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Review

Mitochondria: The Missing Link Between Preconditioning and Neuroprotection

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Abstract. The quote "what does not kill you makes you stronger" perfectly describes the preconditioning phenomenon – a paradigm that affords robust brain tolerance in the face of neurodegenerative insults. Over the last few decades, many attempts have been made to identify the molecular mechanisms involved in preconditioning-induced protective responses, and recent data suggests that many of these mechanisms converge on the mitochondria, positing mitochondria as master regulators of preconditioning-triggered endogenous neuroprotection. In this review, we critically discuss evidence for the involvement of mitochondria within the preconditioning paradigm. We will highlight the crucial targets and mediators by which mitochondria are integrated into neuroprotective signaling pathways that underlie preconditioning, putting focus on mitochondrial respiratory chain and mitochondrial reactive oxygen species, mitochondrial ATP-sensitive potassium channels, mitochondrial permeability transition pore, uncoupling proteins, and mitochondrial antioxidant enzyme manganese superoxide dismutase. We also discuss the role of mitochondria in the induction of hypoxia-inducible factor-1, a transcription factor engaged in preconditioning-mediated neuroprotective effects. The identification of intrinsic mitochondrial mechanisms involved in preconditioning will provide new insights which can be translated into potential pharmacological interventions aimed at counteracting neurodegeneration.

Keywords: Hypoxia inducible factor-1, mitochondria, neuroprotection, preconditioning, reactive oxygen species

INTRODUCTION

In 1964, the concept of "preconditioning" was introduced to describe the stimulation below the threshold of injury which results in subsequent protection [1]. Almost 20 years later, an adaptation to hypoxia by hypoxia in brain tissue *in vitro* was reported [2]. More recent research advances validate a protective role for preconditioning in the brain [3–7], and today it is widely accepted that the preconditioning phenomenon re-

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quires small doses of noxious stimulus to afford neuroprotective responses against future injury [8]. Indeed, stimuli such as ischemia, low doses of endotoxin, and hypoxia have all been shown to be able to induce preconditioning-dependent protective responses [9]. The existence of multiple, diverse preconditioning stimuli that confer protection constitute the well-known phenomenon of "cross-tolerance" [5]. Two distinct forms of preconditioning have been described. Immediate preconditioning occurs within minutes after the preconditioning stimulus and involves cellular changes related to the activity or function of enzymes, secondary messengers, and ion channels. Conversely, delayed preconditioning-induced brain tolerance needs several hours and even days to manifest and is characterized by a dependence on new gene expression and *de novo* protein synthesis [5,10,11]. More recently, it was proposed that preconditioning also elicits stimulus specific genomic reprogramming events, which, in turn, can confer a neuroprotective phenotype [12].

Such monikers aside, the molecular mechanisms responsible for the induction and maintenance of preconditioning-induced brain tolerance are complex and remain largely undefined. That said, mitochondrial-centered mechanisms appear to be important mediators of the preconditioning response [13]. Accumulating data indicates that transient exposure of mitochondria to physiological or pathological stimuli, intracellular events, or pharmacological agents, induces mitochondrial changes that ultimately protect neurons against a variety of lethal insults [14,15]. An inter-relationship between mitochondrial function, the preservation of energy metabolism, and a manifestation of the preconditioning-induced neuroprotective effects has been described in *in vitro* and *in vivo* models of cerebral ischemia [14,16]. Additionally, experimental evidence demonstrates that antioxidants and mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) blockers abolish this preconditioning-induced protection [17, 18], which ascribes a role for mitochondrial reactive oxygen species (ROS) generation and mitoK$_{ATP}$ channel activation in the preconditioning phenomenon.

Mitochondria have been posited to be master integrators of preconditioning-mediated endogenous neuroprotection. Thus, the present review summarizes current knowledge of how mitochondria are involved in preconditioning-induced brain tolerance, putting a focus on mitochondrial respiratory chain and mitochondrial ROS, mitoK$_{ATP}$, mitochondrial permeability transition pore (mPTP), uncoupling proteins (UCPs), and mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) as specific mitochondrial mediators and targets of preconditioning. Finally, we will highlight the role of mitochondria on the induction of hypoxia-inducible factor-1 (HIF-1), a transcription factor believed to play a critical role in the preconditioning-mediated neuroprotective effects. A better understanding of the mechanisms by which mitochondria are involved in preconditioning will help in the development of novel therapeutic strategies aimed to counteract neurodegeneration.

MITOCHONDRIA, PRECONDITIONING, AND NEUROPROTECTION: MECHANISMS

Mitochondria are ubiquitous and dynamic organelles responsible for many crucial cellular processes in eukaryotic organisms. In fact, mitochondria are considered the "gatekeepers of life and death". Major functions of mitochondria include the production of over 90% of cellular ATP, through the TCA cycle and oxidative phosphorylation, the regulation of intracellular calcium (Ca^{2+}) and redox signaling, and the arbitration of apoptosis [19–21]. Given the important role of mitochondria in neuronal physiology, it is not surprising that mitochondria actively participate in preconditioning signaling pathways. In the next subsections, we elaborate on specific mitochondrial mediators and targets implicated in the protective responses of preconditioning.

Mitochondrial respiratory chain and reactive oxygen species

The mitochondrial respiratory chain is one of the primary sources of cellular ROS. Within the four protein complexes associated with the respiratory chain, the primary sites of ROS production and release are complexes I and III [22]. Accumulating data suggests that a slight rise of mitochondrial ROS generation triggers a preconditioning-mediated brain tolerance, suggesting a role for mitochondria in endogenous neuroprotection [23–27]. In fact, preconditioning stimulated by moderate ROS levels protects cultured neurons against different damaging agents and against subsequent massive oxygen radical formation [24]. To this end, an immediate and constant radical scavenger abolishes such ROS-induced neuronal preconditioning [25]. Also, preconditioning with low concentrations of hydrogen peroxide (H_2O_2) protects PC12 cells against apoptosis induced by subsequent lethal oxidative stress [28].

The mechanisms behind H_2O_2-induced neuroprotective effects include attenuation of mitochondrial membrane potential ($\Delta\Psi$m) loss, increase in ROS generation, and overexpression of Bcl-2 [29]. Accordingly, it was demonstrated that the generation of H_2O_2 during brief oxygen-glucose deprivation (OGD) is the main trigger involved in the mechanism of preconditioning-induced neuronal protection [30]. More recently, a preconditioning effect was reported following *in situ* administration of H_2O_2 inside the brain cortex which directly implicates ROS during the triggering phase of cerebral preconditioning [31]. A relationship between mitoK$_{ATP}$ channels and ROS has been postulated, since the protection induced by H_2O_2 against cerebral ischemia-reperfusion injury was blocked by a mitoK$_{ATP}$ channels antagonist, and the antioxidant N-acetyl-cysteine (NAC) blocked protection induced by diazoxide, a mitoK$_{ATP}$ channels opener [31]. This strong and direct relationship between ROS and mitoK$_{ATP}$ further confirms a central stage for mitochondria in the neuroprotection induced by cerebral preconditioning [31].

The inhibition of succinate dehydrogenase (SDH) by 3-nitropropionic acid (3-NPA), an agent known to increase the production of ROS probably at mitochondrial complex I, was shown to promote tolerance to focal cerebral ischemia [32], implicating mitochondrial ROS in cerebral preconditioning. 3-NPA was also able to induce delayed preconditioning in rats when administered 3 days after transient middle cerebral artery occlusion (MCAO) by reducing infarct volume by about 20% [33]. The compound NS1619, which inhibits complex I of the mitochondrial respiratory chain, also induces neuronal preconditioning by increasing ROS production and mitochondrial depolarization [34,35], whereas ROS scavengers during the preconditioning phase significantly abrogates these neuroprotective effects [34]. Furthermore, immediate NS1619 preconditioning decreases Ca^{2+} influx through glutamate receptors, increases superoxide dismutase (SOD) activity, reduces ROS response during glutamate stimulation, and preserves ATP levels [34]. Finally, it has been proposed that "minor" mitochondrial ROS generation induces fission and fusion of mitochondria and relocates the mitochondrial network to form a "mitochondria-free" gap, which may play a role in mitochondrial ROS-mediated protective "preconditioning" by preventing the propagation of ROS during oxidative insult.

Overall these findings point to a critical role for mitochondria and mitochondrial ROS in neuroprotective mechanisms triggered by preconditioning.

Mitochondrial ATP-sensitive potassium channels

There is an ongoing debate concerning the role of mitoK$_{ATP}$ channels in the preconditioning phenomenon of the brain [13]. These channels are localized in the inner mitochondrial membrane and regulate mitochondrial function in several tissues, including the brain [36–38]. Brain mitochondria contain seven times more mitoK$_{ATP}$ channels than liver or heart mitochondria, which reflect the importance of these channels in neuronal functionality and integrity [36]. Recent findings suggest a key role for the mitoK$_{ATP}$ channels as both triggers and end effectors in acute and delayed neuroprotection by preconditioning [13, 23]. Indeed, activation of mitoK$_{ATP}$ channels with pharmacological agents mimics the preconditioning-associated protective effects [39,40]. Conversely, physiological or chemical preconditioning is abrogated by mitoK$_{ATP}$ channels blockers, such as glibenclamide and 5-hydroxydecanoate (5-HD) [40]. Some progress has been made to elucidate the mechanisms underlying the role of mitoK$_{ATP}$ channels in preconditioning protection. For example, opening mitoK$_{ATP}$ channels may decrease $\Delta\Psi$m, promoting an increase in the electron transport chain rate, and, consequently, increasing ATP production [41]. In addition, the activation of mitoK$_{ATP}$ channels was reported to induce neuronal protection by attenuating mitochondrial Ca^{2+} overload and, thus, preventing mPTP induction. More recently, the signal transduction pathways initiated by epsilon protein kinase C (εPKC) were shown to mediate preconditioning-induced neuroprotection through the activation of mitoK$_{ATP}$ channels [42]. Further, abolishment of these beneficial effects of preconditioning was observed following inhibition of both mitoK$_{ATP}$ channels or εPKC [42].

Diazoxide, a selective mitoK$_{ATP}$ channels opener, has been suggested to induce mild oxidative stress and preconditioning-like neuroprotection [43]. However, at high doses diazoxide also inhibits SDH, the complex II of the mitochondrial respiratory chain, leading to the release of ROS in a mitoK$_{ATP}$ channel-independent manner [44]. In a recent review, it was proposed that diazoxide is the most potent inducer of preconditioning-mediated protection due to the combined effects of mitochondrial membrane depolarization and enhanced ROS production through SDH inhibition [13]. The immediate preconditioning induced by low doses of diazoxide was shown to preserve neuronal and vascular function after cerebral ischemia [45]. Additionally, this immediate preconditioning with dia-

zoxide protects against ischemia-reperfusion injury by preventing mitochondrial swelling and Ca^{2+} accumulation in brain cells [46]. Diazoxide also induces delayed preconditioning in neurons via the acute generation of superoxide anion (O_2^-) and activation of protein kinases protecting against the oxidative stress induced by OGD, a well-established *in vitro* model of cerebral ischemia-reperfusion [44]. It was also demonstrated that diazoxide protects neurons against ischemia-induced death by increasing mitochondrial Bcl-2 levels and suppressing the translocation of Bax to the mitochondria and by preventing subsequent cytochrome c release, suggesting that mitoK$_{ATP}$ channel activation may stabilize mitochondrial function by differentially modulating pro-apoptotic and anti-apoptotic proteins [47]. Some *in vivo* studies have also revealed that the acute and delayed preconditioning with diazoxide has a neuroprotective effect against transient focal cerebral ischemia [48,49]. Moreover, diazoxide is effective in protecting hippocampal neurons against oxidative injury induced by exposure to ferrous sulfate ($FeSO_4$) and amyloid-β (Aβ), leading to a suppression of intracellular peroxide formation [50]. Similarly, diazoxide exerts a protective effect against Aβ-induced cytotoxicity in endothelial cells [51] and neurons by suppressing Aβ-mediated increases in $\Delta\Psi$m and intracellular ROS levels [52]. Aβ_{1-42} also enhances the expression of K$_{ATP}$ channel subunits in cholinergic neurons, suggesting that the change in the composition of K$_{ATP}$ channels may contribute to the dysfunction of K$_{ATP}$ channels and disturbances in membrane excitability [53]. Of note, pretreatment with diazoxide reverses the Aβ_{1-42}-induced enhancement in the expression of K$_{ATP}$ channels subunits [53]. In an *in vitro* model of Parkinson's disease (PD), diazoxide protects neurons against MPP^+-induced cytotoxicity via inhibition of ROS overproduction, which improves mitochondrial function [54]. Similarly, this mitoK$_{ATP}$ opener improves both parkinsonian symptoms and neurochemical alterations in rats treated with rotenone, a model of PD [55]. These results suggest that mitoK$_{ATP}$ activation could provide a new therapeutic strategy for the treatment of early PD. 5-HD abolishes all neurorestorative effects of diazoxide [55], which is consistent with previous studies showing that the activation of mitoK$_{ATP}$ channels with diazoxide in PC12 cells induces protection against the neurotoxic effects of rotenone but that this protection being attenuated by 5-HD [56,57]. *In vitro*, diazoxide prevents rotenone-induced microglial activation and the subsequent production of pro-inflammatory factors, such as tumor necrosis factor alpha (TNF-α) and inducible isoform of nitric oxide synthase (iNOS) [58].

BMS-191095, a selective mitoK$_{ATP}$ channels opener, has been shown to induce both immediate and delayed preconditioning in neurons via mechanisms that involve mitochondrial depolarization and PKC activation which attenuate free radical production during neuronal stress [59]. In addition, BMS-191095 depolarizes mitochondria without ROS generation, activates the phosphoinositide 3-kinase (PI3-K) signaling pathway, and increases ATP content and catalase expression; such mechanisms undoubtedly contribute to the neuroprotective effects afforded by this mitoK$_{ATP}$ channels opener [60]. In a similar vein, BMS-191095 affords protection against cerebral ischemia by delayed preconditioning via selective opening of mitoK$_{ATP}$ channels without ROS generation [61].

In summary, the activation of mitoK$_{ATP}$ channels seems to be a key event that elicits neuroprotection by preconditioning and, as such, these channels represent promising therapeutic targets to counteract neurodegeneration.

Mitochondrial permeability transition pore

As already mentioned, mitochondria have a high capacity for Ca^{2+} sequestration, contributing to normal neuronal function [62–64]. Conversely, mitochondrial Ca^{2+} overload leads to the induction of the mPTP, resulting in osmotic swelling and a collapse of the outer mitochondrial membrane. The mPTP, a dynamic multiprotein complex located at the contact site between the mitochondrial inner and outer membranes, is comprised of the voltage-dependent anion channel, the adenine nucleotide translocator, and the regulatory protein cyclophilin D (CypD). Once open, the mPTP allows the release of pro-apoptotic proteins, including cytochrome c and apoptosis-inducing factor, from the mitochondrial intermembrane space into the cytoplasm. Consequently, released cytochrome c binds apoptotic protease-activating factor 1 and activates the caspase cascade [65–67]. While it is unclear whether *bona fide* apoptosis occurs during neurodegeneration [68–70], it is clear that the mPTP is an important inducer of cell death. For instance, mPTP induction has been proposed to be integral to the apoptotic mechanism implicated in ischemia-triggered mitochondrial dysfunction and neuronal cell death [71–74], such that cyclosporine A, which inhibits mPTP, has neuroprotective effects against ischemia-induced brain injury [75,76]. Accordingly, CypD-knockout mice also display a dramatic re-

duction in the size of brain infarcts [77]. Additionally, increased susceptibility to mPTP induction is promoted by Aβ peptides, suggesting an involvement of the mPTP in AD pathophysiology [78,79]. Consistent with this, CypD deficiency substantially improves learning and memory and synaptic function in an AD mouse model and alleviates Aβ-mediated reductions in long-term potentiation [80]. Also, in PD, mPTP may play a role, such that the overexpression of α-synuclein in cell culture and in transgenic mice impairs mitochondrial function and increases susceptibility to mPTP induction [81,82].

Compelling evidence indicates that the inhibition of mPTP opening and its signaling cascade represent crucial events responsible for preconditioning-mediated cytoprotection in both heart and brain [23,83–85]. Despite the fact that the molecular mechanisms underlying these effects are still the objects of investigation, nitrite (NO_2^-), a known endogenous mediator of preconditioning as well as protein kinases, has been proposed as a possible regulator of the mPTP [86,87]. Alternately, a reduction of the oxidation of critical thiol groups of the mPTP, which sensitize pore opening to Ca^{2+}, could represent another potential mechanism involved in preconditioning-mediated inhibition of mPTP opening [85]. When compared with heart, less is known about the functional role of mPTP for preconditioning in the brain. This said, activation of mitoK$_{ATP}$ channels protects the brain against injury through the attenuation of mitochondrial Ca^{2+} overload and, thus, prevents mPTP induction [88]. A signaling pathway linking mitoK$_{ATP}$ and mPTP has been proposed where increased K^+ conductance after mitoK$_{ATP}$ opening alkalinizes the mitochondrial matrix and increases the generation of H_2O_2, which in turn activates an mPTP-associated PKCε. In addition, a pivotal role for PKCε in the induction of tolerance after ischemic preconditioning has been shown in experimental models of cerebral ischemia [89]. Indeed, ischemic preconditioning has been shown to promote a significant increase in respiration and the phosphorylation of respiratory proteins via PKCε in mitochondria located in synaptosomes [90]. Furthermore, it has been shown that preconditioned neurons with short periods of OGD contain large mitochondria with dense matrices, increased respiration and an elevated Ca^{2+} loading capacity [91]. Further, a recent study demonstrated that mitochondrial hyperpolarization following short periods of OGD increased the Ca^{2+} buffering capacity of mitochondria in hippocampal neurons suggesting that enhanced buffering capacity of the mitochondria may be linked to preconditioning after short-term ischemic episodes [91]. Therefore, mPTP is critically involved in preconditioning-induced brain tolerance.

Mitochondrial uncoupling proteins

In the brain, as well as in the heart, preconditioning is related to a moderate uncoupling of the mitochondrial respiratory chain [23,92]. Uncoupling proteins (UCPs) are located in the inner mitochondrial membrane and dissipate the electrochemical proton gradient between the intermembrane space and the mitochondrial matrix to uncouple electron transport from ATP synthesis. As such, UCPs play a critical role in energy balance [93]. One of these proteins, UCP2, expressed in the brain, predominantly in neurons, has been proposed to contribute to neuroprotection by reducing mitochondrial ROS generation [94,95]. Indeed, Mattiasson and colleagues [96] provided evidence of increased expression of brain UCP2, measured as mRNA by *in situ* hybridization, after preconditioning ischemia *in vivo*. It was found that UCP2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma, suggesting that UCP2 is an inducible protein that is neuroprotective by activating cellular redox signaling or by inducing mild mitochondrial uncoupling that prevents the release of apoptogenic proteins [96]. Accordingly, *in vitro* and *in vivo* experiments show that upregulation of UCP2 is part of a neuroprotective set of responses to various cellular stresses involved in preconditioning and protection against free radical-induced cell death is observed in PC12 cells transfected with UCP2 [97]. Moreover, in transgenic mice that expressed UCP2 constitutively in the hippocampus before seizure induction, a robust reduction in cell death was observed. Since UCP2 increases mitochondrial number and ATP levels with a parallel decrease in free radical-induced damage, the authors proposed that mitochondrial UCPs precondition neurons by dissociating cellular energy production from that of free radicals to withstand the harmful effects of cellular stress occurring in a variety of neurodegenerative disorders [97]. More recently, it was confirmed that ischemic preconditioning causes increased expression of UCP2 protein *in vivo*, the timing of which is appropriate for protection against ischemia/reperfusion injury in the hippocampus [98]. In addition, this increase in UCP2 immunoreactivity in preconditioned brains was blocked by ROS scavenging, which demonstrate a dependence of UCP2 expression on ROS production. This finding is in agreement with previous studies that demonstrat-

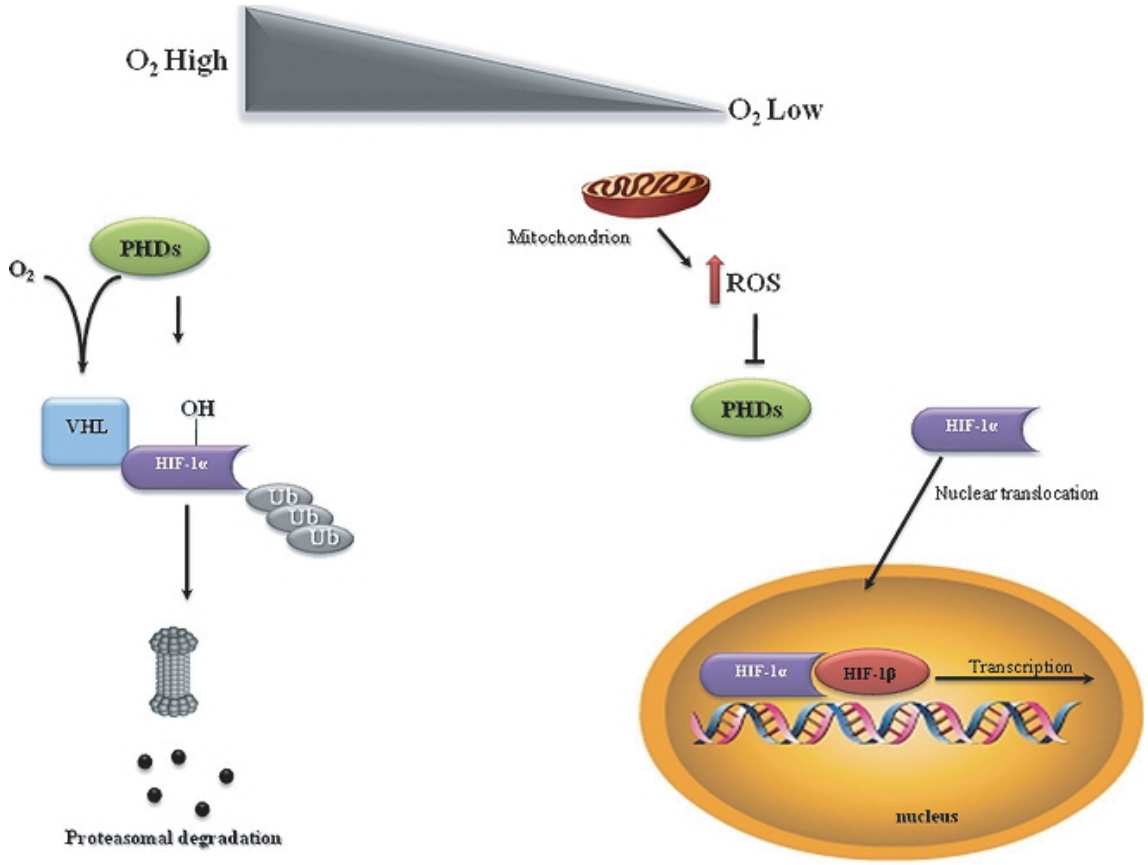

Fig. 1. Schematic illustration of the involvement of mitochondrial reactive oxygen species (ROS) in hypoxia-inducible factor 1 (HIF-1) stabilization. HIF-1 is a heterodimeric protein composed of a constitutively expressed HIF-1β subunit and an inducible HIF-1α subunit. In the presence of molecular oxygen (O_2), HIF-1α is hydroxylated by prolyl hydroxylase enzymes (PHDs) and rapidly degraded by ubiquitin-proteasome system. Under hypoxic conditions, the generation of ROS by the mitochondrial respiratory chain inhibits PHDs activity, preventing the hydroxylation of HIF-1α, resulting in HIF-1α stabilization and translocation to the nucleus. Consequently, HIF-1α dimerizes with HIF-1β, initiating the transcription of HIF-1-responsiveness genes.

ed that excess ROS production can induce UCP2 overexpression and activation [93,99,100]. Finally, it was shown that both resveratrol and ischemic preconditioning induce neuroprotection against cerebral ischemic damage through alterations in mitochondrial function via the sirtuin1 (SIRT1)-UCP2 pathway [101].

Manganese superoxide dismutase

The brain is especially prone to oxidative stress-induced damage due to its high levels of polyunsaturated fatty acids, high oxygen consumption, high content in transition metals, and poor antioxidant defenses [102]. To protect the mitochondria from O_2^--mediated oxidative damage, cells express a nuclear-encoded mitochondrial enzyme, MnSOD, that scavenges O_2^- generated by the electron-transport chain in the mitochondria [103]. Indeed, previous reports demonstrated that overexpression of MnSOD protects mice against focal cerebral ischemia [104,105]. More recently, Scorziello and collaborators [106] proposed that MnSOD may represent the crucial step through which the NO/Ras/ERK1/2 pathway promotes neuroprotection during preconditioning.

MITOCHONDRIA AND HIF-1: GOOD PARTNERS?

A number of key molecules and signaling pathways have been proposed to participate in preconditioning. The induction of the hypoxia signaling pathway with the concomitant stabilization and transcriptional activation of the transcription factor HIF-1 has emerged

as one of the major cellular pathways responsible for preconditioning-induced neuroprotection. HIF-1 is a heterodimeric protein composed of a constitutively expressed HIF-1β subunit and an inducible HIF-1α subunit. Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylase enzymes (PHDs) and rapidly degraded by the ubiquitin-proteasome system (Fig. 1). On the other hand, during hypoxic conditions, enzymatic inhibition of PHDs abrogates HIF-1α proteasomal degradation and results in HIF-1α stabilization and translocation to the nucleus. In the nucleus, HIF-1α recruits HIF-1β and modulates the expression of a wide range of genes involved in angiogenesis, metabolism, apoptosis, and cell survival [107]. HIF-1 induction has been shown to afford neuroprotection in cerebral ischemic stroke and chronic neurodegenerative disorders, such as AD and PD [107]. Indeed, in a neuronal-specific HIF-1α knockout study aimed to evaluate the significance of HIF-1α in the ischemic brain, it was found that loss of HIF-1 increased the extent of brain damage in mice subjected to MCAO and reduced survival rate [108]. Conversely, pharmacologic HIF-1 activators 3,4-dihydroxybenzoic acid, deferoxamine (DFO), and 2,2-dipyridyl significantly reduced ischemic injury in wild-type mice, whereas the effectiveness of these compounds was significantly attenuated in mice with neuron-specific HIF-1α knockdown [108]. Additionally, activation of HIF-1 by the overexpression of a non-degradable HIF-1α was also shown to prevent Aβ_{1-42}-induced neurotoxicity, suggesting a neuroprotective role of HIF-1 in AD [109]. Recently, it was reported that in *in vitro* and *in vivo* models of PD, 3,4-dihydroxybenzoate protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity possibly through the induction of HIF-1α [110].

HIF-1α activation seems to be strictly linked to mitochondrial function. Indeed, under hypoxic conditions, mitochondria act as oxygen sensors and convey signals to HIF-1, with mitochondrial ROS being the putative signaling molecules between a cellular O_2-sensor and HIF-1. ROS generated by the Q_0 site of complex III have been documented to be critical in the hypoxia-mediated survival signaling [111]. Consistent with this, previous studies reported that mitochondrial ROS generation prevent the hydroxylation of HIF-1α, stabilizing HIF-1α and promoting its translocation to the nucleus and dimerization with HIF-1β, initiating the transcription of target genes (Fig. 1) [111–113]. Conversely, mitochondrial DNA-depleted (ρ^0) cells, without a functional mitochondrial respiratory chain, failed to increase ROS generation and HIF-1α accumulation under hypoxic conditions [114,115]. Exogenous application of H_2O_2 has been also shown to induce HIF-1α under normoxic conditions, whereas ROS scavengers block the hypoxic induction of HIF, which further confirms the involvement of ROS in HIF-1 induction [113, 116]. Additionally, in ρ^0 cells, exposure to low levels of H_2O_2 stabilizes HIF-1α protein during normoxia and increases hypoxia responsive element (HRE)-luciferase [115].

A crosstalk between mitochondrial ROS and HIF-1 has been proposed to underlie preconditioning-mediated protective events [107]. Indeed, evidence from the literature demonstrates that hypoxic preconditioning-induced neuroprotection is associated with ROS production and subsequent induction of HIF-1 and its downstream gene erythropoietin [117]. Confirming this, low levels of exogenous H_2O_2 increase HIF-1α expression and protect against ischemia in primary cortical neurons [118].

Overall, these findings provide evidence for the action of mitochondrial ROS on HIF-1α stabilization and activity, a transcriptional regulator of preconditioning-mediated neuroprotective events.

FINAL REMARKS

The experimental data summarized in this review suggest a pivotal role for mitochondria in neuroprotective events provided by preconditioning. Strong evidence demonstrates that the mitochondrial respiratory chain and mitochondrial ROS, mitoK$_{ATP}$, mPTP, UCP2, and mitochondrial antioxidant enzyme MnSOD, are all targets and mediators involved in preconditioning-induced neuroprotective responses. Indeed, "mitochondrial preconditioning" has many neuroprotective effects, both during and following neurotoxic insults, including an improvement of neuronal viability, the attenuation of the intracellular Ca^{2+} influx, suppression of ROS generation, the inhibition of apoptosis, and the maintenance of ATP levels.

Understanding the precise mitochondrial mechanisms involved in preconditioning will provide important information necessary to develop new and more effective therapeutic strategies for neurodegeneration.

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