Expression of P2X7 receptors in primary hippocampal glia: a possible role in cell proliferation and activation

M. Monif,[1] C. Reid,[2] K. Powell[3] and D.A. Williams,[1] [1]Department of Physiology, The University of Melbourne, Vic, Australia, [2]Howard Florey Institute, Melbourne, Vic, Australia and [3]Department of Medicine, The University of Melbourne, Vic, Australia.

The function of extracellular ATP as a neurotransmitter, and as a signaling molecule released during trauma and inflammation has been well documented. ATP acting via purinergic receptors, in particular P2X7 receptor (P2X7R), expressed on glial cells has been shown to have many trophic effects, including cell proliferation, enhanced process formation and alterations in cellular phenotype. The aim of this study was to assess the cellular distribution pattern of P2X7R in primary hippocampal glia in an attempt to understand their role in glial activation and morphological alterations. Standard recombinant DNA technologies were used to generate the DNA coding for a fusion construct, P2X7R-GFP. The fusion construct was transfected into primary hippocampal glia using a modified calcium phosphate transfection technique. The sub-cellular distribution pattern of the chimeric protein was investigated using fluorescence confocal microscopy and immunohistochemistry. Confocal imaging of primary hippocampal cultures expressing P2X7R-GFP indicated that the chimeric protein was exclusively expressed in a subset of hippocampal glia, having the characteristic morphology of activated microglia (n=70), and not astrocytes (confirmed by GFAP staining; n=20). Compared to glial cells expressing the control construct, GFP (n=10), glial cells expressing P2X7R-GFP showed highly fluorescent nodular structures on the plasma membrane (n=30), with presence of fine filamentous fibers projecting from the cell (n=30). Over-expression of P2X7R in primary hippocampal glia that have the characteristic phenotype of microglia, is associated with morphological changes and formation of lamellipodia. These responses are known to be important events in microglial activation and locomotion during neuroinflammatory processes such as Alzheimer’s disease.