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Full Title

Amyloid precursor protein-mediated mitochondrial regulation and Alzheimer's disease

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Short Title

APP regulation of mitochondrial function

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Abstract

Despite clear evidence of a neuroprotective physiological role of amyloid precursor protein (APP) and its non-amyloidogenic processing products, APP has been investigated mainly in animal and cellular models of amyloid pathology in the context of Alzheimer's disease. The rare familial mutations in APP and presenilin-1/2, which sometimes drive increased amyloid beta (Aβ) production, may have unduly influenced Alzheimer's disease research. APP and its cleavage products play important roles in cellular and mitochondrial metabolism, but many studies focus solely on Aβ. Mitochondrial bioenergetic metabolism is essential for neuronal function, maintenance and survival, and multiple reports indicate mitochondrial abnormalities in patients with Alzheimer's disease. In this review, we focus on mitochondrial abnormalities reported in sporadic Alzheimer's disease patients, and the role of full-length APP and its non-amyloidogenic fragments, particularly soluble APP α (sAPPα), on mitochondrial bioenergetic metabolism. We do not review the plethora of animal and *in vitro* studies using mutant APP/presenilin constructs or experiments using exogenous Aβ. In doing so, we aim to invigorate research and discussion around non-amyloidogenic APP processing products and the mechanisms linking mitochondria and complex neurodegenerative disorders such as sporadic Alzheimer's disease.

Keywords

Mitochondria, Amyloid precursor protein, Neuroprotection, Amyloid beta, Metabolism, Bioenergetics, Soluble amyloid precursor protein alpha, Oxidative phosphorylation.

Abbreviations

ABAD, amyloid-binding alcohol dehydrogenase; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; Aβ, amyloid beta; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; CNS, central nervous system; CTF, carboxyl terminal fragment; Cu-ATSM, copper labelled diacetyl-bis N4-methylthiosemicarbazone; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; FAD, flavin adenine dinucleotide; $FADH_2$, reduced flavin adenine dinucleotide; Fis1, mitochondrial fission 1; HSD17B10, 17beta-hydroxysteroid dehydrogenase 10; MAM, mitochondria-associated membrane; MFN1/2, mitofusin 1 and 2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRPP2, mitochondrial RNase P protein 2; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PGC1- α, PPARG co-activator 1 alpha; PET, positron emission tomography; Pi3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; PS-1, presenilin-1; PS-2, presenilin-2; ROS, reactive oxygen species; sAPPα, soluble APP α; sAPPβ, soluble APP β; TIM23, translocase of the inner membrane 23; TOM40, translocase of the mitochondrial outer membrane 40.

Introduction

APP is a transmembrane protein that consists of an extracellular domain, a single transmembrane domain, and a short cytoplasmic fragment. APP is encoded by *APP* (chromosome 21q11.2-q21) (Goldgaber, Lerman, McBride, Saffiotti & Gajdusek, 1987), a member of a gene family that includes APP-like proteins 1 and 2 (APLP1 and APLP2) (Sprecher et al., 1993). Alternative splicing of *APP* generates eight isoforms designated according to their number of amino acids, of which three are the most common: APP_{695} is expressed predominantly in the central nervous system (CNS), while APP_{751} and APP_{770} are more ubiquitously expressed (Sandbrink, Masters & Beyreuther, 1996).

APP is the parent protein of a complex family of peptides. APP is processed from its membrane-bound holoform via two main pathways (Figure 1). The dominant non-amyloidogenic pathway involves cleavage of APP by α-secretase (predominantly ADAM10) (Kuhn et al., 2010), and produces a large N-terminal ectodomain, sAPPα, which is secreted into the extracellular medium, and a carboxyl-terminal fragment (CTF) called CTFα/CTF83 (Kojro & Fahrenholz, 2005). CTFα is endocytosed and undergoes further cleavage by the γ-

secretase complex, yielding a small peptide called p3 and APP intracellular domain (AICD) fragments (Kummer & Heneka, 2014). In contrast, amyloidogenic processing of APP involves cleavage by β-secretase (BACE1) and subsequent release of a shorter ectodomain, soluble APP β (sAPPβ), into the extracellular medium and a 99 amino acid fragment (CTFβ/CTF99) in the membrane (Vassar et al., 1999). Cleavage of CTFβ by the γ-secretase complex generates Aβ peptides (mainly $A\beta_{40}$ and $A\beta_{42}$) and AICD fragments (Kummer & Heneka, 2014). Aβ peptides are a major component of the cerebral plaques found in patients with Alzheimer's disease (Jarrett, Berger & Lansbury, 1993; Mann et al., 1996). Cleavage by α-secretase occurs at a position within the sequence of Aβ, and therefore precludes its formation. Further cleavage of AICD fragments by caspases or caspase-like proteases results in two additional fragments, Jcasp and C31 (Bertrand et al., 2001; Lu et al., 2000).

Together with the intraneuronal tangles of aggregated hyperphosphorylated tau protein, the extracellular accumulation of Aβ into amyloid plaques is a hallmark of Alzheimer's disease. In several pedigrees of early-onset familial Alzheimer's disease, point mutations in *APP* and *PSEN1*, the gene encoding the γ-secretase component presenilin 1, are linked to altered APP processing and abnormal accumulation of Aβ (Hunter & Brayne, 2018; Mullan & Crawford, 1993). Although Alzheimer's disease caused by Mendelian variants in these and other genes constitutes a tiny proportion of disease burden compared with sporadic cases, genes associated with familial Alzheimer's disease have influenced research disproportionately. Consequently, the majority of cellular and animal models in the field involve various combinations of mutant versions of these proteins. A large number of studies have also used synthetic Aβ to show a wide range of toxic effects *in vitro*, which include mitochondrial toxicity. As a result, research has focused largely on the amyloidogenic properties and toxicity of Aβ, and the physiological function of APP and its non-amyloidogenic fragments has received less attention. Extensive reviews focused on animal studies of APP mutants and the role of Aβ in mitochondria are available (Kaminsky, Tikhonova & Kosenko, 2015; Kawamata & Manfredi, 2017; Pagani & Eckert, 2011). Here we focus instead on mitochondrial studies in human Alzheimer's disease, and on potential research gaps looking at non-amyloidogenic APP processing and mitochondrial function.

Mitochondrial bioenergetic metabolism is essential for neuronal function, maintenance and survival, and mitochondrial dysfunction has become an established hallmark of neurodegeneration. Neurons rely on mitochondria for a variety of essential processes, including energy production, through the generation of ATP via the oxidative phosphorylation (OXPHOS) system, which is the main cellular source of reactive oxygen species (ROS) (Lopez Sanchez, Crowston, Mackey & Trounce, 2016). Beyond their bioenergetic role, mitochondria are involved in other important cellular functions, including intracellular calcium homeostasis, iron metabolism and apoptosis signalling amongst others. Disruption of mitochondrial function and dynamics has been associated with a variety of neurodegenerative processes, including those involved in the development of Alzheimer's disease (Lionaki, Markaki, Palikaras & Tavernarakis, 2015; Pei & Wallace, 2018; Zorzano & Claret, 2015), and APP and APP-derived peptides have been reported to affect mitochondrial function. In this review, we focus on the role of full-length APP and its non-amyloidogenic fragments, particularly sAPPα, on mitochondrial bioenergetic metabolism. In doing so, we aim to invigorate research and discussion around non-amyloidogenic APP processing products and the mechanisms linking mitochondria and complex neurodegenerative disorders such as Alzheimer's disease.

Mitochondrial bioenergetics in Alzheimer's disease

Early studies in Alzheimer's disease patients showed decreased brain glucose metabolism using fluorodeoxyglucose positron emission tomography (PET) (Benson, Kuhl, Hawkins, Phelps, Cummings & Tsai, 1983; Chase, Foster, Fedio, Brooks, Mansi & Di Chiro, 1984). This finding has been validated and is an established correlate of the disease, although the extent to which this is a cause or effect continues to be debated (reviewed by (Chen & Zhong, 2013)). One possible cause of decreased glucose utilisation is mitochondrial dysfunction, which has been associated with a variety of neurodegenerative disorders (Johri & Beal, 2012).

The OXPHOS system is responsible for most of the energy production in cells. It consists of five multimeric protein complexes located in the inner mitochondrial membrane (complex I-

IV and the ATP synthase or complex V), encoded by both nuclear and mitochondrial DNA (mtDNA). Briefly, glucose is oxidised to pyruvate in the cytosol through glycolysis. Pyruvate enters mitochondria where it is converted into acetyl coenzyme A, which is further oxidised within the tricarboxylic acid cycle to yield reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$). These two molecules serve as electron donors for OXPHOS complexes I and II. ATP is produced by the shuttling of electrons by carrier molecules along the respiratory complexes, which is accompanied by the pumping of protons from the mitochondrial matrix into the intermembrane space. This electrochemical gradient across the inner mitochondrial membrane is used by the ATP synthase to catalyse the conversion of ADP and inorganic phosphate to ATP (Smeitink, van den Heuvel & DiMauro, 2001).

Mitochondrial OXPHOS metabolism was first associated with Alzheimer's disease through the discovery that cytochrome *c* oxidase (complex IV) enzymatic activity is decreased in some patients with sporadic Alzheimer's disease (Kish et al., 1992; Mutisya, Bowling & Beal, 1994; Parker, Filley & Parks, 1990), which was linked to increased Aβ levels *in vitro* (Parks, Smith, Trimmer, Bennett & Parker, 2001), and contributed largely to the development of the Alzheimer's disease mitochondrial cascade hypothesis (Swerdlow & Khan, 2004). The specificity of complex IV decreases in Alzheimer's disease has been questioned, as cytochrome *c* oxidase activity is also reduced in patients affected by spinocerebellar ataxia type I and Friedreich's ataxia (Kish et al., 1999), and other neurodegenerative disorders (Arnold, 2012; Cavelier et al., 1995). However, the balance of evidence is that cytochrome *c* oxidase is decreased in brain tissue of patients with Alzheimer's disease.

The early demonstration of semi-quantitative histochemical staining for cytochrome *c* oxidase in post-mortem brain samples (Kageyama & Wong-Riley, 1982) led to several studies showing higher numbers of cytochrome *c* oxidase-negative neurons in various regions of Alzheimer's disease brain (Gonzalez-Lima, Valla & Matos-Collazo, 1997; Simonian & Hyman, 1993; Wong-Riley et al., 1997). The veracity of this defect has been reinforced by biochemical studies in peripheral cells (Parker, Filley & Parks, 1990) and brain (Maurer,

Zierz & Moller, 2000; Parker, Parks, Filley & Kleinschmidt-DeMasters, 1994). It has been hypothesised that mitochondrial dysfunction due to complex IV deficiency drives over-production of Aβ (Crouch, Cimdins, Duce, Bush & Trounce, 2007), as the presence of intra-mitochondrial Aβ has been demonstrated in Alzheimer's disease brain (Lustbader et al., 2004). An elegant study addressed this question by using neuron-specific complex IV-deficient mice carrying mutant APP and PS-1, which surprisingly resulted in lower amyloid plaque burden and Aβ levels (Fukui, Diaz, Garcia & Moraes, 2007). Therefore, this suggests that the cytochrome *c* oxidase deficiency in Alzheimer's disease does not drive amyloid production.

Some evidence for other OXPHOS defects has been reported in samples from Alzheimer's disease patients (Johri & Beal, 2012). Complex I and IV transcripts were found to be decreased in one study (Aksenov et al., 1999), while another found evidence for complex I subunit mRNAs to be specifically decreased in Alzheimer's disease brain (Manczak, Park, Jung & Reddy, 2004). Furthermore, complex I subunit protein levels were found to be decreased in different areas of Alzheimer's disease and Down syndrome brain (Kim, Vlkolinsky, Cairns, Fountoulakis & Lubec, 2001), but biochemical studies of enzymatic activity corroborating these findings are lacking in Alzheimer's disease. A meta-analysis of complex I and complex IV findings found that only complex IV was consistently reported to be decreased in Alzheimer's disease (Holper, Ben-Shachar & Mann, 2018). This analysis also showed that decreases of *both* complex I and IV were consistently found in studies of human brain ageing (Holper, Ben-Shachar & Mann, 2018). It is now well-established that ageing is associated with lower OXPHOS capacity in human muscle (Trounce, Byrne & Marzuki, 1989) and in human, non-human primate and rodent brain (Bowling, Mutisya, Walker, Price, Cork & Beal, 1993; Ferrandiz, Martinez, De Juan, Diez, Bustos & Miquel, 1994; Ojaimi, Masters, Opeskin, McKelvie & Byrne, 1999). Since ageing is the strongest risk factor for sporadic Alzheimer's disease, it is clear that an age-related decline in mitochondrial OXPHOS can be a key contributor to Alzheimer's disease pathogenesis, where additional, disease-specific mitochondrial perturbations exist (Crouch, Cimdins, Duce, Bush & Trounce, 2007).

Multiple reports indicate additional mitochondrial abnormalities in Alzheimer's disease, including increased mtDNA oxidative damage (reviewed by (Santos et al., 2013)), pathogenic mtDNA deletions (Corral-Debrinski et al., 1994; Hamblet & Castora, 1997), altered mtDNA methylation (Blanch, Mosquera, Ansoleaga, Ferrer & Barrachina, 2016) and mtDNA gene expression perturbations (Lunnon et al., 2017), which may result in increased oxidative stress. Indeed, imbalanced ROS, oxidative stress and damage are detected in Alzheimer's disease, including increased levels of oxidative markers in mtDNA (Mecocci, MacGarvey & Beal, 1994) and free radicals (Reddy, 2006). This supports the hypothesis that chronic oxidative stress and changes in mtDNA may play a role in neurodegenerative processes. However, the specificity of these mitochondrial changes in Alzheimer's disease is uncertain, given that mtDNA abnormalities and oxidative damage are common features of several neurodegenerative disorders (reviewed in (Cha, Kim & Mook-Jung, 2015)). Similarly, studies have shown decreased protein and mRNA levels of the key transcriptional regulator of mitochondrial biogenesis PPARG co-activator 1 alpha (PGC1-α) in Alzheimer's disease brain, suggesting that mitochondrial biogenesis may also be impaired (Qin et al., 2009; Sheng et al., 2012).

Perturbations in mitochondrial dynamics and in proteins responsible for mitochondrial fusion - optic atrophy 1 (OPA1), mitofusin 1 and 2 (Mfn1/Mfn2), and fission - dynamin-related protein 1 (Drp1, also referred to as DLP1) and mitochondrial fission 1 (Fis1), have also been implicated in Alzheimer's disease. One study showed co-localisation of Drp-1 and Aβ, increased levels of Drp1 and decreased levels of mitochondrial fusion proteins in brain samples (Manczak, Calkins & Reddy, 2011). However, another study reported decreased protein levels of Drp1, OPA1, Mfn1 and Mfn2, and significantly increased Fis1 levels in Alzheimer's disease brain tissue (Wang et al., 2009). Despite discrepancies on the changes in levels of Drp1, these studies suggest that enhanced mitochondrial network fission may be involved in neuronal dysfunction in Alzheimer's disease. This subject has been reviewed by others (Zhu, Perry, Smith & Wang, 2013). Further work is necessary to clarify whether mitochondrial fission/fusion abnormalities in the brain are causal or a product of other upstream perturbations in Alzheimer's disease.

APP and mitochondrial metabolism

The identification of a chimeric mitochondrial targeting sequence suggested that full-length APP could be targeted to mitochondria in neuronal models or transgenic mice over-expressing APP (Anandatheerthavarada, Biswas, Robin & Avadhani, 2003). It has been proposed that APP associates with two components of the mitochondrial protein translocation machinery, TOM40 and TIM23, and its accumulation prevents the import of nuclear-encoded mitochondrial proteins, including OXPHOS subunits, therefore impairing mitochondrial function (Anandatheerthavarada, Biswas, Robin & Avadhani, 2003; Devi, Prabhu, Galati, Avadhani & Anandatheerthavarada, 2006) (Figure 2).

Mitochondria form dynamic physical interactions with the endoplasmic reticulum (ER), known as mitochondria-associated membranes (MAMs). This association constitutes a key signalling hub involved in fundamental cellular processes, including mitochondrial tethering and dynamics, lipid biosynthesis and intracellular calcium signalling (Gomez-Suaga, Bravo-San Pedro, Gonzalez-Polo, Fuentes & Niso-Santano, 2018). There is strong evidence that MAMs are an intracellular site of APP processing (Del Prete et al., 2017; Schreiner, Hedskog, Wiehager & Ankarcrona, 2015). The physical and biochemical apposition of MAMs with mitochondria (Hayashi, Rizzuto, Hajnoczky & Su, 2009) supports the possibility that various APP-derived peptides are able to interact with mitochondria (Figure 2). This may also account for previous reports that indicated that processing of full-length APP occurs in mitochondria (Ankarcrona & Hultenby, 2002; Hansson et al., 2006; Pavlov et al., 2011), which has been revisited and questioned recently, given that mitochondria do not possess the enzymes necessary to produce Aβ peptides from APP (Mamada, Tanokashira, Ishii, Tamaoka & Araki, 2017). Instead, it is thought that Aβ is produced at mitochondria-ER contact sites (Schreiner, Hedskog, Wiehager & Ankarcrona, 2015). The presence and processing of APP in MAMs has led to the hypothesis that alterations in ER-mitochondria connectivity may result in perturbations in lipid homeostasis (Grimm, Rothhaar & Hartmann, 2012), which may contribute to Alzheimer's disease pathology (Area-Gomez et al., 2012; Del Prete et al., 2017).

In addition to full-length APP, APP-derived peptides have been detected in mitochondria, including Aβ (Crouch et al., 2005; Pagani & Eckert, 2011), AICD (Pavlov et al., 2011) and CTF99 (Devi & Ohno, 2012), or in MAMs (Pera et al., 2017), where they may alter mitochondrial function. The presence of AICD in the inner mitochondrial membrane has been linked to disruptions in mitochondrial distribution, morphology and bioenergetics (Ward, Concannon, Whyte, Walsh, Corley & Prehn, 2010), while an accumulation of unprocessed CTF99 has been associated with altered lipid composition of MAMs and mitochondrial membranes that interferes with the assembly of OXPHOS complexes and mitochondrial respiration (Pera et al., 2017) (Figure 2).

Amyloid-binding alcohol dehydrogenase (ABAD), known under a variety of names including 17β-hydroxysteroid dehydrogenase type 10 (HSD17B10) or mitochondrial RNase P protein 2 (MRPP2), has also been linked to mitochondrial perturbations in the context of amyloid pathology. It was shown that pathologic binding of ABAD to Aβ in mitochondria increases leakage of ROS and results in mitochondrial dysfunction (Lustbader et al., 2004). As a key component of the RNase P complex responsible for the 5' processing of mitochondrial tRNAs, ABAD has a direct role in mitochondrial gene expression and respiratory function (Sanchez et al., 2011) and is also involved in the modulation of mitochondrial function by estrogens (Sanchez, Shearwood, Chia, Davies, Rackham & Filipovska, 2015). Inhibition of the ABAD-Aβ interaction significantly reduced mitochondrial Aβ accumulation, leading to improvements in mitochondrial function and attenuation of mitochondrial ROS production (Yao et al., 2011). Surprisingly, despite its direct role in mitochondrial RNA processing, the consequences of ABAD's interaction with Aβ in the regulation of the mitochondrial genome have not been investigated.

Our *in vitro* studies have sought to elucidate the effects of wild-type APP and its non-amyloidogenic fragments, particularly sAPPα, on mitochondrial function, seeking to distinguish the physiological role of APP and its non-amyloidogenic fragments from the known toxic effects of excessive Aβ resulting from over-expression of mutant versions of APP. Over-expression of wild-type APP reduces mitochondrial respiration *in vitro*, while

higher levels of Aβ, either by over-expression of mutant APP harbouring the Swedish mutation (Lopez Sanchez et al., 2017), or via over-expression of BACE1 (Schaefer, von Einem, Walther, Calzia & von Arnim, 2016), do not alter mitochondrial respiration. We found that this reduction in respiratory capacity in neuronal cells over-expressing APP correlated with reduced transcription of mtDNA and a metabolic switch to glycolytic metabolism, suggesting a programmed downregulation of OXPHOS (Lopez Sanchez et al., 2017). Furthermore, these results suggest that non-amyloidogenic APP processing can impact mitochondrial regulation.

It will be important to disentangle epiphenomena arising from over-expression of APP in cellular and mouse models from physiological effects of APP processing. In this regard there are remarkably few studies that have attempted to investigate holo-APP or sAPPα levels in Alzheimer's disease brain. Davidsson et al found lower APP levels in sporadic Alzheimer's disease brain samples compared to age-matched controls (Davidsson, Bogdanovic, Lannfelt & Blennow, 2001). Two other studies support this finding, also showing that both sAPPα and sAPPβ levels were lower in sporadic Alzheimer's disease brain (Wu, Sankaranarayanan, Hsieh, Simon & Savage, 2011) and lower sAPPα levels in sporadic Alzheimer's disease cerebrospinal fluid (Sennvik, Fastbom, Blomberg, Wahlund, Winblad & Benedikz, 2000), and these authors stressed the need for measurement of APP-derived peptides besides Aβ to gain further understanding of Alzheimer's disease pathogenesis. Thus, while Aβ levels are the strongest link between sporadic and Mendelian Alzheimer's disease, it remains to be determined what factors drive the increased β-cleavage of APP in sporadic Alzheimer's disease cases. The possibility that decreased levels of the neurotrophic sAPPα is the key pathogenic driver in Alzheimer's disease remains under-explored (Hunter & Brayne, 2018).

In summary, there is strong evidence that APP and its processing products are present in mitochondria and MAMs. In mitochondria, they interact with the protein import machinery, mtDNA and OXPHOS components, with detrimental consequences on mitochondrial dynamics and bioenergetic function. However, the mechanisms leading to mitochondrial dysfunction are not clear yet. For instance, mitochondria possess systems to prevent damage

caused by misfolded proteins, including their own unfolded protein response mechanism, which is activated when misfolded proteins accumulate in the matrix, and mitophagy, which eliminates damaged mitochondria (Pellegrino & Haynes, 2015). The participation of these protective mechanisms in the context of amyloid pathology warrants further investigation. Furthermore, it is important that APP, sAPPα, Aβ and other APP fragments are measured and reported in studies that employ APP over-expressing models (Hunter & Brayne, 2018). Aβ levels are increased in models of amyloid pathology, and therefore it has become accepted that Aβ accumulation is directly responsible for mitochondrial changes. However, this may reflect the fact that full-length APP and other non-Aβ fragments are not systematically measured, and these may be responsible for cellular and phenotypic changes reported.

Neuroprotective role of APP and sAPPα - metabolic connections

APP has been investigated largely in animal and cellular models that express higher levels of APP, either in its wild-type form or as mutant variants associated with familial Alzheimer's disease (reviewed by (Drummond & Wisniewski, 2017; Sasaguri et al., 2017)). These include first generation transgenic mice over-expressing mutant APP, such as APP V717F (also known as Indiana mutation) (Murrell, Farlow, Ghetti & Benson, 1991) and the commonly-used Swedish mutation (K670N/M671L) (Citron et al., 1992), including Tg2576 (Hsiao et al., 1996) and APP23 mice (Sturchler-Pierrat et al., 1997). However, as discussed above, APP over-expression in these models also results in increased levels of full-length APP and other amyloidogenic and non-amyloidogenic fragments (Sasaguri et al., 2017) that is often overlooked.

Loss of function models have provided important insights into the roles APP plays in neuronal function. APP knockout mice are viable and fertile, however, they display CNS anatomical and behavioural abnormalities (reviewed in (Drummond & Wisniewski, 2017; Sasaguri et al., 2017)), and age-related neurodegenerative symptoms (Dawson et al., 1999; Seabrook et al., 1999) that have been associated with impaired copper homeostasis (Crouch et al., 2005; White et al., 1999), lipid metabolism (Grimm et al., 2005) or iron homeostasis (Ayton et al., 2015), indicating that APP plays a critical role not only during CNS

mammalian development (reviewed in (van der Kant & Goldstein, 2015)), but also in the maintenance of cognitive functions with ageing. Furthermore, APP knockout mice show greater cortical neuronal loss and worsened motor and cognitive defects with mild diffuse brain impact injury compared to wild-type mice (Ayton et al., 2014; Corrigan, Vink, Blumbergs, Masters, Cappai & van den Heuvel, 2012a). The protective fragment has been identified as sAPPα (Corrigan, Vink, Blumbergs, Masters, Cappai & van den Heuvel, 2012b). Interestingly, knock-in of the gene fragment encoding the sAPPα domain is also sufficient to rescue the anatomical and behavioural abnormalities seen in APP knock-out mice (Ring et al., 2007).

APP loss has been linked to impaired iron efflux and iron accumulation in neurons (Wong, Tsatsanis, Lim, Adlard, Bush & Duce, 2014). Lower APP protein levels and higher iron levels were detected in substantia nigra tissue from human patients with Parkinson's disease, and in APP knockout mice, while mice over-expressing APP were resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced substantia nigra neuronal loss (Ayton et al., 2015). This study used Tg2576 mice which, while exhibiting higher levels of Aβ due to the presence of the Swedish mutation in APP, also have higher levels of sAPPα due to the over-expression of APP. This study provides circumstantial evidence for sAPPα being protective of a mitochondrial toxin-induced injury, since MPP^+, the active metabolite of MPTP, is an OXPHOS complex I inhibitor. Similarly, sAPPα has been shown to afford protection post-hypoxia (Hefter et al., 2016), and since hypoxia/reperfusion injury results in part from mitochondrial impairment this is also circumstantial evidence for protection from mitochondrial insult.

Mechanisms of sAPPα-mediated protection – mitochondrial links

Non-amyloidogenic fragments, particularly sAPPα, have been shown to be neuroprotective in a variety of contexts. sAPPα supports normal neuronal function and survival *in vitro* and *in vivo* (Araki et al., 1991; Hayashi, Kashiwagi, Ohta, Nakajima, Kawashima & Yoshikawa, 1994; Meziane et al., 1998; Roch et al., 1994) and confers protection from neurotoxicity, including glucose deprivation, glutamate toxicity and Aβ-induced oxidative injury (Barger &

Harmon, 1997; Goodman & Mattson, 1994; Mattson, Cheng, Culwell, Esch, Lieberburg & Rydel, 1993; Schubert & Behl, 1993).

Despite extensive efforts, the receptor for sAPPα has not been defined, and insights into the mechanism(s) of protection via sAPPα are limited. The phosphatidylinositol 3-kinase/protein kinase B (Pi3K/Akt) survival pathway was identified as one downstream effector of sAPPα in protecting against *in vitro* excitotoxicity (Cheng, Yu, Zhou & Mattson, 2002). sAPPα was also found to protect from trophic factor deprivation *in vitro*, via activation of the Pi3K/Akt pathway that also depended on holo-APP, suggesting that full-length APP acted as a receptor for sAPPα (Jimenez et al., 2011). However, other protective effects of sAPPα have been found to be independent of holo-APP, such as protection from Bcl-2-associated athanogene 3-linked aggresome formation (Kundu et al., 2016).

The insulin receptor (IR) is another candidate for sAPPα binding (Figure 2). Wallace et al. discovered that picomolar levels of sAPPα could potentiate nerve growth factor activity and sustain neurite outgrowth *in vitro*, and that this effect involved both the IR and Pi3k/Akt activation (Wallace, Akar, Lyons, Kole, Egan & Wolozin, 1997). In primary mouse neuronal cultures sAPPα has been shown to increase IR phosphorylation in the absence of insulin, and to protect *in vivo* against features of brain pathology in insulin-depleted diabetes (Aulston, Schapansky, Huang, Odero & Glazner, 2018). One common consequence of Pi3/Akt activation is a metabolic switch from OXPHOS to aerobic glycolysis, as classically seen in the activation of T cells (reviewed by (Finlay, 2012)). We speculate that acute downregulation of OXPHOS via sAPPα-mediated Pi3K/Akt activation is a mechanism of protection from OXPHOS-linked oxidative stress under conditions of neuronal injury. Further work on the interaction of sAPPα with the IR, and downstream effects are warranted.

Mitochondrial function as a biomarker in Alzheimer's disease?

Current Alzheimer's disease biomarkers include fluorodeoxyglucose PET (to quantify regional brain glucose utilisation), structural magnetic resonance imaging (to assess brain tissue volume), amyloid PET (to detect the presence of Aβ plaque and estimate plaque

burden), and CSF Aβ, tau and phosphorylated tau levels, which vary depending on disease progression (Blennow, Hampel, Weiner & Zetterberg, 2010). Recent efforts are now moving towards earlier detection and diagnosis, as it is now accepted that disease progression in Alzheimer's disease takes between 10 and 20 years and pre-clinical pathology may involve mechanisms that are independent of Aβ (Chetelat, 2013).

In vivo measures of mitochondrial function may be worthy of development so that mitochondrial functional changes can be mapped into the time course of prodromal to clinical Alzheimer's disease, as limited methods are currently available. Radioisotope copper labelled diacetyl-bis (N4-methylthiosemicarbazone) (Cu-ATSM) is one candidate (Okazawa, Ikawa, Tsujikawa, Kiyono & Yoneda, 2014). While known in clinical nuclear medicine as a hypoxic imaging agent, it was found that it accumulates in proportion to altered redox state in cells, where nicotinamide adenine dinucleotide $(NAD)^+$ is limited and NADH is in excess, as it occurs when OXPHOS is inhibited (Donnelly et al., 2012). It would be desirable to avoid the expense and challenges of PET however, and more direct measures of cellular NAD^+/NADH (Schaefer et al., 2017) or flavin adenine dinucleotide (FAD/ $FADH_2$) may be possible.

Interest has recently turned to retinal imaging as a non-invasive surrogate measure of mitochondrial function *in vivo*. The retina, the neural layer of the eye, is a developmental extension of the brain and is the only part of the CNS that can be optically imaged *in vivo*. The retina manifests features of CNS diseases and methods to detect retinal biomarkers of Alzheimer's disease are in various stages of development (Hinton, Sadun, Blanks & Miller, 1986; Jentsch et al., 2015; Ko et al., 2018; Koronyo et al., 2017; van Wijngaarden, Hadoux, Alwan, Keel & Dirani, 2017). Several imaging approaches have been postulated to measure NADH and FAD autofluorescence *in vivo* (Elner, Park, Cornblath, Hackel & Petty, 2008; Jentsch et al., 2015). As NADH is fluorescent in its reduced state and FAD is fluorescent in its oxidised form, measurement of the relative intensities of these fluorophores may provide an indication of the metabolic state of the retina (Maleki et al., 2013).

Fluorescence imaging of the eye is confounded by multiple sources of autofluorescence in the cornea, lens, neural retina and retinal pigment epithelium with overlapping excitation and

emission spectra (Spaide, 2009). This is further confounded by substantial variation in autofluorescence due to the influence of cataract, variation in macular pigmentation as well as age- and disease-related variation in lipofuscin accumulation in the retinal pigment epithelium (Spaide, 2009). The recent development of fluorescence lifetime imaging ophthalmoscopy has the advantage of added temporal resolution due to the measurement of fluorescence decay, allowing greater precision in the attribution of fluorescence to a given source in the retina. Preliminary studies in people with Alzheimer's disease indicate that fluorescence lifetime signals may vary between patients with and without the disease, however the source of this difference has not been identified (Jentsch et al., 2015). Further developments in metabolic retinal imaging may enable large-scale prospective studies of Alzheimer's disease due to the ease of obtaining retinal images compared to PET. Such large studies can be expected to differentiate Alzheimer's disease-specific from age-related changes in mitochondrial function, allowing identification of prodromal Alzheimer's disease and discriminating age-related from disease-specific mitochondrial dysfunction in the pathogenic cascade leading to Alzheimer's disease.

Conclusions

- Our current understanding of Alzheimer's disease is evolving and the central role of Aβ toxicity as the disease catalyst is increasingly questioned. Greater understanding of the role of loss of protective non-amyloidogenic APP fragments in the context of Alzheimer's disease, where amyloidogenic APP processing is unquestionably increased, may lead to new insights into efficient therapeutic targets.
- We argue for caution in the interpretation of mitochondrial perturbations in models where APP is over-expressed, as it has been shown that not only Aβ, but also sAPPα or other non-amyloidogenic APP fragments are acute modulators of mitochondrial metabolism. APP is subject to complex processing events that generate several peptides, each of which likely performs specific physiological functions. It is critical that studies using models that over-express APP systematically measure and report the levels of APP, sAPPα, Aβ and other APP-derived fragments, to elucidate their contribution to cellular functions.

- Although energy deficiency and mitochondrial dysfunction have been recognised as early events in Alzheimer's disease, the link between APP or APP-derived peptides, mitochondrial metabolism and pathogenesis remains unclear.
- Novel, non-invasive, surrogate measurements of mitochondrial function *in vivo*, such as retinal imaging may be worthy of development so that mitochondrial functional changes can be mapped into the time course of prodromal to clinical Alzheimer's disease.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

Competing Interests Statement

Peter van Wijngaarden heads Enlighten Imaging, which aims to develop retinal biomarkers in Alzheimer's disease.

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Figure Legends

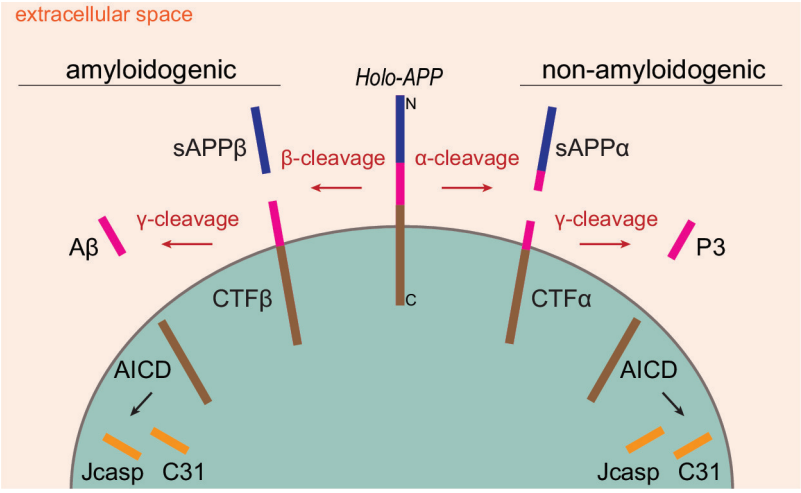
Figure 1. APP processing and cleavage products

Non-amyloidogenic processing of APP (right) involves cleavage by α-secretase, resulting in the generation of sAPPα into the extracellular space, and intracellular CTFα/CTF83. CTFα

undergoes further cleavage by the γ-secretase complex, yielding p3 and AICD fragments, which are further processed by caspases, producing Jcasp and C31. The amyloidogenic pathway (left) involves cleavage by β-secretase, with a subsequent release of sAPPβ into the extracellular medium, and CTFβ/CTF99 in the membrane. Cleavage of CTFβ by the γ-secretase complex generates Aβ peptides, AICD fragments, Jcasp and C31. Cleavage by α-secretase occurs at a position within the sequence of Aβ, and therefore precludes its formation. AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; Aβ, amyloid beta; CTF, carboxyl terminal fragment; sAPPα, soluble APP α; sAPPβ, soluble APP β.

Figure 2. Mechanisms of APP regulation of mitochondrial OXPHOS metabolism

APP accumulates in the protein import channels of mitochondria (TIM/TOM), preventing the import of nuclear-encoded mitochondrial proteins, including subunits of OXPHOS complexes (I-IV, ATP synthase) (1). Binding of sAPPα to an unknown cell membrane receptor, potentially the IR, may activate the Pi3K/Akt pathway to decrease mtDNA transcription of OXPHOS subunits (2). APP and APP-derived peptides can be produced and processed at MAMs (3). An accumulation of unprocessed CTF99 and AICDs in mitochondria has been linked to altered lipid composition and disruption of mitochondrial membranes that interfere with OXPHOS function (4). Pathologic binding of ABAD to Aβ in mitochondria causes leakage of ROS and mitochondrial dysfunction (5), but the effects of this binding in mtDNA processing have not been investigated yet. ABAD, amyloid-binding alcohol dehydrogenase; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; Aβ, amyloid beta; CTF, carboxyl terminal fragment; IR, insulin receptor; MAM, mitochondria-associated membrane; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; sAPPα, soluble APP α; TIM, translocase of the inner membrane; TOM, translocase of the mitochondrial outer membrane.



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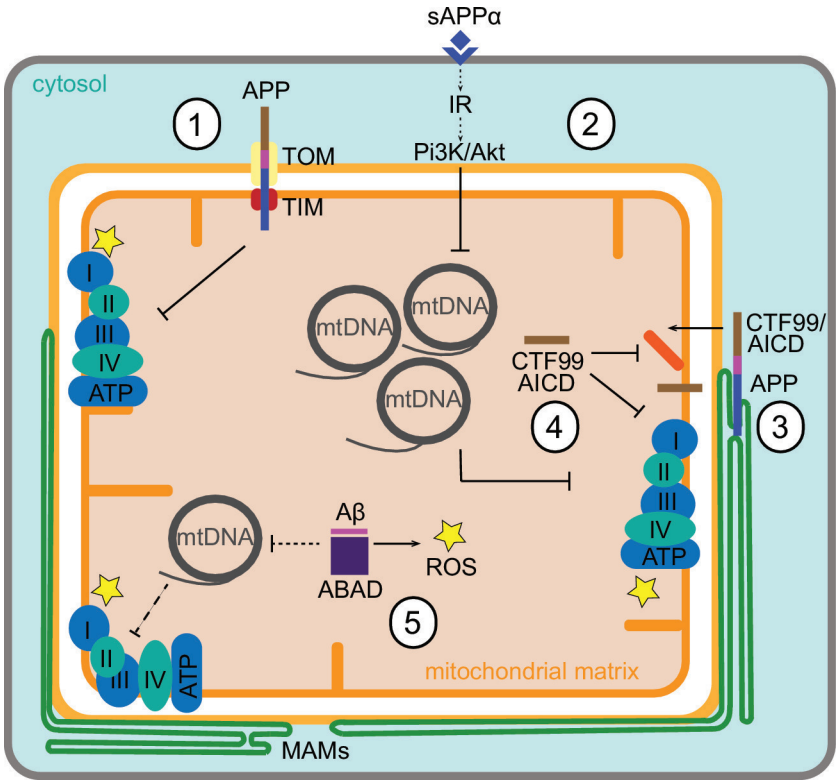

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