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Review article

Mitochondrial calcium imbalance in Parkinson's disease

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ABSTRACT

Multiple factors are involved in the mechanism(s) of neuronal loss in neurodegenerative disorders whilst mitochondria are thought to play a central role in neurodegeneration of Parkinson's disease. Mitochondria are vital to cellular functions by supplying energy in form of ATP and affect cell physiology via calcium, ROS and signalling proteins. Changes in mitochondrial calcium homeostasis and ROS overproduction can induce cell death by triggering mitochondrial permeability transition pore opening. One of the major triggers for PTP is mitochondrial calcium overload. Mitochondrial Ca^{2+} homeostasis is regulated by electrogenic calcium uptake (via Ca^{2+} uniporter MCU) and efflux (in excitable cells via Na^{+}/Ca^{2+} exchanger NCLX). NCLX inhibition has been described in a familial form of Parkinson's disease where PINK-1 deficiency leads to a delayed calcium efflux and mitochondrial Ca^{2+} overload in response to physiological Ca^{2+} stimulation. Overexpression of NCLX in PINK-1 deficient neurons not only protects against mitochondrial calcium overload and calcium induced cell death but also restores mitochondrial bioenergetics in these neurons. Mitochondrial NCLX might therefore play an important role in the mechanism(s) of neurodegeneration in a variety of neurodegenerative disorders and activation of this exchanger may offer a novel therapeutic target.

1. Introduction

The most common neurodegenerative disorders, Alzheimer's disease (AD) and Parkinson's disease (PD), are progressive and incurable diseases affecting elderly people. Considering the ageing population worldwide, this represents a serious cost to society. Many years after these diseases were first described, much has been learnt about the pathology and pathogenesis of these diseases, but a number of gaps in our understanding remain. Only by understanding the pathogenic mechanisms that underlie PD and AD, can therapeutic strategies be designed to halt or slow disease progression, rather than merely treat the symptoms. Chronic elevated Ca^{2+} levels, triggered by altered Ca^{2+} transient handling is a major pathological hallmark of PD. The Ca^{2+} dysregulation has been reported to affect cellular signalling and damage mitochondria resulting in cell death [1,2]. This review will focus on Ca^{2+} dysregulation and the direct consequences for mitochondrial health in PD.

1.1. Physiological calcium homeostasis

Ca^{2+} signalling is fundamentally important to neuronal and glial cells and might represent either a mediator or a manifestation of pathological processes in the CNS [3,4]. Ca^{2+} controls and coordinates a diverse array of physiological functions within the cell such as muscle contraction, proliferation and neurotransmission. The importance of Ca^{2+} as a secondary messenger molecule was first described by Ringer in 1883 who accidentally discovered that isolated hearts require Ca^{2+} for contraction [5]. Subsequent studies in the 20th century underlined the importance of Ca^{2+} in physiology [6]. Ca^{2+} oscillations are vital for synaptic transmission and depolarisation which increases free cytosolic Ca^{2+} through the influx of Ca^{2+} from the extracellular space. For neurons, it is crucial to buffer excessive Ca^{2+} from the cytosol at the time of signal transmission. Therefore, Ca^{2+} levels are tightly controlled by calcium-buffering proteins, such as calbindin and calmodulin, and intracellular stores [7].

Elevated cytosolic Ca^{2+} can be buffered by mitochondria and ER, or extruded in to the extracellular space via Na^{+}/Ca^{2+} exchangers (NCX) and Ca^{2+} ATPase [8,9]. Three plasma membrane NCX isoforms have been identified (NCX1, NCX2 and NCX3) where NCX1 is globally expressed (elevated expression in heart and skeletal muscle) and NCX2/3 are highly expressed in brain tissue. NCX in reverse mode is thought to be neuroprotective under pathophysiological conditions such as ischemia and excitotoxicity [10].

Mitochondria are responsible for the "fine-tuning" of Ca^{2+} transients and mitochondrial Ca^{2+} influx aids the bioenergetic status of the cell [11]. Mitochondria are strategically placed throughout the cell and mitochondrial Ca^{2+} influx stimulates calcium-dependant dehydrogenases, which use NADH/FADH2 and activate the electron

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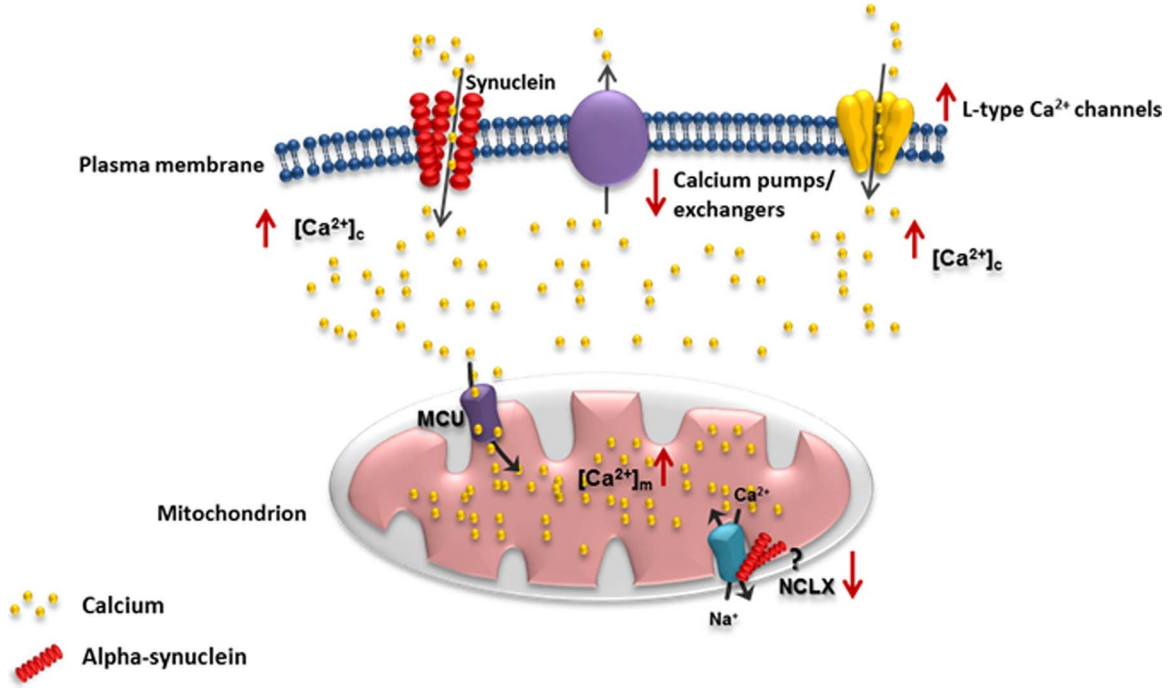

Fig. 1. Ca^{2+} dysregulation in Parkinson's disease. Many mechanisms have been described to induce Ca^{2+} dysregulation observed in PD. Alpha-synuclein itself has been shown to form a pore in the plasma membrane leading to increased calcium influx [35]. Furthermore, inhibition of Ca^{2+} exchangers and plasma membrane pumps may contribute to an elevation of intracellular Ca^{2+} [64]. In PD, a sustained engagement L-type Ca^{2+} channels has been reported that further elevates intracellular Ca^{2+} levels [32]. The elevated cytosolic Ca^{2+} levels are likely to increase mitochondrial Ca^{2+} which has negative downstream effects on mitochondrial health. Furthermore, α-synuclein has also been shown to localise to the mitochondria [63]. It is yet to be established whether there is a direct interaction between α-synuclein and NCLX which could potentiate mitochondrial Ca^{2+} overload.

transport chain and ATP synthesis [12]. Thus, a tight control of Ca^{2+} transients is particularly important in high pacing cells, such as cardiomyocytes and neurons, due to the high energy demand.

The mechanism of mitochondrial Ca^{2+} uptake and efflux has been extensively studied where it was shown that Ca^{2+} is transported across the inner mitochondrial membrane via the electrogenic mitochondrial calcium uniporter (MCU) [13] (Fig. 1). Global ablation of MCU in mice is not lethal and does not result in major cardiac phenotypical suggesting a limited role in cardiac homeostasis [14,15]. However, a conditional MCU knock-out mouse revealed that MCU is required for Ca^{2+}-dependent mitochondrial metabolism during acute stress [16,17]. These studies suggest that under normal physiological conditions mitochondrial Ca^{2+} uptake may take place via other, MCU-independent, mechanism(s).

Mitochondrial Ca^{2+} is extruded in exchange with either H^+ or Na^+. It is well established that Ca^{2+} exchange in excitable cells, such as neurons, is mediated by a Na^+/Ca^{2+} exchanger [18] (Fig. 1). Despite the discovery of the efflux mechanisms, the molecular identity remained elusive for many years. The mitochondrial member of the Na^+/Ca^{2+} exchanger was finally identified and characterised as a member of the superfamily – the $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) [19,20]. NCLX shares a common catalytic core with the NCX superfamily whilst its regulatory domain is shorter and lacks the allosteric Ca^{2+}-binding domain. NCLX controls mitochondrial Ca^{2+} fluxes as the Ca^{2+} efflux is much slower than the MCU-mediated Ca^{2+} influx [20,21]. The importance of NCLX in mitochondrial Ca^{2+} homeostasis and survival of excitable cells has recently been highlighted in a study showing myocardial dysfunction and lethality in a conditional NCLX knock-out mouse [22]. This study provides strong evidence that mitochondrial Ca^{2+} efflux (via NCLX) is indispensable for normal Ca^{2+} homeostasis and cardiac function. Impairment of the mitochondrial influx/efflux leads to a deregulation of mitochondrial Ca^{2+} homeostasis and mitochondrial Ca^{2+} overload. In combination with oxidative stress, Ca^{2+} overload can induce permeability transition pore opening (PTP) which is believed to be an initial trigger for apoptotic and necrotic cell death [23,24]. The role of NCLX in neuronal Ca^{2+} homeostasis is yet to be fully established – this study should ideally be undertaken in a mouse model lacking neuronal NCLX.

Whilst it is well recognised that NCLX is the main Ca^{2+} extrusion mechanism in excitable cells, it should be noted that pharmacological inhibition (and knock-out) of NCLX reduces the efflux by 80%, indicating the presence of other extrusion mechanism(s). NCX2 and NCX3 have been suggested to be play a role in mitochondrial Ca^{2+} efflux as NCX2/3 inhibition by siRNA or antibody-blocking led to a reduced mitochondrial Ca^{2+} efflux [25]. This finding was supported by other studies which demonstrated a possible role for the plasmalemmal NCX in mitochondrial Ca^{2+} efflux [26,27].

1.2. Cellular and mitochondrial pathology in Parkinson's disease

Neurodegenerative diseases are classified as progressive degeneration and selective death of neuronal subtypes. In PD, Lewy body inclusions containing α-synuclein and a loss of dopaminergic neurons of the substantia nigra are the main histopathological hallmarks. On cellular level, oxidative stress and mitochondrial complex I deficiency have been described by many studies investigating PD pathology [28–31]. Neurodegenerative conditions often affect mitochondria and the bioenergetic status of the cell, where mitochondrial Ca^{2+} dysregulation plays a key role in pathogenesis. The underlying molecular mechanism(s) are still debated whilst Ca^{2+} homeostasis and mitochondrial bioenergetics have received more attention in recent years: A global Ca^{2+} dysregulation has been reported in PD and several underlying mechanism for elevated cytosolic Ca^{2+} levels have been proposed. For example, under physiological conditions, voltage-dependent Ca^{2+} channels (L-type) are opened by neuronal plasma membrane depolarization during an action potential and closed during repolarisation [32]. In midbrain neurons, stimulation of the cells with dopamine lead to activation of these channels [33]. In PD, L-type Ca^{2+} channels are autonomously active leading to an increased cytosolic Ca^{2+} influx [34] (Fig. 1). Further, α-synuclein itself has also been

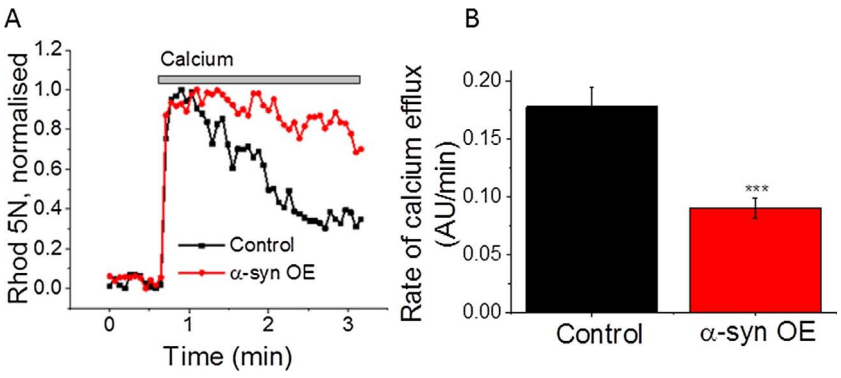

Fig. 2. Impaired mitochondrial Ca^{2+} efflux in neurons overexpressing alpha-synuclein. A) Representative traces of mitochondria loaded with Rhod5N exposed to $CaCl_2$ stimulus. B) The significant delay in mitochondrial Ca^{2+} efflux suggests a role for NCLX in alpha-synuclein pathology. n = 3 experiments; ***$p < 0.001$ Method: Rat primary neuronal co-cultures were prepared as described in [1] and cells were loaded with Rhod5N before being permeabilized as described in [60]. Confocal images were obtained using a Zeiss 710 equipped with a META detection system and a 40 × oil immersion objective. Rhod-5N measurements were undertaken using the 543 nm laser line and 560 nm longpass filter. Statistical analysis and exponential curve fitting were performed using Origin 2017 software (Microcal Software Inc.). Results are expressed as means ± standard error of the mean. Student's T-tests was performed for statistical analysis.

reported to elevate cytosolic Ca^{2+} levels by forming plasma membrane pores allowing extracellular Ca^{2+} to pass [35–37]. The elevated intracellular Ca^{2+} levels require higher ATPase activity to transfer Ca^{2+} across the plasma membrane, resulting in an extra burden to the already bioenergetically impaired neurons in PD. Increased intracellular Ca^{2+} levels also affect Ca^{2+} handling in intracellular organelles such as the endoplasmic reticulum and mitochondria which might potentiate pathological effects [38].

A role for mitochondria in PD pathology was first proposed when the mitochondrial complex I inhibitor MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) was shown to recapitulate the pathophysiology of PD in mice [39]. Further, the discovery of genes affected by mutations in familial PD has not only provided a firm link between mitochondria and PD but also improved our understanding of the underlying mechanism(s). Among those PD-risk genes, discovered by linkage analysis, is α-synuclein itself, the proteins that aggregates in PD Lewy body pathology [40–42]. Alpha-synuclein has been shown to induce oxidative stress and Ca^{2+} dysregulation [43,44]. Further PD risk genes are protein deglycase DJ-1 (DJ-1) and PTEN-induced putative kinase 1 (PINK1) which have both been proposed functions in neuronal stress-response pathways [45,46]. Mutations in PINK1 cause a recessive form of PD and mitochondrial phenotypes have been described by many research groups. For example, limited mitochondrial substrate availability and inhibition of complex I have been reported to result in impaired respiration with elevated levels of reactive oxygen species [47–49]. Inhibition of the mitochondrial calcium efflux in PINK1 KO cells resulted in suppression of the glucose transporter [50]. Furthermore, alterations in mitochondrial metabolism through inhibition of NCLX in pancreatic β-cells of PINK-1 deficient mice lead to changes in glucose-induced Ca^{2+} signal in these cells that result in alteration of glucose sensitivity and insulin secretion [50].

Considering the role of mitochondria in cytosolic Ca^{2+} buffering and the elevated cytosolic Ca^{2+} levels in PD, it is not surprising that mitochondrial Ca^{2+} overload and dysfunction was observed in PINK1-deficient neurons [51–54]. We also found that PINK-1 deficient midbrain neurons are sensitive to dopamine (non-toxic to wild type neurons) where dopamine-induced mitochondrial Ca^{2+} overload triggered PTP opening and cell death [1,54]. Ca^{2+} extrusion in PINK-1 deficient neurons was severely inhibited, resulting in mitochondrial Ca^{2+} overload, increased reactive oxygen species production, PTP opening and ultimately neuronal cell death. Although functional inhibition of the mitochondrial Na^{+}/Ca^{2+} antiport was demonstrated in PINK-1 deficient neurons, a direct proof could not be provided as the molecular identity of NCLX was not established in 2009 [54]. An insight into the question whether mitochondrial Ca^{2+} or dopaminergic dysregulation are early pathogenic processes, Akundi et al. [55] showed that increased Ca^{2+} calcium sensitivity precedes dopamine dysregulations observed in a PINK1-deficient mouse model. This study highlighted again the importance of mitochondrial Ca^{2+} homeostasis in the PINK1-deficient model. Furthermore, we have also shown that mitochondrial Ca^{2+} efflux was also inhibited in PD patients cells bearing PINK-1 mutations [56]. The molecular identification of NCLX opened up a new avenue to study of mitochondrial Ca^{2+} efflux. Indeed, our study in 2015 has shown that protein kinase A phosphorylates NCLX (serine 258) leading to an increase in NCLX activity [57]. This study went on to validate Gandhi et al. [54] findings, showing that pharmacological and genetical activation of NCLX is able to rescue mitochondrial Ca^{2+} overload. Importantly, activation of NCLX was also able to rescue mitochondrial depolarisation and dopamine-induced cell death in PINK-1 deficient neuronal models. Our study provided the first evidence that activation of NCLX-mediated Ca^{2+} efflux is able to rescue mitochondrial phenotypes observed in this PD model [57]. It should be noted that whilst application of respiratory chain complex substrates can restore mitochondrial membrane potential (ΔY_m), it cannot recover mitochondrial Ca^{2+} efflux [54]. However, activation of NCLX is able to rescue the $\Delta\Psi_m$, suggesting that inhibition of NCLX-mediated Ca^{2+} efflux contributes, at least in part, to the mitochondrial depolarisation observed in PINK-1 deficiency [57]. Further, it was reported that PINK-1 is required for the full activation of PKA activity which could explain the reduced NCLX activity observed in PINK1-deficient cells [58,59].

The question remains whether mitochondrial Ca^{2+} efflux is a common phenotype in PD. Preliminary results produced by our laboratory provide further evidence that mitochondrial Ca^{2+} homeostasis may play a central role in PD pathology. Rat primary neuronal co-cultures overexpressing α-synuclein were loaded with a mitochondrial Ca^{2+} dye (Rhod5N) and cells were permeabilized using pseudo-intracellular buffer containing digitonin (+5 mM malate/glutamate). This approach allows direct application of $CaCl_2$ to permeabilized neurons and recording of mitochondrial Ca^{2+} handling (Fig. 2A) [60]. Ca^{2+} efflux in α-synuclein overexpressing neurons was severely impaired when compared to wild type neurons suggesting a role for NCLX in another PD model (Fig. 2B). Interestingly, we and others have previously shown that α-synuclein localises to mitochondria [61–63]. Several mechanisms could explain the inhibition of mitochondrial Ca^{2+} efflux in α-synuclein overexpressing neurons such as 1) direct inhibition of Ca^{2+} efflux through an interaction of α-synuclein with NCLX 2) oxidation of the NCLX by α-synuclein induced reactive oxygen species production; 3) Inhibition of NCLX due to α-synuclein induced mitochondrial depolarisation. Our results warrant further investigations as to how α-synuclein triggers mitochondrial Ca^{2+} accumulation and whether NCLX plays a role in this pathogenic process.

Mitochondrial Ca^{2+} dysregulation impairs mitochondrial health and can lead to cell death. Our data on PINK-1 deficient neurons [57] and alpha-synuclein overexpressing neurons suggest that NCLX may play a common role in the mitochondrial pathogenesis of PD. Therefore, targeting mitochondrial Ca^{2+} and NCLX may represent a novel therapeutic strategy in PD.

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