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Review Article

ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection

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ABSTRACT

Ischemia-reperfusion (IR) injury is central to the pathology of major cardiovascular diseases, such as stroke and myocardial infarction. IR injury is mediated by several factors including the elevated production of reactive oxygen species (ROS), which occurs particularly at reperfusion. The mitochondrial respiratory chain and NADPH oxidases of the NOX family are major sources of ROS in cardiomyocytes. The first part of this review discusses recent findings and controversies on the mechanisms of superoxide production by the mitochondrial electron transport chain during IR injury, as well as the contribution of the NOX isoforms expressed in cardiomyocytes, NOX1, NOX2 and NOX4, to this damage. It then focuses on the effects of ROS on the opening of the mitochondrial permeability transition pore (mPTP), an inner membrane non-selective pore that causes irreversible damage to the heart. The second part analyzes the redox mechanisms of cardiomyocyte mitochondrial protection; specifically, the activation of the hypoxia-inducible factor (HIF) pathway and the antioxidant transcription factor Nrf2, which are both regulated by the cellular redox state. Redox mechanisms involved in ischemic preconditioning, one of the most effective ways of protecting the heart against IR injury, are also reviewed. Interestingly, several of these protective pathways converge on the inhibition of mPTP opening during reperfusion. Finally, the clinical and translational implications of these cardioprotective mechanisms are discussed.

1. Introduction

Reactive oxygen species (ROS) have generally been perceived as toxic byproducts of aerobic metabolism and the primary cause of macromolecular damage. Accordingly, they are thought to underlie many diseases and pathological conditions, including cancer, cardiovascular and neurodegenerative pathologies, and even the aging process [1–5]. In the last two decades, however, it has become increasingly clear that ROS also serve important signaling functions and modulate a wide variety of physiological processes [6,7]. The superoxide anion ($O_2^{\bullet-}$) is the product of a one-electron reduction of oxygen, and its dismutation, either spontaneously or through a reaction catalyzed by superoxide dismutase (SOD), produces hydrogen peroxide (H_2O_2), which is relatively stable *in vivo* compared with other ROS molecules. H_2O_2 is lipid-soluble and can diffuse freely across membranes, acting as a physiological second messenger signaling molecule by selectively oxidizing target proteins [8,9]. In this sense, redox-regulated molecular targets are proteins that respond to alterations in local redox state with a change in conformation, stability, molecular interactions, and activity. Such proteins thus act as signal transducers, and can include ion transporters, receptors, kinases, phosphatases, transcription factors and structural proteins.

Ischemia-reperfusion (IR) injury occurs when the blood supply to an organ is interrupted (ischemia) and then re-established (reperfusion), leading to a "burst" of ROS from mitochondria [10,11]. This review summarizes the main sources of ROS in cardiomyocytes, focusing on

Abbreviations: Akt, protein kinase B or PKB; AMPK, 5′-AMP-activated protein kinase; CcO, cytochrome *c* oxidase; CsA, cyclosporin A; Cx43, connexin 43; CyP-D, cyclophilin D; DMOG, dimethyloxalylglycine; ETC, electron transport chain; GSK-3, glycogen synthase kinase 3; HIF, hypoxia-inducible factor; IPC, ischemic preconditioning; IR, ischemia-reperfusion; Keap1, Kelch-like ECH-associated protein 1; mPTP, mitochondrial permeability transition pore; mitoK$_{ATP}$, mitochondrial ATP-sensitive K^+ channels; mtDNA, mitochondrial DNA; NOX, NADPH oxidase; NRF-1, nuclear respiratory factor-1; Nrf2, nuclear factor erythroid 2-related factor 2; OSCP, oligomycin sensitivity conferral protein; PGC-1α, peroxisome-proliferator-activated receptor-γ (PPARγ) co-activator 1α; PHD, prolyl hydroxylase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PTEN, phosphatase and tensin homologue; RISK, reperfusion injury salvage kinase; ROS, reactive oxygen species; Sirt1, silent information regulator 1; Tfam, mitochondrial transcription factor A; Δ*p*, protonmotive force; $\Delta\psi_m$, mitochondrial membrane potential

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mitochondrial ROS generation and recent findings on ROS production during IR involving succinate accumulation and superoxide production by reverse electron transport (RET) [12,13]. Against this background, key observations on ROS-induced effects in the heart and their potential involvement in the opening of the mitochondrial permeability transition pore (mPTP) are discussed, along with redox-based mechanisms of cardiomyocyte mitochondrial protection, including the HIF (hypoxia-inducible factor) pathway and the antioxidant transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2). Finally, these observations are put into the context of the current knowledge on ischemic preconditioning–a phenomenon whereby transient exposure to ischemia induces protection against subsequent prolonged ischemia–together with the clinical and translational implications of these cardioprotective mechanisms.

2. Main sources of ROS in cardiomyocytes

2.1. Mitochondrial generation of superoxide and hydrogen peroxide

Mammalian mitochondria can generate superoxide and/or hydrogen peroxide from at least eleven different sites associated with substrate catabolism and the electron transport chain (ETC). The distinct topologies, properties and capacities of superoxide and/or hydrogen peroxide production by these sites have been recently reviewed in depth [14]. Here, I briefly describe the best characterized sites of superoxide production in complex I and complex III, and summarize some accepted conclusions about mitochondrial superoxide generation *in vivo*.

Oxidation of substrates releases electrons to cofactors such as NADH and $FADH_2$. These electrons then flow sequentially through a series of redox carriers in respiratory chain complexes, ultimately reducing oxygen to water at cytochrome *c* oxidase (CcO) or complex IV. CcO catalyzes the sequential transfer of four electrons from the reduced cytochrome *c* pool to oxygen, forming water [15]. However, electrons that exit the respiratory chain at complexes upstream of CcO (electron leak), primarily at complexes I and III, partially reduce oxygen [16]. One-electron reduction of oxygen generates superoxide ($O_2^{\bullet-}$), which can be dismutated to hydrogen peroxide (H_2O_2) and this, in turn, can be fully reduced to water or partially reduced to the hydroxyl radical ($^{\bullet}OH$), one of the strongest oxidants in nature. ROS are highly reactive and therefore have short half-lives. Superoxide is unstable, with a lifetime of milliseconds at neutral pH and is thus difficult to quantify. Dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 can occur spontaneously or through a reaction catalyzed by superoxide dismutase (SOD). H_2O_2 is quite unreactive and hence much easier to quantify, but it oxidizes Fe^{2+} to Fe^{3+} to generate hydroxyl radicals through the Fenton reaction [17]. H_2O_2 freely diffuses through membranes and can be detected by highly sensitive and selective probes, providing important information on H_2O_2 production by isolated mitochondria, live cells and *in vivo*.

2.1.1. Complex I

Complex I is the entry point for electrons from NADH into the respiratory chain. This large multi-subunit complex has a hydrophobic arm and a matrix-protruding hydrophilic arm, which contains flavin mononucleotide (FMN) and eight Fe-S (iron-sulfur) clusters [18]. The FMN cofactor accepts electrons from NADH and passes them along a chain of seven Fe-S centers to the coenzyme Q (CoQ) reduction site. The CoQ-binding site is at the junction between the two arms. There are two sites in complex I that can produce superoxide: the FMN domain in the NADH-binding site, site I_F, and the ubisemiquinone in the coenzyme Q-binding site, site I_Q. Superoxide production occurs at the site I_F during conventional forward electron transport when the FMN is in a highly reduced state, such as under conditions of high protonmotive force (Δp) and low rates of ATP synthesis [19–23] (State 4). The proportion of the FMN that is fully reduced depends on the NADH/NAD^+ ratio [20,21]. The CoQ-binding site inhibitor rotenone increases superoxide production as it provokes a backup of electrons onto FMN, which will produce superoxide [24,25]. The production of superoxide in complex I also occurs by RET, with both the FMN and the CoQ reduction sites of complex I proposed as sites of superoxide production during this process [26,27]. RET is caused by the highly reduced state of the CoQ pool, forcing electrons back from $CoQH_2$ into complex I, thus reducing NAD^+ to NADH at the FMN site [23,28–30]. Nevertheless, extensive superoxide production from complex I without reduction of NAD^+ has been reported in the presence of a highly reduced NAD^+/NADH pool [21], since electrons built up on FMN can reduce oxygen to superoxide [27]. Superoxide production by RET is abolished by rotenone, which prevents the entrance of electrons into complex I through the CoQ-binding site [22,23,25]. Maximum superoxide production from complex I during RET is much faster than the maximum rate from the FMN during forward electron transport. As discussed below, RET at complex I has been proposed to be the main source of superoxide upon reperfusion of ischemic tissue [12,13].

2.1.2. Complex III

The pathway of electron flow in complex III is often called the Q-cycle. During this cycle, the complex interacts transiently with CoQ at the Q_i and Q_o sites [31]. Complex III is an important site for ROS formation, and produces large amounts of superoxide from the reaction of oxygen with a ubisemiquinone bound to the Q_o site when supplied with $CoQH_2$ and in the presence of the Q_i inhibitor antimycin A [19,30,32–34]. However, in the absence of antimycin A, the Q_o site ubisemiquinone is not stabilized and superoxide production by complex III is low [35]. Therefore, the production of superoxide by complex III in the absence of antimycin A is negligible compared with that produced by complex I during RET.

Superoxide generated by complex I is released into the matrix, whereas that of complex III is released into both matrix and intermembrane space [36–39]. These different topologies are important for redox signaling and for oxidative damage to macromolecules, especially to mitochondrial DNA (mtDNA). The efficient removal of superoxide is thus essential for survival. As mentioned earlier, this species can be dismutated to H_2O_2 by SOD, or can be protonated to form the lipid-soluble hydroperoxyl radical (HOO•), which can react with polyunsaturated fatty acyl groups to form carbon-centered fatty acyl radicals.

Mitochondria tend to generate superoxide when they are respiring but not making ATP (State 4). Under these conditions, electron carriers, such as CoQ, become highly reduced and the large Δp causes reverse electron flow through complex I and the generation of large amounts of superoxide. This may also prolong the lifetime of ubisemiquinone within complex III, favoring superoxide production. By contrast, mitochondria that are producing ATP (State 3) decrease superoxide production, as the lower Δp increases the oxidation of electron carriers.

A wide variety of antioxidant defense mechanisms have evolved to guard against damage by ROS, or even to repair damage resulting from their reactivity. Clearly, steady-state levels of ROS will depend on the rates of production at different sites and on the antioxidants active in the matrix and the cytosol. Estimates of the steady-state levels of superoxide in cells are 1–250 pM [16].

2.2. Rates of superoxide and hydrogen peroxide production by mitochondria

The rate of superoxide production by mitochondria mainly depends on two factors: first, the concentration of the protein containing the electron carriers that are able to react with oxygen to form superoxide; and second, the proportion of the protein's electron carrier present in a redox form that can react with oxygen. Other factors affecting this rate include the concentration of oxygen and the second-order rate constant for the reaction of the electron carrier with oxygen to form superoxide [16]. The rate of electron leak, therefore, does not depend on the