Pharmacological Research 99 (2015) 410–424

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

# Controlled and Impaired Mitochondrial Quality in Neurons: Molecular Physiology and Prospective Pharmacology

Ivana Matic[a,1], Daniela Strobbe[a,1], Michele Frison[b], Michelangelo Campanella[a,b,c,*]

[a] Department of Biology, University of Rome TorVergata, 00133 Rome, Italy
[b] RVC Department of Comparative Biomedical Sciences, University of London and UCL Consortium for Mitochondrial Research, Royal College Street NW1 0TU, London, United Kingdom
[c] Regina Elena-National Cancer Institute, 00144 Rome, Italy

ARTICLE INFO

*Article history:*
Received 20 February 2015
Received in revised form 27 March 2015
Accepted 27 March 2015
Available online 23 April 2015

*Chemical compounds studied in this article:*
BTB06584 (PubChem CID: 2799764)
Creatine (PubChem CID: 586)
Cyclosporine A (PubChem CID: 5284373)
CoQ10 (PubChem CID: 5281915)
Vitamin C (PubChem CID: 54670067)
Vitamin E (PubChem CID: 2116)
MitoQ (PubChem CID: 11388331)
PMI (tbc)

*Keywords:*
Mitochondria
Autophagy
Neurons
Neurodegeneration

ABSTRACT

Tuned mitochondrial physiology is fundamental for qualitative cellular function. This is particularly relevant for neurons, whose pathology is frequently associated with mitochondrial deficiencies. Defects in mitochondria are indeed key features in most neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS).

When mitochondrial coupling impairs, so does cell metabolism, trafficking and the signaling depending on the homeostasis of the mitochondrial network. Moreover, the quality control of mitochondria – via the process of mitochondrial autophagy – results biased in neurodegeneration stemming major interest on the molecular determinants of this process among neuroscientists.

In this review, we highlight the most notable and acknowledged deficiencies of mitochondrial function and their relationship with diseases occurring in neurons and their transmission.

The physiological aspects of mitochondrial biology in relation to bio-energy, dynamics and quality control will be discussed with the finality to form a comprehensive picture of the mitochondrial contribution to the pathophysiology of neurodegenerative syndromes. In this way we aim to set the scene to conceive novel strategies to better diagnose and target these debilitative conditions.



## 1. Introduction

Mitochondria are dynamic organelles essential for cellular housekeeping functions such as metabolism of amino acids (urea cycle), fatty acids and steroids, Calcium ($Ca^{2+}$) fluxes and apoptosis regulation [1–3] along with their most prominent role [4,5] of cellular energy source via the production of the adenosine triphosphate (ATP).

Quality mitochondria are therefore core to the wellbeing of cells and tissues, particularly in those with delicate bio-energetic equilibrium as the neurons that are post-mitotic, non-proliferating cells highly susceptible to deterioration and decay. The brain is the third most energy-expensive organ in the human body, ranking just below skeletal muscle and liver [6]. Its metabolic requirements are illustrated by a 20% consumption of the body's energy despite it makes up only 2% of the body's weight.

Neurons are heavily dependent on mitochondria to generate the energy [7] required to carry out demanding processes such as: (i) axonal transport of macromolecules and organelles, (ii) maintaining ionic gradients, (iii) loading and releasing neurotransmitters. It is therefore not surprising that impaired mitochondrial function affects neuronal fate and a persisting damaging condition of the organelle results in neurodegeneration.

Neurodegenerative diseases are a heterogeneous group of diseases characterized by gradually progressive, selective loss of anatomically or physiologically related neuronal systems leading to clinical manifestations in the cognition, movement, strength, coordination, sensation, vision, or autonomic control. Hitherto, the link between mitochondrial abnormalities and neurodegeneration has been clinically documented and confirmed, when possible, in

* Corresponding author at: RVC Department of Comparative Biomedical Sciences, University of London and UCL Consortium for Mitochondrial Research, Royal College Street NW1 0TU, London, United Kingdom. Tel.: +44 2074681239; fax: +44 20 7468 5204.
*E-mail address:* mcampanella@rvc.ac.uk (M. Campanella).
[1] These authors have equally contributed.

http://dx.doi.org/10.1016/j.phrs.2015.03.021
1043-6618/

cellular and animal models [8–19]. The unveiling of the hierarchical cascades that upon mitochondrial damage drive neurons to dysfunction and neurodegenerative outbreak yielded a new level of advanced comprehension and identification of molecular targets. However, the advanced understanding of the molecular physiopathology has not been followed by an adequate progression of the pharmacology limiting the full therapeutic exploitation of potential targets [20–22].

Cells have evolved quality preserving systems of their mitochondria which: (a) degrade or export single misfolded proteins from the mitochondrial milieu such as via the ubiquitin-proteasome system (UPS) [23] or (b) remove the entire inefficient population via a process of targeted autophagy known as mitochondrial autophagy or mitophagy [23–25]. Dysregulation of these pathways is thus linked to the pathogenesis of neurodegenerative disorders [26,27] even though the actual role of autophagy per se in neurodegeneration is far from resolved.

This review focuses on this and, by incorporating the most recent findings on the reciprocal interactions between mitochondrial dynamics and mitophagy, aims to provide a summary of the most promising therapeutic mitochondria-related candidates and chemical compounds in neurodegenerative diseases.

## 2. Mitochondrial dysfunction and neurodegeneration

Neurons are very sensitive to damages occurring at mitochondrial level and the hypothesis for which the oxidative stress is a central feature in neurodegeneration is supported by the clinical evidences reporting lipids and proteins peroxidation in post-mortem brain of patients diagnosed with degenerated Central Nervous System (CNS) [28,29]. The mechanisms leading to the mitochondrial impairment in the neurodegenerated brain seem to be various spanning molecular mutations, ablation, inefficient signaling or accumulation of toxic components thus making the recurring oxidative stress a con-causative feature consequent subtle and persistent modifications here outlined.

***Alzheimer's disease (AD)*** is a progressive and irreversible pathology that represents the most common form of dementia among elderly people [30]. It is classified into sporadic AD and familial AD (FAD), where sporadic AD accounts for the majority of case, with aging being the most relevant known risk factor, whereas only 10% are FAD with autosomal-dominant transmission [30]. A major neuropathological feature of AD is the progressive death of neurons in brain regions, which is caused by the presence of intraneuronal neurofibrillary tangle (NFTs) and extracellular β-amyloid plaques. NFTs consist of abnormally phosphorylated tau protein, which is polymerized into paired helical filaments (PHFs), whereas amyloid plaques are composed of β-amyloid peptides ($A\beta_{41-42}$), which polymerize into insoluble fibrils with high β-sheet content. Even though the Aβ-aggregation process has been considered to be the most relevant phenomenon implicated in the etiology of AD, evidences have reported also the role played by specific Aβ accumulation in the mitochondria [10,31]. Aβ is produced from the metabolism of amyloid precursor protein (APP) through sequential cleavage by β- and γ-secretases and several models have documented that APP is targeted to mitochondria, where it interacts with the translocases of the outer and inner mitochondrial membrane (TOM/TIM complex), driving the import of mitochondrial proteins [10,31] (Fig. 1). Intramitochondrial Aβ interacts with amyloid-β-binding alcohol dehydrogenase (ABAD) ending up in producing Reactive Oxygen Species (ROS) that in turn impair the enzymatic activity of complex IV [31–33].

The APPgather in the mitochondria as Aβ aggregation via TIM/TOM complex providing a direct link between AD and the pathological dysfunction of mitochondria implicated in etiology of AD. The latter include the impairment of Complex IV, the inhibition of mitochondrial ABAD, the collapse of mitochondrial membrane potential ($\Delta\Psi$), ROS production and, consequently, lipids peroxidation and oxidation of mitochondrial DNA (mtDNA).

In addition, in both transgenic mouse model and brains isolated from AD patients it has been shown that Aβ peptide interacts with cyclophilin D (CypD). CypD represents an integral part of the mitochondrial permeability transition pore (mPTP) whose opening leads to cell death and its interaction with Aβ potentiates mitochondrial stress damaging neurons and hence synapes [34].

Blockade of CypD may therefore represent a novel therapeutic strategy in AD, since the CypD-deficient cortical mitochondria are resistant to Aβ induced permeability transition and collapse of the mitochondrial membrane potential ($\Delta\Psi_m$) (Fig. 1). Notably, CypD deficiency improves learning and memory, and the synaptic function in an AD mouse model and alleviates Aβ-mediated reduction of long-term potentiation [31,34].

***Parkinson's disease (PD)*** is the second most common neurodegenerative disease after AD, affecting over 4 million people. It exhibits pronounced degeneration of the dopaminergic neurons in the substantia nigra and formation of fibrillar cytoplasmic inclusions, known as Lewy bodies, containing ubiquitin and α-synuclein [35–38]. It is characterized by bradykinesia, tremor, rigidity and impaired postural reflexes, and in addition, mental disorders like depression and psychosis or autonomic and gastrointestinal dysfunction may occur [38]. Roughly 10% of total PD cases are thought to stem from mutations in nuclear genes encoding mitochondrial proteins such as: *SNCA* (also known as *PARK1*) and *LRRK2* (also known as *PARK8*) genes encoding for α-synuclein and leucine-rich repeat serine/threonine-protein kinase 2 respectively. They are responsible for autosomal-dominant form of PD, whereas mutations in *PARK2*, *PINK1* (also known as *PARK6*) and *PARK7* genes encoding for the cytosolic E3 ubiquitin ligase Parkin (), PTEN-induced putative kinase 1 (PINK1), and deglycase protein DJ-1 (), cause the disease in an autosomal-recessive manner. Mutations in *PINK1* and *PARK2* are directly related to impairment of the mitochondrial quality control via autophagy (see paragraph below) reassembled in the idiopathic forms of PD establishing the involvement of mitochondria in the pathogenesis of PD [39]. Quite notably, a number of chemical toxins such as the pesticide rotenone, the herbicide paraquat, ethidium bromide, the 1-methyl-4 phenyl- 1,2,3,6-tetrahydropyridine (MPTP) are all capable of selectively damaging dopaminergic neurons contributing to PD like conditions by inducing an acute ATP deficiency and an increased generation of ROS including superoxide anion radical ($O_2^{\bullet -}$) through inhibition of complex I [37,40–43]. When complex I is inhibited, electrons remain in the matrix site and oxygen ($O_2$) reduced to produce $O_2^{\bullet -}$ and consequent oxidative stress [40,44] (Fig. 2).

The major PD-associated proteins, including the serine peptidase HtrA2, PINK1, Parkin and DJ1, are implicated in mitochondrial functions. HtrA2 precursors contain a mitochondrial-targeting sequence (MTS). In response to stress, HtrA2is phosphorylated by p38 in a PINK1-dependent manner and imported into mitochondria where it binds to the Bcl2-family related protein Hax1. Active HtrA2, which is proteolytically processed by the mitochondrial presenilins-associated rhomboid-like protein PARL, is implicated in the degradation of misfolded proteins and prevents apoptosis (oligomerisation of BAX). Under oxidative conditions PINK1 interacts with and phosphorylates the chaperone TRAP that inhibits cyt*c* release and promotes the correct assembly of proteins. Parkin is also implicated in the mitochondrial stress response targeting dysfunctional mitochondrial for mitophagy. DJ1 could protect Complex I (CI) from oxidative-stress mediated inactivation. Therefore mutations in these genes exhibit decreased complex I activity, an acute