

# Mitochondrial bioenergetics and neurodegeneration: a *paso doble*

Alice Rossi\*, Paola Pizzo\*

**Mitochondria and neuronal activity:** The brain is one of the highest energy demanding organs, consuming ~20% of the total ATP produced by the whole body. Importantly, neurons mainly rely on ATP synthesized by mitochondrial bioenergetics and neuronal activity is strictly dependent on specific mitochondrial localization at synapses, sites consuming a high amount of energy requested for both pre- and post-synaptic processes. Here, mitochondria produce ATP and buffer  $\text{Ca}^{2+}$  rises, two essential processes for neurotransmission and generation of membrane potential along the axon (Magistretti and Allaman, 2015).

Mitochondrial ATP synthesis is driven by two major energy pathways: (1) the tricarboxylic acid cycles (TCA, also known as Krebs cycle), taking place in the mitochondrial matrix and using substrates produced in the cytosol (i.e., throughout glycolysis); (2) the oxidative phosphorylation, fuelled by the respiratory electron transport chain (ETC) at the inner mitochondrial membrane (IMM). ETC activity generates the mitochondrial membrane potential, coupling the activity of the four complexes with that of the ATP synthase, thus allowing ATP production. Importantly, the proper mitochondrial utilization of substrates produced in the cytosol, together with a correct mitochondrial  $\text{Ca}^{2+}$  uptake, are key features essential to sustain the bioenergetics of these organelles. Indeed, the import of substrates into the mitochondrial matrix is crucial for the proceeding of the TCA cycle and, consequently, for the ETC activity; equally, mitochondrial  $\text{Ca}^{2+}$  influx plays a pivotal role in the regulation of mitochondria metabolism, because many mitochondrial enzymes involved in ATP production and several metabolite transporters are regulated by  $\text{Ca}^{2+}$  (Rossi et al., 2019).

Alterations in mitochondrial  $\text{Ca}^{2+}$  handling, mitochondrial substrate import and organelle dynamics, as well as an increase in oxidative stress leading to mitochondrial damage, cause defects in energy production requested by several neuronal activities.

Furthermore, mitochondrial metabolism has been shown to be important for brain health. Especially under stress conditions, when neurons are highly activated and a large amount of energy is needed, impaired mitochondria, unable to supply cellular ATP demand, cause alterations in neuronal excitability eventually leading to  $\text{Ca}^{2+}$  overload and cell death. The accumulation of damaged mitochondria, and consequently of dysfunctional neurons, cause, over the long term, neurodegeneration. Indeed, mitochondrial alterations, and in particular defects in bioenergetic pathways, have been widely reported to be a key factor contributing to neurodegenerative diseases, such as amyloid lateral sclerosis, Parkinson's disease and Alzheimer's disease (AD).

**The “mitochondrial cascade hypothesis” for AD:** AD, a deleterious neurodegenerative disorder, is mainly sporadic; however, a small percentage of cases is due to autosomal dominant mutations in genes encoding the amyloid precursor protein and two homologous

transmembrane proteins, Presenilin 1 (PS1) and 2 (PS2), mainly localized at endoplasmic reticulum (ER), but also Golgi apparatus and endosomal membranes. On one hand, they constitute the catalytic core of the  $\gamma$ -secretase, the enzymatic complex that efficiently cleaves APP generating amyloid- $\beta$  (A $\beta$ ) peptides. These latter products, in pathological conditions due to mutation-driven alterations of  $\gamma$ -secretase enzymatic activity, accumulate with different molecular weights, culminating in an overall cellular toxicity underlying the onset and progression of AD. On the other hand, however, PSs have several independent cellular functions; among them, they have been showed to regulate  $\text{Ca}^{2+}$  homeostasis. In particular, PS2 mutants, linked to familial form of AD (FAD), have been implicated in  $\text{Ca}^{2+}$  homeostasis alterations, reducing the ER  $\text{Ca}^{2+}$  content and consequently affecting the ER to mitochondria  $\text{Ca}^{2+}$  transfer (Zampese et al., 2011; Rossi et al., 2020).

So far, based on the most accepted theory for AD pathogenesis in the last 30 years, the “amyloid cascade hypothesis”, the majority of the proposed therapies for the disease aimed at reducing and slowing the progression of neurodegeneration mainly by targeting the generation/accumulation of toxic A $\beta$  peptides. Unfortunately, all these treatments failed to slow the pathology and to prevent mental decline.

In the last decade, a new hypothesis for disease pathogenesis came to light, the “mitochondrial cascade hypothesis” (Swerdlow et al., 2014). Indeed, several mitochondrial defects have been observed in AD cases, both sporadic and familial, i.e., alterations in mitochondrial morphology, dynamics and movement, oxidative stress, metabolism and mitophagy. Any of these dysfunctional processes could lead to synaptic deficits and cell death with critical consequences not only for single neurons but also for the more complex structure of the brain.

Focusing on mitochondrial metabolism and using different AD models (based on FAD PS1 or PS2 mutations, as well as sporadic AD models), several groups observed impairments in mitochondrial functionality resulting in a defective cell bioenergetics (Swerdlow et al., 2014). Importantly, bioenergetic alterations appear at the early stage of the disease, even before A $\beta$  plaque and neurofibrillary tangle accumulation, the two main hallmarks of this neurodegenerative disorder. However, in this scenario, molecular mechanisms that could explain the observed bioenergetic defects have been poorly investigated. In order to develop effective new therapeutic strategies, unravelling the cascade of events that drives AD progression and, in particular, the events occurring during early phases of the disease, is extremely important.

**Mitochondrial bioenergetics as key factor to sustain neuronal function:** By investigating the mitochondrial dysfunction observed in AD, we recently reported that FAD-PS2 mutants affect the overall mitochondrial bioenergetics.

In details, using several AD models (cell lines expressing FAD-PS2 mutants, primary cortical neurons from FAD-PS2-N141I transgenic mice and FAD-PS2-N141I patient derived-fibroblasts), we observed a reduction in the total ATP produced by mitochondria, as well as in the oxygen consumption rate, an index of ETC activity (Rossi et al., 2020). This energetic impairment was due to a defective pyruvate oxidation by mitochondria caused by two synergic mechanisms: 1) a reduced mitochondrial  $\text{Ca}^{2+}$  uptake, as a consequence of a lower ER  $\text{Ca}^{2+}$  content caused by FAD-PS2 mutants (Zampese et al., 2011), resulting in a dampened TCA cycle progression and ETC activity; 2) an impairment in mitochondrial pyruvate uptake, due to a reduced expression of functional mitochondrial pyruvate carriers (MPC) (Rossi et al., 2020).

Under physiological conditions, pyruvate bridges cytosolic (glycolysis) with mitochondrial metabolism; indeed, once produced in the cytosol through glycolysis, pyruvate crosses first the outer mitochondrial membrane (OMM), through voltage-dependent anion channels (VDACs) that, in turn, can be associated with hexokinase1 (HK1), a cytosolic enzyme that, besides being the first glycolytic enzyme, is dynamically associates with VDAC, likely regulating its channel permeability (Robey and Hay, 2006). Once in the intermembrane space, pyruvate can cross the IMM via MPC, entering the TCA cycle taking place in the matrix. Interestingly, we observed a reduced HK1-mitochondria association in several FAD cellular models, as well as in wild-type cells treated with A $\beta$  oligomers, extending the validity of our results also to sporadic AD cases. Moreover, during our attempts to investigate the mechanism responsible for HK1 detachment from mitochondria, we detected high levels of phosphorylated GSK3 $\beta$  (the active form of the enzyme), a feature constantly reported in AD (Llorens-Martin et al., 2014). Of note, GSK3 $\beta$  has been already shown to phosphorylate VDAC, in this form unable to efficiently bind HK1 (Robey and Hay, 2006). In agreement, we observed a reduced mitochondrial pyruvate utilization in our AD models. In particular, the mitochondrial pyruvate flux was reduced, leading to lower amounts of pyruvate in the matrix and impaired respiration. Noteworthy, the bioenergetic defect could be fully rescued by the use in these cells of methyl-pyruvate, a metabolite analogue able to freely cross both the OMM and the IMM. Molecularly, the defective mitochondrial pyruvate flux was due to lower expression of the MPC2 subunit (but equal levels of MPC1), leading to reduced functional heterodimers of the pyruvate carrier at the IMM (Rossi et al., 2020). Interestingly, GSK3 $\beta$  inhibition by two different drugs (LiCl and AR-014418) rescued the MPC2 expression level and improved mitochondrial ATP synthesis and oxygen consumption rate, proving that the GSK3 $\beta$ -VDAC-HK1-MPC pathway is involved in the mitochondrial defects found in our AD models.

As an important functional consequence of the defective mitochondrial bioenergetics, FAD-PS2 neurons showed a reduced capacity to face high  $\text{Ca}^{2+}$  workflows, with an increased sensitivity to glutamate-induced excitotoxicity (indeed recovered by supplying cells with methyl-pyruvate). Thus, marked glutamate-induced toxicity, a feature at the basis of neuronal death and consistently reported in different neurodegenerative diseases, could be caused by a common, primary and underlying mitochondrial bioenergetic defect induced by

different molecular mechanisms in the diverse neurodegenerative pathologies (Plotegher et al., 2020; Rossi et al., 2020).

As far as AD is concerned, a possible scenario involving mitochondrial associated membranes can be envisaged. These membrane domains have been reported to be altered in AD and represent the place where several proteins actually work causing the mitochondrial defect. Indeed, PS2, VDAC as well as GSK3 $\beta$  are enriched at mitochondrial associated membranes (Szabadkai et al., 2006; Filadi et al., 2016; Bantug et al., 2018), where also A $\beta$  can be produced and accumulated (Schreiner et al., 2015); moreover, both PSs and A $\beta$  oligomers have been reported to interact with GSK3 $\beta$ , favoring its activity (Llorens-Martin et al., 2014). Therefore, we can speculate that, at mitochondrial associated membranes, a pool of hyper-activated GSK3 $\beta$ , induced by PSs or A $\beta$  peptides, causes the phosphorylation of VDAC and the detachment of HK1 from mitochondria. Possibly, HK1-VDAC (localized at the OMM) forms a functional complex with MPC (at the IMM), necessary to stabilize and make the metabolite carrier functional. The HK1 detachment from VDAC will ruin the HK1-VDAC-MPC complex eventually destabilizing MPC heterodimers, thus reducing the capability of mitochondria to take up and utilize pyruvate, leading to bioenergetic crises (Figure 1).

Shedding light on the molecular mechanisms undergoing mitochondrial bioenergetic dysfunctions will open the possibility for the discovery and the development for new therapeutic approaches, aimed at preventing and slowing down the origin and the progression of the specific disease.

Finally, the described AD mitochondrial bioenergetic defect could be linked to a

cellular metabolic rewiring, possibly resulting in systemic alterations in specific metabolic profiles. A detailed metabolic fingerprint characterization of AD patient-derived biofluid samples might offer the possibility to discover new disease biomarkers helpful for AD diagnosis at early stages, years before the appearance of the first cognitive symptoms.

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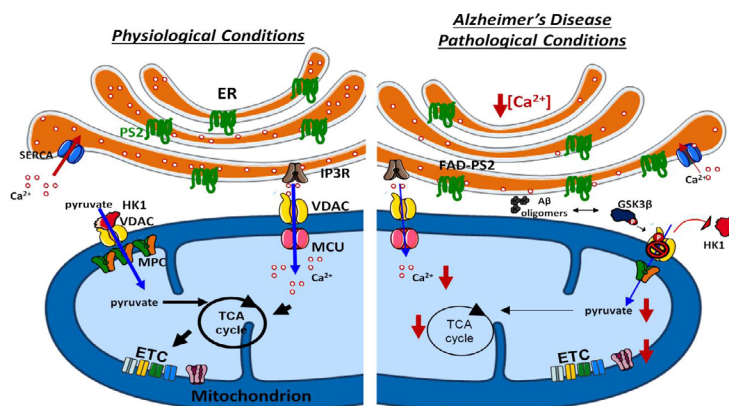
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**Figure 1 | Molecular mechanisms inducing defective mitochondrial bioenergetics in Alzheimer's disease.**

In physiological conditions, mitochondrial ATP production is stimulated by both a correct ER to mitochondria Ca<sup>2+</sup> transfer and mitochondrial import of substrates produced in the cytosol (e.g., pyruvate). In particular, upon ER Ca<sup>2+</sup> release through IP3R, mitochondria can take up Ca<sup>2+</sup> through VDAC (at the OMM) and MCU (at the IMM). In parallel, once pyruvate is produced in the cytosol by glycolysis, it is imported inside mitochondria crossing first the OMM through VDAC associated with HK1, and then the IMM thanks to MPC activity. The amount of both Ca<sup>2+</sup> and pyruvate that can reach the mitochondrial matrix is a key factor for TCA cycle regulation and ETC activity, modulating energy production. In pathological conditions (Alzheimer's disease), the level of ATP produced by mitochondria is reduced due to a dampened Ca<sup>2+</sup> signal and lower pyruvate uptake within mitochondria. Indeed, in FAD-PS2 expressing cells, ER [Ca<sup>2+</sup>], and consequently ER-mitochondria Ca<sup>2+</sup> shuttling, are reduced, negatively regulating bioenergetics. Moreover, both FAD-PS2 mutants and A $\beta$  oligomers, present at the organelle interface, induce GSK3 $\beta$  activation, leading to a higher phosphorylation of VDAC and detachment of HK1. The mitochondrial detachment of HK1, destabilizing MPC functionality, impairs mitochondrial pyruvate uptake and affects mitochondrial activity and ATP production. A $\beta$ : Amyloid- $\beta$ ; ER: endoplasmic reticulum; ETC: electron transport chain; FAD: familial Alzheimer's disease; GSK3 $\beta$ : glycogen synthase kinase 3 $\beta$ ; HK: hexokinase1; IMM: inner mitochondrial membrane; IP3R: inositol trisphosphate receptor; MCU: mitochondrial Ca<sup>2+</sup> uniporter; MPC: mitochondrial pyruvate carrier; OMM: outer mitochondrial membrane; PS2: presenilin 2; SERCA: sarco-endoplasmic reticulum calcium ATPase; TCA cycle: tricarboxylic acid cycle; VDAC: voltage-dependent anion channel.