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# Forty years later: Mitochondria as therapeutic targets in muscle diseases

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ABSTRACT

The hypothesis that mitochondrial dysfunction can be a general mechanism for cell death in muscle diseases is 40 years old. The key elements of the proposed pathogenetic sequence (cytosolic $Ca^{2+}$ overload followed by excess mitochondrial $Ca^{2+}$ uptake, functional and then structural damage of mitochondria, energy shortage, worsened elevation of cytosolic $Ca^{2+}$ levels, hypercontracture of muscle fibers, cell necrosis) have been confirmed in amazing detail by subsequent work in a variety of models. The explicit implication of the hypothesis was that it "may provide the basis for a more rational treatment for some conditions even before their primary causes are known" (Wrogemann and Pena, 1976, Lancet, 1, 672–674). This prediction is being fulfilled, and the potential of mitochondria as pharmacological targets in muscle diseases may soon become a reality, particularly through inhibition of the mitochondrial permeability transition pore and its regulator cyclophilin D.



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## 1. Introduction

The suggestion that cell demise in muscle diseases can be explained by increased net influx of $Ca^{2+}$ triggering a "vicious cycle" of mitochondrial $Ca^{2+}$ overload and energy depletion is now 40 years old [1]. The first event in the proposed sequence was an increase of cytosolic $Ca^{2+}$, which would then be followed by excess mitochondrial $Ca^{2+}$ uptake. In turn, this "$Ca^{2+}$ overload" would cause functional and then structural damage to mitochondria followed by energy shortage, which would worsen the elevation of cytosolic $Ca^{2+}$ and cause hypercontracture of muscle fibers precipitating cell necrosis. The explicit implication of the hypothesis was that it "may provide the basis for a more rational treatment for some conditions even before their primary causes are known" [1]. Relevant progress has been made in defining and testing the role of mitochondria in muscle diseases of various origin. Over the years, additional elements involved in overall $Ca^{2+}$ homeostasis and its pathophysiological regulation have emerged. Key contributions have been the identification of (i) the pathways for $Ca^{2+}$ transport in mitochondria, i.e. the mitochondrial $Ca^{2+}$ uniporter (MCU) com-

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plex and the $Na^+$-$Ca^{2+}$ exchanger NCLX and (ii) the mechanism through which mitochondrial $Ca^{2+}$ – synergistically with oxidative stress – can cause cell death, i.e. the permeability transition pore (PTP). Here we will focus particularly on the latter, as it provides a viable target for pharmacology as anticipated by the insightful hypothesis put forward by Wrogemann and Pena in 1976 [1].

## 2. Mitochondrial $Ca^{2+}$ transport

The ability of isolated mitochondria to catalyze active $Ca^{2+}$ uptake was discovered in the early 1960s [2,3], and evidence suggesting that mitochondrial $Ca^{2+}$ uptake can occur in vivo [4,5] and in cells in situ was obtained a few years later [6]. Direct methods have subsequently demonstrated that mitochondrial $Ca^{2+}$ uptake does occur in situ [7,8], and the recent identification of the mitochondrial $Ca^{2+}$ uniporter (MCU) [9,10] and of its regulatory components [11], as well as of the $Na^+$-$Ca^{2+}$ exchanger NCLX [12], has opened new perpectives to our understanding of the pathophysiology of $Ca^{2+}$ transport. The main physiological role of intramitochondrial $Ca^{2+}$ is regulation of metabolism [13] through stimulation of pyruvate dehydrogenase, $NAD^+$-linked isocitrate dehydrogenase and oxoglutarate dehydrogenase [14]. Intriguingly, the first reported human disorder involving MICU1 – a key component of the MCU complex – causes a proximal myopathy in combination with extrapyramidal movement disorders [15].

The driving force for mitochondrial $Ca^{2+}$ accumulation is the $Ca^{2+}$ electrochemical gradient. Resting cytosolic [$Ca^{2+}$] is below the $K_m$ of the MCU, whose affinity for $Ca^{2+}$ is decreased by cytosolic $Mg^{2+}$ [16,17]. There is no doubt that pathological increases of cytosolic and organellar [$Ca^{2+}$] cause muscle pathology, as shown by genetic manipulation of TRPC3 channels [18], SERCA1 and 2a [19], ORA1/STIM1 [20], NCX1 [21] or by treatment with cardiotoxin [22]. Important pathogenic factors are also calpain activation [23] and reactive oxygen and nitrogen species (ROS and NOS, respectively) which have additive/synergistic effects with those of $Ca^{2+}$ overload [24–26]. Yet, mitochondria can take up $Ca^{2+}$ even under "resting" conditions due to their proximity to the sarcoplasmic reticulum (SR), which creates privileged microdomains where the cation is readily transferred during excitation-contraction coupling, particularly in the proximity of IP3 receptors [8,27–29] in a process that is also sensitive to redox events [30,31]. This set of findings explains why intracellular organelles can be affected by deregulation of $Ca^{2+}$ homeostasis [32] even when cytosolic [$Ca^{2+}$] appears to be normal [33,34], as may be the case in the early stages of muscular dystrophies [35].

In energized mitochondria maintaining a membrane potential of −180 mV (negative inside) the predicted mitochondria-to-cytosol $Ca^{2+}$ accumulation ratio at equilibrium would be a staggering $10^6$, corresponding to an intramitochondrial concentration of 0.1–1 M [36,37]. This is never reached due to the operation of the $Na^+$-$Ca^{2+}$ exchanger NCLX [12,38,39] and of a $Na^+$-independent $Ca^{2+}$ efflux pathway, which could be a $H^+$-$Ca^{2+}$ exchanger [37]. What is then the basis for the "excessive" mitochondrial $Ca^{2+}$ accumulation observed in many muscle diseases? The answer is in the kinetic imbalance between the rates of $Ca^{2+}$ uptake and release. Indeed, the MCU can mediate $Ca^{2+}$ accumulation at an estimated $V_{max}$ of 1400 nmol $Ca^{2+} \times mg^{-1}$ mitochondrial protein $\times$ $min^{-1}$ [17], which exceeds the maximum capacity of $H^+$ pumping by the respiratory chain, while the combined $V_{max}$ of the $Ca^{2+}$ efflux pathways is only about 20 nmol $Ca^{2+} \times mg^{-1}$ mitochondrial protein $\times$ $min^{-1}$ [40]. This is the reason why mitochondria are constantly exposed to the hazards of excessive $Ca^{2+}$ accumulation, and why mitochondrial $Ca^{2+}$ overload could easily occur when acute or chronic increases of cytosolic [$Ca^{2+}$] take place as a result of damage to the sarcolemma and/or to the many proteins involved in cellular $Ca^{2+}$ homeostasis. But what are the $Ca^{2+}$-dependent mechanisms that cause functional and then structural mitochondrial damage, leading to the energetic crisis that precipitates hypercontracture and contributes to fiber death? There is now a consensus that damage may depend on opening of an inner membrane channel, the PTP [41].

## 3. The mitochondrial permeability transition

The occurrence of a $Ca^{2+}$-dependent permeability increase leading to mitochondrial swelling and its detrimental consequences on ATP synthesis had been established and studied before the mechanism of energy conservation was established [42–49]. It is remarkable that in the same years Wrogemann and coworkers had identified a *latent* defect in mitochondria from skeletal muscle and heart in several models of muscular dystrophy. Although the functional properties of mitochondria from myopathic animals were not generally different from those of healthy individuals [50,51], mitochondria from dystrophic animals contained more $Ca^{2+}$ [52] and were particularly sensitive to a $Ca^{2+}$-dependent decrease in ATP production with $NAD^+$-linked substrates [53]. This decrease could be normalized by added $Mg^{2+}$, $NAD^+$, ATP and ruthenium red (which inhibits the MCU); and could be induced by $Ca^{2+}$ in mitochondria from healthy donors [53], a finding that perfectly matched the observations of Vinogradov et al. in rat liver mitochondria [47].

A thorough study of the $Ca^{2+}$-dependent permeability increase was carried out by Haworth and Hunter in 1979 [54–56]. Although these studies capitalized on relevant previous work [42–49], these authors were the first to propose that the permeability increase was due to opening of a *regulated* channel (the PTP), which could have served a yet-unidentified physiological function [54–56]. The argument prevailed, however, that the permeability transition (PT) was an in vitro artifact of little pathophysiological relevance (see [57] and references therein for a detailed account). As already discussed, this may have been an indirect consequence of the demonstration that energy conservation demands a highly impermeable inner membrane, which had just been recognized with the award of the Nobel prize to Peter Mitchell in 1978 [58]. This state of affairs began to change only about 10 years later with the discovery that the PT can be inhibited by nanomolar concentrations of cyclosporin A (CsA) [59–61]. The mitochondrial target of CsA was then shown to be a matrix peptidyl prolyl *cis-trans* isomerase [62] later identified as cyclophilin (CyP) D, the mitochondrial isoform of the CyP family [63,64]. It became soon clear that the only target of CsA was this soluble matrix protein rather than the putative channel itself, and that CsA delayed rather than blocking the PTP. Yet CsA provided a novel tool to address both the molecular nature of the PTP and its role in cellular and organ pathophysiology [65].

After a set of pioneering studies in cells published in the early 1990s [66–69], intense research on the PTP and its role in cell death developed and was rapidly extended to programmed cell death in the immune system and other paradigms [70–75]. In the area of muscle research studies concentrated on the heart, particularly on the role of the PTP in ischemia-reperfusion damage [76], while the PTP hypothesis for the pathogenesis of skeletal muscle diseases appears to have faded away. We found mention to the PTP in this context only in a 1998 review article on apoptosis in metabolic myopathies [77] and in a reappraisal of the Wrogemann-Pena hypothesis from our laboratory in 1999 [78]. As we will discuss more in detail below, this situation was to change a few years later with the unexpected finding that PTP opening is a key determinant in the pathogenic sequence leading to muscle fiber death in the *Col6a1*$^{-/-}$ mouse [79], the animal model of collagen VI muscular dystrophies [80].