Mitochondrial Uncoupling Proteins and Oxidative Stress: Implications for Diabetes and Neurodegeneration

Susana Cardoso[1,2], Renato X. Santos[1,2], Cristina Carvalho[1,2], Sónia Correia[1,2], Maria S. Santos[1,2], Paula I. Moreira[1,3]

[1]Center for Neuroscience and Cell Biology, [2]Department of Life Sciences – Faculty of Sciences and Technology, [3]Faculty of Medicine-Physiology, University of Coimbra, 3000-354 Coimbra, Portugal

ABSTRACT

Uncoupling proteins (UCPs) are a family of mitochondrial anion-carrier proteins located on the inner mitochondrial membrane, where they promote the dissociation of oxidative phosphorylation from respiration. The specific role of neuronal UCPs, UCP2, UCP4 and UCP5/BMCP1, has been widely discussed and although there is no general agreement, there is a strong conviction that these proteins through the mediation of a mild uncoupling may be involved in modulating mitochondrial reactive oxygen species (ROS) production. Mitochondrial ROS dysregulation due to altered production or decomposition of ROS has been linked to diabetes, obesity, neurodegenerative disorders and aging. Several lines of evidence thus suggests that through regulation of mitochondrial ROS production, UCPs activity can have a role as possible important brain damage modifiers in a way to control and limit the formation of free radicals. Here it will be considered the physiology and regulation of neuronal UCPs and their involvement in the regulation of ROS. Finally, neuronal UCPs as potential therapeutic targets especially in diabetes, Alzheimer´s and Parkinson´s diseases will also be discussed.

Keywords: Brain, diabetes, mitochondria, neurodegenerative diseases, oxidative stress, UCPs

Correspondence: Paula I. Moreira, Center for Neuroscience and Cell Biology, Faculty of Medicine-Physiology, University of Coimbra, 3000-354 Coimbra, Portugal.

E-mail: venta@ci.uc.pt/ pismoreira@gmail.com

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INTRODUCTION

Mitochondrial oxidative phosphorylation is responsible for generating cellular energy in the form of ATP. This process occurs via the coupling between the electron transport chain and oxidative phosphorylation. Energy sources such as glucose and fatty acids are metabolized in the cytoplasm and its products are imported into mitochondria originating two energy-rich electron donors, NADH and succinate.[1] Electrons from these molecules then flow along the electron transport chain in mitochondria through Complexes I to IV leading to the final reduction of oxygen to water. Coupled with electrons transport, protons are pumped from the matrix outward generating a potential difference across the inner membrane. The resulting potential energy produced by the proton gradient is used by Complex V (ATP synthase) that channels the protons back to the matrix to drive ADP phosphorylation to ATP.[2] However, this system is not perfect and a small proportion of unpaired electrons, generated from the respiratory chain reactions, interact with molecular oxygen forming superoxide anion ($O_2^{\bullet}$). $O_2^{\bullet}$ itself is relatively unreactive, however, it can directly damage proteins containing Fe–S centers, which includes Krebs cycle and electron transport chain components, and be readily interconvert to other reactive oxygen species (ROS), e.g., hydroxyl ions ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), causing oxidative stress.[3] The majority of free radicals are produced under normal physiological conditions, and they are rapidly sequestered by antioxidant enzyme systems within the mitochondria.[4] Mitochondrial dysfunction causes excessive production of these ROS leading to oxidative stress and cell death.[5]

In a perfect coupled system, protons only enter the mitochondrial matrix through ATP synthase in the presence of ADP; however oxidative phosphorylation

is not fully coupled and there is always a basal proton leak, which accounts for about 20-25% of the basal metabolic rate.[6,7] Mitochondrial uncoupling proteins (UCPs) are solute carriers located in the inner mitochondrial membrane that can uncouple biofuel oxidation from ATP synthesis by modulating the proton gradient across the inner mitochondrial membrane. Through this process, the ATP synthesis is uncoupled from the electron transport, dissipating energy in the form of heat.[6,8] Of five homologues, UCP4 and UCP5 (also known as brain mitochondrial carrier protein-1, BMCP1) are expressed mostly in neurons[9,10] and UCP2 is ubiquitously expressed.[11] UCPs differential expression in tissues suggests a range of physiological functions. The specific role of UCPs has been widely discussed and although there is no general agreement, there is a strong conviction that these proteins may be involved in the defense against ROS therefore protecting from oxidative damage. The first evidence came from a study made by Nègre-Salvayre and co-workers[12] revealing that the inhibition of UCP1 activates the formation of ROS in brown fat mitochondria. Subsequent data suggested that UCP activity may lead to an increase in proton conductance through the interaction with $O_2^{-\bullet}$[13] or ROS products.[14]

Mitochondrial ROS dysregulation due to altered production or decomposition of ROS has been linked to diabetes, obesity, neurodegenerative disorders and aging. The suggestion that an increased ROS production would lead to uncoupling that, in turn, would decrease ROS formation, placed neuronal UCPs as possible important brain damage modifiers in a way to control and limit the formation of free radicals. In this review it will be debated the physiology and regulation of neuronal UCPs. The involvement of these mitochondrial proteins in the regulation of ROS, especially in diabetes, Alzheimer´s and Parkinson´s disease will also be discussed.

MITOCHONDRIAL UNCOUPLING PROTEINS PHYSIOLOGY AND REGULATION

As first proposed by Mitchell,[15] the electrochemical proton gradient between the intermembrane space and the matrix of mitochondria is now known to be the driving force for ATP synthesis, this process being called coupling.[16] However, some mitochondrial carriers harness proton gradient for their function. ADP/ATP and glutamate/aspartate carriers use the electrical component and phosphate carrier and other carriers using substrate-H^+ symport use the pH gradient to dissipate the proton gradient across the inner mitochondrial membrane.[17] Additionally, under normal conditions, a portion of the H^+ gradient is consumed by proton backflow to the matrix via non-protein membrane pores or protein/lipid interfaces.[18] These mechanisms allow H^+ backflow to the matrix bypassing ATP synthase and thereby provoke protein-mediated respiration uncoupling.[19] Thus, uncoupling is an inherent part of mitochondrial physiology. The process of proton leak has been suggested to be involved in thermogenesis, regulation of energy metabolism or carbon fluxes, controlof body mass, and attenuation of ROS production.[20] As said before, UCPs are located in the inner mitochondrial membrane and function as proton carriers, being responsible for basal proton leak. UCP1 was the first UCP described and is almost exclusively expressed in brown adipose tissue (BAT). UCP1 is one of the major mitochondrial proteins responsible for heat generation in the newborn and may be involved in the normal response to cold stress in the adult.[21] There are two main proposed mechanisms to explain how UCP1 modulates proton leak across the mitochondria inner membrane. The first mechanism known as "proton-buffering model" implies a structural component binding and transferring H^+ across the mitochondria membrane; in this case UCP1 works similarly to ion channels.[17,22] The second mechanism, known as "fatty-acid-cycling model", was first proposed by Skulachev in 1991.[23] According to this mechanism, the charged fatty-acid (FA) anion is protonated in the mitochondria intermembrane space, which results in an electrochemically neutral molecule. The neutral FA "flip-flops" across the membrane to the matrix side and because of the pH difference becomes deprotonated. The charged FA anion is thereafter transported by UCP1 back across the membrane to the intermembrane space where the cycle can start all over again.[8,23-25] Nevertheless, these two hypotheses are still under investigation and there are actually arguments for and against both models.[17]

Since 1997, some genes that encode proteins closely related to UCP1 have been discovered: UCP2, UCP3, UCP4, and BMCP1/UCP5.[17] All UCPs are expressed at significantly lower levels in their respective tissues compared with UCP1 in BAT, suggesting that the function of these UCP isoforms may not be thermogenesis.[16] UCP2 is 59% identical to UCP1 and is widely expressed in spleen, lung, stomach, white adipose tissue and also in brain.[26] UCP3 that is mainly expressed in skeletal muscle[27] possesses approximately

57% and 73% of similarity with UCP1 and UCP2, respectively. Finally, UCP4[28] and BMCP1[29] are mainly expressed in the central nervous system being 34% and 30% identical to UCP1, respectively, and BMCP1 is the only UCP found in the parenchymal cells of the liver.[30] All the UCPs share in common a tripartite structure that consists of three repeats of approximately 100 amino acids, each one containing two hydrophobic stretches that correspond to transmembrane alpha helices that span the phospholipids bilayer of the mitochondrial inner membrane. The two attached alpha helices are linked by a long hydrophilic loop, which is oriented toward the mitochondrial matrix side. UCPs have a monomer molecular weight of about 30 kDa with both the N- and the C-terminal ends oriented toward the cytosolic side of the inner mitochondrial membrane. The functional unit of UCPs is believed to be a homodimer formed by two identical subunits that contain 12 transmembrane helices.[31-33] One important concern is that the regulation of UCP mRNA expression may not correspond to the levels of protein expression, and so mRNA data cannot be used as predictive of UCP protein levels or activity.[16] Evidence has revealed that although UCP2 is well expressed in many tissues at the mRNA level, this is not followed by changes in protein expression.[9] The reason for this discrepancy between mRNA and protein levels is not known but it suggests posttranscriptional regulation of UCP expression.

The proton-conductance of UCP1 is tightly regulated through inhibition by purine nucleotides at physiological concentrations, with GDP being the experimentally favored nucleotide (but ADP and ATP are also active). This inhibition is overcome by FA released from intracellular triacylglycerol stores following adrenergic activation in response to cold.[34,35] The two most UCP1-like proteins, UCP2 and UCP3, were also assumed to be involved in proton transport and nucleotide binding,[4] especially because all amino acid residues involved in GDP binding in UCP1 are conserved in UCP2/UCP3 and, indeed, nucleotides do experimentally inhibit UCP2/UCP3.[36] Further studies demonstrated that proton-conductance of UCP2 and UCP3 is only catalyzed when specifically activated[37] by $O_2^{-\bullet}$,[13] hydroperoxy FA[35] or other activators.[38] Indeed, it has been previously proposed by Echtay and colleagues[13] a simple feedback cycle in which the protons transport of UCPs is acutely upregulated by mitochondrial oxidative stress to lower the membrane potential and thus decrease $O_2^{-\bullet}$ production (Figure 1). Additional data reported that mice lacking UCP2 have higher $O_2^{-\bullet}$ production in islets and other tissues.[39-41] Evidence from studies of UCP2 function also suggest that UCP2 is not activated directly by $O_2^{-\bullet}$ but rather, the peroxidation of lipids initiated by $O_2^{-\bullet}$ leads to an increase in UCP2-mediated proton translocation.[42] The lipid peroxide-mediated activation of UCP2 has been proposed to promote egress of FA

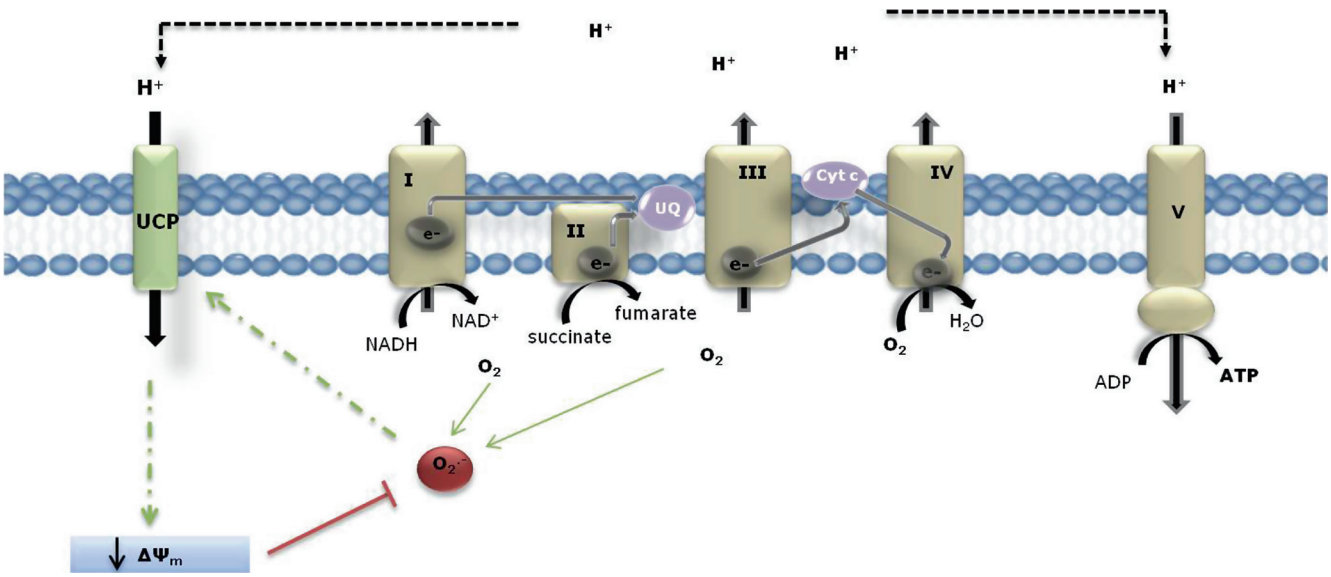

Figure 1. Potential neuroprotective mechanism of neuronal UCPs. The increased production of superoxide anion ($O_2^{-\bullet}$) activates the mitochondrial uncoupling proteins (UCPs) that function as uncouplers by acting as a channel for proton entry into the mitochondrial matrix dissipating the transmembrane potential ($\Delta\Psi_m$) generated by respiratory Complexes I-IV. This mild uncoupling will lead to diminished $O_2^{-\bullet}$ production, protecting the cell against oxidative stress and damage. UQ: coenzyme Q; Cyt c: cytochrome c; Complex I: NADH ubiquinone oxidoreductase; Complex II: succinate ubiquinone oxidoreductase; Complex III: ubiquinone-cytochrome c reductase; Complex IV: cytochrome c oxidase; Complex V: ATP synthase.

peroxides from the matrix to the intermembrane space of the mitochondria, where they cause less damage to mitochondrial DNA, aconitase and other mitochondrial matrix-localized components.[25] This was supported by data from Jaburek and colleagues,[35] who reported (a) that linoleic acid hydroperoxide induces purine nucleotide-sensitive H^+ uniport in liposomes containing UCP2, and (b) that the same compound caused a fast flip-flop-dependent acidification of liposomes. Authors thus suggested that UCP2 may transport peroxidized FAs and mediate a FA peroxide-cycling mechanism.[35]

The lipid hydroperoxide derivative, 4-hydroxynonenal (HNE), induced by high ROS levels, can act as a signal to induce UCPs-mediated uncoupling since it was demonstrated that HNE effects were inhibited by GDP.[14] Interestingly, HNE effects were also inhibited by the ANT specific inhibitor atractyloside (CAT) and the inhibitory effect of GDP and CAT were not additive allowing the authors to conclude that HNE-induced increase in H^+ conductance activates both UCP and ANT to form a heterodimer, which is sensitive to both inhibitors.[14] Therefore, it is assumed that through regulation of mitochondrial ROS production, uncoupling activity can have a role in cellular metabolism and signal transduction, since several critical signaling pathways have redox-sensitive components.

MITOCHONDRIA, OXIDATIVE STRESS AND NEURONAL UNCOUPLING PROTEINS

Mitochondria are essential organelles for neuronal function because the limited glycolytic capacity of these cells makes them highly dependent on aerobic oxidative phosphorylation for their energetic needs.[43] However, it has been established that ROS production is also inherent to mitochondrial oxidative metabolism.[44,45] In the brain, the Krebs cycle mainly generates NADH and succinate, which in turn are oxidized in reactions catalyzed by several enzyme complexes located in the inner membrane of mitochondria.[45] A small proportion of the electrons flowing through Complexes I and III react with oxygen forming $O_2^{-\bullet}$,[46] a primary ROS that is rapidly dismutated into H_2O_2 utilizing both manganese superoxide dismutase (MnSOD), which is located to the mitochondria and copper-zinc superoxide dismutase (Cu-ZnSOD) mainly found in the cytosol. H_2O_2 is promptly converted to H_2O via catalase and glutathione peroxidase, but has the potential to be converted to the highly reactive $^\bullet OH$ via the Fenton reaction, which will damage all classes of biomolecules underlying ROS-mediated neurotoxicity.[4,44,47] Mitochondrial ROS formation is controlled by factors affecting and reflecting the metabolic state of intact mitochondria.[45] Within mitochondrial phospholipid bilayer, the antioxidants coenzyme Q and vitamin E are involved in lipid peroxidation prevention, coenzyme Q being also involved in vitamin E recycling and it is regenerated by the respiratory chain.[4] When antioxidant defense system, comprised by enzymantic and non-enzymatic antioxidant defenses, is unable to neutralize excessive ROS production, an imbalance favoring oxidative stress occurs. Excessive ROS formation will attack unsaturated FAs in membranes leading to lipid peroxidation and the production of HNE that conjugates to membrane proteins impairing their function.[48] Mitochondrial bioenergetics and redox state are also influenced by intracellular Ca^{2+} levels being mitochondria capable of promoting Ca^{2+} uptake into the matrix, driven by mitochondrial membrane potential ($\Delta\Psi_m$).[49] Within mitochondrial matrix increased levels of Ca^{2+} can affect mitochondrial ROS release and subsequent oxidative stress.

Aging is a universal process and the major risk factor for several neurodegenerative disorders including Parkinson´s (PD), and Alzheimer´s (AD) diseases. Mitochondrial $O_2^{-\bullet}$ is the first free radical generated by the oxidative phosphorylation system and its production has been associated to oxidative damage underlying several degenerative diseases and aging.[50] There are two main hypotheses regarding oxidative stress and aging; the "rate of living" theory[51] and the "uncoupling to survive" theory.[52] In 1956, Harman proposed the free radical theory of aging postulating that free radicals play a central role in the aging process. According to this theory, mitochondria are damaged by ROS and therefore produce more ROS, creating a vicious cycle that increases in function of age. The primary targets of ROS that show accumulated oxidative damage or dysfunction over time include macromolecules (e.g., nuclear and mitochondrial DNA, lipids, and proteins), which, in turn, affect mechanisms such as apoptosis, protein turnover, and multiple mitochondrial functions.[3]

The other theory regarding oxidative stress and aging, the "uncoupling to survive" theory[52] is based on the hypothesis that ineffective ATP production must exist to decrease mitochondria ROS production. This theory relies upon the existence of basal proton leak across the inner mitochondrial membrane and the fact that mice with long lifespan and high metabolic rates also

present a higher degree of mitochondrial uncoupling and decreased $\Delta\Psi_m$.[53] Indeed, it has been demonstrated that human UCP2 targeted expression in mitochondria of adult fly neurons promotes an increase in state 4 of respiration, a decrease in ROS production and oxidative damage accompanied with an extension in life span without the compromise of fertility or physical activity.[54] Nevertheless, as discussed by Friederich and colleagues,[7] both theories can be interconnected into one theory defending that the protection against ROS production and decreased formation by the mitochondria are key factors in determining lifespan.

Mitochondrial ROS production is closely linked to $\Delta\Psi_m$ such that hyperpolarization (high $\Delta\Psi_m$) increases and promotes ROS production.[55] While long-term, complete uncoupling of mitochondria would be detrimental, it has been hypothesized that mild uncoupling could be beneficial since it causes a decrease in ROS production.[55] The evidence for the relation of $\Delta\Psi_m$ and ROS generation came from a study in isolated succinate-supported heart mitochondria, where the addition of uncouplers decreased the rate of ROS emission.[56] Furthermore, this effect was evident only in a narrow $\Delta\Psi_m$ range and only in well coupled, highly polarized mitochondria, where decrease in $\Delta\Psi_m$ by only 10 mV resulted in 80% decrease in the rate of ROS generation.[56]

Neuronal mitochondrial uncoupling proteins have been shown by some *in vitro* and *in vivo* studies to play an important role in the regulation of $O_2^{\bullet-}$ formation protecting the cells from oxidative injury. Despite the presence of three UCPs isoforms in brain, UCP2 has been the isoform analysed in the major part of studies concerning brain damage and neurodegeneration.

In vitro *studies*

It has been demonstrated that neuronal UCP2 has an important role as an uncoupler of oxidative phosphorylation limiting oxidative injury to brain tissue. In isolated mitochondria, purine nucleotides (ATP, ADP, GTP, and GDP) have been shown to inhibit $O_2^{\bullet-}$-induced and UCP2- or UCP3-dependent proton leak.[13] However, purine nucleotides are not cell permeable and therefore are unable to inhibit UCPs when added to intact cells. To overcome this issue, it was recently found a compound, genipin that is able to inhibit UCP2 in intact cells thus allowing the investigation of UCP2 biology.[57] Although, UCP2-mediated genipin effects in neuronal cells have not been reported yet, its addition to pancreatic islet cells was shown to inhibit UCP2-mediated proton leak, increase $\Delta\Psi_m$ and ATP levels, plasma membrane K-ATP channels closure, and insulin secretion stimulation in a UCP2-dependent manner.[57] In cancer cells, mitochondria are metabolically abnormal and the induction of UCP2 represents one of the many adaptive mechanisms underlying chemotherapeutic resistance whereas genipin, through interaction with UCP2 rendered drug-resistant cancer cells more sensitive to ROS-producing chemotherapeutic agents.[58]

Liu and colleagues[59] demonstrated that UCP4 has an important role in promoting neuronal survival. Indeed, the authors observed that PC12 cells overexpressing UCP4 present a reduction in mitochondrial oxidative phosphorylation and ROS production that result from the UCP4-induced metabolic shift associated with enhanced glucose uptake and glycolysis to compensate for reduced mitochondrial ATP production.[59] Thus, UCP4 activity makes neural cells less reliant on mitochondrial respiration for maintenance of energy levels. In order to establish the function of UCP4 in neurons, UCP4 gene silencing by RNA interference (RNAi) was performed in cultured rat hippocampal neurons. It was observed that UCP4 silencing decreased neuronal survival.[59] Additionally, Chan and colleagues[60] demonstrated that human UCP4 expression in PC12 cells regulates mitochondrial Ca^{2+} sequestration and entry but not its release. Furthermore, UCP4 expression decreased the magnitude of sustained elevation in intracellular Ca^{2+} concentration after cellular Ca^{2+} depletion, inhibited mitochondrial Ca^{2+} overload and oxidative stress thereby preventing cell death.[60]

In the brain BMCP1/UCP5 is present in the cortex, basal ganglia, substantia nigra, cerebellum, and spinal cord. Data show that neuronal cell lines transfected with BMCP1/UCP5 had higher state 4 of respiration and lower $\Delta\Psi_m$, revealing greater mitochondrial uncoupling.[61] It was also observed a reduction in mitochondrial ROS production.[61] Moreover, the exposure of neurons overexpressing BMCP1 to linoleic acid further enhanced state 4 of respiration while the addition of bovine serum albumin prevented this augmentation,[61] supporting the notion of UCPs activation by free FAs.

In vivo *studies*

In normal brain, UCP2 mRNA expression is localized mainly in specific brain regions, which is up-regulated after injury.[62] Previous studies show an increased UCP2 gene expression, both in brain and cerebellum after

lipopolysaccharide (LPS) administration whereas no changes were observed in BMCP1 mRNA levels in LPS-injected mice.[63] Mattiason and co-workers[64] found that transgenic mouse overexpressing human UCP2 protein subjected to ischemic preconditioning are protected against a severe ischemic insult presenting enhanced neurological recovery. The authors found that UCP2 promote a shift in H_2O_2 release from the mitochondrial matrix to the extramitochondrial space, where it can be degraded by some antioxidant enzymes.[64] Recently, Liu and colleagues[65] also demonstrated that ischemic preconditioning caused increased expression of UCP2 in rat hippocampus that conferred protection against ischemia/reperfusion injury. Treatment with SOD at the time of ischemia preconditioning attenuated the increase of UCP2 staining, therefore implying a role for $O_2^{\bullet-}$-induced UCP2 expression.[65] Interestingly, UCP2 knockout mice have an increased resistance to cerebral ischemia that is associated with reduced oxidative injury and an increase of neuronal antioxidant state.[66] It was also shown that UCP2 mRNA induction was temporally associated with changes of mitochondrial glutathione levels following ischemia in mice. The authors suggested that a chronic adaptation to the lack of UCP2 occurs, which may contribute to the reduction of ischemic and oxidative injury in UCP2 knockout mice.[66] Therefore, interventions aimed to maintain mitochondrial homeostasis may provide new directions to prevent or attenuate acute central system injury. Accordingly, the reduction of dietary fat in immature animals rapidly reduces neuronal UCP2 expression/activity leading to increased mitochondrial ROS production.[67] These changes decreased animals resistance to excitotoxic insults resulting in increased neuronal death.[67] It has also been reported an increased UCP2 and UCP5 expression in ischemic lesions in brain slice sections obtained from embolic stroke and multiple infarction brains.[68] Moreover, UCP5 expression in the lesions was higher in multiple infarction cases than in embolic stroke brain suggesting that UCP5 may respond to repetitive ischemic stresses or have a long-term effect,[68] thus implicating a neuroprotective role for UCPs in neuronal injury.

DIABETES AND NEURODEGENERATION: UCPS AS POTENTIAL THERAPEUTIC TARGETS AGAINST OXIDATIVE STRESS

The brain is extremely sensitive to oxidative damage due to its high oxygen demand, high content of oxidisable polyunsaturated FAs, the presence of redox-active metals and low activity of antioxidant enzymes.[11,69,70] Despite the fact that neurodegenerative disorders have disparate clinical features, they are characterized by mitochondrial dysfunction and oxidative stress.[2,70,71] Due to UCPs ability to regulate both mitochondrial metabolic efficiency and free radical generation, they are of special interest in diabetes and neurodegenerative pathologies, namely AD and PD.

Diabetes

When the β-cells of the pancreas "sense" an increase in glucose levels they synthesize and secrete insulin into the circulation.[72] The binding of insulin to its receptors regulate the uptake of glucose from the circulation in order to be stored or used directly as fuel. Any alteration in this process will critically lead to diabetes mellitus. In diabetic patients, the occurrence of chronic hyperglycemia and/or hypoglycemia has deleterious effects in the brain, being associated with a decline in cognitive performance[73,74] and directly implied in the aging process and age-related disorders.[75,76] Indeed, hyperglycemia, impaired insulin and insulin-like growth factor type 1 (IGF-1) signaling have been proposed as pathogenic factors contributing to AD.[77] Due to this characteristics AD was named "Type 3 diabetes".[77] Diabetes, namely hyperglycemia, leads to an oversupply of electrons in the electron transport chain that results in mitochondrial membrane hyperpolarization and ROS formation,[78,79] being mitochondrial energy metabolism dysfunction and oxidative stress recognized as main players in diabetes and related complications.[80,81] Previous studies also indicate that hypoglycemia potentiates brain injury by inducing mitochondrial dysfunction and oxidative stress and damage.[82,83] The suggestion that UCPs have a role in diabetes, perhaps acting as a protective mechanism against excessive $O_2^{\bullet-}$ production came from studies in pancreatic β-cells,[84] endothelial cells,[85] kidney[86,87] and heart.[88,89] Concerning the role of neuronal UCPs in diabetes-induced brain damage, the knowledge is scarce. Although UCP3 expression was thought to occur mainly in the muscle, a recent study shows that UCP3 is also normally present in dorsal root ganglion (DRG) neurons.[90] Moreover, UCP3 levels were decreased in DRG neurons isolated from streptozotocin-induced diabetic animals while UCP3 overexpression in cultured DRG neurons was able to prevent glucose-induced mitochondrial hyperpolarization, ROS formation and induction of programmed cell death.[90] Also, the human neuroblastoma

cell line SH-SY5Y when exposed to high concentrations of glucose present a down-regulation of UCP3 protein expression and an increase in $\Delta\Psi_m$ and intracellular ROS.[91] In opposite, the addition of IGF-1, which positively regulates UCP3 expression,[92,93] prevented glucose-induced neurite degeneration and UCP3 down-regulation leading to ROS levels and $\Delta\Psi_m$ normalization.[93] Thus, it seems that the modulation of UCPs expression and/or activity could be a promising strategy to decrease ROS production and/or prevent their damaging effects in hyperglycemia-induced neuronal injury. More studies must be done to clarify this matter.

Alzheimer´s disease

AD is a progressive age-dependent neurodegenerative disorder and the most common form of dementia, accounting for 50-70% of dementia cases. While less than 5% of AD cases are familial and associated with mutations in amyloid β precursor protein (APP) and presenilins 1 and 2 (PS1 and PS2), the majority of AD cases are sporadic in origin and involve genetic and environmental factors that taken alone are not sufficient to develop the disease.[94] This neurodegenerative disease is characterized by progressive cognitive decline and the presence of Aβ plaques and neurofibrillary tangles constituted mainly by hyperphosphorylated tau protein.[95,96] Abnormal APP processing is believed to play a central role in AD. APP may be metabolized along two distinct pathways: the amyloidogenic and the non-amyloidogenic pathways. In the latter, APP is cleaved by an α-secretase producing non-amyloidogenic proteins. In the amyloidogenic pathway, APP is cleaved by β- and γ-secretases producing Aβ peptides.[97] It has been reported that Aβ oligomers are potent synaptotoxins, which block the proteasome function, inhibit mitochondrial activity, increase oxidative stress, and alter intracellular Ca^{2+} levels leading to synaptic dysfunction.[98]

Recent data from postmortem brain tissue from AD patients with different degrees of severity demonstrated that this neurodegenerative disease is associated with impairments in mitochondrial gene expression, namely in complex IV of the mitochondrial respiratory chain, increased levels of p53 gene expression and increased molecular indexes of oxidative stress, such as up-regulation of nitric oxide synthase (NOS) and NADPH-oxidase (NOX). Additionally, the authors performed real time quantitative RT-PCR studies and found that in the brain, UCP4 and UCP5 expression is approximately 200 and 800-fold higher, respectively, than UCP 2 expression,[99] thus suggesting a protective role of UCP4 and UCP5 against oxidative stress under normal circumstances. However, in AD brains UCPs gene expression decreased significantly relative to control brains and the mean levels tended to be lower in AD brains with higher grades of neurodegeneration.[99] So, the failure to maintain normal levels or increase the expression of UCPs may potentiate oxidative stress leading to progressive mitochondrial DNA damage and energy depletion.

It was also shown an increase in UCP4 expression levels in the cortex and hippocampus of mice undergoing dietary restriction.[59,100] Since dietary restriction has been shown to be neuroprotective in animal models of AD,[101] it was suggested that neuronal UCPs may have a role against chronic neurodegenerative diseases.[102,103]

Parkinson´s disease

PD is the second most common neurodegenerative disorder that begins by causing motor dysfunction but ultimately affects the mind and personality.[95] This disease is clinically characterized by progressive rigidity, bradykinesia and tremor and pathologically by the degeneration of pigmented neurons in the substantia nigra and by the presence of intraneuronal proteinaceous cytoplasmic inclusions that immunostain for α-synuclein and ubiquitin, designated Lewy Bodies.[47,95] It is well established that oxidative stress and mitochondrial dysfunction are associated with the degeneration of dopaminergic neurons in PD.

The involvement of mitochondrial dysfunction in PD arose from the finding that 1-methyl 4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a synthetic opiate, cause parkinsonism in drug addicted individuals.[104] MPTP is metabolized to MPP^+ in glial cells and this metabolite inhibits the Complex I of the mitochondrial respiratory chain.[104] Horvath and colleagues[105] have previously shown that coenzyme Q (CoQ) therapy in a PD animal model induces mitochondrial uncoupling in the substantia nigra preventing MPTP-induced cell death. Moreover, it seems that CoQ protective effects is mediated by UCP2 activation[105] Later on, Andrews and co-workers[106] demonstrated that dopamine neurons sensitivity to MPTP is increased in UCP2 knockout mice, whereas UCP2 overexpression decreased MPTP-induced nigral dopamine cell loss by increasing mitochondrial uncoupling and decreasing ROS production. Furthermore, electron microscopic analysis revealed that the substantia nigra from UCP2 knockout mice had significantly lower

number of mitochondria compared with control.[106] Similarly, Conti and colleagues[107] showed that transgenic mice overexpressing UCP2 in catecholaminergic neurons present increased uncoupling of their mitochondria and a reduction in oxidative stress markers, which contributed to neuroprotection and retention of locomotor functions after MPTP exposition. These results suggest that UCP2 is an essential homeostatic protein regulating cell survival and vulnerability to harmful toxins. The role of UCP2 in PD was also explored in normal nigrostriatal dopamine function and studies show that mice lacking UCP2 exhibited reduced dopamine turnover in the striatum, tyrosine hydroxylase immunoreactivity in the substantia nigra, striatum and nucleus accumbens and dopamine transporter immunoreactivity in the substantia nigra.[108] Accordingly, UCP2 knockout mice exhibited reduced total movement distance, movement velocity and increased rest time compared with wild type rats[108] suggesting UCP2 involvement in the maintenance of normal nigrostriatal dopamine neuronal function. Recently, the same authors reported that UCP2 mediates ghrelin-induced neuroprotection against the loss of dopaminergic neurons of the substantia nigra after MPTP treatment through alterations in mitochondrial respiration, ROS production, and biogenesis.[108]

The role of neuronal UCP5 was investigated in a neuroblastoma cell line exposed to MPTP.[109] The authors observed that UCP5 knockdown increased caspase 3 levels and cell death. UCP5 knockdown also increased cytotoxicity induced by low doses of MPTP but had no effects in oxidative stress and membrane depolarization.[109] However, in the presence of high doses of MPTP, UCP5 knockdown exacerbated cytotoxicity, and increased oxidative stress and mitochondrial membrane polarization,[109] demonstrating the protective role of UCP5 in oxidative stress-induced neurodegeneration.

CONCLUSION

Mitochondrial free radical production has been pointed out as an underlying cause of many pathological processes and aging. The production of ATP through the oxidative phosphorylation of intermediate substrates is coupled to production of ROS that in excess will have a deleterious role in cellular function. Several lines of evidence suggest that the interaction between ROS and UCPs represents a protective mechanism aimed to decrease the levels of free radicals inside the mitochondria and reduce its consequent deleterious effects. Research involving neuronal UCPs is still an emerging field and more studies are needed to clarify the role of these proteins. Nevertheless, the results obtained in experimental models indicate that their neuroprotective and neuromodulatory roles open a promising avenue to develop better therapies to prevent or treat diabetes and neurodegenerative disorders.

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