The involvement of β-amyloid precursor protein proteolytic processing 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 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 promote its retention on the cell surface.

APP is a type 1 transmembrane protein more commonly known as the precursor to the toxic β-amyloid peptide that accumulates in the AD brain. However, regulation of APP expression by iron regulatory protein implies a relationship 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
During the course of recent studies we have modified our original hypothesis in how APP is involved in neuronal iron efflux. Using a newly developed multiplex assay that is able to more accurately measure iron oxidation under physiological conditions, we have not only now published revised enzymatic kinetics for ceruloplasmin (Fig. 1), but also identified that in a normal physiological environment, ferric iron incorporation into transferrin is sufficiently enabled by the biological polyanions that are prevalent within extracellular fluids. Using this assay we have now determined that APP ferroxidase activity originates 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 to that previously reported; potentially either using the high anion, or soluble Ceruloplasmin, content within the surrounding extraneuronal environment. Biological evidence of a role for APP in iron efflux via ferroportin continues to be strengthened by our research illustrating: (1) correlation between surface presented APP and FPN in neurons, (2) evidence of altered iron homeostasis caused by α- and β-secretase cleavage of APP from the cell surface (Fig. 2 A&B), (3) altered iron levels caused by familial mutations in APP around the β-secretase cleavage site (Fig. 2 C&D), and (4) our previously published parallel work on tau requirement to transport APP to the cell surface to facilitate iron efflux. Findings on how the proteolytic processing of APP can regulate iron homeostasis have obvious implications for iron accumulation in familial AD as well as potentially hazardous side effects of β-secretase inhibitors currently being used as a therapeutic candidate in AD clinical trials.

Outlook.
By continuing to support a novel candidate function for APP we now begin to explain the diverse trophic and morpho-regulatory activities of the protein and elucidate the vulnerability of the body to age-associated iron accumulation.

Current Aβ modulatory compounds such as β-secretase inhibitors attenuate Aβ production but also initiate a number of detrimental side effects, such as the iron dyshomeostasis suggested by our research, when administered at a dose that is too high. However, we propose that these drugs may still have therapeutic potential in AD if the administered dose is carefully monitored. Measuring iron in blood during β-secretase inhibitor administration may provide a more accurate biomarker for dose response in future clinical trials. Mediating the therapeutic dose of β-secretase inhibitor in response to plasma iron changes during administration could allow a better outcome for future clinical trials.

Publications


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