND DEVELOPMENT OF AGE- AND NOISE-RELATED HEARING LOSS IN HUMANS

This ATP-mediated adaptive mechanism also occurs in humans, and its absence through functional mutations in the *P2rx2* gene leads to early hearing loss and increased susceptibility to noise exposure (Yan et al., 2013). The absence of cochlear $P2X_2$ receptor signalling in two Chinese families with an autosomal dominant progressive hearing loss (DFNA41) is attributable to the dominant negative

mutation *P2rx2* c.178G > T (p.V60L). This mutation disabled the $P2X_2$ receptor functionally and presumably removed the ATP-mediated otoprotection. Among family members with DFNA41, hearing loss was progressive with age, evident in the first decade and progressing across all frequencies to a severe loss (60-70dB) by 30 years of age. Exposure to noise significantly enhanced the loss of hearing at high frequencies. A mutation of the *P2rx2* gene has also been identified in an Italian family leading to a similar form of progressive hearing loss (Faletra et al., 2013).

DISCUSSION AND CONCLUSIONS

In summary, there is clear evidence that the cochlea adapts to moderate levels of noise and that this adaptation, mediated by ATP-gated ion channels in cochlear tissues, is manifest in mice as a noise-induced TTS with similar time course of recovery to human TTS. This TTS adaptive response appears to confer protection on the cochlea, and its absence leads to enhanced PTS in animals. In humans, it leads to rapidly progressing hearing loss (DFNA41), exacerbated by noise exposure.

These findings have implications for our understanding of the mechanisms and sound exposure laws governing the development of noise injury in the cochlea. Clearly some TTS, especially from moderate sustained noise exposure, is not strictly an injury response but a manifestation of a normal physiological adaptive process that enables the cochlea to maintain sensitivity in background noise. The fact that higher noise levels lead to TTS even in animals without the $P2X_2$ receptors, and hence lacking the adaptive response, indicates that this normal adaptation has limits, and if exceeded leads to TTS by some other mechanism, which may reflect cellular injury. The obvious consequence of these findings is that some noise-induced TTS could be regarded as a physiological response and the corollary is that the presence of such a TTS would be indicative of an ear that is potentially protected from noise exposure. It may also explain differences in individual susceptibility to noise and the view that some people have “tough” ears and others “tender” ears. There are several transcript variants of the *P2rx2* gene that result from splice variation (Linan-Rico et al., 2012) and more than a dozen human allelic variants are listed in the Database of Genomic Variants (Zhang et al., 2006), which could support variation in $P2X_2$ receptor protein function across populations.

Based on these studies, we may need to rethink the classification of noise-induced cochlear injury based on the functional (audiometric) responses to the noise exposure. The evidence from animal studies demonstrates that some neural injury can occur even with the full recovery of thresholds (Furman et al., 2013, Serveyenko et al., 2013), supporting the view that the temporary audiometric shifts from noise cannot be regarded as an accurate index of injury to the cochlea. Our data show that the adaptive mechanism mediated by ATP release represents an important constituent of cochlear response to noise, but further studies are required to fully understand the temporary basis of noise-induced hearing loss,

REFERENCES

Chen Z, Kujawa SG, & Sewell WF (2007) Auditory sensitivity regulation via rapid changes in expression of surface AMPA receptors. Nat Neurosci 10:1238-1240.

Faletra F, Girotto G, D'Adamo AP, Vozzi D, Morgan A, Gasparini P6 (2013) A novel P2RX2 mutation in an Italian family affected by autosomal dominant nonsyndromic hearing loss. Gene.534:236-9.

Furman AC, Kujawa SG, Liberman MC (2013). Noise-induced cochlear neuropathy is selective for fibers with low spontaneous ratesJ Neurophysiol. 110:577-586.

Housley, G.D., Kanjhan, R., Raybould, N.P., Greenwood, D., Salih, S.G., Järlebark, L., Burton, L.D., Setz, V.C.M., Cannell, M.B., Soeller, C., Christie, D.L., Usami, S., Matsubara, A., Yoshie, H., Ryan, A.F., Thorne PR (1999) Expression of the P2X2 receptor subunit of the ATP-gated ion channel in the cochlea: implications for sound transduction and auditory neurotransmission. J Neurosci 19:8377-8388.

Housley GD, Luo L, & Ryan AF (1998) Localization of mRNA encoding the P2X2 receptor subunit of the adenosine 5'-triphosphate-gated ion channel in the adult and developing rat inner ear by in situ hybridization. J Comp Neurol 393:403-414.

Housley GD, Morton-Jones R, Vlajkovic SM, Telang RS, Paramananthasivam V, Tadros SF, Wong AC, Froud KE, Cederholm JM, Sivakumaran Y, Snguanwongchai P, Khakh BS, Cockayne DA, Thorne PR, Ryan AF (2013) ATP-gated ion channels mediate adaptation to elevated sound levels. Proc Natl Acad Sci U S A, 110:7494-7499.

Huang L.C., Thorne P.R., Vlajkovic S.M., Housley G.D. (2010) Differential expression of P2Y receptors in the rat cochlea during development. Purinergic Signaling 6:231-248.

King M, Housley GD, Raybould NP, Greenwood D, & Salih SG (1998) Expression of ATP-gated ion channels by Reissner's membrane epithelial cells. Neuroreport 9:2467-2474.

Liñan-Rico A, Jaramillo-Polanco J, Espinosa-Luna R, Jiménez-Bremont JF, Liñan-Rico L, Montaño LM, Barajas-López C. (2012) Retention of a new-defined intron changes pharmacology and kinetics of the full-length P2X2 receptor found in myenteric neurons of the guinea pig. Neuropharmacol 63: 394-404.

Maison SF, Usubuchi H, Liberman MC (2013) Efferent feedback minimizes cochlear neuropathy from moderate noise exposure J Neurosci.33: 5542-5552.

Muñoz DJ, Kendrick IS, Rassam M, & Thorne PR (2001) 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 121(1):10-15.

Patuzzi R (1998) Exponential onset and recovery of temporary threshold shift after loud sound: Evidence for long-term inactivation of mechano-electrical transduction channels. Hear Res 125:17–38.

Puel JL, Ruel J, Gervais d'Aldin C, & Pujol R (1998) Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. Neuroreport 9:2109-2114

Sergeyenko Y, Lall K, Liberman MC, Kujawa SG (2013)Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. J Neurosci. 33;13686-13694.

Sliwinska-Kowalska M, A Davis, A. (2012) Noise-induced hearing loss. Noise and Health, 14:274-280

Telang R.S., Paramananthasivam V., Vlajkovic S.M., Munoz D.J.B., Housley G.D., Thorne P.R. (2010) Reduced P2X2 receptor-mediated regulation of endocochlear potential in the ageing mouse cochlea. Purinergic Signal 6:263-272.

Thorne PR, Munoz DJ, & Housley GD (2004) Purinergic modulation of cochlear partition resistance and its effect on the endocochlear potential in the guinea pig. J Assoc Res Otolaryngol 5:58-65

Vlajkovic SM, Thorne PR, Housley GD, Munoz DJ, & Kendrick IS (1998) Ecto-nucleotidases terminate purinergic signalling in the cochlear endolymphatic compartment. Neuroreport 9:1559-1565.

Wong AC-Y, Kristina E Froud, KE and Yves Shang-Yi Hsieh YS-Y (2013). Noise-induced hearing loss in the 21st century: A research and translational update. World J Otorhinolaryngol.3: 58-70.

Yan D, Xie D, Walsh T, Yuan H, Sirmaci A, Zhu Y, Fujikawa T, Wong ACY, Loh TL, Du L, Grati M, Vlajkovic SM, Blanton S, Chen Z-Y, Thorne PR, Kachar B, Tekin M, Zhou H, Housley GD, King M-C, Liu X-Z. (2013) Mutation of the ATP-gated P2X2 receptor leads to progressive hearing loss and increased susceptibility to noise. Proc Natl Acad Sci USA 110:2228-2233.

Zhang J, Feuk L, Duggan GE, Khaja R, Scherer SW. (2006) Development of bioinformatics resources for display and analysis of copy number and other structural variants in the human genome. Cytogenet Genome Res. 2006;115: 205-214.