Chapter 3

Oxidative Stress, Mitochondrial and Insulin Signaling Dysfunction: A Redoubtable Trio in Alzheimer's Disease Pathogenesis

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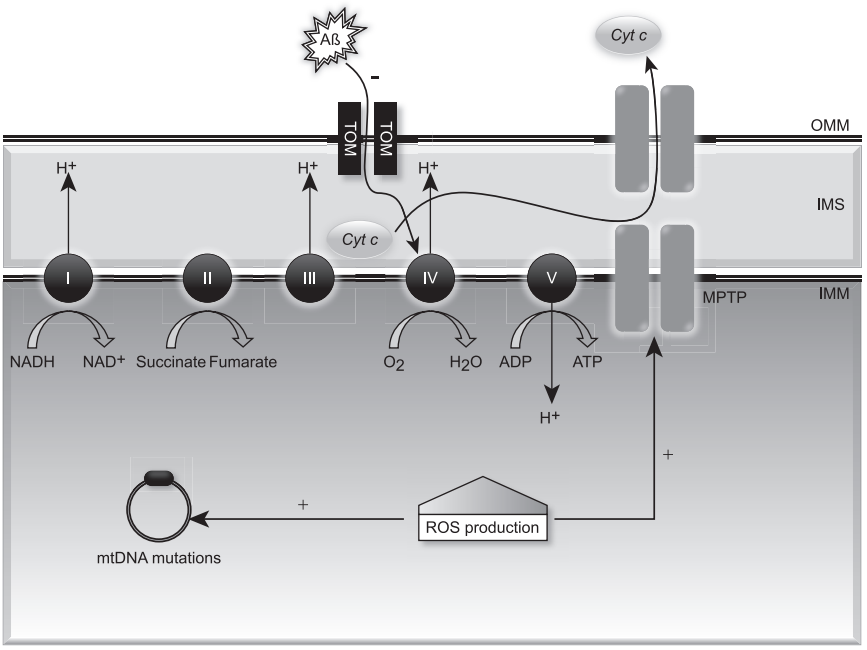
Alzheimer's disease (AD) represents the most common form of dementia among people age 65 and older, affecting more than 35 million people worldwide and representing 50–56% of cases at autopsy and in clinical series. Clinically, AD is characterized by a progressive cognitive deterioration, together with impairments in behavior, language, and visuospatial skills, culminating in the premature death of the individual typically 3–9 years after diagnosis (Querfurth and LaFerla 2010, 329). The great majority of AD cases are sporadic in origin with a late onset, while a small proportion (< 1%) has genetic origin and involves mutations in amyloid β protein precursor (AβPP) and presenilins 1 and 2 (PS1 and PS2), leading to autosomal dominant familial AD with an early onset. Additionally, the allelic abnormalities of the apolipoprotein E (APOE) gene on chromosome 19 are responsible for both anticipated onset and increase in severity of inherited and sporadic AD (Rocchi et al. 2003, 1). Neuropathologically, AD has as main hallmarks the selective neuronal and synaptic loss, the deposition of

extracellular senile plaques, mainly composed of amyloid-β (Aβ) peptide and the presence of intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein (Selkoe 2001, 75; Moreira et al. 2006, 97; Moreira et al. 2007a, 1621; Moreira et al. 2009, 741). Aβ peptide consists of 39–43 amino acid residues, derived from the proteolytic cleavage of AβPP by the β- and γ-secretases (Uemura, Kuzuya and Shimohama 2004, 1). The most common resulting fragments are either 40 or 42 amino acids in length ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$).

Over the last decades many efforts have been made to uncover the molecular mechanisms underlying the pathogenesis of AD with several hypothesis being proposed to answer to one of the most exciting question of the actuality: What is the trigger/early event(s) of AD? In 2004, the "mitochondrial cascade hypothesis" emerged to explain many of the biochemical, genetic and pathological features of sporadic AD (Swerdlow and Khan 2004, 8). This hypothesis postulates that (1) inheritance determines mitochondrial baseline function and durability; (2) mitochondrial durability influences how mitochondria change with age; and (3) when mitochondrial alterations reaches a threshold, AD histopathology and symptoms ensue (Swerdlow and Khan 2009, 308). In addition, mitochondria have been shown to be targets of the deleterious effects of Aß (LaFerla, Green, and Oddo 2007, 499), potential sites of Aß production (Hansson et al. 2004, 654; Keil et al. 2004, 50310) and triggers of the disease (Nunomura et al. 2001, 759; Pratico et al. 2001, 4183; Hauptmann et al. 2009, 1574), which provide stronger evidence supporting the "mitochondrial cascade hypothesis." Thus, it is plausible that mitochondrial-dependent pathogenic mechanisms have a central stage in the onset and progression of AD (fig. 3.1). Oxidative stress has also been implicated in the pathogenesis of AD occurring prior to the onset of symptoms, the oxidative changes being pervasive throughout the body and detected peripherally (Ghanbari et al. 2004, 41; Moreira, Harris et al. 2007, 195; Perry et al. 2003, 552) and associated with the vulnerable regions of the brain affected in disease (Nunomura et al. 1999, 1959; Nunomura et al. 2001, 759). The complex nature and genesis of oxidative damage in AD could be the result of mitochondrial abnormalities that can trigger oxidative stress. Interestingly, also disturbances in insulin metabolism, especially insulin resistance, have been suggested to be involved in AD, attributing a role to the disruption of insulin signaling in AD pathophysiology (Moreira, Santos et al. 2007, 1621; Cardoso et al. 2009, 483).

In light of this evidence, the present review is devoted to discuss the current knowledge concerning the role of oxidative stress, mitochondria, and insulin signaling deregulation in the onset and progression of AD.

Figure 3.1



Mitochondrial dysfunction in Alzheimer's disease. Mitochondrial dysfunction is intimately involved in the pathogenesis of AD. Amyloid β peptide (Aβ) has been documented to impair the activity of respiratory chain complex IV, leading to increased reactive oxygen species (ROS) levels. Indeed, Aβ peptide was shown to be imported into mitochondria via the translocase of the outer membrane (TOM) import machinery and localized to mitochondrial cristae, thus promoting mitochondrial dysfunction and oxidative damage. Furthermore, Aβ also interacts with cyclophilin D, a critical molecule involved in mitochondrial permeability transition pore (MPTP) formation and cell death. The opening of MPTP leads to the release of pro-apoptotic factors such as cytochrome c (Cyt c) to the citosol, and consequently to the induction of the apoptotic cell death. Additionally, mitochondrial DNA (mtDNA) mutations have also been implicated in mitochondrial dysfunction that occurs in AD. ADP- adenosine diphosphate; Cyt c- cytochrome c; IM- inner membrane; IMS- intermembrane space of mitochondria; NAD+- oxidized nicotinamide adenine dinucleotide; NADH- reduced nicotinamide adenine dinucleotide; H+- proton; OM- mitochondrial outer membrane

Unraveling the mechanisms involved in the etiopathogenesis of AD could provide new insights that can be translated to potential pharmacological interventions aimed to treat this neurodegenerative disease.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION AS TRIGGERS OF NEURODEGENERATION IN ALZHEIMER'S DISEASE

Mitochondria are ubiquitous and dynamic organelles that house many crucial cellular processes in eukaryotic organisms being considered "gatekeepers of life and death." Major functions of mitochondria include the production of over 90% of cellular ATP through the tricarboxylic acid cycle (TCA) cycle and oxidative phosphorylation, regulation of intracellular calcium (Ca^{2+}) and redox signaling and the arbitration of apoptosis (Green and Kroemer 2004, 626; Beal 2005, 495; Mattson, Gleichmann, and Cheng 2008, 748). Therefore, the importance of mitochondria for neuronal function and survival is notorious since neurons are cells with extremely high energy demands, mitochondrial oxidative phosphorylation being essential for neurons to meet their high energy requirements. In line with this, neurons are very vulnerable to bioenergetic crisis and dysfunction of mitochondrial machinery (Murphy, Fiskum, and Beal 1999, 231; Moreira et al. 2009, 741). Indeed, dysfunction of mitochondrial energy metabolism culminates in ATP production and Ca^{2+} buffering impairment, and exacerbated generation of reactive oxygen species (ROS) (Beal 2005, 495). ROS, in turn, cause cell membranes damage through lipid peroxidation and accelerates the high mutation rate of mitochondrial DNA (mtDNA). Additionally, accumulation of mtDNA mutations enhances oxidative damage, causes energy crisis and increases ROS production, in a vicious cycle (Petrozzi et al. 2007, 87). Moreover, the brain is especially prone to oxidative stress-induced damage due to its high levels of polyunsaturated fatty acids, high oxygen consumption, high content in transition metals and poor antioxidant defenses (Nunomura et al. 2006, 82323). The next subsections explore the role of oxidative stress in AD as well as the contribution of mitochondrial malfunctions to the pathophysiology of the disease.

Oxidative Stress: A Critical Player in Alzheimer's Disease

For a long time, oxidative stress was defined as the imbalance between the formation of ROS and the antioxidant defense mechanisms. Meanwhile, a new concept of oxidative stress emerged to account for two different mechanistic outcomes, macromolecular damage and disruption of thiol redox circuits, which lead to aberrant cell signaling and dysfunctional redox control (Jones 2006, 1865). Increased oxidative stress has been observed in age and age-related neurodegenerative diseases, mitochondria being both targets and sources of ROS (Lin and Beal 2006, 787). In

fact, accumulating evidence demonstrates that oxidative damage marked by high levels of lipid, protein, and nucleic acid oxidation is increased in vulnerable neurons in AD (Castellani et al. 2001, 175; Nunomura et al. 1999, 1959; Nunomura et al. 2001, 759; Smith et al. 1997, 2653; Straface et al. 2005, 2759). Nucleic acid oxidation is marked by increased levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG) (Nunomura et al. 1999, 1959; Nunomura et al. 2001, 759). Protein oxidation is marked by elevated levels of protein carbonyl and widespread nitration of tyrosine residues in the susceptible neurons (Smith et al. 1996, 120; Smith et al. 1997, 2653). Lipid peroxidation is marked by higher levels of thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), isoprostanes and altered phospholipid composition (Sayre et al. 1997, 2092). Also, modifications to sugars are observed via increased glycoxidation and glycation (Smith et al. 1994, 5710; Smith et al. 1995, 172) that are responsible for the formation of advanced glycation endproducts (AGEs) such as N^{ε}-(carboxymethyl) lysine (CML), pentosidine and pyralline.

Additionally, it has been proposed that oxidative stress precedes all the other pathological hallmarks of AD pathogenesis. Indeed, the secretion and deposition of Aβ within vulnerable AD neurons have been suggested to be compensatory mechanisms developed by cells to protect themselves against oxidative damage (Hayashi et al. 2007, 1552; Nakamura et al. 2007, 12737; Smith et al., 2002, 1194). Accordingly, Aβ was demonstrated to follow the appearance of oxidative stress markers in AD (Petersen et al. 2007, 143) and it was shown that this peptide protects lipoproteins from oxidation in cerebrospinal fluid and plasma (Atwood et al. 1998, 12817; Atwood et al. 2003, 249–266; Cuajungco et al. 2000, 19439; Kontush et al., 2001, 119). Similarly, in AβPP transgenic mouse models of AD (Tg2576), it was also observed that oxidative stress appears before than Aβ deposition (Pratico et al. 2001, 4183; Smith et al., 1998, 2212).

In light of this evidence, Zhu and colleagues proposed the "Two-Hit hypothesis," which postulates that the early and progressive oxidative damage to neurons elicits a compensatory response such that the cell can exist in the overly oxidizing environment. Furthermore, this "oxidative steady state," with the initial purpose to afford protection, makes the cell more vulnerable to additional insults, such as Aβ deposition and NFT formation (Zhu et al. 2001, 39; Zhu et al. 2004, 219; Zhu et al. 2007, 494). Cellular oxidative damage also promotes cell cycle aberration and tau hyperphosphorylation, leading to the NFT formation (Castegna et al. 2002, 1524; Castegna et al. 2003, 1394; Lee et al. 2004, 1; Lee et al. 2005, 164; Mark

et al. 1997, 255). Consequently, damaged cells succumb to the degenerative process, or exist in a dysfunctional state, the ultimate manifestation of which is the cognitive decline and dementia descriptive of AD.

Along with increased oxidative damage, impaired antioxidant defenses have also been proposed to be prominent features of AD (Smith et al. 1997, 2653; Straface et al. 2005, 2759). Indeed, decreased activities of the antioxidant enzymes copper/zinc superoxide dismutase (Cu/ZnSOD) and catalase (CAT) were found in the frontal and temporal cortex of AD patients (Marcus et al. 1998, 40). The total antioxidant capacity was also significantly decreased in AD as well as in mild cognitive impairment (MCI) but not in patients with vascular dementia (Straface et al. 2005, 2759), being shown a negative correlation between the total antioxidant capacity and disease duration, in AD patients (Guidi et al. 2006, 262). It has also been proposed that oxidative stress-mediated neuronal loss could be initiated by a decline in glutathione (GSH), which acts as a scavenger of free radicals and is the most abundant thiol-reducing agent in mammalian tissues (Bains and Shaw 1997, 335). In fact, altered GSH levels were observed in specific regions of the central nervous system of AD patients (Gu et al. 1998, 24). Similarly, it was found a reduced GSH content in lymphoblasts carrying AβPP, PS1, and PS2 gene mutations when compared to controls (Cecchi et al. 1999, 152). More recently, it has been reported that erythrocytes of AD and MCI patients present a decrease in GSH levels and GSH/GSSG ratio compared to age-matched control subjects (Bermejo et al. 2008, 162). Accordingly, a study from our laboratory showed low levels of GSH in the triple transgenic model of AD (3xTg-AD), accompanied by a decrease in vitamin E levels and high levels of lipid peroxidation (Resende et al. 2008, 2051).

Mitochondrial abnormalities have also been implicated in the etiopathogenesis of AD, which can be triggered by oxidative disturbances. Compelling evidence demonstrates that AD patients present reduced metabolic activity, which is believed to be the result of oxidative damage to vital mitochondrial components (Aksenov et al. 1998, 151; Aliev et al. 2003, 209–238; Anderson, Cummings, and Cotman 1994, 286; Hirai et al. 2001, 3017). As mentioned above, besides being essential ATP-producing organelles, mitochondria are also one of the major intracellular sources of potentially pathogenic ROS, including hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\cdot}$) and superoxide ($O_2^{\cdot -}$), particularly in highly metabolically active organs such as the brain (Wallace 1999, 1482). Excessive mitochondrial ROS generation damages several cellular targets including mitochondrial components themselves (lipids, proteins, and DNA) (Moreira et al. 2009, 741). Indeed, mitochondrial ROS induce mutations

in the mitochondrial DNA (mtDNA), which in turn impair the oxidative phosphorylation system. This impairment results in an exacerbation of ROS generation, promoting the augment of the number of mtDNA mutations in a vicious positive feedback cycle (Fukui and Moraes 2008, 251). Also the lack of histones in mitochondrial DNA (mtDNA) and diminished capacity for DNA repair render mitochondria an easy target to oxidative stress events (Moreira et al. 2009, 741).

Overall, oxidative stress-induced cellular damage has been long recognized as a culprit in degenerative processes that occur in AD. The next subsection is devoted to explore the intimate connection between mitochondrial impairment and oxidative stress.

Mitochondrial Anomalies and Oxidative Stress: Side by Side in Alzheimer's Disease

Accumulating data from *in vitro, in vivo* and human studies argue that mitochondrial dysfunction and bioenergetics failure are early events implicated in AD pathogenesis (Moreira et al. 2010, 2) (Fig. 3.1). Impaired activities of the three key TCA enzyme complexes, pyruvate dehydrogenase (PDH), isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase (KGDH) have been documented in postmortem AD brain and fibroblasts from AD patients (Huang et al. 2003, 309; Bubber et al. 2005, 695). Data from our laboratory also demonstrated that the levels of both PDH and KGDH are decreased in AD brains (Moreira et al. 2007, "Autophagocytosis of mitochondria," 525). Furthermore, Bubber and collaborators (2005, 695) tested whether impairments in TCA cycle enzymes correlate with disability in AD brains. The authors observed that all the changes in TCA cycle activities (specifically that of PDH complex) correlated with the clinical state, suggesting a coordinated mitochondrial alteration (Bubber et al. 2005, 695). These enzymes are known to be highly susceptible to oxidative modification and are altered by exposure to a range of pro-oxidants (Tretter and Adam-Vizi 2000, 8972). In addition, a decline in respiratory chain complexes I, III, and IV activities was found in platelets and lymphocytes from AD patients and postmortem AD brain tissue (Kish et al. 1992, 776; Parker et al. 1994, 1086; Bosetti et al. 2002, 371; Valla et al. 2006, 323), further emphasizing that mitochondrial abnormalities are present at the earliest symptomatic stages of the disease. Similarly, *in vitro* studies demonstrated that pheochromocytoma cells (PC12) exposed to $A\beta_{1\text{-}40}$ and $A\beta_{25\text{-}35}$ present mitochondrial dysfunction characterized by the inhibition of complexes I, III, and IV of the mitochondrial respiratory chain (Pereira, Santos and Oliveira 1998, 1749).

More recently, Fattoretti and collaborators (2009), in order to establish a link between AD and mitochondrial dysfunction, investigated succinic dehydrogenase (SDH) (mitochondrial respiratory complex II) activity in mitochondria of hippocampal CA1 pyramidal neurons obtained from 3xTg-AD mice. The authors observed a decreased density (number of mitochondria/μm^3 of cytoplasm) of SDH-positive mitochondria in 3xTg-AD mice. Data from our laboratory also revealed that AD fibroblasts present high levels of oxidative stress and apoptotic markers when compared with young and age-matched controls. Moreover, AD-type changes could be generated in control fibroblasts using N-methylprotoporphyrin to inhibit cytochrome c oxidase (COX) assembly, which indicates that the observed oxidative damage was associated with mitochondrial dysfunction. Additionally, the effects promoted by the N-methylprotoporphyrine were reversed or attenuated by lipoic acid and *N*-acetyl cysteine (Moreira, Harris et al. 2007, 195). Overall, these findings suggest that mitochondria are important in oxidative damage that occurs in AD and that antioxidant therapies may be promising.

mtDNA mutations have also been implicated in mitochondrial dysfunction in the pathogenesis of AD (see fig. 3.1). For instance, 20 point mutations were detected in the mitochondrial-encoded cytochrome c oxidase subunits I, II, and III genes in AD patients (Hamblet et al. 2006, 398). Qiu and collaborators (2001, 261) also identified two missense mutations in the mtDNA of COX in a patient with AD. Further, a high aggregate burden of somatic mtDNA mutations was observed in postmortem brain tissue from AD patients (Lin et al. 2002, 133; Coskun, Beal and Wallace, 2004, 10726).

Accumulating evidence also indicates that Aβ and AβPP could directly target mitochondria. For instance, Aβ was found to impair cellular respiration, energy production, and mitochondrial electron chain complexes activity in human neuroblastoma cells (Rhein, Baysang et al. 2009, 1063). Moreover, cultured neurons isolated from Tg mice that overexpress a mutant form of AβPP and Aβ-binding alcohol dehydrogenase (ABAD) (Tg mAβPP/ABAD) display spontaneous generation of H_2O_2 and $O_2^{(-}$, decreased ATP, release of cytochrome c and induction of caspase 3-like activity followed by DNA fragmentation and loss of cell viability. Furthermore, generation of ROS is associated with dysfunction at the level of COX (Takuma et al. 2005, 597). Similarly, Crouch and colleagues (2005, 672) found that $A\beta_{1\text{-}42}$ can disrupt mitochondrial COX activity in a sequence- and conformation-dependent manner. In an *in vitro* study designed to explore the effect of the AβPP Swedish double mutation (K670M/N671L) on oxidative stress-induced cell death mechanisms in PC12 cells, increased activity of caspase 3 was observed due to an enhanced activation of both

intrinsic and extrinsic apoptotic pathways, including activation of JNK pathway. Moreover, apoptosis was attenuated by SP600125, a JNK inhibitor, through protection of mitochondrial dysfunction and reduction of caspase 9 activity (Marques et al. 2003, 28294). These findings corroborate the hypothesis that the massive neurodegeneration at an early age in familial AD patients could be a result of an increased vulnerability of neurons through the activation of different apoptotic pathways as a consequence of elevated levels of oxidative stress. In addition, mitochondrial dysfunction was also linked to the accumulation of full-length and carboxy-terminally truncated AβPP across mitochondrial import channels in brain tissue from AD patients. The authors observed that this accumulation of AβPP inhibited the entrance of nuclear-encoded COX subunits IV and Vb proteins, which was associated with decreased cytochrome c oxidase activity and increased H_2O_2 levels (Devi et al. 2006, 9057). Similarly, Anandatheerthavarada and colleagues (2003) reported an accumulation of full-length AβPP in the mitochondrial compartment in a transmembrane-arrested form that impaired mitochondrial functionality and energy metabolism. Also, a progressive accumulation of Aβ monomers and oligomers was detected within the mitochondria of both transgenic mice overexpressing mutant AβPP and postmortem brain from AD patients (Caspersen et al. 2005, 2040; Crouch et al. 2005, 672; Devi et al. 2006, 9057; Manczak et al, 2006, 1437). A direct link between Aβ-induced toxicity and mitochondrial dysfunction in AD pathology has been suggested by the interaction between mitochondrial Aβ and ABAD (Yan and Stern 2005, 161; Lustbader et al. 2004, 448). Moreover, this interaction was found to induce mitochondrial failure via changes in mitochondrial membrane permeability and a reduction in the activities of enzymes involved in mitochondrial respiration (Lustbader et al. 2004, 448).

More recently, Hansson Petersen and collaborators (2008, 13145) showed that Aβ peptide is imported into mitochondria via the translocase of the outer membrane (TOM) import machinery and localized to mitochondrial cristae (see fig. 3.1). Thus, it has been proposed that Aβ species transport to mitochondria cause mitochondrial dysfunction and oxidative damage, and consequently damage neurons both structurally and functionally (Caspersen et al. 2005, 2040; Crouch et al. 2005, 672; Devi et al. 2006, 9057; Manczak et al. 2006, 1437; Hansson Petersen et al. 2008, 13145). Previous studies from our laboratory also reported an increased susceptibility to mitochondrial permeability transition pore (MPTP) induction promoted by Aβ peptides (Moreira et al. 2001, 789; Moreira et al. 2002, 257) (fig. 3.1). In accordance, it was provided a plausible mechanism underlying Aβ-induced mitochondrial dysfunction, in which Aβ interacts with cyclophilin D, a critical

molecule involved in MPTP formation and cell death. Du and collaborators (2008) showed that the interaction of cyclophilin D with mitochondrial Aβ potentiates mitochondrial, neuronal and synaptic stress. Conversely, cyclophilin D ablation protects neurons from Aβ-induced MPTP formation and the resultant mitochondrial and cellular stresses. Additionally, cyclophilin D deficiency substantially improves learning and memory and synaptic function in an AD mouse model and alleviates Aβ-mediated reduction of long-term potentiation (LTP) (Du et al. 2008, 1097).

Another study reported that the presequence protease (PreP) is responsible for the degradation of the accumulated Aβ in mitochondria, further supporting the association of Aβ with mitochondria and mitochondrial dysfunction in AD (Falkevall et al. 2006, 29096). However, the key role of mitochondria in AD pathogenesis was recently highlighted, as well as the close interplay of this organelle with the two main pathological features of the disease. Rhein, Song, and collaborators (2009, 20057) demonstrated that Aβ and tau synergistically impair mitochondrial function and energy homeostasis in 3xTg-AD mice. Accordingly, a previous study demonstrated that Tg mice overexpressing the P301L mutant human tau protein present alterations of metabolism-related proteins including mitochondrial respiratory chain complexes, antioxidant enzymes and synaptic proteins that are associated with increased oxidative stress. Moreover, mitochondria from these Tg mice displayed increased vulnerability toward Aβ insult, which reinforce a possible synergistic action of tau and Aβ pathology on the mitochondria. The authors also suggest that tau pathology involves a mitochondrial and oxidative stress disorder possibly distinct from that caused by Aβ (David et al. 2005, 23802). These findings may contribute to a better understanding of the biochemical pathways underlying mitochondrial dysfunction in AD and may help lead to the development of novel mitochondrial-targeted therapeutic strategies. Ultrastructural alterations in mitochondrial morphology such as reduced size and broken internal membrane cristae were also documented in brains from AD patients (Hirai et al. 2001, 3017; Baloyannis 2006, 119). One reasonable explanation for these observations could be the increased mitochondrial autophagy found in AD (Moreira, Moreira et al. 2007, "Autophagocytosis of mitochondria," 525; Moreira et al. 2007, "Increased autophagic degradation," 614). Another consequence of Aβ on mitochondria is the induction of dynamic changes, including mitochondrial fission/fusion perturbations. Wang and collaborators (2008, "Dynamin," 470) reported abnormal mitochondrial fission and fusion in fibroblasts from sporadic AD patients, marked by lower levels of dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission. The authors also observed that AD fibroblasts display elongated

mitochondria which form collapsed perinuclear networks (Wang et al. 2008, "Dynamin," 470; Wang et al. 2009, "Role of abnormal mitochondrial dynamics," 153). Accordingly, AβPP overexpression in M17 neuroblastoma cells resulted in predominantly fragmented mitochondria, decreased Drp1 and optic atrophy protein 1 (OPA1) levels, and a defect in neuronal differentiation (Wang et al. 2008, "Amyloid-beta overproduction," 19318). Moreover, reduced expression levels of Drp1, OPA1, mitofusin (Mfn)1 and 2 and increased mitochondria fission protein 1 (Fis1) levels were found in hippocampal tissues from AD patients compared with age-matched controls (Wang et al. 2009, "Impaired balance," 9090). These results suggest that AD is characterized by mitochondrial fission/fusion imbalance, and consequently mitochondrial fragmentation and abnormal distribution, which potentiates mitochondrial and neuronal dysfunction in this neurodegenerative disease.

BRAIN GLUCOSE TRANSPORT AND METABOLISM AND INSULIN SIGNALING DEREGULATION IN ALZHEIMER'S DISEASE

Glucose is the main source of energy required for normal brain function. Since neurons are incapable to synthesize or store glucose, they are dependent on glucose transport across the blood-brain barrier (BBB), which is mediated by glucose transporters (GLUTs) (Scheepers, Joost, and Schurmann 2004, 364). An impairment of glucose metabolism in the brain of AD patients has been observed by positron emission tomography (PET) imaging studies (Azari et al. 1993, 438; Small et al. 1996, 70; Davis et al. 1997, 4526). Moreover, this impairment seems to be a cause, rather than a consequence, of neurodegeneration in AD (Hoyer 2004, 541).

Insulin-mediated neuronal insulin receptor (IR) and/or insulin-like growth factor-1 receptor (IGF-1R) activation (Kahn et al. 1993, 291; Noh et al. 1999, 263) underlies a complex and important role in the regulation of brain metabolism (Gasparini and Xu 2003, 404; Santos, Pereira, and Carvalho 1999, 33; Shah and Hausman 1993, 151; Yang, Raizada, and Fellows 1981, 1050), neuronal growth and differentiation (Schechter et al. 1998, 270; Gasparini and Xu 2003, 404; Plitzko, Rumpel, and Gottmann 2001, 1412) or neuromodulation (Gasparini and Xu 2003, 404; Kremerskothen et al. 2002, 153; Shuaib et al. 1995, 809; Vilchis and Salceda 1996, 1167). Originally, the brain was believed to be an insulin-insensitive organ. However, biochemical evidence for the presence of insulin and IRs in the brain and expression of insulin-sensitive GLUT-4 in neurons confirm the idea that the brain is in fact a target organ for insulin (El Messari et al.

2002, 225). Indeed, IRs are widely expressed throughout the brain in both neurons and glia (Wozniak et al. 1993, 1), with highest levels in the olfactory bulb, cerebral cortex, hippocampus, cerebellum, and hypothalamus (Havrankova, Roth, and Brownstein 1978, 636; Van Houten et al. 1979, 666). In the adult brain, insulin derives primarily from its synthesis in pancreatic β-cells, being transported by cerebrospinal fluid (CSF) into the brain. This transport occurs mainly via a carrier-mediated, saturable, regulatable, and temperature-sensitive active process (Erol 2008, 241; Salkovic-Petrisic and Hoyer 2007, 217; Banks 2004, 5; Burns et al. 2007, 1094). Additionally, previous studies showed that insulin could be synthesized *de novo* in the brain. This idea was confirmed by the observation of the existence of preproinsulin I and II mRNA within rat fetal brain and in cultured neurons, and insulin immunoreactivity in the endoplasmatic reticulum (ER), Golgi apparatus, cytoplasm, axon, dendrites and synapses of neuronal cells (Adamo, Raizada, and LeRoith 1989, 71; Craft et al. 1996, 123; Schechter et al. 1996, 16; Schechter et al. 1998, 270; Zhao et al. 1999, 34893). This was further supported by the high levels of insulin detected in brain extracts (Havrankova et al. 1979, 636), the presence of insulin in immature nerve cell bodies (Schechter et al. 1992, 27; Schechter et al. 1996, 16), the observation that, despite peripherally injected, insulin can enter the CSF rapidly (Freude et al. 2005, 3343), and that less than 1% of the hormone crosses the BBB in dogs and rodents (Banks and Kastin 1998, 883).

Hoyer (2004, 135) suggested that the impaired glucose utilization observed in AD brains is a consequence of diminished glucose breakdown in brain tissue, caused by a disturbance in the control of glucose utilization at the level of insulin signal transduction. Furthermore, it has been shown that type 2 diabetes mellitus is a risk factor for AD and that AD patients have a higher risk to develop type 2 diabetes (Cole and Frautschy 2007, 10; Moreira et al. 2009, 741). More recently, it was proposed that AD can be an "insulin-resistant brain state" or even a "type 3 diabetes" (Rivera et al. 2005, 247; Steen et al. 2005, 63; Craft et al. 1998, 164). Indeed, it was observed an age- and AD-related decrease in insulin mRNA and protein levels (Lester-Coll et al. 2006, 13; Rivera et al. 2005, 247; Steen et al. 2005, 63), IR and IGF-1R expression (Frolich et al. 1999, 290; Moloney et al. 2010, 224), insulin receptor substrate-1 (IRS-1) and IRS-2 levels and phosphatidylinositol 3-kinase (PI3-K) and extracellular-regulated kinase 1/2 (ERK1/2) activities. Furthermore, AD patients show increased fasting plasma insulin levels, decreased CSF insulin levels, and/or decreased CSF/plasma insulin ratio, besides increased Aβ levels (Watson and Craft 2004, 97), suggesting a decrease in insulin clearance, which may provoke

an elevation of plasma Aβ levels (Li and Holscher 2007, 384). It was also found that insulin modulates AβPP processing both *in vivo* and *in vitro.* Insulin has also been proposed to increase the extracellular concentration of Aβ by two independent mechanisms: stimulation of Aβ secretion by the enhancement of its trafficking from the ER and trans-Golgi network, the main site for Aβ generation, to the plasma membrane, which significantly reduces the intracellular concentration of Aβ derivatives (Aβ40 and Aβ42); or inhibition of extracellular degradation of Aβ by insulin-degrading enzyme (IDE), a metalloprotease enzyme responsible for insulin degradation and is also the main soluble Aβ degrading enzyme at neutral pH (Gasparini et al. 2001, 2561). This last hypothesis is supported by (1) a decrease in IDE activity and mRNA and protein levels in AD brain; (2) impaired brain Aβ and insulin degradation in knockout mice lacking IDE (Frolich et al. 1999, 290; Hong and Lee 1997, 19547; Lucas et al. 2001, 27); (3) increased IDE immunoreactivity around senile plaques; and (4) enhanced IDE activity in IDE and AβPP double transgenic mice associated with a decrease in Aβ and prevention of AD (Leissring et al. 2003, 1087). Meanwhile, brain IR does not desensitize, thus IDE may constitute a negative feedback loop that controls insulin action (van der Heide, Ramakers, and Smidt 2006, 205; Zhao et al. 2004, 71).

Since AD has been recognized as an "insulin-resistant brain state," the intracerebroventricular (icv) injection of diabetogenic streptozotocin (STZ) has been shown to produce neurochemical and brain glucose metabolism changes, as well as long-term and progressive deficit in learning, memory, and cognitive behavior, that resemble those found in the brain of patients with AD (Grünblatt et al. 2007, 757; Salkovic-Petrisic and Hoyer 2007, 217). It was found that icvSTZ administration promotes a significant decrease in IRs expression in cortex and hippocampus, insulin-1 mRNA in hippocampus, insulin-2 mRNA in cortex and a significant increase of tau phosphorylation in hippocampus, these alterations being associated with the impairment of memory and learning. These findings suggest that alterations of neuronal insulin signaling severely affect learning and memory processes. Additionally, icvSTZ administration was shown to induce brain atrophy, mainly due to neuronal and oligodendroglial cell loss mediated by apoptosis, mitochondrial dysfunction, neuroinflammation, and oxidative stress (Lester-Coll et al. 2006, 13). Furthermore, icvSTZ administration causes abnormalities in brain glucose metabolism, including reduction of glucose utilization in 17 of 35 brain areas (Duelli et al. 1994, 737) and decreased activities of glycolytic enzymes, leading to a decline in the levels of energy-rich compounds, ATP and creatine phosphate (Lannert and

Hoyer 1998, 1199). De la Monte and collaborators (2006, 89) also reported an increase of AβPP and acetylcholinesterase expression, GSK-3β activity, phospho-tau and ubiquitin levels and decreased expression of choline acetyltransferase in icvSTZ-treated animals. Collectively, these findings support the idea that dysfunctional insulin signaling is critically involved in the pathogenesis of AD.

Disturbance of tau phosphorylation seems to be another mechanism by which insulin is implicated in AD pathology. Indeed, insulin has been shown to activate the major kinases involved in tau phosphorylation, including glycogen synthase kinase 3β (GSK-3β), ERK1/2 and cyclin-dependent kinase 5 (Cdk-5) (Li and Hölscher 2007, 384; de la Monte and Wands 2005, 23802; van der Heide, Ramakers, and Smidt 2006, 205). Conversely, it has been reported that insulin and IGF-1 also inhibit abnormal tau hyperphosphorylation by stimulating Akt-induced phosphorylation/inactivation of GSK-3β in both human and animal neurons (Li and Holscher 2007, 384; de la Monte and Wands 2005, 23802; Moloney et al. 2010, 224; Hong and Lee 1997, 19547). Accordingly, it was previously shown that insulin and IGF-1 reduce tau phosphorylation promoting its binding to microtubules by inhibition of GSK-3β via the PI-3K pathway (Ho et al. 2004, 902). In primary cortical neurons it was also observed that insulin or IGF-1 transiently increases phosphorylation of specific tau residues by activation of GSK-3β (Lesort and Johnson 2000, 305). Thus, disturbed insulin and/or IGF-1 signaling pathways could potentiate abnormal tau hyperphosphorylation leading to NFT formation (Cheng et al. 2005, 5086). Furthermore, hyperphosphorylated tau fails to be transported into axons, accumulating and aggregating into NFTs in neuronal perikarya, which promote mitochondrial dysfunction, oxidative stress, apoptotic or necrotic death (de la Monte and Wands 2005, 45).

FINAL REMARKS

Oxidative stress and mitochondrial abnormalities have been proposed to play a central role in the pathogenesis of AD. Mitochondria are pivotal in controlling cell survival and death, since they generate the majority of cellular ATP, buffer intracellular Ca^{2+}, integrate apoptotic signaling pathways and represent one of the major sources of pathogenic ROS. Thus, perturbations in the physiological functions of these organelles inevitably culminate in oxidative damage and disturbed mitochondrial and neuronal function. Oxidative stress, in turn, potentiates mitochondrial and neuronal dysfunction in a vicious cycle. Converging evidence also posits that impaired brain glucose metabolism and abnormalities in the insulin

signaling cascade are intimately involved in the onset and establishment of AD pathology. As such, a detailed understanding of the involvement of oxidative stress, mitochondrial anomalies, impaired brain glucose metabolism, and dysfunctional insulin signaling will be of paramount importance in the context of AD.

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