NZMSJ ACADEMIC Writing Prize ISSUE 7 RUNNER UP

Characterisation of ecto-5'-Nucleotidase (CD73) and nucleoside triphosphate diphosphohydrolase-8 (NTPDase8) expression in rat cochlea

Son T. Pham
PhD Candidate
University of New South Wales
Australia

Son is a Vietnamese citizen who came to New Zealand as an international student in 2002. He graduated from the University of Auckland in 2007 with a Bachelor of Technology in Biology with First Class Honours, and was awarded a three-year PhD scholarship at the end of 2007 to study the nature of Hepatitis C Virus at the University of New South Wales, Australia. After completion of his PhD study, he wishes to pursue a science career in infectious diseases, possibly becoming a virologist.

Research Project Summary

The purinergic signalling pathway provides an important homeostatic regulation of sound transduction and neurotransmission in the cochlea[4]. This pathway involves extracellular nucleotides as signalling molecules acting on P2 receptors (use adenosine 5'-triphosphate (ATP) as the ligand) and nucleosides acting on P1 receptors (use adenosine as the ligand), and the enzymes collectively known as ectonucleotidases. Many pathophysiological and therapeutic potential issues regarding the cochlea are directly linked with this signalling pathway[2]. ATP and adenosine are extracellular signalling messengers that can modulate a number of physiological processes in the cochlea (auditory neurotransmission, electrochemical homeostasis, signal transduction) and also protect the cochlea from oxidative stresses (noise, ototoxicity)[2,8,11]. However, there is still insufficient understanding of the involved mechanisms, especially of the ectonucleotidase enzymes involved in regulating this extracellular ATP to adenosine in the cochlea.

There is strong evidence from previous studies[8-11] for the expression of certain members of these ectonucleotidases, the ecto-nucleoside triphosphate diphosphohydrolase family (NTPDase1, 2, 3) in the cochlea. Before these projects were conducted, however, there had never been any evidence for NTPDase8 expression. In addition, although the evidence for ecto-5'-nucleotidase (CD73) activity has been provided in studies showing the hydrolysis of AMP to adenosine following its perfusion through the cochlear fluid compartments, there had been no confirmation on the expression of this enzyme in the cochlea. Due to these reasons, the Deafness Research Foundation funded this project to characterise both NTPDase8 and CD73, in terms of their mRNA expression and distribution in the cochlea. An advanced imaging technique - confocal immunofluorescence microscopy - was used to investigate the localisation of these enzymes in rat cochlea.

The project was performed in the Auditory Neurobiology Laboratory, in ten weeks from November 2006 to February 2007, under the supervision of Dr. Srdjan Vlajkovic, an expert in purinergic signalling. The study represents the first attempt to investigate the expression and distribution of these two key enzymes' likely involvement in regulating ATP signalling in the cochlea. These findings, along with previous reports, provided good evidence for the enzyme cascade involved in the hydrolysis of ATP to adenosine. At the health significance end of this project, the confirmed findings highlighted novel molecular mechanisms involved in regulation of hearing. Furthermore, the results from this project provide deeper insight into the purinergic control of sound transduction and neurotransmission in the cochlea, and serve as an important reference point for future gene therapy and other hearing-loss treatments.

ABSTRACT

In the cochlea, ectonucleotidases regulate the signalling levels of extracellular nucleotides by hydrolysing adenosine 5'-triphosphate (ATP) and the related purine nucleotides. The surface-located enzymes include members of the E-NTDase family (CD39/NTPDase 1, 2, 3 and 8) and the ecto-5'nucleotidase (CD73). In previous studies, three members of the E-NTPDase (NTPDase-1, -2 and -3) were localised. Activity of CD73 in the cochlea was also reported. The present study focuses on the mRNA expression of CD73 and NTPDase8. Western blotting confirmed the expression of these enzymes in the cochlea. Confocal immunofluorescence localised CD73 to the basal and intermediate cells of the stria vascularis, capillaries and small blood vessels throughout the cochlear compartments. NTPDase8 was immunolocalised to the interdental cells of the spiral limbus, to Claudius cells and to the root region of spiral ligament. In the past, NTPDase8 was reported to have a higher affinity for ATP than for ADP[1], while CD73 was known to break down AMP to adenosine. These differential expressions, functions and localisation of NTPDase8 and CD73 along with previously reported NTDase-1, -2, -3 together suggest a possible interaction cascade between these enzymes and the spatial, as well as temporal, regulation of P2 receptor signalling in the cochlea.

INTRODUCTION

ATP is well-known for its role as a cellular energy-driving molecule. In the nervous system, particularly in the cochlea, there is strong evidence that extracellular ATP and its corresponding nucleoside, adenosine, have multiple physiological and pathophysiological roles in purinergic signaling, acting via P2 receptors (ATP as ligand) and P1 receptors (adenosine as ligand)[2]. Ectonucleotidases are important in regulating the effects of P2 receptors by hydrolyzing extracellular nucleotides[7]. The expression and distribution, as well as their roles in hydrolyzing cochlear ATP and ADP, of the three cell surface-located members of the ecto-nucleoside triphosphate diphosphohydrolase family (NTPDase1, 2, 3) have been previously reported [8-11]. There is also evidence for ecto-5'-nucleotidase (CD73) activity involved in the hydrolysis of AMP to adenosine following perfusion through the cochlear fluid compartments[12]. The present project aimed to address the mRNA expression and provides the immunocytochemical localisation of the membrane-bound E-NTPDase enzymes NTPDase8 and CD73 in the cochlea, and to extend the characterisation of tissue-specific P2 receptor signalling.

MATERIAL AND METHODS

Animal and cochleae preparation

The experiments were carried out on adult male Wistar rats weighing around 250 g. All procedures were approved by the University of Auckland Animal Ethics Committee. The animals were deeply anesthetized and then perfused with 0.9% NaCl and 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB: Na_2HPO_4 77.5 mM, NaH_2PO_4 22.5 mM, pH 7.4). The cochleae were isolated and trimmed under a dissecting microscope. They were then used directly for mRNA extraction or post-fixed overnight in 4% PFA at 4°C.

Cloning and sequencing

NTPDase8 and CD73 cDNA were amplified from rat cochleae. Primer pairs, specific for each cDNA, were designed based on the reported sequence of NTPDase8 (GeneBank® accession # NM_001033565) and CD73 (GeneBank® accession # NM_021576). Primer sequences are shown: (NTPDase8F: GCCTTTGGTTGGATCACTGT / NTPDase8R: CAATCCTCTTGGCCC TTACA) and (CD73F: GACCAGCAACTCAATGAGGCA / CD73R: CATTGGCAGGAAGAC AGGAG). Rat cochleae were removed and mRNA extracted using the Dynabeads mRNA DIRECT Kit® (Dynal Biotech Ltd, Oslo, Norway). The mRNA extract was reversely transcribed to cDNA (5µ L mRNA, 1µ L depc water, 1µ L random hexamers, 10µ L 2X 1st strand reaction mix, 2µ L Superscript III / RNAse OUT enzyme mix, Invitrogen) using standard reverse transcription protocols. The first cDNA strand was then used for PCR amplification.

PCR was performed using standard procedures. Platinum® DNA polymerase High Fidelity was purchased from Invitrogen with 3'-5' proofreading property to minimize the chance of getting errors. The PCR was run at 40 cycles. The PCR product was then purified using the Roche High Pure® PCR Product Purification Kit with supplied protocol.

The purified PCR product was cloned to pCR®2.1 plasmid using Invitrogen TA® cloning kits with TA overhangs for improved cloning efficiency. Clones were screened and selected based on white/blue selection on XGal and Ampicilin-treated agar plates. Plasmids DNA from positive clones were purified using the Qiagen Plasmid Midiprep Kit®. The plasmids were subjected to restriction analysis with EcoRI to confirm the size and orientation of the insert. Purified DNA plasmids were sent to the sequencing facility at the School of Biological Science, the University of Auckland, to confirm the identity of the insert.

Western blotting

Cochlear and liver proteins were dissolved in both non-reducing Laemmli's sample buffer (1% SDS, 20% glycerol, 0.1% bromophenol blue, 125mM Tris at pH 6.8) and reducing buffer (45µ L 0.1M DTT, 7.5µ L mecaptoethanol in 97.5µ L non-reducing buffer). The treated protein mixtures were then separated by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane (Roche Diagnostics, Auckland, New Zealand). Blocking (5% skim milk, 2% normal goat serum in TBS-T - 20mM Tris-base, 137mM NaCl, 0.1% Tween20) was followed by probing using the primary antibodies (with concentration of 1:500 titre for polyclonal guinea pig anti-rat NTPDase8 antibodies and pre-immune serum control; 5µ L/g for monoclonal mouse anti-rat CD73 antibodies and mouseIgG control). The NTPDase8-blotted and CD73-blotted membranes were incubated for one hour with rabbit-peroxidase anti-guinea pig IgG (1:2000 dilutions) and goat-peroxidase anti-mouse IgG (1:3000 dilution), respectively, before the bandings were visualised by chemiluminescence (ECL Western Blotting Analysis System, Amersham Bioscience).

Confocal Immunofluorescence

Polyclonal NTPDase8 and monoclonal CD73 (0.5mg/mL, BD Pharmingen) primary antibodies were raised in guinea pigs and mice respectively. Pre-immune serum controls (for NTPDase8) and Mouse IgG controls (for CD73) were used to test the specificity of these antibodies. Specificity was re-confirmed using Western blotting against proteins extracted from liver tissue, where the expression of NTPDase8 and CD73 had been confirmed previously.

The rat cochleae fixed in 4% PFA were decalcified in 5% EDTA / PB solution (pH 7.4) for seven days then cryoprotected in 30% sucrose / PB solution overnight at 4°C. They were then rinsed in 0.1 M PBS and snap-frozen in ice-cold N-Pentane. Sectioning was carried out at 30µ m in a cryostat and the sections were placed in a 24-well plate. Antibody concentrations were: NTPDase-8 (1:500 and 1:1000) and CD73 (1µ g/mL and 5µ g/mL). Each well was then rinsed with 0.5 mL sterile PBS 0.1 M (Na_2HPO_4 77.5 mM, NaH_2PO_4 22.5mM, NaCl 154mM, pH 7.4), and permeabilised with 1% Triton-X for one hour. Non-specific binding sites were blocked using 1.5% normal goat serum (NGS). Primary antibodies were incubated overnight at 4°C.

Secondary antibodies for NTPDase-8 (Alexa 594 goat anti-guinea pig IgG conjugate) and CD73 (Alexa 488 goat anti-mouse IgG conjugate) were applied for two hours in the dark at room temperature. The tissues were then rinsed and mounted on microscope slides using anti-fading reagent (CITIFLUOR). They were screened for NTPDase-8- and CD73-specific immunofluorescence using a confocal microscope (Leica Leisertechnik, Heidelberg, Germany)

RESULTS

Expression of NTPDase8 and CD73 in the cochleae

The agarose gel electrophoresis of PCR products (figure 2A, 2B) revealed the expression of both CD73 and NTPDase8 enzymes in the cochleae. Cloning and sequencing of the purified plasmids was carried out in the Sequencing Facility (School of Biological Sciences, University of Auckland), using the AB Applied Biosystem 96 sequencing machine. Sequence analysis confirmed the exact identities of the submitted DNA sequences. In western blotting, NTPDase-8 antibodies bound to the 70-75 kDa protein (figure 2D) in both rat cochlear and rat liver tissues, under both reducing (R) and non-reducing (NR) conditions, suggests that NTPDase-8 exists in monomer form. The size is consistent with the predicted size[3]. On the other hand, although CD73 western blot result showed weak bands in rat cochlear extracted protein lanes (figure 2C) - possibly due to tissue size, expression and protein degradation - CD73 antibodies did bind to 60-65 kDa bands in the liver lanes and in both reducing and non-reducing conditions. This is consistent with the predicted size[14,15]. The observed smearing bands in figure 2C are explained by protein fragmentation due to the freeze-thaw treatment of proteins prior to western blotting. No negative controls (pre-immune serum control and mouseIgG control) showed banding, which indicates no unspecific binding of primary antibodies. Also, recall that rat liver expresses both NTPDase-8 and CD73[3,15]; binding of primary antibodies to the expected cochlear and liver proteins confirmed the specificity of these primary antibodies for NTPDase-8 and CD73.

NTPDase8 Immunolocalisation

The cochlear spiral ligament, the interdental cells of the spiral limbus and the Claudius cells exhibited prominent NTPDase8 immunofluorescence (figure 3E-F) consistently in all three cochlear turns - basal, middle and apical (data not shown). The strong NTPDase8-specific immunofluorescence in the spiral ligament showed branching, characteristic of cells in the root region (figure 3E). No immunofluorescence was observed in the organ of Corti, spiral ganglion or blood vessels (figure 3F).

CD73 Immunolocalisation

CD73-specific immunofluorescence was prominent in the basal and intermediate cell layer of the stria vascularis (figure 3H). CD73 was also localised in capillaries of the stria vascularis, spiral ligaments, spiral limbus and spiral ganglion (figure 3G). Immunofluorescence was consistent in all

Figure 1

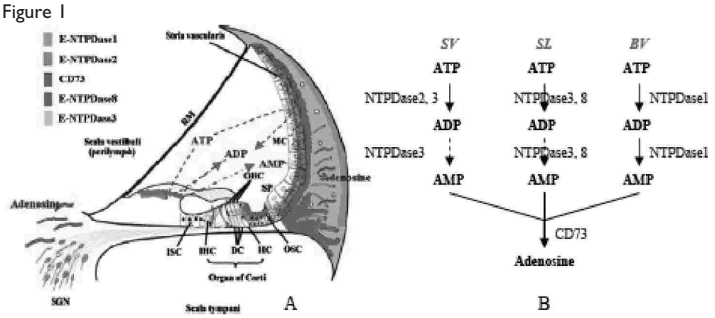

Figure 2

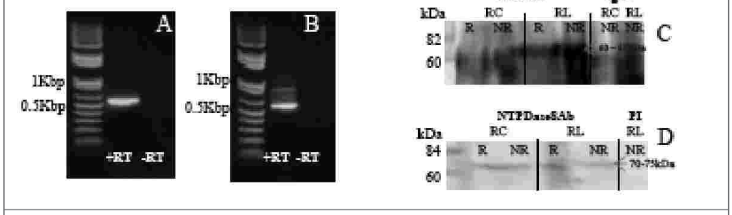

Figure 3

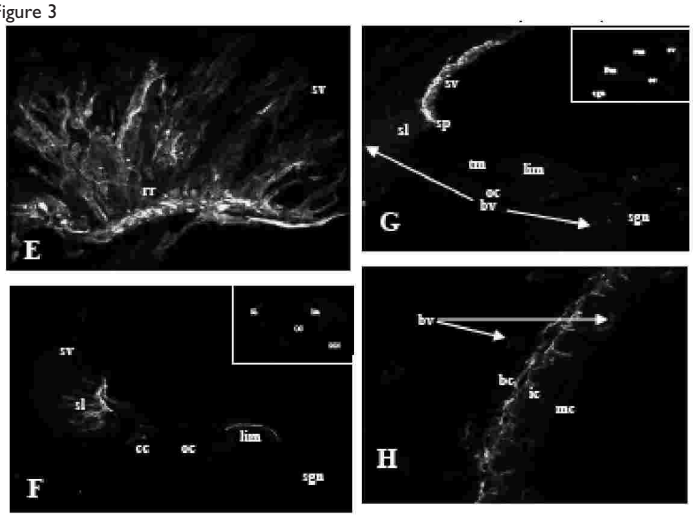

Figure 1: (A) Summary of the differential distribution of E-NTPDase8 and CD73 in conjunction with the previously reported E-NTPDase1 and -2 and -3 in the rat cochleae. NTPDase8 and CD73 show distinct tissue-specific localisation in the cochleae. Only spiral ligament localisation of NTPDase3 is shown in this figure. NTPDase2, -3 and CD73 show partly overlapping localisation in the stria vascularis (expression of NTPDase3 in this region is not shown in this figure), while NTPDase1 and CD73 overlap only in the vasculature. NTPDase3, -8 shows overlapping localisation in the root region of the spiral ligament. However, NTPDase8 does not express elsewhere in the spiral ligament. A possible pathway for ATP regulation is also depicted. Modified with permission from Vlajkovic SM (2002)[8]. **(B)** The three hypothetical ATP hydrolysis cascades in the spiral ligament (SL), blood vessels (BV) and the stria vascularis (SV): solid arrows.

Figure 2: Expression of NTPDase8 and CD73 in rat cochlear tissues.
RT-PCR: CD73 **(A)** and NTPDase8 **(B)** cDNAs generated by RT-PCR from rat cochlear mRNA (in SYBR safe agarose gel). The molecular size of CD73 PCR products were 539bp and of NTPDase8 PCR products were 522bp. Western blot characterisation of anti-peptide antisera used for immunohistochemistry for CD73 **(C)** and NTPDase8 **(D)** proteins. See results section for further explanation.

Abbreviations: RC: rat cochleae; RL: rat liver; R: reducing condition; NR: non-reducing condition; PI: pre-immune serum; IgG: MouseIgG control

Figure 3: Confocal Immunofluorescence: (E-F) NTPDase8; **(G-H)** CD73. Insets F and G show no primary antibody control with the same confocal microscope setting. Good signal-background differences indicate specific binding of antibodies both primary and secondary. **(E)** 100X magnification of the fluorescent signal in the root of the spiral ligament shows branching, characteristic of the root cells. **(F)** 1:500 titer of anti-NTPDase8 primary antibodies: strong signal in the root region of spiral ligament, Claudisu cells and the spiral limbus was observed. **(G)** 5μ g/mL of anti-CD73 primary antibodies; **(H)** 100X magnification on stria vascularis shows the fluorescent signal in the basal and intermediate layer of this region.

three turns (data not shown). CD73-specific immunofluorescence was not observed in the organ of Corti, the spiral limbus or the spiral ligament (figure 3G).

DISCUSSION

In the central and peripheral nervous system ATP acts as a neurotransmitter and as a neuromodulator[1]. It has been reported to be released during cochlear injury such as noise trauma[2], when it acts on P2 receptors, particularly the ATP-gated ion channels which may trigger cytotoxic cascades[4]. Inactivation of ATP in the cochleae involves a variety of enzymes that dephosphorylate ATP to adenosine and the subsequent reuptake of adenosine. These are important steps in the regulation of ATP signalling. The hydrolysis of ATP to adenosine also serves to salvage purines within the cochlear compartments[12]. Our study addresses the distribution, expression and possible functions of the two ectonucleotidases that may be involved in regulation of cochlear sensitivity by hydrolysing extracellular ATP and its metabolic products.

NTPDase8 expression in the scala media: putative role in electrochemical homeostasis

NTPDase8 shows specific expression in the tissues bordering the scala media: strongly in the root cells of spiral ligament, in Claudius cells and in the interdental cells of the spiral limbus (figure 3F). These regions are involved in K^+ cycling and may have a role in pathophysiology of noise-induced hearing loss[6]. This role is accomplished by regulating the activation of several P2X and P2Y receptor subunits expressed in these regions. As NTPDase8 hydrolyses ATP and ADP, with a stoichiometry of (ATP:ADP) of 3:1[1], this region-specific localisation of NTPDase8 suggests roles for NTPDase8 in both the spatial and temporal regulation of ATP and ADP levels, and in the differential P2 receptor activation in these compartments. Another suggested role for NTPDase8 is actually in common with NTPDase3 which hydrolyses ATP to AMP in the stria vascularis and spiral ligament. The expressions and co-localisation of NTPDase8 and NTPDase3 in the spiral ligament of rat cochleae[11] suggest that tight regulation of $P2X^2$ subunit activation is likely to be involved in K^+ cycling. Each of these enzymes has different nucleotide-hydrolysis profiles. This again indicates complex regulation of the endocochlear potential which is the driving force for sensory transduction. Especially the sole expression of NTPDase8 in the root cells of the spiral ligament may imply an important, but yet fully-understood, role in this region (figure 3E).

CD73 expression in cochlear blood vessels and stria vascularis: putative role in adenosine production, cochlear blood flow, haemostasis and thrombogenesis

In contrast with the function of NTPDases, the ecto-5'nucleotidase (CD73) is evidenced to be involved in the final step of the inactivation and catabolism of ATP and the formation of adenosine by hydrolysing AMP to adenosine[12]. The expression of CD73 in the capillaries in various compartments (stria vascularis, spiral ligament, spiral limbus and spiral ganglion) suggests spatial regulation of adenosine production in cochlear tissues (figure 3G). Along with the presence of NTPDase1 which hydrolyses both ATP and ADP equally in blood vessels[1], vascular CD73 would complete the ATP-adenosine hydrolysis cascade in the cochlear vasculature (figure 1B). Moreover ATP can also modulate blood flow in the cochlea via G-protein-coupled P2Y receptors. Extracellularly released ATP and ADP

during inner ear injury modulates thrombogenesis at the site of vascular injury via G-protein-coupled $P2Y^{1}$ and $P2Y^{12}$ receptors on platelets[5,10]. This possible cascade for ATP hydrolysis via CD73 and NTPDase1 in the blood stream may be crucial for maintaining purine-regulated cochlear blood flow, vascular haemostasis and thrombogenesis. This additionally implicates vascular CD73 as another possible target for the therapy of various blood-flow related disorders in the inner ear such as sudden deafness and Meniére's disease. On the other hand, because extracellularly formed adenosine may also modulate neurotransmission by inhibiting the release of other neurotransmitters such as glutamate[12], vascular CD73 may also involve indirect adenosine-mediated regulation of sensory transduction and serve to salvage purines. Finally, recall that NTPDase2 hydrolyses ATP 30-fold preferentially with respect to ADP, and NTPDase3 hydrolyses ATP two-fold more rapidly than ADP[1]. The co-localisation of these enzymes[8,11] in the basal and intermediate cells of the stria vascularis, which is important in providing the cochlear potential, would imply another cascade for ATP hydrolysis via NTPDase2, NTPDase3 and CD73 in this compartment (figure 1B). This also reveals the role of CD73 in cochlear electrochemical homeostasis. Nevertheless, due to limitations of this study further investigation is needed as discussed below.

Possible ATP-regulation pathways

This study revealed two possible ATP-hydrolysis cascades: via NTPDase1 and CD73 in the blood stream, and via NTPDase2,-3 and CD73 in the stria vascularis (figure 1B). On the larger scale, assuming that ATP is not completely region-specific, inactivation of ATP would start with NTPDase2, -3 and 8 in the tissues bordering the scala media, when the ATP level is high. While ATP is a limiting factor for NTPDase2[1], transiently accumulated ADP would then be hydrolysed by NTPDase3 and NTPDase8. The transient accumulation of ADP delays the formation of adenosine by inhibiting CD73[1]. This would help complete the hydrolysis of ATP all the way down the cascade to AMP before AMP is hydrolysed by CD73. This is consistent with previous publications showing that rapid hydrolysis of ATP to ADP is followed by slow AMP accumulation due to feed-forward inhibition of CD73[1,13]. However, the rate of ADP hydrolysis is also facilitated solely by the presence of NTPDase1 in the blood vessels, suggesting that ADP hydrolysis is tightly regulated in this cellular compartment. In general, the difference in expression and hydrolysis profiles of all of these enzymes suggests spatial, and importantly, temporal regulation of the rate of ATP hydrolysis: rapid hydrolysis of ATP to ADP by NTPDase2, accumulation of ADP then followed by rapid ADP hydrolysis by NTPDase1, -3, and -8, and production of adenosine by CD73 (ecto-5'-nucleotidase). The overall hypothetical pathway is fully illustrated in figure 1A and B.

LIMITATIONS AND FUTURE STUDIES

This study did not employ any quantitative methods to explore the capacity for gene expression. The study also did not involve any in-vivo analysis to study the temporal activity of such enzymes. Immunohistochemical data have not been collected due to time constraints and may need to be in any future studies to re-confirm the confocal immunofluorescent data. Within the allowed timeframe, findings from this study can still serve as a reference point for future studies and perhaps as important implications for inner ear therapy. In order to provide more information on the temporal effects of these enzymes as well as their other possible roles, future studies would need to involve further quantitative investigation such as real-time PCR or noise-induced experiments and in vivo controlled ATP concentration experiments.

CONCLUSION

In conclusion, this study demonstrated the expressions and distributions of NTPDase8 and CD73 in rat cochlea, and implicated roles for NTPDase8 and CD73 in purinergic regulation of cochlear electrochemical homeostasis, sensory transduction, blood flow, vascular haemostasis and thrombogenesis. This study also implies the spatial and temporal organisation of extracellular ATP hydrolysis, which directly influences cochlear electrochemical homeostasis.

ACKNOWLEDGEMENT

Thanks to the Deafness Foundation for the funding and to Dr Vlajkovic for providing supervision and the facilities required for the project.

Abbreviations used in the article

oc: Organ of Corti; sv: Stria vascularis; sgn: Spiral Ganglion; sl: Spiral ligament; lim: Spiral Limbus; cc: Claudius cells; rr: root region; bv: Blood vessel; mc: marginal cells; ic: intermediate cells; bc: basal cells; tm: tectorial membrane; rm: Reissner membrane; is: inner sulcus; isc: inner sulcus cells; os: outer sulcus; osc: outer sulcus cells; sp: spiral prominence; osl: osseous spiral lamina; ihc: inner hair cells; hc: Hensen's cells; dc: Deiter's cells; ohc: outer hair cells;

REFERENCES

1. Bigonnesse F, Levesque S A, Kukulski F, Lecka J, Robson SC, Fernandes JG and Sevigny J.
Cloning and characterisation of mouse nucleoside triphosphate diphosphohydrolase-8.
Biochemistry 2004; 43: 5511-5519

2. Burnstock G.
Pathophysiology and therapeutic potential of Purinergic Signalling.
Pharmacology Review 2006; 55: 58 - 86

3. Fausther M, et al.
Cloning, purification and identification of liver canalicular ecto- ATPase as NTPDase8.
Am J Physiol Gastrointest Liver Physiol, Mar 2007; 292: G785 - G795.

4. Housley GD, Jagger DJ, Greenwood D, Raybould NP, Salih SG, Jarlebark LE, Vlajkovic SM, Kanjhan R, Nikolic P.
Purinergic regulation of sound transduction and auditory neurotransmission.
Audiology Neurootology 2002. 7: 55-61

5. Munoz DJB, McFie C, Thorne PR.
Modulation of cochlear blood flow by extracellular purines.
Hearing Research 1999; 172: 55-61

6. Raphael Y, Altschuler RA.
Structure and innervation of the cochleae.
Brain Research Bulletin 2003; 60: 397 - 422

7. Robson SC, Sevigny J, Zimmermann H.
The E-NTPDase family of ectonucleotidases: Structure function relationship and pathophysiological significance.
Purinergic signalling 2006; 2: 409 - 430

8. Vlajkovic SM, Housely GD, Munoz DJB, Robson SC, Sevigny J, Wang CJH, Thorne PR.
Noise exposure induces upregulation of ecto-nucleoside triphosphate diphosphohydrolases 1 and 2 in rat cochleae.
Neuroscience 2004; 126: 763 - 773

9. Vlajkovic SM, Thorne PR, Sevigny J, Robson SC, Housley GD.
Distribution of ectonucleoside triphosphohydrolases 1 and 2 in rat cochleae.
Hearing Research 2002; 170: 127 - 138

10. Vlajkovic SM, Thorne PR, Sevigny J, Robson SC, Housley GD.
NTPDase1 and NTPDase2 immunolocalisation in mouse cochleae: Implication for regulation of P2 receptor signalling.
The Journal of Histochemistry and Cytochemistry 2002; 50 (11): 1435 - 1441

11. Vlajkovic SM, Vinayagamoorthy A, Thorne PR, Robson SC, Wang CJH, Housley GD.
Noise-induced up-regulation of NTPDase3 expression in the rat cochleae: Implication for auditory transmission and cochlear protection.
Brain Research 2006. 1104: 55- 63