The involvement of β-amyloid precursor protein in neuronal iron homeostasis in dementia

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Introduction

Iron is an essential element required as a cofactor in metabolic processes throughout the body and specifically in tissues of high oxygen consumption, such as the central nervous system. High levels of unbound iron are detrimental as this may catalyze the production of toxic reactive oxygen species. It is clear that increased cellular susceptibility to oxidative stress associated with iron accumulation leads to neurodegeneration. Age-related increases in neuronal iron, altered iron-related protein expression and increased susceptibility to oxidative stress have all been documented in neuropathological regions from patients with Alzheimer's disease (AD), Parkinson's disease and tauopathies.

One regulatory route in regulating cellular iron homeostasis is through proteins required to facilitate the efflux of iron from the cell. β-Amyloid precursor protein (APP), Ceruloplasmin and Hephaestin are all able to facilitate the movement of iron across the plasma membrane, partly through their ability to complex with the iron exporter ferroportin and promoting its retention on the cell surface.

APP is a type 1 transmembrane protein more commonly known as the precursor to the toxic ß-amyloid peptide that accumulates in the AD brain. However, regulation of APP expression by iron regulatory protein implies an interaction with iron status. Our group strengthened this iron relationship through the discovery of the requirement for APP in promoting the efflux of iron via ferroportin in cells such as neurons. Prior to our discovery no mechanism was known for neuronal iron export as within the brain a membrane-associated form of Ceruloplasmin is only expressed on astrocytes and Hephaestin is only expressed in oligodendrocytes.

Results

Biological evidence of a role for APP in iron efflux via ferroportin continues to be strengthened both by our own work and research from independent groups. While concerns have recently arisen as to some of the original findings on the ability of APP to oxidize Fe^{2+} to Fe^{3+}, we have revisited our original data to establish in more detail the causes of such variability in APP ferroxidase activity. During the course of these studies we have now established that the ability of APP to oxidize iron originate from phosphate. We suggest that the presence of this physiologically abundant anion raises the possibility that APP facilitates the efflux of intraneuronal iron through an alternative mechanism; potentially either using the high anion, or soluble Ceruloplasmin, content within the surrounding extraneuronal environment.

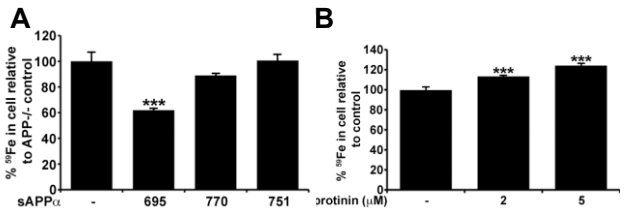

Figure 1: the KPI domain is responsible for larger APP isoforms differentially affect neuronal iron response. **(a)** APP-/- primary neuronal cultures preincubated with Fe^{59} before the administration of each isoform of sAPP illustrate that only $sAPP_{695}$ was able to reduce the intracellular accumulation of iron after 6hrs. **(b)** Retention of Fe^{59} within the cell is dose dependently increased upon the addition of the KPI homologous peptide Aprotinin. Data are means ± SEM, N=3, *=p<0.05, **=p<0.01, ***=p<0.001 compared to control by 2-tailed Ttest.

Despite the modification to the original hypothesis proposed on how APP was able to efflux neuronal iron, a significant understanding for the necessity of APP in neuronal iron homeostasis is now

provided. This is in part through a substantially greater understanding of the effect each of the 3 major APP isoforms (i.e. APP_{695}, APP_{751} and APP_{770}) has on neuronal iron regulation. Supporting our previous primary publication on the iron homeostatic role of APP and independent validation by other researchers, the increased presence of APP_{695} appears to lower the intracellular labile iron pool (Fig. 1A). However, intriguingly, a similar pattern does not appear to be evident in the presence of the APP_{751} and APP_{770} isoforms (Fig. 1A). The inhibitory role in cellular iron efflux of both APP_{751} and APP_{770} suggests the Kunitz Protease Inhibitor (KPI) domain present in both to be responsible and similar results with the KPI homologous peptide Aprotinin support this theory (Fig. 1B). Altering the proteolytic processing of neuronal APP_{695} has also been recently identified to modify neuronal iron. By either utilizing mutations within the β-secretase cleavage site of APP, or suppression of α- and β-secretase activity by chemical inhibition (Fig. 2), promotion of the amyloidogenic pathway has been identified to increase intracellular iron whereas the non-amyloidogenic processing of APP negates this response. These findings have implications for all AD associated pathogenic mutations of APP and their potential to compound upon neuronal vulnerability to increased iron levels and oxidative stress within the disease.

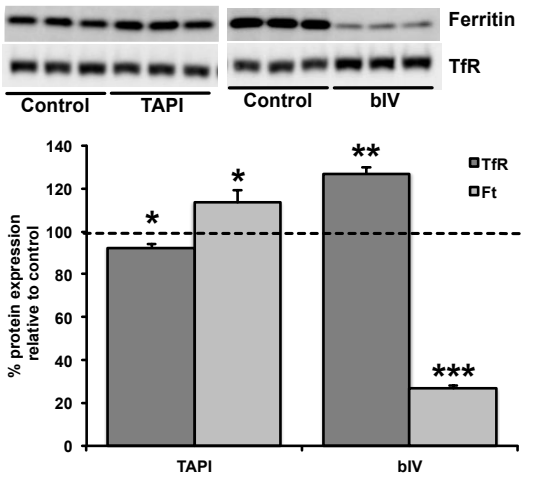

Figure 2: inhibition of α-secretase or β-secretase activity alters neuronal iron homeostasis. SH-SY5Y neuroblastomas incubated with the α-secretase inhibitor TAPI, previously reported (and confirmed by ourselves) to reduce α-cleavage of APP, have decreased TfR and increased ferritin expression representing an increased intracellular labile iron pool. Inversely, β-secretase inhibition by bIV dramatically increases TfR and reduces ferritin expression in SH-SY5Ys signifying an increased labile iron pool. Data are means ± SEM, N=3, *=p<0.05, **=p<0.01, ***=p<0.001 compared to control by 2-tailed Ttest.

Outlook.

By continuing to support a novel candidate function for APP we now begin to explain the diverse trophic and morphoregulatory activities of the protein and elucidate the vulnerability of the body to age-associated iron accumulation. Restoring or replacing the ability of APP to control neuronal iron homeostasis may be a novel mechanism of action for new therapeutics targeting a range of neurodegenerative diseases. Since investigating the therapeutic value of metal chelating compounds in a range of transgenic models of AD and PD pathology it has become gradually clearer that the therapeutic capability of these compounds may not only be through their ability to isolate small amounts of labile iron but also partially through their ability to use the brain's own iron regulatory system to rebalance iron tissue levels.

Publications

Chen, J., Marks, E., Lai, B., Zhang, Z., Duce, J., Lam, L., Volitakis, I., Bush, A., Hersch, S., & Fox, J. (2013) Iron accumulates in Huntington's disease neurons: protection by Deferoxamine. *PLoS One.* **8:** e77023.

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