Localisation of P2X6 receptor protein expression in the adult rat cochlea

D. Greenwood, L.K. Yang, P.R. Thorne and G.D. Housley, Department of Physiology, University of Auckland, Private Bag 92 019, Auckland 1009, New Zealand.

The P2X6 receptor (P2X6R) is one of seven P2X receptors forming a homologous family of mammalian channel proteins (ATP-gated ion channels). P2X6R cDNA was first isolated from a superior cervical ganglion cDNA library (Collo *et al.*, 1996). mRNA expression of this subtype is highly distributed throughout the brain. There is extensive overlap in localisation of P2X6R mRNA with the other subunits, especially P2X4R and P2X2R, indicating potential for forming heteromeric P2X receptors (Collo *et al.*, 1996). Co expression of P2X6R with P2X2R (King *et al.*, 2000), or P2X4R (Le *et al.*, 1998) can produce modified channel phenotypes whereby the original P2X2R or P2X4R properties are altered, supporting the likely occurrence of P2X6R as a heteromer and extending the functional diversity of these receptors. In the mammalian cochlea extracellular ATP acting at P2X receptors has a major role in auditory function, including sound transduction and auditory neurotransmission. All seven P2XR subunit mRNAs are represented in the cochlea (Greenwood *et al.*, 1999). Previously, P2X6R expression has been localised to the spiral ganglion (Brandle *et al.*, 1999; Xiang *et al.*, 1999). In this study we used immunoperoxidase histochemistry and confocal immunofluoresence to investigate the sites of P2X6R protein expression in adult cochlea. Given the propensity for P2X6R to co localise with P2X2R (Collo *et al.*, 1996) and a recent report demonstrating an up-regulation of P2X2R protein expression in auditory hair cells and spiral ganglion neurones in response to noise (Wang *et al.*, 2003) we investigated whether P2X6R expression was also modified by noise exposure.

Adult Wistar rats were subjected to either ambient noise (control) or white noise at 110dB for 72 hours. Paraformaldehyde fixed cochleae were isolated. Floating 50 mm sections were labelled using a rabbit anti-rat antibody raised against an intracellular C-terminus peptide of the P2X6R coding sequence (Roche, Palo Alto). P2X6R immunoreactivity was detected using a Vectastain Elite ABC kit (Vector Laboratories) and visualised after incubation with the chromogenic substrate 3, 3-diaminobenzidine tetrahydrochloride (Vector Laboratories). A Cy3-conjugated anti-rabbit IgG goat antibody (Chemicon) was utilised as the secondary antibody in the immunofluorescence study. Specificity of the antibody was confirmed by (i) omission of the primary antibody and (ii) with a peptide block of the P2X6R epitope. Intensity of fluorescent P2X6R labelling was analysed using Image Pro Plus software (Adobe).

In this study, the spiral ganglion was confirmed as the principal site of P2X6R expression. Other notable structures showing P2X6R expression included supporting cells of the organ of Corti, and the stria vascularis. P2X6R protein expression was unchanged by noise exposure unlike P2X2R, a potential co-subunit. This finding suggests that P2X6R expression may contribute to heteromultimeric assembly with other P2XR in the cochlea. Possible phenotype modulation conferred by P2X6R may be diminished by noise-induced up-regulation of co-assembled P2XR.

Brandle, U., Zenner, H.P. & Ruppersberg, J.P. (1999) *Neuroscience Letters,* 273: 105-108.

Collo, G., North, R.A., Kawashima, E., Merlopich, E., Neidhart, A., Surprenant, A. & Buell, G. (1996) *Journal of Neuroscience,* 16: 2495-2507.

Greenwood D., Burrows M., Kanjhan R., Raybould N.P., Salih S.G. & Housley G.D. (1999) *Proceedings of the Physiological Society of NZ,* 18: 55p.

King, B.F., Townsend-Nicholson, A., Wildman, S.S, Thomas, T., Spyer, K.M. & Burnstock, G. (2000) *Journal of Neuroscience,* 20: 4871-4877.

Le, K.T., Babinski, K. & Seguela, P. (1998) *Journal of Neuroscience,* 18: 7152-7159.

Wang J.C.C., Raybould N.P., Lin L., Ryan A.F., Cannell M.B., Thorne P.R. and Housley G.D. (2003) *NeuroRepor,t 14(6:) 817-23.*

Xiang, Z., Bo, X. & Burnstock, G. (1999) Hearing Research, 128 190-196.

Studies approved by University of Auckland Animal Ethics Committee. Funded by: Health Research Council, Marsden Fund, Maurice and Phyllis Paykel Trust. I. Oglesby at Roche Palo Alto is thanked for supplying the P2X6 antisera