Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes

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Abstract

It has been argued that in late-onset Alzheimer's disease a disturbance in the control of neuronal glucose metabolism consequent to impaired insulin signalling strongly resembles the pathophysiology of type 2 diabetes in non-neural tissue. The fact that mitochondria are the major generators and direct targets of reactive oxygen species led several investigators to foster the idea that oxidative stress and damage in mitochondria are contributory factors to several disorders including Alzheimer's disease and diabetes. Since brain possesses high energetic requirements, any decline in brain mitochondria electron chain could have a severe impact on brain function and particularly on the etiology of neurodegenerative diseases. This review is primarily focused in the discussion of brain mitochondrial dysfunction as a link between diabetes and Alzheimer's disease.
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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a selective neuronal cell death associated with two hallmark pathological lesions: the intracellular neurofibrillary tangles (NFTs) and extracellular amyloid deposits in the form of senile plaques. The etiological events leading to AD pathogenesis are unclear. Although age and the inheritance of predisposing genetic factors appear to play a major role, more recent evidence suggests that the development and progression of AD is subject to a wide variety of both environmental and genetic modifiers [1,2]. There is no single gene that accounts for AD heritability, despite some clues that have been provided by genetic analysis of the rare cases of early-onset familial AD which are caused by missense mutations in the amyloid β precursor protein (AβPP) and presenilin-1 and -2 genes. The vast majority of late-onset AD cases are sporadic in origin. Mutations and polymorphisms in multiple genes are likely to contribute to sporadic AD pathogenesis together with non-genetic factors. The specific accumulation of neurotoxic amyloid-β (Aβ) [3] derived from the post translational proteolysis of AβPP [4] in the central nervous system (CNS) appears to represent a major pathological step in the evolution of AD [5]. AD has been thought to occur due to the accumulation of aggregated neurotoxic Aβ appearing in specific brain regions (hippocampus and cerebral cortex), triggering an inflammatory response, neuronal cell death and gradual cognitive decline [5].

Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia. In type 1 diabetes, which generally develops at a young age, the principal defect is an auto-immune-mediated destruction of pancreatic cells, leading to insulin deficiency. In type 2 diabetes the principal defect is insulin resistance, leading to a relative insulin deficiency .The islest of Langerhans in type 2 diabetes is characterized by β-cell loss [6,7] and islet amyloid derived from islet amyloid polypeptide (IAPP) [8–10], a protein coexpressed and secreted with insulin by β-cells. Similarly

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to Aβ peptide, IAPP spontaneously forms into amyloid aggregates in an aqueous environment [11]. Furthermore, it has been reported that degeneration of pancreatic islets is also associated with NFTs formation (for review see [12]). Similarly to AD, the incidence of type 2 diabetes strongly increases with age. Altogether these findings implicate a close biological relationship between type 2 diabetes and AD.

In addition to complications that affect the eyes, kidneys, heart, blood vessels and nerves, diabetes mellitus is associated with damage to the CNS and cognitive deficits [13,14]. Impairment of learning and memory has been documented in both type 1 and type 2 diabetes. CNS deficits range from moderate to severe, depending on the quality of glycemic control, and involve mainly verbal memory and complex information processing [15–17].

Furthermore, it has been shown that insulin affects several brain functions including cognition and memory, and several studies have established links between insulin resistance, diabetes mellitus and AD [18]. Recent evidence indicates that insulin regulates the metabolism of Aβ and tau proteins [19–21]. Hoyer [22] was the first to suggest that desensitization of the neuronal insulin receptors and signalling events in AD, leads to a reduction in acetylcholine and a corresponding decrease in cerebral blood flow. These abnormalities result in chronic and increasing deficits in brain oxidative metabolism.

Due to the increasing number of data demonstrating a connection between diabetes and AD, efforts have been developed to elucidate the exact mechanism(s) underlying this connection. Although both disorders possess several overlapping features, mitochondrial dysfunction is one of the most relevant rendering mitochondrion an important target of scientific research. This review starts by given an overview about the involvement of insulin signal transduction in AD pathophysiology followed by the discussion of glucose/ energetic metabolism deficiency in this disease. The last part of this review culminates with the discussion of mitochondrial dysfunction as a link between diabetes and AD.

2. Insulin and Alzheimer's disease pathophysiology

Abnormalities in insulin metabolism, pertinent to type 2 diabetes, are among the central factors thought to mechanistically influence the onset of AD via their influence on synthesis and degradation of Aβ. For example, there is evidence indicating that insulin itself may significantly promote extracellular amyloidogenic Aβ peptides through mechanisms that involve the acceleration of AβPP/Aβ trafficking from the *trans*-Golgi network, a major cellular site for Aβ generation, to the plasma membrane [21]. Additionally, recent studies have indicated that certain signal transduction pathways downstream of the insulin receptor, may also promote the generation of Aβ peptides by modulating the cleavage of the parent AβPP at the γ-secretase site [23], a site determinant of Aβ amyloidogenicity. Although

this evidence tentatively suggests that type 2 diabetes might play an important role in AD through mechanisms that involve Aβ peptide generation, alternate studies suggest that insulin may also provoke amyloid accumulation by limiting Aβ degradation via direct competition for the insulin-degrading enzyme (IDE). IDE is a zinc-metallopeptidase that preferentially cleaves proteins with a propensity to form β-pleated sheet-rich amyloid fibrils [24,25], such as Aβ peptides [26,27]. This relationship of IDE with Aβ is supported by recent evidence indicating that IDE activity in the brain is negatively correlated with Aβ content [26,27], and that IDE expression is decreased in the AD brain [28,29].

Furthermore, it has been reported that Aβ40 and Aβ42 reduce insulin binding and insulin receptor autophosphorylation. The reduction in binding seems to be caused by a decrease in the affinity of insulin to the insulin receptor, which suggests that Aβ is a direct competitive inhibitor of insulin binding and action [30].

Recently, Steen and collaborators [31] demonstrated the existence of extensive abnormalities in insulin and insulin-like growth factor type I and II (IGF-I and IGF-II) signalling mechanisms in AD brains. These abnormalities were associated with reduced levels of insulin receptor substrate (IRS) mRNA, tau mRNA, IRS-associated phosphotidylinositol 3-kinase, and phospho-Akt (activated), and increased glycogen synthase kinase-3β activity and AβPP mRNA expression. The strikingly reduced CNS expression of genes encoding insulin, IGF-I, and IGF-II, as well as the insulin and IGF-I receptors, led the authors to suggest that AD may represent a neuro-endocrine disorder that resembles diabetes mellitus. In addition, the same research group demonstrated that insulin and insulin-like growth factor expression and function deteriorate with progression of AD being these effects linked to brain reductions in acetylcholine [32]. Therefore, the authors proposed the term, "Type 3 Diabetes" to reflect this pathogenic mechanism of neurodegeneration.

Furthermore, insulin has been shown to regulate the phosphorylation state of tau protein by regulating the activity of phosphorylating enzymes. Insulin concentration deficit increases the activity of glycogen synthase-3 kinase [33], which was found to cause tau hyperphosphorylation [19]. ATP acts in similar way, reduction of ATP activates both protein kinases erk36 and erk40 [34], which in turn causes tau hyperphosphorylation [35].

These data provide clear evidence that the metabolism of AβPP, Aβ degradation and tau protein phosphorylation are under control of insulin signal transduction.

3. Glucose/energetic metabolism deficiency in Alzheimer's disease

Normal brain function requires a steady supply of energy substrate to carry out all of its cellular and molecular needs. Glucose is the primary source of fuel for any energy-demanding activity in brain that together with oxygen is delivered by the circulation for the metabolic chores that

keep brain cells healthy [36]. When glucose delivery to the brain stops, catastrophic neurological consequences or even death can develop. There is increasing amount of evidence suggesting that insulin present in CNS is a regulator of central glucose metabolism, similar to that observed in the periphery, even if it is considered that glucoregulation is not the main function of insulin in the brain (for review see [37]).

Early and severe abnormalities of cerebral glucose metabolism parallel worsening of the symptoms of dementia [38,39]. Late-onset AD is associated with glucose abnormalities distributed over all cortical areas, and particularly in parietotemporal and frontal association cortices [40,41]. This hypometabolism in the cerebral cortex is particularly pronounced in structures with both high glucose demands and insulin sensitivity (for review see [42]).

Recently, Kim et al. [43] reported that glucose hypometabolism of early onset AD patients is much greater in magnitude and extent than that of late onset patients, though both groups are similar in dementia severity. When the authors compared the decline of glucose metabolism with the Clinical Dementia Rating (CDR) stage, the slope was steeper in early onset than in late onset AD suggesting that the greater hypometabolism in early onset patients is required to reach the same severity of dementia.

These abnormalities in cerebral glucose utilization include a diminished activity of key enzymes involved in intermediary metabolism notably the activity of glutamine synthetase, creatine kinase, aconitase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [44–46]. These enzymes are highly susceptible to oxidative modification and are altered by exposure to a range of pro-oxidants [47]. Reduced pyruvate dehydrogenase activity results in a decreased level of acetyl-CoA, and together with the diminished activity of choline acetyltransferase, the synthesis of acetylcholine in the presynaptic neuron is markedly reduced [48]. In this respect, it is noteworthy that the degeneration of the cholinergic system correlates with the progression of mental disturbances in patients with AD [49]. A decreased concentration of acetyl-CoA may also decrease the formation of intracellular cholesterol [50]. Cholesterol is the main sterol in membranes and is important for normal cell function. Cholesterol levels are markedly decreased in brain membranes and in the cerebrospinal fluid of AD patients [51–53]. Another decisive pathophysiological consequence of the markedly perturbed glucose metabolism is a decrease in ATP production from glucose by around 50% in the beginning of sporadic AD [54]. A fall in ATP formation in the sporadic AD brain has also been demonstrated by other investigators [55,56]. This energy deficit may compromise ATP-dependent processes in a hierarchical manner [57] including cellular and molecular mechanisms.

The most consistent defect in mitochondrial electron transport enzymes in AD has been a deficiency in cytochrome oxidase. There are several reports indicating a reduced cytochrome oxidase activity in AD platelets [58,59] and in *post mortem* brain tissue from patients with AD,

particularly in neurofibrillary tangle-bearing neurons [60,61]. Previous studies have also demonstrated a perikaryal accumulation of cytochrome oxidase protein, immunolocalized to cytosol by immunoelectron microscopy in the face of reduced numbers of intact mitochondria. These results suggested that enhanced degradation of mitochondria occurs in AD, leaving behind lysosomal detritus containing non-functioning mitochondrial components [62]. Studies with cybrid cells demonstrated that deficits in cytochrome oxidase in AD platelets could be transferred to Rho cells, which retain the cytochrome oxidase deficit [63,64]. Additionally the resulting cybrid cells showed markedly increased free radical production, impaired intracellular calcium buffering, elevated basal cytosolic calcium concentration, and enhanced sensitivity to inositol 1,4,5-triphosphate-mediated calcium release [63,64]. Recently, Crouch and colleagues [65] found that Aβ42 specifically inhibited cytochrome oxidase of human mitochondria in a dose-dependent manner this effect being dependent on the presence of Cu^{2+}. Altogether these data indicate that mitochondria dysfunction is a relevant event occurring in AD pathophysiology.

4. Mitochondrial dysfunction as a trigger of neuronal degeneration and death

Although the brain represents only 20% of the body weight; it receives 15% of cardiac output and accounts for 20% of total body oxygen consumption. This energy requirement is largely driven by neuronal demand for energy to maintain ion gradients across the plasma membrane that is critical for the generation of action potentials. This intense energy requirement is continuous; even brief periods of oxygen or glucose deprivation result in neuronal death.

Mitochondria are increasingly recognized as subcellular organelles that are essential for generating the energy that fuels normal cellular function while, at the same time, they monitor cellular health in order to make a rapid decision (if necessary) to initiate a programmed cell death. As such, the mitochondria sit a strategic position in the hierarchy of cellular organelles to continue the healthy life of the cell or to terminate it. These organelles are essential for neuronal function because the limited glycolytic capacity of these cells make them highly dependent on aerobic oxidative phosphorylation for their energetic needs. However, oxidative phosphorylation is a major source of endogenous toxic free radicals, including hydrogen peroxide (H_2O_2), hydroxyl (HO$^\cdot$) and superoxide (O_2^-) that are products of normal cellular respiration [66]. With the inhibition of electron transport chain, electrons accumulate in complex I and coenzyme Q, where they can be donated directly to molecular oxygen to give O_2^- that can be detoxified by the mitochondrial manganese superoxide dismutase (MnSOD) to give H_2O_2 that, in turn, can be converted to H_2O by glutathione peroxidase (GPx). However, O_2^- in the presence of nitric oxide (NO$^\cdot$), formed during the conversion of

arginine to citrulline by nitric oxide synthase (NOS), can originate peroxynitrite ($ONOO^-$). Furthermore, H_2O_2 in the presence of reduced transition metals can be converted to toxic $HO^.$ via Fenton and/or Haber Weiss reactions. Inevitably, if the amount of free radical species produced overwhelms the neuronal capacity to neutralize them, oxidative stress occurs, followed by mitochondrial dysfunction and neuronal damage. Reactive species generated by mitochondria have several cellular targets including mitochondrial components themselves (lipids, proteins and DNA). The lack of histones in mitochondrial DNA (mtDNA) and diminished capacity for DNA repair render mitochondria an easy target to oxidative stress events.

Mitochondria also serve as high capacity Ca^{2+} sinks, which allow them to stay in tune with changes in cytosolic Ca^{2+} loads and aid in maintaining cellular Ca^{2+} homeostasis that is required for normal neuronal function [67–69]. Conversely, excessive Ca^{2+} uptake into mitochondria has been shown to increase ROS production, inhibit ATP synthesis, release cytochrome c, and induce mitochondrial permeability transition [70–72]. The mitochondrial permeability transition (MPT) is defined as the sudden increase of inner mitochondrial membrane permeability to solutes of molecular mass less than 1500 Da [73,74]. Strong evidence now exists that the MPT is due to the opening of a nonselective megachannel (estimated to be 2–3 nm in diameter) [75,76]. Because the chemiosmotic theory is based on the inner membrane being impermeable to solutes that are not specifically transported, MPT would collapse the mitochondrial membrane potential ($\Delta\Psi m$) and uncouple the electron transport system from the production of ATP. Additionally MPT results in mitochondrial swelling and can lead to the release of proapoptotic proteins. Importantly, Ca^{2+}, Pi, oxidative stress, and low inner membrane potential promote the onset of MPT, whereas cyclosporin A (CsA), Mg^{2+}, ADP, and the existence of a high membrane potential oppose the onset [74,77].

Mitochondrial dysfunction and the resulting energy deficit trigger the onset of neuronal degeneration and death.

5. Mitochondrial impairment links diabetes to Alzheimer's disease

Increased oxidative stress has been implicated in the pathology of several diseases including diabetes and AD [78,79]. Evidence from the literature indicates that there is an increase in oxidative stress in human [80] and experimental diabetes [81,82] and a decrease in the antioxidant capacity [83,84].

Oxidative damage in rat brain is increased by experimentally induced hyperglycemia [85]. Schmeichel et al. [86] suggested that oxidative stress leads to oxidative injury of dorsal root ganglion neurons, mitochondria being a specific target. Recently, we observed that brain mitochondria isolated from streptozotocin (STZ) diabetic rats, a model of type 1 diabetes, possess a lower content of coenzyme Q9

(CoQ9) indicating a deficit in antioxidant defenses in diabetic animals and, consequently, an increased probability of oxidative stress occurrence [87]. The reduced form of CoQ may function as an antioxidant, protecting membrane phospholipids and serum low-density lipoprotein from lipid peroxidation by quenching lipid radicals or lipid peroxidation initiating species and, it also protects mitochondrial membrane proteins and DNA from free radical-induced oxidative damage [88–90].

Diabetes and AD are associated with impaired glucose utilization, deficits in mitochondrial activity and metabolic dysfunction [91–93]. Inhibition of cellular energy production has been shown to reduce or abolish both insulin secretion and action [94]. In addition, the decrement in oxidative phosphorylation (OXPHOS) efficiency is related to a loss in the control of glucose homeostasis as evidenced by the increase in tissue and blood lactate levels, as well as by the change in glucose tolerance. Cybrid cells constructed from individuals with maternally inherited diabetes exhibited lactic acidosis, poor respiration and marked defects in mitochondrial morphology and respiratory chain complex I and IV activities [95].

Diabetes mellitus leads to functional and structural changes in the brain, which appear to be most pronounced in the elderly. Furthermore, increased age is associated with insulin resistance [96]. Increasing data support the idea that mitochondrial function declines with aging and in age-related diseases such as diabetes and AD [92,97]. Data from our laboratory show the existence of an age-related impairment of the respiratory chain and an uncoupling of OXPHOS in brain mitochondria isolated from Goto-Kakizaki (GK) rats, a model of type 2 diabetes [98]. Furthermore, we also show that aging exacerbates the decrease in the energetic levels promoted by diabetes [98]. The maintenance of OXPHOS capacity is extremely important in the brain since about 90% of the ATP required for the normal functioning of neurons is provided by mitochondria. Because CNS depends so heavily on ATP production, the inhibition of OXPHOS will affect this system before any other system. For example, CNS requires a large amount of ATP for the transmission of impulses along the neural pathway, thus mitochondrial function impairment will result in neurodegeneration and loss in neuronal metabolic control [92,97].

$\Delta\Psi m$, which normally accounts for 80% of the proton-motive force, contributes for the high degree of reduction of the matrix $NADPH/NADP^+$ pool and, in turn, this pool helps to maintain the matrix glutathione pool in the reduced state. We observed that the maintenance of $\Delta\Psi m$ in mitochondria isolated from STZ rats is correlated with the unchanged content of reduced glutathione (GSH) [87]. GSH is abundant in mitochondria and is a first-line defense in the cellular antioxidant system. Baydas et al. [99] reported that although STZ diabetic rats present higher levels of lipid peroxidation in hippocampus, cortex and cerebellum as compared to control rats, no significant alterations are found in GSH levels in the same brain regions.

As previously discussed, mitochondria are also important cytoplasmic Ca^{2+} buffers since they avoid the increase of Ca^{2+} above a critical value termed "set-point". In oxidative stress conditions, a sustained increase in intracellular Ca^{2+} concentration occurs [100] and the cytosolic Ca^{2+} levels play a role in the modulation of several intracellular signalling pathways, including protein kinase C-α and calmodulin-dependent signalling [101], which have also been implicated in apoptotic processes. The cytosolic Ca^{2+} level can be increased by ROS in various cell types through the mobilization of intracellular Ca^{2+} stores and/or through the influx of extracellular Ca^{2+} [102]. The maintenance of Ca^{2+} homeostasis represents a major expenditure within neurons and, through respiratory control mechanisms, is tightly coupled to the rates of OXPHOS and the generation of ROS. We observed that diabetes decreases the capacity of mitochondria to accumulate Ca^{2+}, a favourable intracellular environment for MPT opening [87,98]. Furthermore, our data are in agreement with the "Calcium hypothesis" which first proposes that among the many biochemical and histological changes involved in brain aging and in age-related diseases, Ca^{2+} alteration is a central defect [103,104]. Accordingly, we observed that brain mitochondria of GK rats present an age-related susceptibility to Ca^{2+}, indicating that aging predisposes the diabetic rats' mitochondria to the opening of MPT. The MPT opening might be also associated with osmotic swelling of mitochondria leading to structural changes of these organelles. Indeed, in peripheral nerves of diabetic humans, the existence of mitochondrial ballooning and disruption of internal cristae is observed, although this is localized to Schawnn cells and is rarely observed in axons [105]. Similar structural abnormalities in mitochondria have been described in Schawnn cells of galactose-fed rats [105] and dorsal root ganglion neurons of long-term STZ diabetic rats [106]. One current hypothesis is that high glucose concentrations induce elevated levels of OXPHOS, resulting in damaging amounts of ROS that lead to changes in mitochondrial structure and function [107].

Accumulating evidence suggests that mitochondrial dysfunction is intimately associated with AD pathophysiology. Furthermore, Lustbader et al. [108] reported that Aβ interacts with Aβ-binding dehydrogenase (ABAD) in mitochondria obtained from AD patients and transgenic mice brains, which suggests that ABAD is a direct molecular link from Aβ to mitochondrial toxicity. More recently, the same group reported that ABAD enhances Aβ-induced cell stress via mitochondrial dysfunction [109]. Another study also showed that Aβ is present in mitochondria and, in the presence of copper, inhibits cytochrome oxidase [65]. AβPP has also been associated with the outer mitochondrial membrane [110]. Furthermore, it has been shoen that an IDE isoform, which regulates Aβ levels, is targeted to mitochondria [111]. There is also evidence that β-secretase is present in these organelles [112]. In addition, we have demonstrated that a functional mitochondria is required for

Aβ-induced neurotoxicity, as investigated using ρ+ and ρ0 mitochondrial DNA depleted cells [113].

Studies from our laboratory show that Aβ inhibits the respiratory chain complexes and reduces ATP levels in PC12 cells [114,115]. We also showed that Aβ40 and Aβ25-35 impair the respiratory chain, uncouple OXPHOS, decrease the energetic levels and exacerbate the susceptibility of isolated brain mitochondria to MPT opening [87,98,116]. However, we observed that Aβ exacerbates Ca^{2+}-induced opening of MPT without inducing the permeability *per se* [117,118]. Recently, we observed that CoQ10 treatment attenuates the decrease in OXPHOS efficiency induced by Aβ40 [116]. CoQ10 is a key component of the mitochondrial electron transport chain (ETC) that not only serves as the electron acceptor for complexes I and II of the ETC but is also a potent antioxidant. Indeed, recent findings from our laboratory show that CoQ10 treatment avoids the increase in H_2O_2 production induced by Aβ40 [116]. Previously, *in vitro* studies have shown that Aβ-mediated cell death in both neuronal and non-neuronal cells is mediated in part by the increase in cellular H_2O_2 [119] and that catalase has a protective role as an H_2O_2-degrading enzyme [120]. Furthermore, we observed that several other antioxidants (vitamin E, idebenone, and reduced glutathione), melatonin and nicotine showed protective effects by improving the activity of the respiratory chain complexes and maintaining $\Delta\Psi m$ and cellular energetic levels [113].

Recent findings [121] indicate that insulin is a major regulating factor of mitochondrial OXPHOS in human skeletal muscle. Previously, Boirie and collaborators [122] reported that insulin selectively stimulates mitochondrial protein synthesis in skeletal muscle and activates mitochondrial enzyme activity. However, a direct stimulatory action on ATP production was not shown. Our results are in accordance with these data because although we do not observe any significant change on ATP content, insulin treatment increases mitochondrial OXPHOS efficiency [87]. In this line, Gustafsson et al. [123] reported that (IGF-1) protects from hyperglycemia-induced oxidative stress and neuronal injuries by regulating $\Delta\Psi m$, possibly by the involvement of uncoupling protein 3 (UCP3). Similarly, Huang et al. [124] reported that insulin prevents depolarization of the mitochondrial inner membrane in sensory neurons of type 1 diabetic rats. Furthermore, insulin was capable to increase mitochondrial antioxidant defenses (CoQ9 content) that had been reduced by diabetes. Growing evidence suggests the importance of insulin and (IGFs) in intracellular antioxidant status by playing a pivotal role in protein kinase B-mediated expression of Bcl2 protein, that prevents the escape of ROS by opposing the oxidative-stress-induced pro-apoptotic action of Bax [125]. Another study showed that pre-treatment of cells with IGF-1 suppresses H_2O_2-induced apoptosis by subsequent inhibition of Bax expression [125,126]. Recently, Duarte et al. [127] reported that insulin protects cortical neurons against oxidative stress

this effect being due to the modulation of glutathione redox cycle.

Our data also indicate that insulin is capable to increase the capacity of mitochondria to accumulate Ca^{2+} suggesting a role of insulin in Ca^{2+} homeostasis. Moreover, it has been shown that insulin modulates the cellular clearance of Aβ [21] and IGF-1 protects neurons against its neurotoxic effects [128]. Recently, Rensink and colleagues [129] reported that insulin inhibits Aβ-induced cell death in cultured human brain pericytes. In accordance, our data indicate that insulin treatment also protects against mitochondrial injury induced by Aβ40 [87].

Data discussed above are consistent with the view that diabetes-related mitochondrial dysfunction is exacerbated by aging and/or by the presence of neurotoxic agents, such as Aβ, suggesting that diabetes and aging are risk factors for the neurodegeneration induced by these peptides. An association between diabetes and AD has long been recognized. Here we presented evidence that the association between diabetes and AD signifies a common underlying pathology, in this case, mitochondrial dysfunction. However, we also showed that mitochondrial dysfunction can be avoided/reduced by insulin and antioxidants. Although insulin does not affect basal mitochondria function, in the presence of Aβ insulin prevents a drastic decline in mitochondrial OXPHOS efficiency and avoids an increase in the oxidative stress, improving and/or preserving the function of neurons under adverse conditions. Given the importance of mitochondria as primary source of oxidative stress in AD and diabetes, the use of antioxidants may also be useful. However, the broad occurrence of both diseases, the non-regenerative nature of the CNS and the fact that AD diagnosis often does not occur until late in disease progression, suggest that the ideal antioxidant should be used as prophylactic treatment in aged population.

References

[1] Tanzi RE. A genetic dichotomy model for the inheritance of Alzheimer's disease and common age-related disorders. J Clin Invest 1999;104:1175–9.

[2] Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 2005;120:545–55.

[3] Glenner GG, Wong CW, Quaranta V, Eanes ED. The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. Appl Pathol 1984;2:357–69.

[4] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987;325:733–6.

[5] Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. J Alzheimers Dis 2001;3:75–80.

[6] Westermark P, Wilander E. The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia 1978;15:417–21.

[7] Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, et al. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in pancreas in type 2 diabetes. Diabetes Res 1988;9:151–9.

[8] Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci U S A 1987;84:8628–32.

[9] Westermark P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, Johnson KH. Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. Proc Natl Acad Sci U S A 1987;4:3881–5.

[10] Johnson KH, O'Brien TD, Hayden DW, Jordan K, Ghobrial HK, Mahoney WC, et al. Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic beta cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques. Am J Pathol 1988;130:1–8.

[11] Glenner GG, Eanes ED, Wiley CA. Amyloid fibrils formed from a segment of the pancreatic islet amyloid protein. Biochem Biophys Res Commun 1988;155:608–14.

[12] Nicolls MR. The clinical and biological relationship between type II diabetes mellitus and Alzheimer's disease. Curr Alzheimer Res 2004;1:47–54.

[13] Gispen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci 2000;23:542–9.

[14] Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, et al. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 2001;56:42–8.

[15] Ryan CM. Neurobehavioral complications of type I diabetes. Examination of possible risk factors. Diabetes Care 1988;11:86–93.

[16] Strachan MWJ, Deary IJ, Ewing FME, Frier BM. Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. Diabetes Care 1997;20:438–45.

[17] Brands AMA, Biessels GJ, De Haan EHF, Kappelle LJ, Kessels RPC. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 2005;28:726–35.

[18] Gasparini L, Netzer WJ, Greengard P, Xu H. Does insulin dysfunction play a role in Alzheimer's disease? Trends Pharmacol Sci 2002;23:288–93.

[19] Mandelkow EM, Drewes G, Biernat J, Gustke N, Van Lint J, Vandenheede JR, et al. Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. FEBS Lett 1992;314:315–21.

[20] Solano DC, Sironi M, Bonfini C, Solerte SB, Govoni S, Racchi M. Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. FASEB J 2000;14:1015–22.

[21] Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, et al. Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. J Neurosci 2001;21:2561–70.

[22] Hoyer S. The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade trigger late-onset sporadic Alzheimer disease (SAD). A mini-review. J Neural Transm 2002;109:991–1002.

[23] Phiel CJ, Wilson CA, Lee VM, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature 2003;423:435–9.

[24] McDermott JR, Gibson AM. Degradation of Alzheimer's beta-amyloid protein by human and rat brain peptidases: involvement of insulin-degrading enzyme. Neurochem Res 1997;22:49–56.

[25] Vekrellis K, Ye Z, Qiu WQ, Walsh D, Hartley D, Chesneau V, et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci 2000;20:1657–65.

[26] Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci U S A 2003;100:4162–7.

[27] Miller BC, Eckman EA, Sambamurti K, Dobbs N, Chow KM, Eckman CB, et al. Amyloid-beta peptide levels in brain are inversely

correlated with insulysin activity levels in vivo. Proc Natl Acad Sci U S A 2003;100:6221–6.

[28] Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ, Ericksen S, Roth RA, et al. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. Am J Pathol 2003;162:313–9.

[29] Perez A, Morelli L, Cresto JC, Castano EM. Degradation of soluble amyloid beta-peptides 1-–40, 1-42, and the Dutch variant 1-40Q by insulin degrading enzyme from Alzheimer disease and control brains. Neurochem Res 2000;25:247–55.

[30] Xie L, Helmerhorst E, Taddei K, Plewright B, Van Bronswijk W, Martins R. Alzheimer's beta-amyloid peptides compete for insulin binding to the insulin receptor. J Neurosci 2002;22:RC221.

[31] Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease-is this type 3 diabetes? J Alzheimers Dis 2005;7:63–80.

[32] Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. J Alzheimers Dis 2005;8:247–68.

[33] Hong M, Lee VM. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. J Biol Chem 1997;272:19547–53.

[34] Röder HM, Ingram VM. Two novel kinases phosphorylate tau and the KSP site of heavy neurofilament subunits in high stoichiometric ratios. J Neurosci 1991;11:3325–42.

[35] Bush ML, Miyashiro JS, Ingram VM. Activation of a neurofilament kinase, a tau kinase, and a tau phosphatase by decreased ATP levels in nerve growth factor-differentiated PC12 cells. Proc Natl Acad Sci U S A 1995;92:1962–5.

[36] Erecinska M, Silver IA. ATP and brain function. J Cereb Blood Flow Metab 1989;9:2–19.

[37] Gerozissis K. Brain insulin and feeding: a bi-directional communication. Eur J Pharmacol 2004;490:59–70.

[38] Hoyer S. Abnormalities of glucose metabolism in Alzheimer's disease. Ann N Y Acad Sci 1991;640:53–8.

[39] Fukuyama H, Ogawa M, Yamauchi H, Yamaguchi S, Kimura J, Yonekura Y, et al. Altered cerebral energy metabolism in Alzheimer's disease: a PET study. J Nucl Med 1994;35:1–6.

[40] Mielke R, Herholz K, Grond M, Kessler J, Heiss WD. Differences of regional cerebral glucose metabolism between presenile and senile dementia of Alzheimer type. Neurobiol Aging 1992;13:93–8.

[41] Herholz K, Schopphoff H, Schmidt M, Mielke R, Eschner W, Scheidhauer K, et al. Direct comparison of spatially normalized PET and SPECT scans in Alzheimer's disease. J Nucl Med 2002;43:21–6.

[42] Henneberg N, Hoyer S. Desensitization of the neuronal insulin receptor: a new approach in the etiopathogenesis of late-onset sporadic dementia of the Alzheimer type (SDAT)? Arch Gerontol Geriatr 1995;21:63–74.

[43] Kim EJ, Cho SS, Jeong Y, Park KC, Kang SJ, Kang E, et al. Glucose metabolism in early onset versus late onset Alzheimer's disease: an SPM analysis of 120 patients. Brain 2005;128:1790–801.

[44] Sorbi S, Bird ED, Blass JP. Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. Ann Neurol 1983;13:72–8.

[45] Kish SJ. Brain energy metabolizing enzymes in Alzheimer's disease: alpha-ketoglutarate dehydrogenase complex and cytochrome oxidase. Ann N Y Acad Sci 1997;826:218–28.

[46] Gibson GE, Park LC, Sheu KF, Blass JP, Calingasan NY. The alpha-ketoglutarate dehydrogenase complex in neurodegeneration. Neurochem Int 2000;36:97–112.

[47] Tretter L, Adam-Vizi V. Inhibition of Krebs cycle enzymes by hydrogen peroxide: A key role of α-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J Neurosci 2000;20:8972–9.

[48] Sims NR, Bowen DM, Allen SJ, Smith CCT, Neary D, Thomas DJ, et al. Presynaptic cholinergic dysfunction in patients with dementia. J Neurochem 1983;40:503–9.

[49] Baskin DS, Browning JL, Pirozzolo FJ, Korporaal S, Baskin JA, Appel SH. Brain choline acetyltransferase and mental function in Alzheimer disease. Arch Neurol 1999;56:1221–3.

[50] Michikawa M, Yanagisawa K. Inhibition of cholesterol production but not of nonsterol isoprenoid products induces neuronal cell death. J Neurochem 1999;2:2278–85.

[51] Svennerholm L, Gottfries CG. Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). J Neurochem 1994;62:1039–47.

[52] Mulder M, Ravid R, Swaab DF, de Kloet ER, Haasdijk ED, Julk J, et al. Reduced levels of cholesterol, phospholipids, and fatty acids in cerebrospinal fluid of Alzheimer disease patients are not related to apolipoprotein E4. Alzheimer Dis Assoc Disord 1998;12:198–203.

[53] Eckert GP, Cairns NJ, Maras A, Gattaz WF, Muller WE. Cholesterol modulates the membrane-disordering effects of beta-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease. Dement Geriatr Cogn Disord 2000;11:181–6.

[54] Hoyer S. Oxidative energy metabolism in Alzheimer brain. Studies in early-onset and late-onset cases. Mol Chem Neuropathol 1992;16:207–24.

[55] Sims NR, Bowen DM, Neary D, Davison AN. Metabolic processes in Alzheimer's disease: adenine nucleotide content and production of 14CO2 from [U-14C]glucose in vitro in human neocortex. J Neurochem 1983;41:1329–34.

[56] Brown GG, Levine SR, Gorell JM, Pettegrew JW, Gdowski JW, Bueri JA, et al. In vivo 31P NMR profiles of Alzheimer's disease and multiple subcortical infarct dementia. Neurology 1989;39:1423–7.

[57] Buttgereit F, Brand MD. A hierarchy of ATP-consuming processes in mammalian cells. Biochem J 1995;312:163–7.

[58] Parker Jr WD, Mahr NJ, Filley CM, Parks JK, Hughes D, Young DA, et al. Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. Neurology 1994;44:1086–90.

[59] Cardoso SM, Proença MT, Santos S, Santana I, Oliveira CR. Cytochrome c oxidase is decreased in Alzheimer's disease platelets. Neurobiol Aging 2004;25:105–10.

[60] Kish SJ, Bergeron C, Rajput A, Dozic S, Mastrogiacomo F, Chang LJ, et al. Brain cytochrome oxidase in Alzheimer's disease. J Neurochem 1992;59:776–9.

[61] Mutisya EM, Bowling AC, Beal MF. Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. J Neurochem 1994;63:2179–84.

[62] Castellani R, Hirai K, Aliev G, Drew KL, Nunomura A, Takeda A, et al. Role of mitochondrial dysfunction in Alzheimer's disease. J Neurosci Res 2002;70:357–60.

[63] Davis RE, Miller S, Hermstadt C, Ghosh SS, Fahy E, Shinobu LA, et al. Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer's disease. Proc Natl Acad Sci U S A 1997;94:4526–31.

[64] Swerdlow RH, Parks JK, Cassarino DS, Maguire DJ, Maguire RS, Bennett Jr JP. Cybrids in Alzheimer's disease: a cellular model of the disease? Neurology 1997;49:918–25.

[65] Crouch PJ, Blake R, Duce JA, Ciccotosto GD, Li QX, Barnham KJ, et al. Copper-dependent inhibition of human cytochrome c oxidase by a dimeric conformer of amyloid-1-42. J Neurosci 2005;25:672–9.

[66] Wallace DC. Mitochondrial diseases in man and mouse. Science 1999;283:1482–8.

[67] Ichas F, Mazat JP. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low-to high-conductance state. Biochim Biophys Acta 1998;1366:33–50.

[68] Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, Pozzan T. Mitochondria as biosensors of calcium microdomains. Cell Calcium 1999;26:193–9.

[69] Rizzuto R, Bernardi P, Pozzan T. Mitochondria as all-round players of the calcium game. J Physiol 2000;529:37–47.

[70] Jiang D, Sullivan PG, Sensi SL, Steward O, Weiss JH. Zn(2+) induces permeability transition pore opening and release of pro-apoptotic peptides from neuronal mitochondria. J Biol Chem 2001;276: 47524–9.

[71] Brustovetsky N, Brustovetsky T, Jemmerson R, Dubinsky JM. Calcium-induced cytochrome c release from CNS mitochondria is associated with the permeability transition and rupture of the outer membrane. J Neurochem 2002;80:207–18.

[72] Sullivan PG, Rabchevsky AG, Keller JN, Lovell M, Sodhi A, Hart RP, et al. Intrinsic differences in brain and spinal cord mitochondria: implication for therapeutic interventions. J Comp Neurol 2004;474: 524–34.

[73] Bernardi P. The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. Biochim Biophys Acta 1996;1275:5–9.

[74] Bernardi P, Broekemeier KM, Pfeiffer DR. Recent progress on regulation of the mitochondrial permeability transition pore; a cyclosporin-sensitive pore in the inner mitochondrial membrane. J Bioenerg Biomembr 1994;26:509–17.

[75] Szabo I, Bernardi P, Zoratti M. Modulation of the mitochondrial megachannel by divalent cations and protons. J Biol Chem 1992;267: 2940–6.

[76] Szabo I, Zoratti M. The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin A. J Biol Chem 1991;266: 3376–9.

[77] Hansson MJ, Mansson R, Mattiasson G, Ohlsson J, Karlsson J, Keep MF, et al. Brain-derived respiring mitochondria exhibit homoge-neous, complete and cyclosporin-sensitive permeability transition. J Neurochem 2004;89:715–29.

[78] Eckert A, Keil U, Marques CA, Bonert A, Frey C, Schüssel K, et al. Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease. Biochem Pharmacol 2003;66:1627–34.

[79] Yorek MA. The role of oxidative stress in diabetic vascular and neural disease. Free Radic Res 2003;37:471–80.

[80] Desco MC, Asensi M, Marquez R, Martinez-Valls J, Vento M, Pallardo FV. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. Diabetes 2002;51:1118–24.

[81] Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405–12.

[82] Reagan LP, Magarinos AM, Yee DK, Swzeda LI, Van Bueren A, McCall AL, et al. Oxidative stress and HNE conjugation of GLUT3 are increased in the hippocampus of diabetic rats subjected to stress. Brain Res 2000;862:292–300.

[83] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599–622.

[84] Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003;17:24–38.

[85] Aragno M, Brignardello E, Tamagno E, Gatto V, Danni O, Boccuzzi G. Dehydroepiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. J Endocrinol 1997;155: 233–40.

[86] Schmeichel AM, Schmelzer JD, Low PA. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. Diabetes 2003;52:165–71.

[87] Moreira PI, Santos MS, Sena C, Seiça R, Oliveira CR. Insulin protects against amyloid β-peptide toxicity in brain mitochondria of diabetic rats. Neurobiol Dis 2005;18:628–37.

[88] Beyer RE. The participation of coenzyme Q in free radical production and antioxidation. Free Radic Biol Med 1990;8:545–65.

[89] Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta 1995;1271: 195–204.

[90] Lenaz G. Role of mitochondria in oxidative stress and ageing. Biochim Biophys Acta 1998;1366:53–67.

[91] Kristal BS, Jackson CT, Chung HY, Matsuda M, Nguyen HD, Yu BP. Defects at center P underlie diabetes-associated mitochondrial dysfunction. Free Radic Biol Med 1997;22:823–33.

[92] Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. Neurochem Res 2001;26:739–64.

[93] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–25.

[94] Gerbitz KD, Gempel K, Brdiczka D. Mitochondria and diabetes. Genetic, biochemical, and clinical implications of the cellular energy circuit. Diabetes 1996;45:113–26.

[95] van den Ouweland JM, Maechler P, Wollheim CB, Attardi G, Maassen JA. Functional and morphological abnormalities of mitochondria harboring the tRNA (Leu) (UUR) mutation in mitochondrial DNA derived from patients with maternally inherited diabetes and deafness (MIDD) and progressive kidney disease. Diabetologia 1999;42:485–92.

[96] Hollenbeck CB, Reaven GM. Treatment of patients with non-insulin-dependent diabetes mellitus: diabetic control and insulin secretion and action after different treatment modalities. Diabet Med 1987;4:311–6.

[97] Orth M, Schapira HA. Mitochondria and degenerative disorders. Am J Med Genet 2001;106:27–36.

[98] Moreira PI, Santos MS, Moreno AM, Seiça R, Oliveira CR. Increased vulnerability of brain mitochondria in diabetic (Goto-Kakizaki) rats with aging and amyloid-beta exposure. Diabetes 2003;52:1449–56.

[99] Baydas G, Nedzvetskii VS, Tuzcu M, Yasar A, Kirichenko SV. Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. Eur J Pharmacol 2003;462:67–71.

[100] Biessels GJ, ter Laak MP, Hamers FPT, Gispen WH. Neuronal Ca(2+) disregulation in diabetes mellitus. Eur J Pharmacol 2002;447:201–9.

[101] Clapham DE. Calcium signaling. Cell 1995;80:259–68.

[102] Drögue W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.

[103] Khachaturian ZS. Calcium hypothesis of Alzheimer's disease and brain aging. Ann N Y Acad Sci 1994;747:1–11.

[104] Kostyuk E, Voitenko N, Kruglikov I, Shmigol A, Shishkin V, Efimov A, et al. Diabetes-induced changes in calcium homeostasis and the effects of calcium channel blockers in rat and mice nociceptive neurons. Diabetologia 2001;44:1302–9.

[105] Kalichman MW, Powell HC, Mizisin AP. Reactive, degenerative, and proliferative Schwann cell responses in experimental galactose and human diabetic neuropathy. Acta Neuropathol (Berl) 1998;95: 47–56.

[106] Sasaki H, Schmelzer JD, Zollman PJ, Low PA. Neuropathology and blood flow of nerve, spinal roots and dorsal root ganglia in longstanding diabetic rats. Acta Neuropathol (Berl) 1997;93:118–28.

[107] Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404: 787–90.

[108] Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, et al. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. Science 2004;304:448–52.

[109] Takuma K, Yao J, Huang J, Xu H, Chen X, Luddy J, et al. ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. FASEB J 2005;19:597–8.

[110] Anandatheerthavarada HK, Biswas G, Robin MA, Avadhani NG. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. J Cell Biol 2003;161:41–54.

[111] Leissring MA, Farris W, Wu X, Christodoulou DC, Haigis MC, Guarente L, et al. Alternative translation initiation generates a novel isoform of insulin-degrading enzyme targeted to mitochondria. Biochem J 2004;383:439–46.

[112] Hansson CA, Frykman S, Farmery MR, Tjernberg LO, Nilsberth C, Pursglove SE, et al. Nicastrin, presenilin, APH-1, and PEN-2 form active gamma-secretase complexes in mitochondria. J Biol Chem 2004;279:51654–60.

[113] Cardoso SM, Santos S, Swerdlow RH, Oliveira CR. Functional mitochondria are required for amyloid beta-mediated neurotoxicity. FASEB J 2001;15:1439–41.

[114] Pereira C, Santos MS, Oliveira C. Mitochondrial function impairment induced by amyloid beta-peptide on PC12 cells. Neuroreport 1998;9: 1749–55.

[115] Pereira C, Santos MS, Oliveira C. Involvement of oxidative stress on the impairment of energy metabolism induced by Abeta peptides on PC12 cells: protection by antioxidants. Neurobiol Dis 1999;6: 209–19.

[116] Moreira PI, Santos MS, Sena C, Nunes E, Seica R, Oliveira CR. CoQ10 therapy attenuates amyloid beta-peptide toxicity in brain mitochondria isolated from aged diabetic rats. Exp Neurol 2005;196: 112–9.

[117] Moreira PI, Santos MS, Moreno A, Oliveira C. Amyloid beta-peptide promotes permeability transition pore in brain mitochondria. Biosci Rep 2001;21:789–800.

[118] Moreira PI, Santos MS, Moreno A, Rego AC, Oliveira C. Effect of amyloid beta-peptide on permeability transition pore: a comparative study. J Neurosci Res 2002;69:257–67.

[119] Behl C, Davies JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 1997;77:817–27.

[120] Milton NGN. Inhibition of catalase activity with 3-amino-triaz-ole enhances the cytotoxicity of the Alzheimer's amyloid-β peptide. Neurotoxicology 2001;22:767–74.

[121] Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. Proc Natl Acad Sci U S A 2003;100:7996–8001.

[122] Boirie Y, Short KR, Ahlman B, Charlton M, Nair KS. Tissue-specific regulation of mitochondrial and cytoplasmic protein synthesis rates by insulin. Diabetes 2001;50:2652–8.

[123] Gustafsson H, Soderdahl T, Jonsson G, Bratteng JO, Forsby A. Insulin-like growth factor type 1 prevents hyperglycemia-induced uncoupling protein 3 down-regulation and oxidative stress. J Neurosci Res 2004;77:285–91.

[124] Huang TJ, Price SA, Chilton L, Calcutt NA, Tomlinson DR, Verkhratsky A, et al. Insulin prevents depolarization of the mitochondrial inner membrane in sensory neurons of type 1 diabetic rats in the presence of sustained hyperglycemia. Diabetes 2003;52: 2129–36.

[125] Hong F, Kwon SJ, Jhun BS, Kim SS, Ha J, Kim S-J, et al. Insulin-like growth factor-1 protects H9c2 cardiac myoblasts from oxidative stress-induced apoptosis via phosphatidylinositol 3-kinase and extracellular signal-regulated kinase pathways. Life Sci 2001;68: 1095–105.

[126] Napier JR, Thomas MF, Sharma M, Hodgkinson SC, Blass JJ. Insulin-like growth factor-I protects myoblasts from apoptosis but requires other factors to stimulate proliferation. J Endocrinol 1999;163:63–8.

[127] Duarte AI, Santos MS, Oliveira CR, Rego AC. Insulin neuroprotec-tion against oxidative stress in cortical neurons—involvement of uric acid and glutathione antioxidant defenses. Free Radic Biol Med 2005;39:876–89.

[128] Niikura T, Hashimoto Y, Okamoto T, Abe Y, Yasukawa T, Kawasumi M, et al. Insulin-like growth factor I (IGF-I) protects cells from apoptosis by Alzheimer's V642I mutant amyloid precursor protein through IGF-I receptor in an IGF-binding protein-sensitive manner. J Neurosci 2001;21:1902–10.

[129] Rensink AAM, Otte-Höler I, de Boer R, Bosch RR, ten Donkelaar HJ, de Waal RMW, et al. Insulin inhibits amyloid β-induced cell death in cultured human brain pericytes. Neurobiol Aging 2004;25:93–103.