Role of Phosphoinositides in the Biology of the Amyloid Precursor Protein

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ETHICS STATEMENT

The research associated with this thesis abides by the international and Australian codes on animal experimentation, and the rulings of the Animal Ethics Committee of the University of Tasmania

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Abstract

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. In countries with aging populations, such as Australia, the prevalence of AD is projected to increase substantially. AD is characterised by two distinctive pathological lesions in the brain, amyloid plaques and neurofibrillary tangles. The major component of amyloid plaques is an aggregating protein termed the beta-amyloid protein (Aβ). Aβ is formed normally from a larger precursor protein, known as the beta-amyloid precursor protein (APP). Although APP is centrally involved in the pathogenesis of Alzheimer's disease and the production of Aβ, relatively little is known about its normal function. Deciphering the function of APP in the brain may be essential for the development of effective AD therapeutics.

APP is a type I transmembrane glycoprotein that can be proteolytically processed by α, β- and γ-secretases to produce a number of secreted ectodomain fragments termed sAPPβ, sAPPα, Aβ and p3. Many studies have suggested that sAPPα may act in the maintenance and development of the central nervous system, by acting as a paracrine factor. In vitro, sAPPα has been reported to modulate the proliferation and differentiation of a variety of cell types. However, the mechanistic basis for these effects is unclear. In part, this uncertainty has arisen because the cell-surface receptor molecules that interact with sAPPα are not known.

Previous studies have reported that sAPPα may interact with a novel lipid-raft type membrane domain in the cell. Furthermore, sAPPα has been reported to bind to the lipid GM1-ganglioside. On the basis of these reports, the work in this thesis explored the hypothesis that an interaction of APP with cell surface lipids could facilitate binding and/or signalling by sAPPα.

To determine if sAPPα is able to interact with a sub-group of lipids. The relative ability of sAPPα to bind to 27 physiological lipids was examined using a protein-lipid overlay assay. This assay identified that sAPPα could bind selectively to phosphoinositide lipids (PIPs). Further, a recombinant fragment of APP corresponding to the E1 N-terminal domain (APP-E1) also bound selectively to PIPs, suggesting there is a PIP-binding region within the E1 domain of APP.

To investigate whether APP and PIP could interact on the cell surface, it was first necessary to demonstrate that PIPs are present on the cell surface. A live cell immunolabelling method was used to examine the location of cell surface PIPs. Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) immunoreactivity was found to be present on the surface of cells in primary murine hippocampal cultures in discrete puncta <1 μm in size. This observation was also confirmed using a recombinant $PI(4,5)P_2$ biosensor protein.

To examine whether APP could interact with cell-surface PIP, studies were performed to examine the degree of colocalisation of exogenous APP-E1 and cell-surface $PI(4,5)P_2$. APP-E1 that was added to primary hippocampal cultures bound to the surface of neurons in discrete puncta <1 μm in size. The cell-bound APP-E1 and the cell-surface $PI(4,5)P_2$ were highly co-localised on the surface of neurons. However, cell-surface $PI(4,5)P_2$ was also present on glial cells in culture where APP-E1 did not bind. Furthermore the binding of APP-E1 to cells could not be inhibited using a water soluble analogue of $PI(4,5)P_2$. Therefore, these data suggested that APP-E1 interacts with cell-surface $PI(4,5)P_2$, but the interaction was not sufficient to explain why APP-E1 binds to the cell surface.

As the APP E1 domain contains a heparin-binding site, the role of this region was investigated in the binding of APP-E1 to PIP and also the binding of APP-E1 to cells. Heparin did not block the binding of APP-E1 to PIP in vitro, suggesting the heparin-binding region and the PIP-binding region in the APP E1 domain are

distinct. However, heparin did inhibit the binding of APP-E1 to cells, suggesting that the heparin-binding region of APP is required for binding to cells. Furthermore, heparitinase treatment of cells significantly reduced cell surface heparan sulfate immunoreactivity, but did not affect the binding of APP-E1 to cells. These results suggest that APP may interact with PIP on the cell surface along with another cell surface component that binds to the heparin-binding site, which is not heparan sulfate.

As PIPs are involved in many aspects of cellular physiology, it was hypothesized that APP may signal through modulation of levels of PIPs. To address this hypothesis, levels of PIPs were measured in primary cortical cultures by two methods. Firstly, a mass-spectroscopy based method was developed to measure total levels of cellular PIP. No change in total PIP levels upon sAPPα treatment could be detected using this method. Secondly, levels of cell-surface PIPs were determined using an array of anti-PIP biosensors and antibodies. Under resting conditions, only $PI(4,5)P_2$ was present on the surface of cells. However, in the presence of APP-E1, there was an increase in the level of cell surface $PI(3,4,5)P_3$ and an increase in the level of $PI(4,5)P_2$, indicating that APP binding to cells may result in an increase level of cell surface PIPs.

The data presented in this thesis demonstrate that APP has a novel N-terminal PIP-binding domain. This domain may play a role in the normal function of APP, by facilitating PIP-dependent signalling.

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Publication and presentation record

Peer-reviewed publications:

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Small, D. H., Hu, Y., Bolos, M., **Dawkins, E**., Foa, L., and Young, K. M. (2013). Beta-amyloid precursor protein: Function in stem cell development and Alzheimer's disease brain. Neurodegener Dis (DOI: 10.1159/000353686)

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Dawkins, E. and Small, D.H. The N-terminal region of the amyloid precursor protein of Alzheimer's disease binds to PI(4,5)P_2-rich microdomains on the surface of primary neurons.

Dawkins, E. and Small, D.H. Functions of the β-amyloid precursor protein.

Conference presentations:

Dawkins, E., Gasperini, R., Vincent, A.J., Foa, L. and Small, D.H. Analysis of Aβ cell interactions and uptake in hippocampal culture. Oral Presentation; Australian Neuroscience Society Conference, Gold Coast, 2012

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Hu Y., Hung A.C., Cui H., **Dawkins E**., Foa L., Young K.M. and Small D.H. APP stimulates neural stem/progenitor cell proliferation by increasing cystatin C secretion. Poster; Australian Neuroscience Society Conference, Melbourne, 2013