Imaging ATP Release in the Cochlea: The Development of a Novel Cellular Biosensor

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ABSTRACT

The aim of this study was to develop a novel biosensor that can be used to detect ATP release from cochlear tissues. Chinese hamster ovary (CHO) cells were transformed by cotransfection with linearised pcDNA3.1 plasmids containing an enhanced Green fluorescent protein (eGFP) reporter gene (pcDNA3.1–eGFP) and the endogenous $P2X_2$ subunit which assembles to form ATP-gated ion channels (pcDNA3.1–$P2X_{2-1}$). Expression of the eGFP was detected using confocal microscopy and expression of the P2X receptors was detected using a whole cell patch clamp.

Transfected cells showed a 11 per cent response rate (n = 46) following exposure to 100μM ATP under whole cell patch clamp, which produced inward ATP-gated currents that ranged from –31 to –1350 pA (mean current response = –934.03 ± 416.38pA) with a mean reversal potential ($V_{z\ ATP}$) of –21 ± 7mV. This corresponded closely with the proportion of cells which showed GFP fluorescence.

Confocal imaging of intracellular calcium levels ($[Ca^{2+}]$) was also considered as a means of detecting ATP activation of the $P2X_2$ receptors. CHO cells were loaded with a $[Ca^{2+}]_i$ indicator dye (Fluo-4AM) and then exposed to 40μM ATP. In both untransfected and $P2X_{2-1}$ transfected CHO cells, this revealed a large, rapid increase in $[Ca^{2+}]_i$ (mean peak F/F_0 = 6.0 and 4.2 respectively, where F = fluorescence at peak and F_0 = control fluorescence prior to ATP application). This is attributable to P2Y receptor coupling of intracellular calcium release from the endoplasmic reticulum as previously reported.

In summary, this study has shown that CHO cells can be used as a biosensor to detect extracellular ATP, either by transfection of P2X receptors for sensitive detection of ATP-gated membrane currents, or by imaging of calcium release using confocal microscopy.

KEYWORDS

Cochlea, CHO cells, P2X receptor, ATP, Noise-induced hearing loss

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Brian Grainger is currently in the third year of a Bachelor of Science degree in Biomedical Science and hoping to enter medicine as a graduate in 2006. This research was conducted in the Molecular Physiology laboratory, part of the Auckland Auditory Neuroscience Group, as a summer studentship over the summer of 2004/2005.

INTRODUCTION

Noise Induced Hearing Loss (NIHL) is a significant cause of disability and is estimated to affect approximately 120 million people worldwide[1] including 390,600 New Zealanders[2]. The effects of NIHL are known to compound with age, resulting in sensory deprivation, loss of communication skills, social isolation and loss of self-esteem[3,4,5] all of which have a compounding detrimental effect on the patient's overall quality of life.

Protection of the cochlea from damage caused by exposure to excessive noise levels is believed to involve a two tiered system. The first level of protection is provided by the olivocochlear bundle (OCB), the efferent neural fibres of the cochlea which reduce the signal transduction activity of the hair cells through cholinergic-synapse-induced hyperpolarisation[6,7]. However, this mechanism becomes less effective at sound levels beyond about 80dB and consequently, sound levels approaching 90dB result in temporary threshold shift (TTS) and those in excess of 110dB result in permanent threshold shift (PTS)[8].

The second level of protection involves ATP release and signalling to specific ATP-gated ion channels, assembled from two broad molecular classes of receptor subunit; P2X and P2Y[9]. ATP levels in the cochlea are known to increase in response to noise exposure[10] following release from vesicular stores in the stria vascularis[11], and the supporting cells (Hensen's cells) of the organ of Corti[12]. ATP molecules subsequently bind to and activate the P2X and P2Y receptors leading to a reduction in the endocochlear ionic potential which provides the driving force for signal transduction at the sensory hair cells[9] and thus a consequential preservation of hearing function.

This study aims to develop a cellular biosensor to detect the release of ATP within the cochlea by using genetic engineering technology to express the gene for the $P2X_2$ receptor in a cultured mammalian cell line and then positioning these cells at various sites within the cochlea and using the magnitude of $P2X_2$ receptor response to ATP as a measure of endogenous ATP release within the cochlea. The potential of knowledge of this homeostatic process to provide opportunities for the clinical treatment of NIHL has been enhanced by the discovery that administration of ATP to hearing impaired guinea pigs resulted in detectable improvements in cochlea function[13].

METHODS

CHO Cell Transfection

Chinese hamster ovary (CHO) cells (Gibco) were maintained in 200mL Dulbecco's modified eagle medium (D-MEM) (Invitrogen) supplemented with 1 per cent non-essential amino acids (NEAA) (Invitrogen), 1 per cent streptomycin and penicillin (S/P) (Invitrogen) and 10 per cent fetal bovine serum (FBS) (Invitrogen). Prior to transfection, 2×10^5 cells were seeded into 24-well plates and made up to a total volume of 1mL using modified Eagle's minimal essential medium (Opti-MEM) (Invitrogen) and then incubated at 37°C in 5 per cent CO_2 for 24 hours.

Cell transfection was carried out with cotransfection of pcDNA3.1–$P2X_{2-1}$ (Invitrogen; kindly supplied by Mrs. Denise Greenwood) and pcDNA3.1–eGFP (Invitrogen; kindly supplied by Mrs. Carol Wang), with the identity of the $P2X_{2-1}$ insert validated by restriction endonuclease digest (see Fig. 1A). This combined mixture was then aliquoted into the wells of a 24-well cell plate seeded with 2×10^5 CHO cells (see Fig. 1B) in 500μL Opti-MEM media to obtain a final concentration of 800ng DNA in each well. The cells were subsequently incubated at 37°C in 5 per cent CO_2 for 24 hours. Transformation of CHO cells with the gene constructs was confirmed by fluorescence microscopy, based on detection of expression of the eGFP reporter.

Electrophysiology

Whole cell patch clamp recordings of successfully transformed CHO cells were made as previously described for cochlear hair cells[14] 24, 48 and 72 hours after transfection. Cells were placed in a 200μL recording bath mounted on an inverted microscope (Nikon, Japan). The bath contained a standard external solution (composition in mM: NaCl 150, KCl 4, Na_2HPO_4 8, NaH_2PO_4 2, $CaCl_2$ 1.5, $MgCl_2$ 1.0, D-glucose 1.0, pH 7.25 with 1M NaOH, osmolarity = 320mOsmol). Bath solutions were exchanged through rapid bath superfusion (300-500μL/min^{-1}) via a peristaltic pump (Gilson, France). Recordings were made using glass electrodes (3–5MΩ; filamented borosilicate glass, Harvard Apparatus GC120TF-10, U.K.) were used containing an high K^+ solution (composition in mM KCl 150, $MgCl_2$ 2, NaH_2PO_4 1, Na_2HPO_4 8, EGTA 0.5, $CaCl_2$ 0.001 pH 7.25 with 1M KOH, osmolarity = 310mOsmol). The recording electrode was linked to a patch clamp amplifier (Axopatch 200, Axon Instruments, USA) connected to a computer running Clampex 8.0 (Axon Instruments) via an Tecmar TL1 interface (Labmaster Scientific Solutions, USA).

Cell selection was based on morphology, with emphasis on those with small to medium diameter and a smooth surface (selection based on GFP expression was not feasible as the microscope was not equipped with fluorescence imaging). The cell membrane inside the pipette was voltage clamped at a holding potential of –60mV (V_h) and then ruptured by gentle suction and a brief hyperpolarising pulse to –150mV to obtain whole-cell recordings. A voltage ramp (–150 to +100mV; V_h = –60mV; 1s) was used to determine the zero current potential (V_z) and slope conductances between –90mV and –60mV (G_{-75}) and –10mV to +10mV (G_0). A 20mV hyperpolarising pulse was applied for post hoc determination of cell membrane series resistance (R_s) and capacitance (C_m) and the membrane time constant (_). Rapid bath superfusion (300-400μL.min^{-1}) of 100μM ATP (Sigma) provided inward currents in CHO cells voltage clamped at –60mV. ATP was typically applied for a duration of 60 seconds. Chronic purinergic receptor desensitisation was assessed by repeated applications separated by wash of at least 180 seconds duration. Analysis of data was carried out using Clampfit 8.0 (Axon Instruments) as previously described[14].

Calcium Imaging

CHO cells were plated onto 5cm culture dishes which had 2cm holes drilled in their bases affixed with microscope slide coverslips treated with 1:10 Poly D-Lysine (Sigma): PBS to promote cell adhesion. Prior to imaging, cells were loaded with 4μM Fluo-4 AM (Molecular Probes, USA) diluted from a 1mM stock containing 50μg Fluo-4 AM 5 per cent (w/v) pluronic F-127 in 50μL dimethyl disulfoxide and incubated at 37°C in 5 per cent $CO_{2(g)}$ for 60 minutes before washing in PBS and reincubation for a further 30 minutes.

Imaging was carried out using an inverted confocal microscope (Zeiss LSM410 Axiovert 100TV) with bath superfusion (275-280μL.min^{-1}) via a peristaltic pump (Gilson). The Fluo-4 indicator was excited by an argon ion laser at 488nm (Uniphase, USA) and light emitted was collected by a 40x water immersion objective (NA 1.2; Zeiss, Germany) and detected at 535 ± 25nm. 400μM ATP was applied to the untransfected (control) cells and 40μM ATP was applied to both the transfected and the pcDNA3.1–$P2X_{2-1}$ and pcDNA3.1–eGFP cotransfectants for durations ranging from 32 seconds to 113 seconds. Each application of ATP was separated by washes of at least 90s duration. Analysis of data was carried out using interactive data language (IDL).

RESULTS

CHO Cell Transfection

Transfection efficiency was approximately 20-30 per cent. Specifically, using FACS at 24 hour intervals post transfection revealed the following percentages of each cell population were exhibiting green fluorescence levels above that of untransfected CHO cells; pcDNA3.1–eGFP transfectants; 34.18 per cent at 24 hours, 22.18 per cent at 48 hours and 11.98 per cent at 72 hours, pcDNA3.1–$P2X_{2-1}$ and pcDNA3.1–eGFP cotransfectants; 17.41% at 24h, 17.82% at 48h and 8.67% at 72h (See Fig. 1C). Most of the data for $P2X_{2-1}$ expression were obtained using transient transfection. In addition, CHO cells which had been selected for stable transfection using G418 were also studied. Selection for singly transfected pcDNA3.1–$P2X_{2-1}$ cells was solely achieved through G418 resistance as there was no GFP selectable marker to indicate transformation of the cell genome. Transient transfection of pcDNA3.1–$P2X_{2-1}$ with pcDNA3.1–GFP and later pcDNA3.1–$P2X_{2-1}$ with pcDNA3.1–eGFP was confirmed using FACS.

Electrophysiology

Recordings were made from a total of 46 CHO cells which, in the absence of ATP in the bath, exhibited a mean zero current potential (V_z) of –62 ± 5.4mV, with a mean electrode series resistance (R_s) of 15.8 ± 1.4MΩ and a mean membrane capacitance (C_m) of 8.7 ± 1.1pF (Fig. 1D).

Rapid bath superfusion of 100μM ATP produced inward current responses in 5/46 cells (11 per cent) with an average current response of –934 ± 416pA. Of these current responses, two were obtained in pcDNA3.1–$P2X_{2-1}$ singly transfected cells; one (I_{ATP} = –517pA) was a stable transfectant which had undergone a total of 17 days G418 exposure, the other (I_{ATP} = –1350pA) was a transient transfectant which was patch clamped 24 hours after transfection. Analysis of ATP responses with repeated applications separated by 120 seconds,

demonstrated desensitisation which was unexpected given reports of sustained ATP current responses in other $P2X_2$ expression studies[15,16]. Analysis of the current-voltage relationship (Fig. 1E) showed that the CHO cells had no intrinsic voltage-dependent conductances whereas the ATP-activated current showed inward rectification. The mean membrane slope conductance at –75mV (G_{-75}) of 1.6 ± 0.3pS was comparable to the mean conductance at 0mV(G_0) of 1.4 ± 0.4pS. Hence there was no voltage dependency. In fact, this conductance converts to an input resistance of 714 GΩ at 0mV. In contrast, the ATP-gated current was inwardly rectifying (Fig. 1E), with a slope conductance at –60mV of approximately 8nS.

The remaining three current responses were recorded from cells cotransfected with pcDNA3.1–$P2X_{2-1}$ and pcDNA3.1–eGFP and patch clamped 48 hours after transfection. The initial current responses obtained from both these cells were more slowly desensitising and thus more characteristic of the typical $P2X_2$ response[15,16]. The first cell which responded (I_{ATP} = –302pA) exhibited chronic desensitisation of the inward current amplitude with successive ATP applications separated by 180 second washes (I_{ATP} = –41, –40, –32pA). The effect of ATP-gated currents on the whole cell current potential was also recorded (mean $V_{z\ ATP}$ = –21 ± 7.0mV). The second cell (I_{ATP} = –307pA) also exhibited chronic desensitisation of the inward current amplitude with successive ATP applications (I_{ATP} = –127, –65, –49, –53pA). The effect of ATP-gated currents on the whole cell current potential was also recorded (mean $V_{z\ ATP}$ = –38 ± 17.3mV). The third cell (I_{ATP} = –708pA) also exhibited chronic desensitisation of the inward current amplitude with successive ATP applications (I_{ATP} = –208 pA).

The effect of ATP-gated currents on the whole cell current potential was also recorded (mean $V_{z\ ATP}$ = –64± 32.5mV). The overall chronic desensitisation of the inward current amplitude is shown in Fig. 1F.

Calcium Imaging

In pilot experiments, 400μM ATP induced an elevation in intracellular calcium ($[Ca^{2+}]_i$) in untransfected CHO cells after 32 seconds. CHO cells express endogenous $P2X_7$ receptors[18,17] with an ATP EC_{50} of 1252.3μM and a detection threshold of 94.6μM[17], so this result could possibly be attributed to the ATP activation of these channels and subsequent calcium influx from the external solution. ATP was then applied at 40μM in an attempt to exclude the $P2X_7$-induced response (as $P2X_7$ receptors are insensitive to ATP at this concentration). 40μM ATP produced an F/F_0 of 6.0 (Fig. 1G), which should be compared with an F/F_0 of 4.2 observed in the pcDNA3.1–$P2X_{2-1}$ and pcDNA3.1–eGFP. As 40μM ATP is below activation for threshold for $P2X_7$ receptors, this calcium response to ATP is likely to arise from endogenous P2Y receptors which have an EC_{50} of 2.3 ± 0.5 am[19].

DISCUSSION

This study showed that CHO cells could be developed as a biosensor to detect extracellular ATP. The eGFP reporter gene provides the opportunity for fluorescence-based selection of transformants, while expression of the $P2X_{2-1}$ receptor provides the facility to detect ATP release from tissues based on the activation of inward currents that can be recorded using the sensitive whole cell patch clamp technique. The existence of endogenously expressed P2Y receptors that are strongly coupled to intracellular calcium stores indicates the potential to use calcium imaging as another means of detecting ATP release in tissues, which would involve distributed imaging of a number of CHO cells scattered over the target tissue.

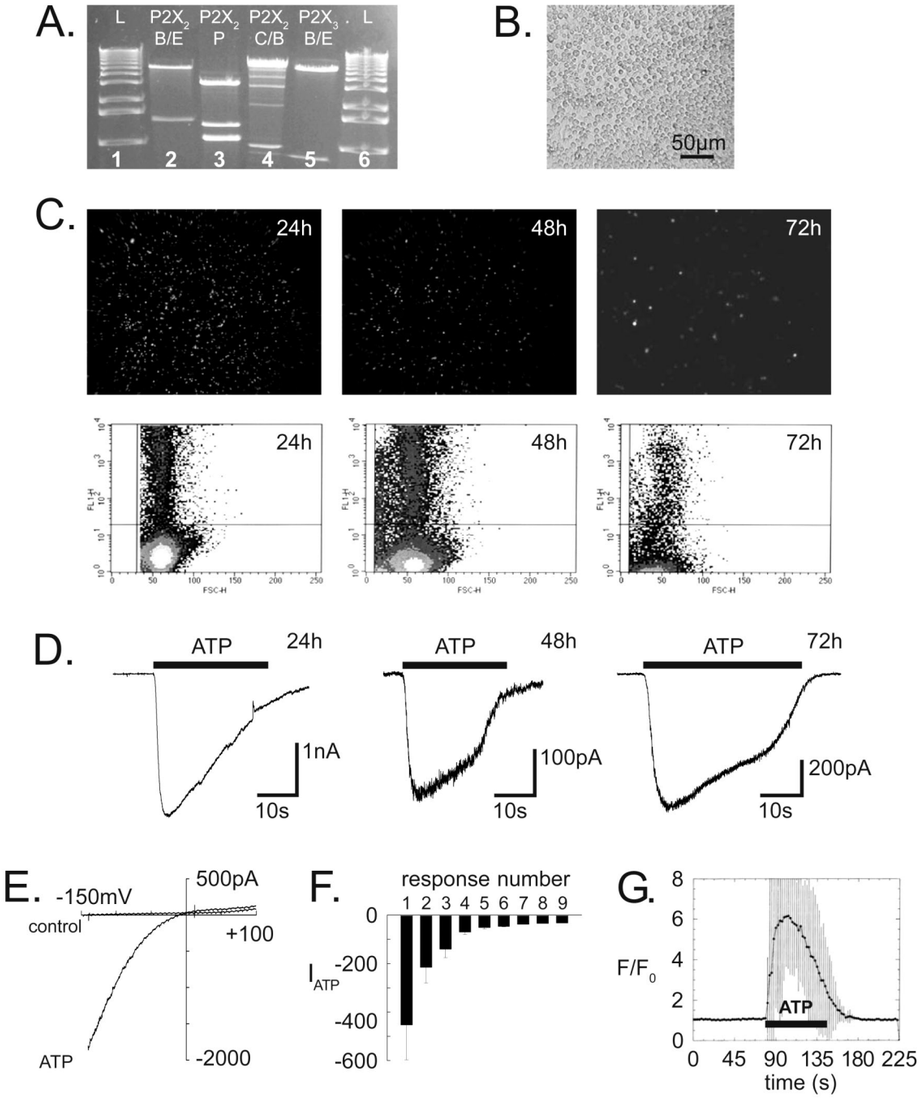
The apparent low expression rate for the both transfected $P2X_{2-1}$ protein and eGFP reporter gene as evidenced by only 11 per cent of cells displaying current responses to 100mM ATP and FACS measurements of eGFP expression showing only 20-30 per cent efficiency may be attributed to the cotransfection paradigm. Although studies exist which describe efficient CHO cell cotransfection using lipofectamine[20], studies involving GFP cotransfection of G(s) alpha cells[21] describe low transfection efficiencies (3.6±1.5 per cent) comparable to that observed here. In order to develop an efficient biosensor, the cells would need to be selected for stable transfection. Given that earlier attempts with a pcDNA3.1/NT-GFP-TOPO-$P2X_{2-1}$ chimera were unsuccessful (data not shown), future experiments could use FACS to enrich the transfected cells for G418 selection. This would then enable patch clamp recording from clonal selection to isolate cells stably transfected with both $P2X_{2-1}$ and eGFP.

Whole cell patch clamp recordings from a P2X receptor would allow the positioning of individual CHO cell biosensors adjacent to target cells within the cochlear tissue, including suspected sites of ATP release bordering the scala media. Luciferin-Luciferase assays from extracted guinea pig cochlear fluids indicate increases in extracellular ATP levels ranging from ten to several hundred nM[10] when the cochlea is exposed to stress such as hypoxia and noise stress. Given that heterologous expression of $P2X_2$ receptors generates ATP-gated currents with an EC_{50} of 1-60μM[15,22], the sensitivity of the $P2X_2$ biosensor may be insufficient to detect endogenous ATP. However, a splice variant of the $P2X_{2-3}$ receptor would provide the required detection sensitivity. Thus, CHO cells expressing P2X receptors with high nM to low μM sensitivity offer a high resolution biosensor for detection of ATP release sites in the cochlea, which would allow investigation of the mechanism of ATP release.

While there was a good correlation between transfection efficiency of the eGFP determined by FACS, and the success of whole cell patch clamp recording from the $P2X_2$ receptors, the experimental yield of only five cells out of 46 transfectants displaying ATP-gated current responses was low. Additional factors mitigating this may include the time required to translate the $P2X_{2-1}$ gene sequence, then transport and correctly assemble the resulting protein in the cell membrane. This is suggested by the ATP-induced current recordings obtained at 48-60 hours post-transfection displaying slower acute desensitisation kinetics more characteristic of the typical slowly desensitising $P2X_2$ current responses [15,24]. Although studies of P2X receptor expression in HEK 293 cells have shown a >90 per cent response rate to ATP in recordings made 12-48 hours after transfection[23]. No studies of $P2X_2$ receptor expression in CHO cells [17, 24] have reported any details on the time required after transfection to obtain functional channel expression. The successfully transfected CHO cells exhibited ATP-activated membrane conductances around 8nS. Given the reported unitary conductance of $P2X_2$ receptors in CHO cells of 22pS[16] this indicates that approximately 230 trimeric $P2X_2$ channels were correctly assembled in the cell membrane. The ability of immunocytochemistry to elucidate protein expression independent of functionality or membrane localisation provides scope for further experiments to evaluate the feasibility of a $P2X_2$-based CHO cell biosensor for ATP release in the cochlea.

Previous studies have reported the ATP EC_{50} conferred by the endogenous P2Y receptors to be in the low μM range[19]. It is reasonable to suggest that a suitable biosensor for cochlear ATP release may be developed simply from imaging the ATP induced increase in $[Ca^{2+}]_i$ mediated by these endogenous receptors expressed in CHO cells placed beneath a cochlear slice preparation from which ATP release has been induced. However, such a setup would not allow individual CHO cells to be positioned immediately adjacent to sites of ATP release and consequently the biosensor may not achieve the level of sensitivity required to detect localised fluctuations in endolymphatic ATP. There is

Figure 1



A. Multiple restriction enzyme digests oflinearised pcDNA3.1 containing either the $P2X_{2-1}$ fragment used forCHO cell transfection (Lanes 2.4) or a $P2X_3$ fragment which was used as a control (Lane 5). Restriction enzymes are as follows: B/E = *Bam*HI and *Eco*RI; P = P*vu*I1; C/B = *Cla*I and B*am*HI.

B. Bright field image of CHO cells.

C. (top row) Fluorescent images of eGFP expression in cotransfected CHO cells at indicated times of analysis following transfection; (bottom row) percentage of cotransfected CHO cells expressing eGFP. FACS analysis of eGFP expression shown as the number of cells whos fluorescence exceeded the null flouresecence levels of untransfected CHO cells (indicated by the horizontal line).

D. ATP (l00μM) induced an inward current in CHO cells expressing $P2X_{2-1}$ receptors that changed in profile over time. from a rapidly (24 h) to a more slowly desensitising current (48 and 72h).

E. The current voltage relationship of the ATP-gated current response exhibited inward rectification and reversed around OmV.

F. Repeated applications of ATP (l00μM) revealed run down in the amplitude of theATP-gated current.

G. Fluorescent Ca^{2+} imaging of ATP(40μM) induced rises in intracellular Ca^{2+} levels from ATP-gated currents and activation of endogenous P2Y receptors.

also the additional problem of run-down of intracellular calcium stores associated with P2Y receptor – PLC – IP_3 receptor-gated signalling. Thus while having potential, the feasibility of calcium imaging of CHO cells during prolonged and repeated ATP exposure would need to be investigated further. However as the pharmacological threshold and EC_{50} for P2Y receptor activation is comparable to the most sensitive of the P2X receptors, this component of the study has developed an interesting new element of the proposal to develop the CHO cell as a biosensor.

REFERENCES

1. World Health Organisation 2001
Fact Sheet 258: *Occupational and Community Noise*
URL: http://www.who.int/inf-fs/en/fact258.html

2. Greville KA.
Population numbers and characteristics
Greville Consulting 2001
URL: http://www.grevilleconsulting.co.nz/nz_hearing_impaired_population.htm

3. Rosenhall U.
The influence of ageing on noise-induced hearing loss
Noise Health 2003;5(20): 47-53

4. Campo P, Pouyatos B, Lataye R, Morel, G.
Is the aged rat ear more susceptible to noise or styrene damage than the young ear?
Noise Health 2003;5(19):1-18

5. Gates GA, Schmid P, Kujawa SG, Nam B, and D'Agostino R.
Longitudinal threshold changes in older men with audiometric notches
Hear Res 2000;141(1-2): 220-8

6. Maison SF, Liberman MC.
Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength
J Neurosci 2000;20(12): 4701-7

7. Desmedt JE, Lagrutta V.
Function of the uncrossed efferent olivo-cochlear fibres in the cat
Nature 1963;200: 472-473.

8. Zheng XY, Henderson D, McFadden SL, Hu BH.
The role of the cochlear efferent system in acquired resistance to noise-induced hearing loss
Hear Res 1997;104(1-2):191-203

9. Housley GD Jagger DJ, Greenwood D, Raybould NP, Salih SG, Jarlebark LE *et al.*
Purinergic Regulation of Sound Transduction and Auditory Neurotransmission
Audiol NeuroOtol 2002;7:55-71

10. Muñoz DJ, 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): 10-5

11. 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

12. Gale JE, Piazza V, Ciubotaru CD, Mammano F.
A mechanism for sensing noise damage in the inner ear
Curr Biol 2004;14(6): 526-9

13. Sugahara K, Shimogori H, Okuda T, Takemoto T, Hashimoto M, Yamashita H.
Cochlear Administration of Adenosine Triphosphate Facilitates Recovery from Acoustic Trauma (Temporary Threshold Shift)
ORL 2004;66:80-84

14. Raybould NP, Jagger DJ, Housley GD.
Positional Analysis of Guinea Pig Inner Hair Cell Membrane Conductances: Implications for Regulation of the Membrane Filter
JARO 2001;02: 362-376

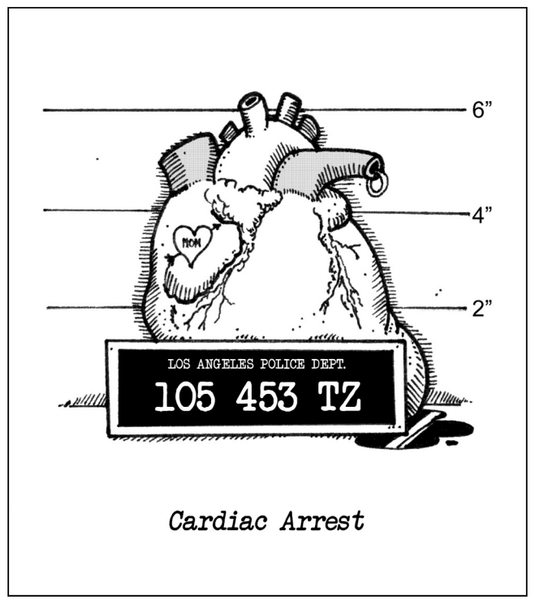
15. Brake AJ, Wagenbach MJ, Julius D.
New Structural Motif for ligand gated ion channels defined by an ionotropic ATP receptor
Nature 1994;371: 519-523

16. Evans RJ.
Single channel properties of ATP-gated cation channels (P2X receptors) heterologously expressed in Chinese hamster ovary cells
Neurosci Lett 1996;19;212(3):212-4

17. Michel AD, Chessell IP, Hibell AD, Simon J, Humphrey PP.
Identification and characterization of an endogenous P2X7 (P2Z) receptor in CHO-K1 cells
Br J Pharmacol 1998;125: 1194-1201

18. Lundy PM, Nelson P, Mi L, Frew R, Minaker S, Vair C *et al.*
Pharmacological differentiation of the P2X7 receptor and the maitotoxin-activated cationic channel
Eu J Pharmacol 2004;487: 17-28

19. Iredale PA, Hill SJ.
Increases in intracellular calcium via activation of an endogenous P2 purinoceptor in cultured CHO-K1 cells
Br J Pharmacol 1993;110: 1305-1310



Cardiac Arrest

FEATURE : OPINION

A Fijian Experience

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The author has a strong interest in Pacific Island and indigenous health as his mother was born in Tonga. He has also been involved in student politics and education for last 3 years and is the NZMSA president for 2006. He is involved in rural health with NZMSA and Matagouri Rural Club and he was the OUMSA president in 2003/2004.

Sigatoka Hospital

At the end of fourth year in 2004, I decided to spend my summer holidays working in hospital in Fiji to gain some more clinical experience. I have always had a strong interest in Pacific Island health as my mother was born in Tonga and moved over to Auckland when she was young. I decided to work in Fiji because our family had a timeshare in Sigatoka and there was nearby hospital.

There are two main public hospitals in Fiji, one in Suva and one in Lautoka. I worked in Sigatoka District Hospital, about 1hr drive from Nadi along the beautiful Coral Coast. The Sigatoka valley is known as the "salad bowl" of Fiji because of the many crops, fruit and vegetables that are grown. These products would be on sale at the local markets along with freshly caught fish and shellfish and imported spices and curry mixtures from India. Food is an important part of life in Fiji and for a Dunedin student this was paradise!

Sigatoka District Hospital has about 60 beds, with 4 doctors, nurses and midwives. There are two separate men's and women's wards, obstetric unit, emergency surgery theatre, emergency department (ED) and an outpatient's clinic. The beauty of this set up was that I could do a bit of everything. I mainly stayed in the emergency department, where I would be with another doctor or often just with a nurse! It was a bit daunting at first to be responsible for admitting patients and their management. All I can say is that I learnt very quickly and became very good friends with the staff who I learnt much from. In Fiji, GPs are private so people either come to the ED or go to the outpatients, this meant we dealt with many people and problems that varied from trivial to life threatening.

When most people think about Fiji, they think about the resorts and lazy cocktails on the beach. After working there it soon became apparent that the reality was far different, as 25.5% of Fijian's live below the poverty line. Many Fijians live in villages in concrete or corrugated houses. Power is usually by generator, with outside showers and toilets. There are often many people living in one house. These factors impact hugely with the control of infection and disease. We would often see a whole family or part of a village for vomiting and diarrhoea. This would be related to a common water or food source. Scabies and skin abscesses were very common because of the poor hygiene, humid conditions and the shortage of water. The skin abscesses would develop as folliculitis or small abscesses and because of the reluctance to see a doctor would develop into carbuncles (large abscesses) with surrounding cellulitis. For severe cases some needed surgery. These late presentations were common with indigenous Fijians. They would normally see the doctor after they have tried herbal or local remedies. Other examples of late presentations included diabetic patients with 2 year old foot sores, dislocated shoulders that had been out for 2 months, pregnant women that had not been to any antenatal appointments and turning up the day before delivering. Fijian Indians on the other hand came more readily to the hospital. It was sometimes difficult to judge their level of pain and how sick they were. The lack of quick lab tests and reliable imaging made decisions on transferring patients to the main hospital in Lautoka very difficult.

We would often see a whole family or part of a village for vomiting and diarrhoea.

The ED was always busy, with no triage system and only 4 beds. It was hard to work out who you had and hadn't seen. We would often be rehydrating 10 patients for gastroenteritis and then mixed in between, have some very serious cases. One particular occasion in the space of 20 minutes while working with a nurse, we were stabilizing a patient for transfer with heavy vaginal bleeding, managing a patient with BP of 230/150 and then a pick up truck pulls up with a man who fell out of a large tree while pruning it! Another busy occasion when the ED was overflowing, I noticed an Indian girl, about 13 who I hadn't seen. I asked her to get up from her chair and onto the bed. She was markedly