SciMedCentral

Annals of Cardiovascular Diseases

Review Article

Mitochondrial Sources of ROS in Cardio Protection and Ischemia/Reperfusion Injury

Francesca Tullio, Maria-Giulia Perrelli, Saveria Femminò, Claudia Penna, and Pasquale Pagliaro*

Department of Clinical and Biological Sciences, University of Turin, Italy

***Corresponding author**

Pasquale Pagliaro, Department of Clinical and Biological Sciences, University of Turin, Regione Gonzole, Regione Gonzole 10, 10043 Orbassano, Italy, Tel: 396705450; Fax: 39-9038639; Email: pasquale.pagliaro@unito.it

Submitted: 23 May 2016

Accepted: 16 June 2016

Published: 20 June 2016

Keywords

- Ischemia/reperfusion injury
- Cardioprotection
- Reactive oxygen species
- Redox signaling; Mitochondria

Abstract

Several lines of evidence suggest that the reactive oxygen species (ROS) generatated by mitochondria have a pivotal role in determining activation of cardioprotective pathways and cell survival or, alternatively, in damaging cellular components and initiating cell death. In this article, we report the role of some important components of mitochondria in determining protective ROS signaling or deleterious ROS stress. We discuss studies showing the mechanisms whereby ROS generation occurs in pre (PreC) and postconditioning (PostC) and myocardial ischemia/reperfusion (I/R) injury. In particular, we consider mitochondrial ROS production that target respiratory complexes, mitochondrial ATP-sensitive potassium (mK_{ATP}) channels or connexin 43 (Cx43) to activate cell survival programs, in conditioning by ischemic and pharmacologic agents. ROS signaling renders cells more resistant to the detrimental effects of I/R, via activation of enzymes and limitation of mitochondrial permeability transition pore (mPTP) opening. Importantly, this pore plays a crucial role in determining ROS production and cell death. Moreover, we consider the role of other mitochondrial components which mainly contribute to deleterious ROS generation, namely monoamine oxidase (MAO) and growth factor adaptor Shc ($p66^{Shc}$), which may interact with other components to generate ROS and may be the putative target(s) of therapeutic agents.

INTRODUCTION

Ischemic preconditioning (PreC) of myocardium is an adaptive response in which brief (a few minutes) episodes of ischemia/reperfusion (I/R), prior to sustained ischemia, lead to cardioprotection. Postconditioning (PostC) is obtained with brief (a few seconds) cycles of I/R at the immediate onset of reperfusion after sustained ischemia. Myocardial protection can also be obtained with brief (a few minutes) episodes of I/R applied to an organ remote to the heart, before (remote PreC), after (remote PostC), or even during (remote Per-conditioning, PerC) the sustained episode of myocardial ischemia. Intriguingly, ischemic and remote PreC are characterized by a biphasic period of protection: a first window of protection (early PreC), which starts immediately after the PreC stimulus and last for a couple of hours, as well as a second window of protection (delayed PreC), which starts 12 hours after the PreC stimulus and last for a 48-72 hours.

Protection by PreC, PostC and remote conditionings comprises a reduction of infarct size, cardiac dysfunction and arrhythmias incidence. This cardioprotective effect have been proven in different species including patients; however it seems that the presence of cardiovascular risk factors, comorbidities, and their medications may interfere with cardioprotective signaling pathways (see for extensive reviews: [1-3]). The mechanisms involved in the cardioprotective effect have not been fully elucidated; however several signal transduction cascades have been proposed [1-4]. Better comprehension of the intracellular mechanisms underlying the ischemic conditioning strategies may offer an important protocol for cardioprotection and their translation to clinical use for therapeutic interventions [2,4].

Potentially cooperative intracellular signaling cascades are recruited by phosphorylation/ dephosphorylation processes in mechanisms of cardioprotection. Several biofactors bind to specific receptors present on the cellular surface leading to an activation of intracellular signal transduction which includes *redox signaling* by reactive oxygen species (ROS), *S-nitrosylation* by NO and its derivatives, *S-sulfhydration* by hydrogen sulfide, and *O-Linked glycosylation* by beta-N-acetylglucosamine. All these modalities interact and control an entire pathway, thus influencing each other. For instance, enzymes can be nitrosylated and/or phosphorylated in specific and/or multiple site(s) with consequent increase or decrease of their specific activity. The signaling pathways that lead to cardioprotection are thought to converge on mitochondria, and various mitochondrial proteins

have been identified as targets of these post-transductional modifications (see for an extensive review: [5, 6]).

Mitochondria represent about 40% of cardiomyocytes mass; they are rich in enzymes responsible of ROS production and in anti-oxidants and play a role of paramount importance in determining both cardiac protection and injury [6]. Mitochondria have at least eleven potential sources of superoxide radical and hydrogen peroxide. In the present review we will analyze the role of some mitochondrial components in determining ROS production, bearing in mind that small amounts of mitochondrial ROS are necessary for normal life. For example they are necessary to mediate metabolic vasodilatation. Moreover ROS may induce cardioprotection or alternatively they may contribute to determine the damage by ischemia/reperfusion. This dual role is not only due to different amount or type of ROS (a ROS is not always good or bad), but it depends on a plethora of conditions, including the site of production and the timing co-presence of appropriate/inappropriate targets and the co-presence of pro- or anti-oxidants.

RESPIRATORY CHAIN

In cardiomyocytes mitochondria are considered the principal source of ROS and the electron transport chain (ETC) represents the main site of ROS formation [7-9]. Mitochondria also contain the anti-oxidative defense systems, that include enzymatic systems, as superoxide dismutases (SODs), catalase (CAT) and peroxidases, and non-enzymatic mechanisms, as glutathione, thioredoxin-2, vitamins (A, E, and C), ubiquinone, urate, lipoic acid, making them a central player in cellular redox homeostasis [6,10].

During mitochondrial respiration, superoxide radical ($O_2^{\bullet-}$) is the first ROS produced at the ETC by incomplete reduction of O_2. The classical concept of $O_2^{\bullet-}$ production at the ETC is that in the presence of substrates and in the absence of ADP (i.e., respiratory state 4), the ETC is highly reduced, which increases the probability that electrons "slipping" from the ETC to O_2, reducing O_2 with one electron to $O_2^{\bullet-}$. In other words $O_2^{\bullet-}$ is generated by singlet electron reduction of di-oxygen. Instead, when ADP accelerates electron flux and $O_2^{\bullet-}$ consumption (state 3), the ETC becomes more oxidized, which decreases its redox potential and thus $O_2^{\bullet-}$ formation [11,12].

Under physiological conditions, during electrons flow from NADH and $FADH_2$ to O_2, the $O_2^{\bullet-}$ production is converted to hydrogen peroxide (H_2O_2) by SODs, either in the matrix by Mn^{2+}-dependent SOD (Mn-SOD) or intermembrane space (Cu/Zn-SOD) (Figure 1). The SODs immediately converts $O_2^{\bullet-}$ to H_2O_2 that diffuses freely across organelle and cellular membranes.

H_2O_2 may, thus, influence the cell function by reacting with thiol residues of the redox-sensitive proteins in both the cytoplasms, nucleus and/or other organelles, including mitochondria [6]. Moreover, H_2O_2 can diffuse and affect other cell function in a paracrine fashion. Yet, the presence of metal cofactors and iron-sulfur clusters in ETC proteins can lead to an increase in oxidative stress through the Fe^{2+}-dependent Fenton reaction or the Fe^{3+}-dependent Haber–Weiss reaction in which H_2O_2 is converted in a highly reactive radical species (hydroxyl radical, $^{\bullet}OH$) [13].

Among the proteins that form the ETC, complex I (NADH: ubiquinone oxidoreductase) and III (ubiquinol: cytocrome c oxidoreductase; cytocrome bc complex) are considered the main ROS producers because directly involved in $O_2^{\bullet-}$ formation [7-9,14,15] (Figure 1). ROS production from complex I can occur in two different ways: 1) in a forward mode when the downstream passage of electrons from NADH is blocked by the binding of inhibitors, such as rotenone, to Q-site or by inhibitors of complex III or IV, and 2) in a reverse electron transfer, when electrons from succinate of complex II are transferred to complex I via ubiquinol. Recently succinate accumulation during ischemia and subsequent succinate oxidation after reperfusion has been proposed as principal mechanisms to explain the burst of mitochondrial ROS production [16]. In fact, recent studies revealed that another relevant source of ROS is represented by complex II (succinate: ubiquinone oxidoreductase; succinate dehydrogenase). It is well known that ROS formation by complex I and III can be modulated by activity of complex II. High concentration of succinate and high membrane potential are associated with a massive $O_2^{\bullet-}$ production by complex I, while complex II inhibitors slow ROS formation; an inhibition of complex II also can lead to the $O_2^{\bullet-}$ formation at the ubiquinol oxidation site of complex III. Recently Quinlan et al. found that complex II of mitochondria derived by rat skeletal muscle cells can produce high levels of $O_2^{\bullet-}$ or H_2O_2, in the presence of an inhibition of complex I and complex III and a low concentration of succinate [17-19]. Ubisemiquinone represents the electron donor for $O_2^{\bullet-}$ formation by complex III of heart mitochondria during Q-cycle mechanism excluding a role for ubiquinol and cytochrome b [20]. However experiments in vitro show that, if inhibitors as antimycin A block ubiquinone reduction site, ROS production by complex III can occur at the ubiquinol oxidation site [21]. Under physiological conditions, it seems that the complex IV does not produce ROS, however Prabu et al., report that in ischemic conditions $O_2^{\bullet-}$ formation is mediated by hyperphosphorylated complex IV [22] demonstrating an involvement in pathological conditions. For extensive review about ROS formation in ETC see [11].

An imbalance between an increase of ROS formation and

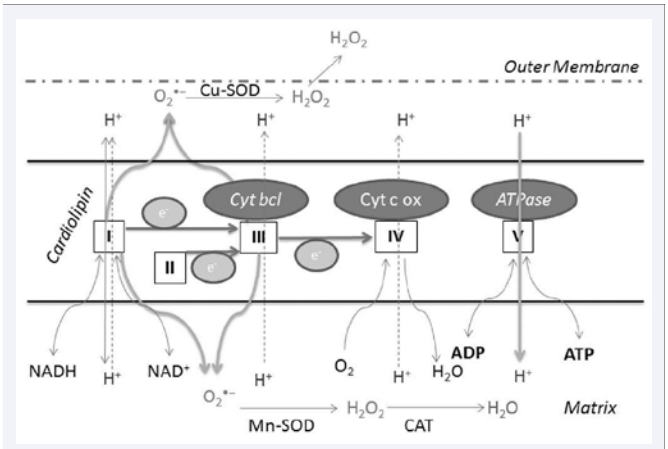

Figure 1 Respiratory chain and main sites of ROS production. Anion superoxide is mainly formed along the ETC on complex I and III. It is transformed by Mn-SOD or Cu-SOD to peroxide, which diffuses freely out of mitochondria. For acronyms and further explanation see the text.

reduced anti-oxidant defenses can lead to various deleterious processes. Oxidative stress related to the excessive ROS generation plays a crucial role in I/R injury [23] in which an impairment of ETC complexes was described in different model system (isolated cardiomyocytes, Langhendorff-perfused heart) and in animal disease models [17].

Chen et al. have evaluated whether increased ROS formation could be induced by ischemic damage of ETC alone and whether the production site following ischemia coincided with the sites of ischemic damage. These authors report that cardiac ischemia leads to an impairment of ETC and increases the net production of H_2O_2 from complex I and III; thus contributing to myocardial injury during reperfusion. Complex I activity is reduced by ischemia without alteration of NADH ferricyanide oxidoreductase, supporting that impairment happens to the proximal as well as the distal ETC [24]. Importantly, this lowered activity has been observed in the two populations of mitochondria existing in cardiomyocytes, namely subsarcolemmal (SSM) and interfibrillar mitochondria (IFM). In isolated rat heart it was demonstrated that 25min of ischemia decrease complex III activity, cytochrome c (Cyt c) content, and oxidation through Cyt c oxidase in both SSM and IFM [25]. It is proposed that rat hearts subjected to I/R protocol present defects in complex I, III and IV activity that could be attributed to a ROS-induced damage of cardiolipin, which is required for optimal activity of complex I [26].

Nevertheless, the pharmacological administration of a reversible inhibitor of complex I, e.g. amobarbital, immediately prior to ischemia protects distal ETC function and attenuates cardiac injury in a dose-dependent manner [27]. Moreover, *in vivo,* the administration of different complex I activity inhibitors, such as volatile anesthetics, HMR098, nitrosyl-modifiers of cysteine and cell permeable nitric oxide (NO) donors. Attenuate I/R injury [28].

Mitochondrial permeability transition pore (mPTP), whose composition is still controversial (see below) interacts with respiratory complexes to produce massive quantity of ROS. Recently Gharib group demonstrated that the pharmacological association of cyclosporine-A, an inhibitor of the putative component of mPTP Cyclophillin D (CypD), with rotenone, an inhibitor of complex I, in presence of protective maneuvers (PreC and PostC) reduces cell death in adult mice cardiomyocytes and HL- cardiac cell-line after hypoxia/reoxygenation (H/R). These observations led to the conclusion that this protection is dependent by CypD and are associated with mPTP opening prevention and with an enhanced mitochondrial resistance to Ca^{2+} overload [29]. Moreover, in isolated mouse heart the maintenance of an intracellular low pH in the early minutes of reperfusion reduces myocardial injury preventing mPTP opening through lowered ROS generation via a reversible and partial blockade of electron transport at complex I [28].

Szczepanek et al, using transgenic mice with cardiomyocyte-specific over expression of mitochondria-targeted STAT3 with a mutation in the DNA-binding domain, demonstrated that genomic modulation of complex I activity limits mitochondrial injury during ischemia and subsequent myocardial injury during reperfusion [30]. Using genetic *in vivo* and *in vitro* models, other authors demonstrated that mice deficient for the second isoform of sphingosine kinase, due to a pre-existing defect in complex IV assembly depending by sphingosine--phosphate, cannot be protected by PreC [26]. Thus, it is tempting to speculate that ROS from complex I are deleterious, whereas those from complex IV are protective. Whether this is due to the amount of ROS and/or to co-localization of anti-oxidants is not clear at moment. It has been reported that specific complex II inhibitors, such as atpenin A5 or malonate, have been used to mimic PreC and PostC-like cardioprotective effects through activation of mitochondrial ATP-sensitive potassium channels [31,32] in different model systems (see also below). This cardioprotective effect is due to the decrease of the membrane potential that is necessary for the formation of deleterious ROS at complex I via reverse electron transfer during reperfusion and to the promotion of production of signaling ROS at the Q_o site of complex III by shifting the redox state of the Q-pool to an intermediate state [17]. Thus, attenuation of electron flux through complex during ischemia and early reperfusion reduces myocardial injury and enhances functional recovery.

POST-TRANSLATIONAL MODIFICATIONS OF MITOCHONDRIAL COMPLEXES IN PREC AND POSTC

Many authors report that posttranslational modifications, such as an increase of protein tyrosine nitration of complex I and complex II, a decrease of protein *S*-glutathionylation of complex II, and an increase of hyperphosphorylation of complex IV are involved in I/R injury mechanism [11,21,22,33]. Liu and colleagues demonstrated that in mouse heart after I/R there is an important increment in protein tyrosine nitration. In particular nitrated mitochondrial proteins include 4 subunits from the oxidative phosphorylation system (the 24 and the 30 kDa subunits of complex I, the Rieske ISP of complex III, and the α subunit of ATP synthase), five enzymes in the matrix, and voltage-dependent anion channel (VDAC), another putative component of mPTP [34] (see also below the paragraph on Mitochondrial permeability transition pore). Reversible S-nitrosylation of Cys39 on the ND3 subunit of complex I moderates ROS production, oxidative injury and tissue necrosis slowing the reactivation of mitochondria at the beginning of reperfusion of ischemic tissue [16]. Furthermore in the myocardium subjected to I/R oxidative impairment, decreased protein S-glutathionylation, and increased protein tyrosin nitration at the 70 kDa subunit was described in the complex II [33,35]. Overall, data suggest that S-nitrosylation prevails in protection, whereas nitration prevails in injury.

Recently, Sun et al., have shown that, pretreating rat neonatal cardiomyocytes with a H_2S donor, the amount of ROS in the course of a simulated I/R results reduced through inhibition of mitochondrial complex IV activity and increase of the activities of SODs, via their S-sulfhydration [13]. Moreover, Ran et al. report that delayed PreC induced by leaf extract of Gingkgo biloba reduces cardiac I/R injury through an up-regulation of Cyt c oxidase expression in rats [36]. Delayed PreC, which occurs 2-24 hours after PreC stimuli, requires modulation of gene expression. In fact, Chen group, evaluating bioenergetics in isolated rat hearts subject to I/R, demonstrated a biphasic modulation of electron transfer activity and ETC protein expression during I/R, which appears to be correlated to dynamic biosynthesis of peroxisome

proliferator-activated receptor-γ coactivator- α (PGC- α), a coactivator of the PPARγ, and regulated by gene transcription and translational control [37].

Besides PreC, mitochondrial ETC is also involved in PostC protection. In fact, in a pig model of regional myocardial I/R Heusch et al demonstrated the activation of mitochondrial STAT3 with better conservation of mitochondrial complex I, calcium retention capacity and reduction of infarct size by ischemic PostC [38]. Very recently Lou demonstrated that PostC cardioprotection can be obtained using Intralipid®, a clinically available fat emulsion, and involves the fatty acid intermediate palmitoylcarnitine, an inhibition of complex IV, an increase in signaling ROS production and an activation of the so-called RISK (reperfusion injury salvage kinases) pathway, highlighting a new mechanism of PostC cardioprotection. [39].

In summary, in the heart the mitochondrial respiratory chain may be the main source of ROS and the beneficial role of mitochondrial ROS has been demonstrated by several lines of evidence, ranging from their role as signaling molecules in heart rest conditions to their modulator role during increased metabolic demand. The various mitochondrial complexes intervene in different conditions of oxygenation to determine an increase of ROS production.

Thus, high mitochondrial ROS production may be responsible of redox stress and damage, especially in ischemia and reperfusion. However, the bland activation of complexes III and IV has been implicated in protective pathways, and the lower ROS production may trigger protective redox sensible signaling, whereas the reversible inhibition of ETC complexes, particularly complex I, has been involved in cardioprotection.

MITOCHONDRIAL ATP-SENSITIVE K^+ CHANNEL

The exact composition of mitochondrial ATP-sensitive K^+ (mK_{ATP}) channels is unknown. However channel complexes seem formed by KCNJ-encoded Kir6. 2 pore subunits co-assembled with the regulatory ATP-binding cassette sulfonylurea receptor. These channels are located in the inner mitochondrial membrane (IMM), and they may be considered like checkpoints that perform a rheostat-like operation adjusting mitochondrial membrane potential dependent functions to match energetic demands of the working heart [40-42]. It is also likely that these channels operate as biosensors that enable high-fidelity read-out of metabolic distress signals [43-45].

Specifically, mK_{ATP} channels may directly contribute in regulating mitochondrial function via effects on membrane potential and on mitochondrial matrix volume regulation. It seems that mK_{ATP} channel opening allows K^+ entry within mitochondrial matrix which, together K^+/H^+ exchange, leads to matrix swelling, thus enhancing the rate of oxidative metabolism and the formation of ROS [46-49]. Actually, the activity of the mK_{ATP} channel may be linked to the cellular energetic state *via* its inhibition by ATP/ADP [48,49]. Changes in mitochondrial matrix volume, mitochondrial potential, oxygen consumption and ROS production have been described by study that used mK_{ATP} channel inhibitors and openers [46-49]. Only when molecular approaches will be available for studying these channels more information on their physiological function may be acquired.

Channel deficit impairs tolerance to endurance challenge, hemodynamic load, or sympathetic discharge [6,50,51]. mK_{ATP} channels are considered targets of protective signaling pathways and controller of the ROS signaling that are essential for cardioprotection [6,49]. In fact mK_{ATP} channels opening has been found to augment ROS levels in the mitochondrial matrix of perfused heart [52] and in cardiomyocytes [53]. mK_{ATP} channel activation comprises the PreC and PostC protective effect [10,54,55], whereas disruption of the mK_{ATP} channels blunts this protective response [56,57].

Channel opening allows K^+ to enter the matrix along its electrochemical gradient that then is counter balanced by electrogenic efflux of H^+ directed by respiratory chain. mK_{ATP} dependent matrix alkalinization affects complex I and/or III, thus leading to an increased generation of $O_2^{\bullet -}$ and its byproducts H_2O_2 and $^{\bullet}OH$ [47] (Figure 2). Yet, the K^+ uptake and the activity of the K^+/H^+ exchanger may improve matrix volume regulation, which, as said above, is important in the regulation of the rate of oxidative phosphorylation and associated ROS production [47,49]. In this ROS production a role may be also played by Cx43 (see below). Nevertheless, the mechanism of mK_{ATP}-dependent elevated synthesis of ATP and ROS has been proven in mitochondria isolated from preconditioned hearts [47,49], but remains to be ascertained in vivo. The redox coupling of mK_{ATP} channel opening and PKC activation may represent an important link in the cardioprotective signaling. Forbes and colleagues were the first to recognize this association, observing that anti-oxidants, such as N-acetylcysteine (NAC) or N-2-mercaptopropionylglycine (MPG), could interfere with the protection induced by diazoxide (DZO), a putative mK_{ATP} channels opener [58]. In fact, pharmacological opening of mK_{ATP} channels by DZO contributes to the generation of small quantity of ROS [59]. Studies performed by Garlid and collaborators showed that the ionophore valinomycin, at a concentration yielding the same K^+ flux as DZO, produced the same ROS augment as DZO demonstrating that the mK_{ATP} -dependent ROS increase is due specifically to K^+ influx into the

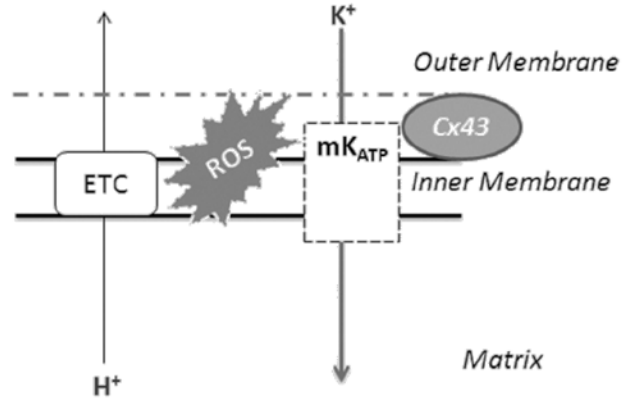

Figure 2 Cx43 and mK_{ATP} channel interaction. Cx43 and mK_{ATP} channels may cooperate in enhancing ROS production by mitochondria. mK_{ATP} channels are regulated by Cx43 and by other factors (see text) to open and allow K^+ entry. This entry regulates mitochondrial volume and favors ROS formation by ETC. For acronyms and further explanation see the text.

matrix [46]. We evidenced that ROS signalling is downstream of mK_{ATP} channel opening in isolated rat hearts after a I/R protocol with an intermittent infusion of DZO or DZO+MPG at the beginning of reperfusion, since MPG attenuated DZO-induced protection [60].

In the context of cardioprotection, it was demonstrated that PKG and/or Akt-dependent phosphorylation leads the mK_{ATP} channels opening, promoting K^+ entry into mitochondria with consequent alkalinisation of the mitochondrial matrix and production of ROS with a protective signalling role. Indeed, activated PKG induces the phosphorylation of a protein on the outer mitochondrial membranes (OMM), which then causes the mK_{ATP} channels on the IMM to open, suggesting that the signal of protection is transfered from the OMM to the IMM. This is completed by a series of intermembrane signalling steps that includes PKCε activation. The resulting small ROS burst activates a second PKC pool which, through another signal transduction pathway, causes mPTP inhibition (the putative end effector) and cell death reduction [61]. The production of this small burst of ROS occurs at the re-introduction of oxygen in the myocardium during the phase of reflow of the PreC and PostC cycles. Then ROS can directly activate PKC by triggering release of Zn^{2+} from the regulatory domain [62]. Schemes and diagrams describing these protective pathways converging on mitochondria have been previously published by us, e.g. [61], and other groups, e.g. [63].

Many authors have proposed H_2O_2 as the messenger of ROS-dependent mK_{ATP} channels opening [46,64]. Conversely, recent studies demonstrated that the signaling ROS responsible for activating mitochondrial PKCε is a downstream oxidation product of $^{\bullet}OH$ and that superoxide and H_2O_2 are not the direct signaling ROS. These studies also showed that the main action of submillimolar MPG on mitochondria and perfused heart was the inactivation of PKCε by thiol reduction, rather than ROS scavenging [48]. These studies strongly support the concept that a reactive species is not always bad or always good, but that the final result always depends on many variables in play.

Also $NO^{\bullet}$ donors can activate mK_{ATP} channels in ventricular myocytes of rabbit and can promove the protective effect of mK_{ATP} channels opener DZO[65]. Besides cGMP/PKG-dependent phosphorylation, mK_{ATP} could be opened by direct reaction of $NO^{\bullet}$ and derivatives via S-nitrosylation, as well as by the action of H_2S via S-sulfhydration [5,61]. However, controversy exists on the nature, existence and opening of mK_{ATP} channels, which may also be a toxic process [66,67]. PKC activation leading to the opening of mK_{ATP} channels has been challenged by Halestrap group: they demonstrated that PreC inhibits mPTP opening in situ, through an indirect mechanism that probably involves a decreased ROS production and Ca^{2+} overload at reperfusion [41,67].

In summary, mK_{ATP} channels opening could be triggered by a variety of stimuli and it seems a fundamental step of mitochondrial ROS signalling in PreC and PostC cardioprotection.

MITOCHONDRIAL CONNEXIN 43

Although the mechanism by which Connexin 43 (Cx43) is necessary for cardioprotection in myocardium or mitochondria has not been elucidated, we briefly discuss this prominent and intriguing feature of cardioprotection. Connexin 43 is a transmembrane protein, fundamental for the formation of gap junctions in the ventricular myocardium and essential for cell-cell communication. Electrical coupling of cardiomyocytes in the health heart or the spread of death signals among neighboring cells in post-ischemic myocardium is determined by the number of gap junctions and by the conductance of each gap junction. One of the mechanism that regulates the permeability of these junctions is the phosphorylation at Ser or Tyr residues by protein kinases of Cx43 [68,69]. Cx43 is formed by four transmembrane domains, one intracellular and two extracellular loops, as well as by cytosolic amino- and carboxy-termini.

However Cx43, depending on circumstances and cell type, can play important role in different mechanisms as survival and death [68,70]. A series of studies demonstrated the significant role of Cx43 in ROS production in mitochondria for redox signaling [54,59,71-73].

In cardiomyocytes, Cx43 is also present at the mitochondrial membranes and in particular at IMM of SSM subpopulation (Figure 2). Cx43 presence has not been detected at IMM of cardiomyocytes of IFM subpopulation [71,72]. The pathway by which Cx43 is imported into SSM with cardioprotection seems to involve Hsp90 and Tom20 [74].

It has been reported that the decrease in mitochondrial Cx43 quantity is sufficient to inhibit the cardioprotection induced by DZO PreC, reducing ROS formation [59]. Moreover, mitochondrial Cx43 has been described to be fundamental for PreC protection, but not for PostC protection in mice heterozygous for Cx43 (Cx43+/-) [74-76]. Actually, Cx43, as said before, is a target of different protein kinases, and mitochondrial Cx43 is highly phosphorylated under physiological conditions [61,77]; it seems that the phosphorylated portion of Cx43 is increased with ischemia and reduced with PostC, but the role of Cx43 in PostC has not yet been clarified [71,78]. We proposed that the lower level of Cx43 phosphorylation in PostC may contribute to have low levels of ROS in early reperfusion with a protective role [79].

Cx43 has been linked with the structure and function of the mK_{ATP} channels and with their opening; thus contributing to cardioprotection through the production of a modest amount of ROS, which acts as trigger biofactors of PreC and PostC. Mitochondrial PKCε, which is a mediator of the activation of mK_{ATP} channels, phosphorylates also Cx43 and the activation of mK_{ATP} channels is closely associated with Cx43 presence [6,47]. It was demonstrated that if Cx43 is absent or inhibited mitochondrial K^+ influx is reduced, which might affect mitochondrial respiration [72,73]. In fact, in murine mitochondria in which Cx43 was replaced by Cx32, a connexin which forms channels with lower K^+ conductance than Cx43 [80], mitochondrial K^+ influx is reduced [72]. It has been proposed by Ishikawa et al. that in cardiomyocytes Cx43 is necessary for the activation of class I_B PI3K by GPCRs, leading to a protection against necrosis mediated by Akt-GSK3β pathway. The authors suggested that PI3K-Akt signaling is essential to promote the ROS formation by the opening of the mK_{ATP} channels.

In fact, this function of the mK_{ATP} channels requires a certain level of Cx43 and its putative cofactor, β-subunit of G protein, in mitochondria [81]. It has also been observed that mitochondrial

Cx43 forms a co-immunoprecipitate with GSK3β. Interestingly; GSK3β phosphorylation at Ser9 is considered the point of convergence of several cardioprotective pathways, leading to mPTP inhibition [71].

Recently, an analysis of the influence of Cx43 on mitochondrial function has underlined the importance of Cx43 for O_2 consumption and ATP production. Inhibition or reduction of mitochondrial Cx43, by 8αGA or Cx43 mimetic-peptides, specifically decreases complex I respiration [82]. Moreover, Boengler and collaborators showed that both the genetic ablation of Cx43 in conditional knockout mice and the acute inhibition of mitochondrial Cx43 by Gap 9, a specific Cx43 inhibitor, reduce velocities of mitochondrial K^+ uptake, underlining the substantial impact of Cx43 on mitochondrial K^+ fluxes [83]. Yet, Ruiz-Meana and colleagues have shown that the protective effect of PreC against mitochondrial respiratory failure in reperfused myocardium is independent of the most widely accepted mechanisms of PreC protection, including mK_{ATP} channels and mPTP, but requires the presence of mitochondrial Cx43. In fact, this study shows that this protective effect of PreC is unaffected by inhibition of mK_{ATP} channels and mPTP, but is lost in mitochondria from genetically-modified animals in which Cx43 is replaced by the low conductance connexin Cx32 [84].

In summary, it seems that mitochondrial Cx43 in SSM has a role of essential importance for the correct functioning of mK_{ATP} channels and mitochondrial respiration and may influence the formation of protective ROS.

MITOCHONDRIAL PERMEABILITY TRANSITION PORE

Although the molecular structure of mPTP is controversial, there are no doubts that it is a mitochondrial feature that may play a crucial role in life and death of cells dissipating the transmembrane proton/electrochemical gradient. This pore is a high-conductance mega channel

which may play an important role in regulating the Ca^{2+} homeostasis in physiological, pathological and pathophysiological conditions [85]. The opening of mitochondrial Ca^{2+} uniporter (MCU) [86], located in the inner membrane, is responsible for Ca^{2+} entry in the mitochondrial matrix, because of to the driving force of the negative mitochondrial membrane potential. Calcium exit may occur via the mitochondrial Na^+/Ca^{2+} exchanger [87], the H^+/ Ca^{2+} exchanger [88] and/or the mPTP opening [85]. This Ca^{2+} handling is a key mechanism for the transduction of life or death signals to mitochondria. It is clear that prolonged mPTP opening requires matrix Ca^{2+} overload and is modulated by ROS, inorganic phosphate, cyclophilin D and pH. It seems that mPTP oscillate rapidly between open and closed states in normal cells, where this transient mPTP opening may be a physiological mechanism responsible of "Ca^{2+} spitting out" that can prevent dangerous Ca^{2+} overload in the matrix. It has been also proposed that the transient opening is involved in ROS-dependent signaling responsible of preconditioning cardioprotection [89]. Actually, in the I/R and cardioprotection scenarios the modulation of mPTP is crucial in determining the life or death of cells. When matrix Ca^{2+} overload occurs, together the other above mentioned modulators, a prolonged mPTP opening may result in the release of pro-apoptotic factors (i.e. cytochrome c), collapse of oxidative phosphorylation and cell death for apoptosis or necrosis (see also below).

Studies on cardiomyocytes performed by Paillard and collaborators [90] have reported that the mitochondrial CypD, a putative component of mPTP, modulates the Ca^{2+} crosstalk between endoplasmatic reticulum and mitochondria via the VDAC/Grp75/IP3R complex. This regulation has a significant function in reperfusion injury. Genetic or pharmacological inhibition of every partner of the complex modifies the Ca^{2+} fluxes and blunts lethal reoxygenation injury.

The formation of mPTP plays a pivotal role in the so-called ROS-induced ROS release (RIRR) [91], where an excess of ROS induces the opening of mPTP, which in turn facilitates the formation of ROS through inhibition of the respiratory chain because of the loss of Cyt c and pyridine nucleotide induced by mPTP. [10,92]. Many studies have indeed revealed a relevant participation of mPTP opening and have associated cellular death with the release of Cyt c after pro-apoptotic Bax and enhanced ROS levels. This vicious cycle begins with the rapid reperfusion when a great increment in ROS production could be observed due to the recovery of pH and Ca^{2+} overload, thus inducing the amplification of the damage [61].

The pore was described for the first time by Hunter et al. in 976 and it is positioned between IMM and OMM [93]. Its opening consents a communication between the cytoplasm and the mitochondrial matrix. The molecular identity of the proteins that constitute this pore has not yet been clarified, however, it has been suggested that it is formed by the VDAC in the OMM, the adenine nucleotide transporter (ANT) in the IMM, and CypD in the matrix of the mitochondria (Figure 3). In the closed state, CypD is separated from the IMM, whereas hexokinase II (HK II) is connected to OMM components of the pore. Instead, the opened state seems to be promoted by the binding of CypD to the IMM in a process controlled by both Ca^{2+} and inorganic phosphate (Pi) [6,10,61].

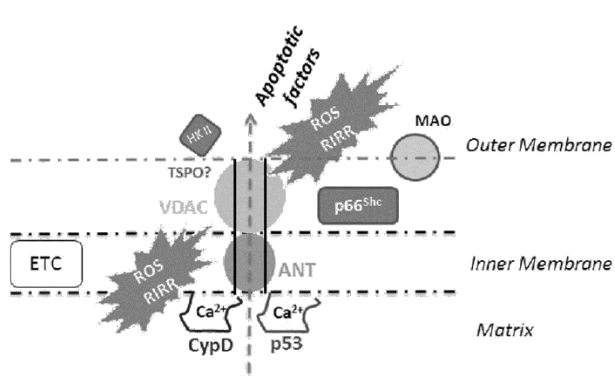

Figure 3 Putative components of mPTP and other mitochondrial enzymes. mPTP and enzymes are involved in the production of ROS and in triggering the mechanisms leading to RIRR phenomenon and to the release of pro-apoptotic factors. Also MAO and $p66^{Shc}$ are located between the two membranes and contribute to form H_2O_2. For acronyms and further explanation see the text.

Importantly, after an oxidative stress, mPTP opening induced by Cyp D translocation from the matrix to the IMM and consequent interaction with target protein can occur through direct and indirect mechanisms.

Oxidative stress can trigger a direct binding of CypD to a target protein in the IMM inducing a conformational modification of the target protein through chemical changes and/or variation in the inner membrane topography due to elevated swelling of the matrix.

Most, if not all, studies in the late 990s and early 2000s years have put the focus on ANT as a target protein that cooperating with CypD triggers the pore opening.

First studies have offered large evidences that a key role in mPTP opening is played by the Ca^{2+}- triggered conformational modification of the ANT which is promoted by CypD binding [94-96].

The interaction of CypD with other proteins present in the matrix can lead to an indirect binding with the target protein in the IMM. In fact, recent studies demonstrated a Ca^{2+}-dependent mPTP opening by p53. In fact, after a brain IR injury the oxidative stress can lead to an increase in the mitochondrial matrix of p53, a tumor suppressor protein that triggers mPTP opening and necrosis through physical interaction with CypD. Conversely, diminuition of p53 levels or mice treatment with CsA prevented p53-CypD complex opening which was correlated with effective stroke protection [97]. It is likely that p53 triggers translocation of CypD to the IMM and therefore favors the pore opening through association interaction with a pore protein. However, no regulation of Ca^{2+}-dependent mPTP opening by p53 has been observed and it is not clear how p53-CypD interaction senses and promotes mPTP opening in a Ca^{2+}-independent mode [95,98].

Genetic studies conducted in knockout mice revealed that mitochondria containing neither VDAC nor ANT were still sensible to Ca^{2+}-dependent mPTP opening by p53-induced mPTP formation, therefore excluding the role of these proteins as the central structural elements of the mPTP. Furthermore, these investigation have also clearly proved that CypD is a key modulator of mPTP [56,95,99,100]. However, mitochondria isolated from CypD-/- mice were more resistant to mPTP opening with respect to wild-type mice and showed mPTP induction at highe Ca^{2+} concentration, and lower cell death in response to oxidative stress [57,95,101]. Moreover, mPTP-mediated cell death preferably happened through necrosis rather than apoptosis as $CypD^{-/-}$ cells were resistant to necrotic stimuli but showed analogous sensitivity to apoptotic factors as wild-type cells [57,95]. Yet, current results indicate that the mPTP is composed by a unique Ca^{2+}-dependent arrangement of dimers of the ATP synthase [102,103].

Furthermore, several studies clearly demonstrated that mitochondrially-bound HK II is indeed a significant determinant of infarct size and may represent one of the end-effectors of PreC. In fact, HK II seems a necessary component of mPTP multiprotein complex. Mitochondrial HK II, besides to regulate mPTP, affects mitochondrial membrane potential and ROS production, and may also define the direction of cardiac metabolic flux [104]. Mitochondrial translocator protein (TSPO), previously called peripheral benzodiazepine receptor, is an 8-kDa protein located in the OMM and is considered another putative component of the mPTP complex [105,106]. It has been reported that ligands of TSPO, 4′-chlorodiazepam and TRO40303, prevent the doxorubicin-induced alterations in contractility and improve cardiomyocytes viability. These cardioprotective effects were correlated with both a potent decrease in ROS formation and inhibition of mPTP opening. Thus, administration of TSPO ligands has been proposed as a novel pharmacological strategy to protect the heart. However, recent evidences have shown that TSPO has no role in the modulation or structure of the mPTP. These experiments, conducted in mice in which the Tspo gene has been conditionally eliminated or employing natural and synthetic ligands of TSPO, suggest that TSPO ligands do not regulate mPTP activity. Further, hearts lacking TSPO are as sensitive to I/R injury as hearts from control mice, underlining that TSPO is not involved in heart I/R injury, a condition where the mPTP is causally involved [103]. Future studies may ascertain the real role of TSPO on mPTP function.

Phosphorylation of putative mPTP components by Akt, PKC, GSK or STAT3 has been proposed as mechanism(s) involved in modifying pore opening. Also the post-transcriptional modification induced by NO and derivative, namely S-nitrosylation, may involve direct protein modifications or indirect effects through protein kinases. Indeed, a mitochondria-selective S-nitrosylating agent has been shown to reduce infarct size in mice [16]. Moreover, pharmacological PostC with DZO induced a redox sensible phosphorylation/translocation of Akt, ERK/2 and GSK3β into the mitochondria and increased mitochondrial S-nitrosylated-protein components of mPTP (e.g., VDAC) in rat hearts [6, 32].

Recently, studies confirmed that also classical NO-cGMP modulates the pore opening by inactivating GSK3β, in fact the protective effect of cGMP on the mPTP opening is lost in cells transfected with the constitutively active GSK3β mutant plasmid [107]. Importantly, inactivation of GSK3β is considered the final step of cardioprotective pathways. Accordingly, GSK3β inhibitors given at reperfusion mimic conditioning protection [108].

Opening of mPTP is regulated by ions (Pi,H^+,Ca^{2+},Mg^{2+}), ROS, adenine nucleotides, ubiquinones [50, 109,110], and many other biofactors. During a prolonged ischemia, when pH decreases below 7.4 value, the probability of opening of mPTP of the de-energized mitochondria is greatly reduced. Low pH also diminishes mitochondrial Ca^{2+} uptake and promotes Ca^{2+} expulsion from mitochondrial matrix, due to mitochondrial NHE and NCE activation [79]. Yet, low pH may activate uncoupling proteins (UCPs), carriers of H^+ that uncouple ATP synthesis from O_2 consumption [111]. Low pH may also repress glycolysis and pyruvate formation, leading to a slower feeding of the respiratory complex chain. Therefore, low pH mainly limits the opening of the mPTP during the ischemia. At the onset of reperfusion, quite various conditions are produced depending on whether mitochondrial membrane potential quickly recovers. In case of energized respiring mitochondria, a low pH can encourage Pi uptake growing its intra-mitochondrial quantity, thus acting as an opener of the mPTP. On the contrary, when the mitochondrial

membrane is depolarizated, long-lasting opening of the mPTP occurs when quick normalization of tissue pH occurs in the presence of Ca^{2+} overload, Pi, ROS formation, and/or lower levels of NO• [61]. The pr oapoptotic signals Bax and p53 also promote mPTPopening. It allows entry of water and solutes (<.5 kDa) favouring mitochondrial swelling, which lead to rupture of the MOM and to Cyt c release. Thus, mPTP opening is included in a vicious cycle as it is increased in conditions associated with mitochondrial dysfunction, leading to increased ROS production and depletion of ATP, which in turn favour mPTP opening.

The long lasting mPTP opening is a critical determinant of myocardial I/R injury [112] and inhibition of the pore opening serves as an important mechanism by which PreC [113] and PostC [114] confer cardioprotection. Opening of the mPTP is associated with the loss of the membrane potential of mitochondria and proton gradient across the IMM. Depending on the complicated equilibrium between cellular antagonists and inducers, mPTP can be subjected to a transient or intermediate/long-lasting opening [115]. The mPTP opening for brief period is likely to produce reversible cellular changes, so that this transient opening has been indicated to be implicated in physiological processes and cardioprotection, such as intracellular NAD^+ traffic, and transient production of ROS. Actually, the transient increase in mPTP opening probability is involved in ROS-dependent triggering of cardioprotection by PreC [61,89, 116]. However as said mitochondria are important determinants and regulator of cell death. Besides mPTP formation, apoptotic stimuli converging on Bax and Bak may oligomerize to form pores mediating OMM permeabilization. Cyt c is then released and can bind to the adapter protein Apaf- to activate caspase-9, leading to apoptosis. Cyt c release and apoptosis can be amplified by the opening of mPTPs; however, prolonged mPTP opening results in collapse of oxidative phosphorylation and cell death for necrosis.

In summary, although the molecular structure of mPTP is controversial, when formed it is a high conductance pore that may dissipate the transmembrane proton/electrochemical gradient. The long lasting opening of the pore leads to ATP depletion, failure of membrane ion pumps, enhanced ROS production, solute exit/entry, organelle swelling and ultimate mitochondrial rupture leading to death of cardiomyocytes for necrosis and/or apoptosis. Importantly, acidosis inhibits mPTP formation while calcium and ROS promote its formation. During ischemia the low pH inhibits the formation of the pore, but at the beginning of reperfusion its quick restoration, associated with the rapid increase in mitochondrial calcium concentration and ROS formation causes the pore formation. The low pH in early reperfusion allows redox signaling to activate protective kinases, including PKC, while mPTP formation is still inhibited. Then the cardioprotective signaling pathways converging on GSK3β to keep mPTP closed, even after pH is normalized, and hence cell death is reduced. This seems a common mechanism for both PreC and PostC protection.

$p66^{SHC}$

Different reports highlight the relevance of the growth factor adaptor Shc ($p66^{Shc}$) as a further mitochondrial component which contribute to ROS generation (see for an extensive review: [7, 8]). This protein is a $p52^{Shc}$ /$p46^{Shc}$ splice variant, two cytoplasmic adaptor proteins containing a C-terminal SH2 domain nearby to a collagen homology (CH) region [8] and implicated in the transmission of intracellular signals from activated tyrosine kinases to Ras [9]. $p66^{Shc}$ shows the equivalent $p52^{Shc}$ /$p46^{Shc}$ (SH2-CH-PTB) modular conformation and includes a unique N-terminal region (CH2). A fraction of $p66^{Shc}$, located within the mitochondrial inter-membrane space, oxidizes reduced Cyt c leading to the reduction of molecular oxygen and consequently to the formation of hydrogen peroxide, thus contributing to the opening of mPTP and to the triggering of apoptosis (Figure 4) [120, 121].

Several studies pointed out that the genetic ablation of $p66^{Shc}$ has as effect a substantial decrease of oxidative stress. In fact, cells derived from $p66^{Shc}$ knockout ($p66^{Shc-/-}$) mice showed no changes in reduced ROS levels [122,123]. Moreover, in *in vivo* experiments, $p66^{Shc-/-}$ tissues showed less intracellular and systemic oxidative damage [123,124]. In addition, *in vitro* studies demonstrated that $p66^{Shc\ -/-}$ cells were resistant to apoptosis caused by a variety of diverse signals, including H_2O_2 [125]. Similarly, *in vivo* experiments on $p66^{Shc-/-}$ mice indicated that these mice were resistant to apoptosis induced by hypercholesterolemia and ischemia [123,124].

Several studies relate $p66^{Shc}$ to cardiovascular pathophysiology (see for an extensive review: [10, 126]). In particular, it has been shown that $p66^{Shc}$ has an important role in a wide range of pathological alterations related to oxidative stress [127]. Some works have suggested that PKCβ phosphorylation of $p66^{Shc}$ on Ser36 could induce its translocation to mitochondria [128] increasing mitochondrial ROS formation and amplifying deleterious PKCβ signaling triggered by an initial oxidative stress [0] (Figure 4). Studies conducted in $p66^{Shc}$ knockout mice underlined an association of the lack of $p66^{Shc}$ with myocardial hyperplasia [129]. Moreover, oxidative stress and ROS formation appeared to be relevant also for cells other than differentiated cardiomyocytes. In fact, it has been shown that the lack of $p66^{Shc}$ protects against diabetic cardiomyopathy by preventing the senescence of cardiac progenitor cells that interfered with cardiac and vascular cell turnover [130].

Currently, few information on the possible involvement

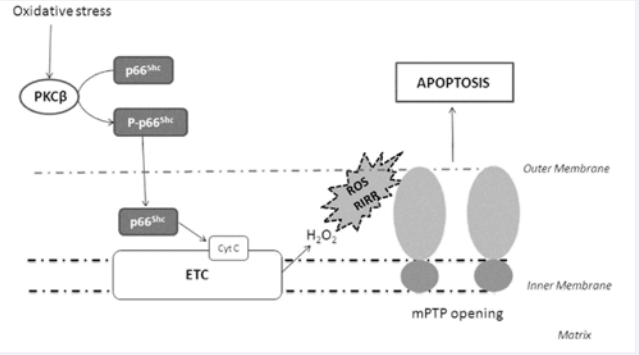

Figure 4 Action of $p66^{Shc}$ to form peroxide and its effects on mPTP. $p66^{Shc}$ in the mitochondrial inter-membrane space through Cyt c induces ROS formation, contributing to the opening of mPTP and to the triggering of apoptosis. For acronyms and further explanation see the text.

of $p66^{Shc}$ in myocardial I/R are available. Recently Carpi and collaborators showed that hearts lacking of $p66^{Shc}$ are more resistant to I/R injury preventing the oxidative damage of structural components of cardiomyocytes as lipids and proteins [120]. Interestingly, this cardioprotective efficacy due to $p66^{Shc}$ ablation was similar to that associated with monoamine oxidase (MAO) inhibition; these effects were not additive, but are not clear whether MAO and $p66^{Shc}$ interact to generate ROS or their final ROS products cumulate (for MAO role in ROS production see below).

In summary, $p66^{Shc}$ is a mitochondrial ROS generator, specifically peroxide, which may play a role in determining susceptibility to cardiac I/R injury. Whether it may play a in some circumstance a physiological ROS-signaling role is unknown.

MONOAMINE OXIDASES

MAOs are flavoenzymes and are present in two isoforms, MAO-A and -B, with different substrate specificity and inhibitors sensitivity. They show in their primary sequence 70% homology [131, 132], and contain the FAD cofactor covalently linked by a thioether bond to Cys406 in MAO-A and to Cys397 in MAO-B [133,134]. Both isoforms present a helix within the carboxy-terminal domain that serves to anchor them to the OMM with a different orientation for the two isoforms [135,136]. However fractions of enzymes are also been observed in the nuclear membrane, endoplasmic reticulum and also in the plasma membrane.

MAOs are necessary for the oxidative deamination of neurotransmitters and dietary amines with the formation of H_2O_2 as byproduct. Oxidative deamination substrates are preferentially norepinephrine and serotonin (5-HT) for MAO-A and phenylethylamine and benzylamine for MAO-B. Both isoforms catalyze the deamination of tyramine, dopamine, octopamine, 3-iodothyronamine and tryptamine. The aldehyde intermediate, produced by the reaction of oxidative deamination, is immediately converted to the corresponding acid by the action of aldehyde dehydrogenases. A failure of this latter enzyme may favor the production of toxic aldehyde intermediate compounds, and may exacerbate the injury determined by MAO-induced H_2O_2 formation. This is the case of either acute cardiac damage due to I/R injury or the passage from compensated hypertrophy to ventricular dilation/pump failure, on chronic conditions (see for an extensive review: [137,138]). It has been described that MAO-A is the prevalent isoform in the human and rat hearts, which do not display appreciable MAO-B activity. However, exactly the opposite is true in mice [139]. Parini and collaborators, which were among the firsts to described MAO-A as an important source of ROS in myocardium, reported that ROS generated by MAO-A are able to trigger different signaling pathways leading either to cell proliferation, hypertrophy or apoptosis depending on the concentrations of substrate available [140].

While low concentrations of 5-HT were able to induce cardiomyocytes hypertrophy in MAO-A dependent manner, including also the activation of ERK/2 [141], the lack of MAO-A activity showed higher cardiac levels of 5-HT, norepinephrine and epinephrine in mice [142,143]. Studies performed in mice knockout for MAO-A showed hypertrophy of cardiomyocytes and LV dilation at baseline, though LV dysfunction was not present and no hemodynamic changes were observed. These results observed in MAO-$A^{-/-}$ mice could be associated to raised cardiac 5-HT levels and hyperactivation of $5\text{-}HT_{2A}$ receptors [143]. Accordingly, experiments performed in dogs with congestive heart failure (CHF) induced by tachypacing showed an elevated MAO-B expression within myocardium [144]. This elevated MAO-B expression may contribute to cardiac decompensation. Moreover, in hearts of mice in which transverse aortic constriction (TAC) induced CHF subsequently to pressure overload, norepinephrine degradation by MAO-A was increased and associated with raised oxidative stress, LV remodeling and apoptosis. Thus, genetic ablation of either MAO-A or -B enzyme alleviated ROS burden and LV maladaptive remodeling, as well as mitochondrial dysfunction and cardiomyocytes apoptosis in hearts of mice subjected to TAC [138].

ROS produced by MAOs, in the presence of high 5-HT concentrations, induce the release of Cyt c, upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2 proteins. Indeed, it was demostrated in an *in vivo* rat model that infarct size was lower in animals treated with pargyline or clorgyline prior to I/R with respect to untreated rats [145].

However, another study on isolated Langendorff perfused mouse hearts reported that MAO inhibition after treatment with pargyline is equally efficacious in limiting I/R injury despite 5-HT was absent in that model [120].

Recently, the important role of MAOs in vasculature has been demonstrated, showing that MAOs expression in mouse aorta was induced through PI3K and NFκB pathway subsequent a treatment with angiotensin II and lipopolysaccharide. MAOs greatly participated to vascular generation of H_2O_2 after these treatments and the pharmacological inhibition of both MAO-A and MAO-B completely prevented this increase in oxidative stress [146]. More recently, a clinical study in human atrial myocardium underlined, for the first time, the contribution of MAOs as source of ROS in this tissue and suggested that atrial MAOs activity serves as an independent predictor of post-operative atrial fibrillation [147].

Some recent studies pointed out that cardiac-specific overexpression of MAO-A was accompanied by ultrastructural defects of cardiac mitochondria, ATP depletion and ultimately led to cardiomyocytes necrosis and heart failure [138,148]. In particular, Di Lisa's group observed that H_2O_2 formation occurs much earlier at the mitochondrial level rather than in the cytosol, supporting the idea that mitochondria are "early targets" of endogenously produced oxidative stress that leads to mitochondrial dysfunction. Yet, MAOs activation was associated with the loss of mitochondrial membrane potential. These researchers also demonstrated the basic role of aldehydes generated by amine catabolism via MAO, in the MAO-mediated mitochondrial dysfunction in cardiomyocytes [138].

In summary, MAOs activity affects cardiac biology and mitochondrial function and is important sources of mitochondrial ROS. It is likely that MAOs inhibition has therapeutic value for treating cardiac ROS-dependent injury of ischemic and non-ischemic origin.

MITOCHONDRIAL NO SYNTHASE

Nitric oxide (NO) is a free radical with important regulatory functions, which in some circumstance may behave as an anti-oxidant. It can exert a complex influence on cardiovascular system not only by regulating vascular smooth muscle tone, but also by adapting several cell function to the different conditions cells afford during their life. In particular, NO may regulate energy metabolism and response to I/R insult. Specific NO synthase (NOS) isoforms are confined to distinct locations in cardiomyocytes. For instance, endothelia NOS (eNOS) is located in the caveolae of the sarcoplasmic membrane, whereas neuronal NOS (nNOS) seems mainly confined onto sarcoplasmic reticulum membrane, where they play specific role [149,150]. Whether a specific mitochondrial NOS (mtNOS) exists is a matter of controversy. Recent data seem to suggest the presence of a NOS isoform within mitochondria, which regulates both chronic hypoxia and I/R. Its function seems affected by metabolic states and mitochondrial environment, including concentrations of Ca^{2+}, O_2, L-arginine, NADPH, and mitochondrial membrane potential ($\Delta\Psi$) [151-153]. Nitric oxide alone or in concert with ROS, forming reactive nitrogen species (RNS), may act through several mechanisms, including guanylyl cyclase activation and/or other protein activity regulation via processes of nitration and/or S-nitrosylation [154]. Therefore, NO and RNS within mitochondria act as pathophysiological regulator of function of Cyt c oxidase and regulates the affinity of components of respiration for oxygen [151-153,155].

In summary, NO and RNS regulates the mitochondrial capacity to act as sensors of oxygen over a large pathophysiological range and it seems that these organelles have their own enzyme (mtNOS) to produce NO.

CONCLUSIONS

ROS are produced by cells during normal life to regulate several physiological functions and to protect themselves by injury, but the production can escape control and may be deleterious. In fact several components of mitochondria contribute with respiratory chain complexes to produce ROS. It appears that they can produce either anion superoxide or peroxide. However the mitochondria comprise several anti-oxidant enzymes (not considered in this minireview) transforming superoxide to peroxide. In fact, the production of the diffusible H_2O_2 by these organelles serves as an significant signaling component that associates cellular metabolism to cell function and needs, as well as to blood flow control. Mitochondrial ROS generation may also play a pivotal role in triggering cardioprotective pathways and evoking the expression of cell survival programs that reduce post-ischemic myocardial injury and cell death by PreC and PostC stimuli. The cardioprotective mechanisms induced by conditioning involve activation of mK_{ATP} channels, and perhaps Cx43, which lead to ROS generation by respiratory chain complexes. ROS may activate downstream components of signaling pathways to drive the expression/activation of cardioprotective enzymes that restrict I/R injury and cell death. Moreover, the different composition of subpopulations of mitochondria suggests that mitochondrial ROS production at signaling levels may be localized at specific intracellular compartments and within subpopulation of mitochondria to activate cell survival programs and to enhance tolerance to I/R. Nevertheless, overproduction of mitochondrial oxidants, to which may contribute MAO and $p66^{Shc}$ sand other enzymes (here not considered), leads to RIRR to exacerbate oxidative stress via mPTP long-lasting opening. This, in turn, can progress to death by necrosis and/or apoptosis during I/R. Therefore, we must focus our attention on these mitochondrial elements to learn increasingly more on the myocardial tissue redox balance in physiology and pathophysiology, and in order to understand more on altered ROS production in the various components of the cardiovascular systems in acute and chronic pathologies. These are prerequisites for an enrichment of our therapeutic armamentarium against many invalidating cardiovascular conditions, such as ischemia/reperfusion injury and chronic heart failure.

ACKNOWLEDGMENTS

The authors of this paper are supported in part by Progetti di Ateneo RILO (ex60%) MeccaSaric to PP and CP. We are indebted to Prof Donatella Gattullo for the invaluable support.

REFERENCES

1. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev. 2007; 59: 418-458.
2. Hausenloy DJ, Erik Botker H, Condorelli G, Ferdinandy P, Garcia-Dorado D, Heusch G, et al. Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res. 2013; 98: 7-27.
3. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ, et al. Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res. 2010; 87: 406-423.
4. Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. Lancet. 2013; 381: 166-175.
5. Pagliaro P, Moro F, Tullio F, Perrelli MG, Penna C. Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell signaling. Antioxid Redox Signal. 2011; 14: 833-850.
6. Penna C, Perrelli MG, Pagliaro P. Mitochondrial pathways, permeability transition pore, and redox signaling in cardioprotection: therapeutic implications. Antioxid Redox Signal. 2013; 18: 556-599.
7. Dröse S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol. 2012; 748: 145-169.
8. Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. Free Radic Biol Med. 2009; 47: 333-343.
9. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009; 417: 1-13.
10. Di Lisa F, Kaludercic N, Carpi A, Menabò R, Giorgio M. Mitochondrial pathways for ROS formation and myocardial injury: the relevance of p66(Shc) and monoamine oxidase. Basic Res Cardiol. 2009; 104: 131-139.
11. Chen YR, Zweier JL. Cardiac mitochondria and reactive oxygen species generation. Circ Res. 2014; 114: 524-537.
12. Nickel A, Kohlhaas M, Maack C. Mitochondrial reactive oxygen species

production and elimination. J Mol Cell Cardiol. 2014; 73: 26-33.

13. Sun WH, Liu F, Chen Y, Zhu YC. Hydrogen sulfide decreases the levels of ROS by inhibiting mitochondrial complex IV and increasing SOD activities in cardiomyocytes under ischemia/reperfusion. Biochem Biophys Res Commun. 2012; 421: 64-69.

14. Brand MD. The sites and topology of mitochondrial superoxide production. Exp Gerontol. 2010; 45: 466-472.

15. Jastroch M, Divakaruni AS, Mookerjee S, Treberg JR, Brand MD. Mitochondrial proton and electron leaks. Essays Biochem. 2010; 47: 53-67.

16. Chouchani ET, Methner C, Nadtochiy SM, Logan A, Pell VR, Ding S, et al. Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. Nat Med. 2013; 19: 753-759.

17. Dröse. Differential effects of complex II on mitochondrial ROS production and their relation to cardioprotective pre- and postconditioning. Biochim Biophys Acta. 2013; 1827: 578-587.

18. Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA, Brand MD. Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. J Biol Chem. 2012; 287: 27255-27264.

19. Siebels I, Dröse S. Q-site inhibitor induced ROS production of mitochondrial complex II is attenuated by TCA cycle dicarboxylates. Biochim Biophys Acta. 2013; 827: 1156-1164.

20. Turrens JF, Alexandre A, Lehninger AL. Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. Arch Biochem Biophys. 1985; 237: 408-414.

21. Drose S, Brandt U, Wittig I. Mitochondrial respiratory chain complexes as sources and targets of thiol-based redox-regulation. Biochim Biophys Acta. 2014; 1844: 1344-1354.

22. Prabu SK, Anandatheerthavarada HK, Raza H, Srinivasan S, Spear JF, Avadhani NG. Protein kinase A-mediated phosphorylation modulates cytochrome c oxidase function and augments hypoxia and myocardial ischemia-related injury. J Biol Chem. 2006; 28: 206-270.

23. Tullio F, Angotti C, Perrelli MG, Penna C, Pagliaro P. Redox balance and cardioprotection. Basic Res Cardiol. 2013; 108: 392.

24. Chen Q, Moghaddas S, Hoppel CL, Lesnefsky EJ. Ischemic defects in the electron transport chain increase the production of reactive oxygen species from isolated rat heart mitochondria. Am J Physiol Cell Physiol. 2008; 294: 460-466.

25. Lesnefsky EJ, Gudz TI, Migita CT, Ikeda-Saito M, Hassan MO, Turkaly PJ, et al. Ischemic injury to mitochondrial electron transport in the aging heart: damage to the iron-sulfur protein subunit of electron transport complex III. Arch Biochem Biophys. 2001; 385: 7-28.

26. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM. Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: involvement of reactive oxygen species and cardiolipin. Circ Res. 2004; 94: 53-59.

27. Chen Q, Hoppel CL, Lesnefsky EJ. Blockade of electron transport before cardiac ischemia with the reversible inhibitor amobarbital protects rat heart mitochondria. J Pharmacol Exp Ther. 2006; 36: 200-207.

28. Xu A, Szczepanek K, Maceyka MW, Ross T, Bowler E, Hu Y, et al. Transient Complex I Inhibition at the Onset of Reperfusion by Extracellular Acidification Decreases Cardiac Injury. Am J Physiol Cell Physiol. 2014; 306:1142-1153.

29. Teixeira G, Abrial M, Portier K, Chiari P, Couture-Lepetit E, Tourneur Y, et al. Synergistic protective effect of cyclosporin A and rotenone against hypoxia-reoxygenation in cardiomyocytes. J Mol Cell Cardiol. 2013; 56: 55-62.

30. Szczepanek K, Chen Q, Derecka M, Salloum FN, Zhang Q, Szelag M, et al. Mitochondrial-targeted Signal transducer and activator of transcription 3 (STAT3) protects against ischemia-induced changes in the electron transport chain and the generation of reactive oxygen species. J Biol Chem. 2011; 286: 2960-2920.

31. Wojtovich AP, Brookes PS. The complex II inhibitor atpenin A5 protects against cardiac ischemia-reperfusion injury via activation of mitochondrial KATP channels. Basic Res Cardiol. 2009; 104: 121-129.

32. Penna C, Perrelli M, Tullio F, Angotti C, Camporeale A, Poli V, et al. Diazoxide postconditioning induces mitochondrial protein S-Nitrosylation and a redox-sensitive mitochondrial phosphorylation/translocation of RISK elements: no role for SAFE. Basic Res Cardiol. 2013; 108: 371.

33. Chen YR, Chen CL, Pfeiffer DR, Zweier JL. Mitochondrial complex II in the post-ischemic heart: oxidative injury and the role of protein S-glutathionylation. J Biol Chem. 2007; 282: 32640-32654.

34. Liu B, Tewari AK, Zhang L, Green-Church KB, Zweier JL, Chen YR. Proteomic analysis of protein tyrosine nitration after ischemia reperfusion injury: mitochondria as the major target. Biochim Biophys Acta. 2009; 794: 476-485.

35. Chen CL, Chen J, Rawale S, Varadharaj S, Kaumaya PP, Zweier JL, et al. Protein tyrosine nitration of the flavin subunit is associated with oxidative modification of mitochondrial complex II in the post-ischemic myocardium. J Biol Chem. 2008; 283: 2799-8003.

36. Ran K, Wan J, Yang D, Xiao Y, Chang Y. [Effect of Gingkgo biloba leaf extract induced delayed preconditioning on cytochrome c oxidase expression during myocardial ischemia-reperfusion in rats]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2012; 37: 89-93.

37. Lee HL, Chen CL, Yeh ST, Zweier JL, Chen YR. Biphasic modulation of the mitochondrial electron transport chain in myocardial ischemia and reperfusion. Am J Physiol Heart Circ Physiol. 2012; 302: 410-422.

38. Heusch G, Musiolik J, Gedik N, Skyschally A. Mitochondrial STAT3 activation and cardioprotection by ischemic postconditioning in pigs with regional myocardial ischemia/reperfusion. Circ Res. 2011; 109: 302-308.

39. Lou PH, Lucchinetti E, Zhang L, Affolter A, Schaub MC, Gandhi M, et al. The mechanism of Intralipid(R)-mediated cardioprotection complex IV inhibition by the active metabolite, palmitoylcarnitine, generates reactive oxygen species and activates reperfusion injury salvage kinases. PLoS One. 2014; 9: 87205.

40. Grover GJ, Atwal KS, Sleph PG, Wang FL, Monshizadegan H, Monticello T, et al. Excessive ATP hydrolysis in ischemic myocardium by mitochondrial FF0-ATPase: effect of selective pharmacological inhibition of mitochondrial ATPase hydrolase activity. Am J Physiol Heart Circ Physiol. 2004; 208: 1747-1755.

41. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. Cardiovasc Res. 2004; 61: 372-385.

42. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev. 2008; 88: 581-609.

43. Golstein P, Kroemer G. A multiplicity of cell death pathways. Symposium on apoptotic and non-apoptotic cell death pathways. EMBO Rep. 2007; 8: 829-833.

44. Takagi H, Matsui Y, Sadoshima J. The role of autophagy in mediating cell survival and death during ischemia and reperfusion in the heart. Antioxid Redox Signal. 2007; 9: 373-381.

45. Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F. Postconditioning--A new link in nature's armor against myocardial ischemia-reperfusion injury. Basic Res Cardiol. 2005; 100: 295-310.

46. Andrukhiv A, Costa AD, West IC, Garlid KD. Opening mitoKATP increases superoxide generation from complex I of the electron transport chain. Am J Physiol Heart Circ Physiol. 2006; 29: 2067-2074.

47. Costa AD, Garlid KD. Intramitochondrial signaling: interactions among mitoKATP, PKCepsilon, ROS, and MPT. Am J Physiol Heart Circ Physiol. 2008; 295: 874-882.

48. Garlid AO, Jaburek M, Jacobs JP, Garlid KD. Mitochondrial reactive oxygen species: which ROS signals cardioprotection? Am J Physiol Heart Circ Physiol. 2013; 305: 960-968.

49. Garlid KD. Opening mitochondrial K(ATP) in the heart-what happens, and what does not happen. Basic Res Cardiol. 2000; 95: 275-279.

50. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J. 999; 34: 233-249.

51. Piper HM, Abdallah Y, Schäfer C. The first minutes of reperfusion: a window of opportunity for cardioprotection. Cardiovasc Res. 2004; 6: 365-371.

52. Pasdois P, Beauvoit B, Tariosse L, Vinassa B, Bonoron-Adèle S, Dos Santos P. Effect of diazoxide on flavoprotein oxidation and reactive oxygen species generation during ischemia-reperfusion: a study on Langendorff-perfused rat hearts using optic fibers. Am J Physiol Heart Circ Physiol. 2008; 294: 2088-2097.

53. Oldenburg O, Qin Q, Krieg T, Yang XM, Philipp S, Critz SD, et al. Bradykinin induces mitochondrial ROS generation via NO, cGMP, PKG, and mitoKATP channel opening and leads to cardioprotection. Am J Physiol Heart Circ Physiol. 2004; 286: 468-476.

54. Boengler K, Heusch G, Schulz R. Mitochondria in postconditioning. Antioxid Redox Signal. 2011; 4: 863-880.

55. Heusch G, Büchert A, Feldhaus S, Schulz R. No loss of cardioprotection by postconditioning in connexin 43-deficient mice. Basic Res Cardiol. 2006; 101: 354-356.

56. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, et al. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature. 2004; 427: 461-465.

57. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature. 2005; 434: 652-658.

58. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ Res. 2001; 88: 802-809.

59. Heinzel FR, Luo Y, Li X, Boengler K, Buechert A, García-Dorado D, et al. Impairment of diazoxide-induced formation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. Circ Res. 2005; 97: 583-586.

60. Penna C, Mancardi D, Rastaldo R, Losano G, Pagliaro P. Intermittent activation of bradykinin B2 receptors and mitochondrial KATP channels trigger cardiac postconditioning through redox signaling. Cardiovasc Res. 2007; 75: 168-177.

61. Perrelli MG, Pagliaro P, Penna C. Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species. World J Cardiol. 2011; 3: 186-200.

62. Korichneva I, Hoyos B, Chua R, Levi E, Hammerling U. Zinc release from protein kinase C as the common event during activation by lipid second messenger or reactive oxygen. J Biol Chem. 2002; 277: 44327-44331.

63. Cohen MV, Downey JM. Ischemic postconditioning: from receptor to end-effector. Antioxid Redox Signal. 2011; 14: 821-831.

64. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem. 1998; 273: 18092-18098.

65. Sasaki Y, Noguchi T, Seki J, Giddings JC, Yamamoto J. Protective effects of imidapril on He-Ne laser-induced thrombosis in cerebral blood vessels of stroke-prone spontaneously hypertensive rats. Thromb Haemost. 2000; 83: 722-727.

66. Daiber A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. Biochim Biophys Acta. 2010; 797: 897-906.

67. Das M, Parker JE, Halestrap AP. Matrix volume measurements challenge the existence of diazoxide/glibencamide-sensitive KATP channels in rat mitochondria. J Physiol. 2003; 547: 893-902.

68. Miura T, Miki T, Yano T. Role of the gap junction in ischemic preconditioning in the heart. Am J Physiol Heart Circ Physiol. 2010; 298: 1115-1125.

69. Solan JL, Lampe PD. Connexin43 phosphorylation: structural changes and biological effects. Biochem J. 2009; 419: 261-272.

70. Decrock E, De Vuyst E, Vinken M, Van Moorhem M, Vranckx K, Wang N, et al. Connexin 43 hemichannels contribute to the propagation of apoptotic cell death in a rat C6 glioma cell model. Cell Death Differ. 2009; 16:151-163.

71. Boengler K, Stahlhofen S, van de Sand A, Gres P, Ruiz-Meana M, Garcia-Dorado D, et al. Presence of connexin 43 in subsarcolemmal, but not in interfibrillar cardiomyocyte mitochondria. Basic Res Cardiol. 2009; 104:141-147.

72. Miro-Casas E, Ruiz-Meana M, Agullo E, Stahlhofen S, Rodriguez-Sinovas A, Cabestrero A, et al. Connexin43 in cardiomyocyte mitochondria contributes to mitochondrial potassium uptake. Cardiovasc Res. 2009; 83:747-756.

73. Rottlaender D, Boengler K, Wolny M, Michels G, Endres-Becker J, Motloch LJ, et al. Connexin 43 acts as a cytoprotective mediator of signal transduction by stimulating mitochondrial KATP channels in mouse cardiomyocytes. J Clin Invest. 2010; 120: 1441-1453.

74. Rodriguez-Sinovas A, Boengler K, Cabestrero A, Gres P, Morente M, Ruiz-Meana M, et al. Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90-dependent TOM pathway and its importance for cardioprotection. Circ Res. 2006; 99: 93-101.

75. Li H, Samouilov A, Liu X, Zweier JL. Characterization of the effects of oxygen on xanthine oxidase-mediated nitric oxide formation. J Biol Chem. 2004; 279: 16939-16946.

76. Schwanke U, Konietzka I, Duschin A, Li X, Schulz R, Heusch G. No ischemic preconditioning in heterozygous connexin43-deficient mice. Am J Physiol Heart Circ Physiol. 2002; 283: 1740-1742.

77. Totzeck A, Boengler K, van de Sand A, Konietzka I, Gres P, Garcia-Dorado D, et al. No impact of protein phosphatases on connexin 43 phosphorylation in ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2008; 295: 106-112.

78. Penna C, Mancardi D, Rastaldo R, Pagliaro P. Cardioprotection: a radical view Free radicals in pre and postconditioning. Biochim Biophys Acta. 2009; 1787: 781-793.

79. Duchen MR. Mitochondria and calcium: from cell signalling to cell death. J Physiol. 2000; 529: 57-68.

80. Harris AL. Voltage-sensing and substate rectification: moving parts of

connexin channels. J Gen Physiol. 2002; 119: 165-169.

81. Ishikawa S, Kuno A, Tanno M, Mssiki T, Kouzu H, Itoh T, et al. Role of connexin-43 in protective PI3K-Akt-GSK-3β signaling in cardiomyocytes. Am J Physiol Heart Circ Physiol. 2012; 302: 2536-2544.

82. Boengler K, Ruiz-Meana M, Gent S, Ungefug E, Soetkamp D, Miro-Casas E, et al. Mitochondrial connexin 43 impacts on respiratory complex I activity and mitochondrial oxygen consumption. J Cell Mol Med. 2012; 6: 1649- 1655.

83. Boengler K, Ungefug E, Heusch G, Leybaert L, Schulz R. Connexin 43 impacts on mitochondrial potassium uptake. Front Pharmacol. 2013; 4: 73.

84. Ruiz-Meana M, Núñez E2, Miro-Casas E, Martínez-Acedo P, Barba I, Rodriguez-Sinovas A, Inserte J. Ischemic preconditioning protects cardiomyocyte mitochondria through mechanisms independent of cytosol. J Mol Cell Cardiol. 2014; 68: 79-88.

85. Bernardi P, Petronilli V. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. J Bioenerg Biomembr. 1996; 28: 131-138.

86. De Stefani D, Raffaello A, Teardo E, Szabò I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature. 2011; 476: 336-340.

87. Castaldo P, Cataldi M, Magi S, Lariccia V, Arcangeli S, Amoroso S. Role of the mitochondrial sodium/calcium exchanger in neuronal physiology and in the pathogenesis of neurological diseases. Prog Neurobiol. 2009; 87: 58-79.

88. Villa A, Garcia-Simon MI, Blanco P, Sese B, Bogonez E, Satrustegui J. Affinity chromatography purification of mitochondrial inner membrane proteins with calcium transport activity. Biochim Biophys Acta. 1998; 1373: 347-359.

89. Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. Basic Res Cardiol. 2009; 104: 189-202.

90. Paillard M, Tubbs E, Thiebaut PA, Gomez L, Fauconnier J, Da Silva CC, et al. Depressing mitochondria-reticulum interactions protects cardiomyocytes from lethal hypoxia-reoxygenation injury. Circulation. 2013; 128: 1555-1565.

91. Zorov DB, Juhaszova M, Yaniv Y, Nuss HB, Wang S, Sollott SJ. Regulation and pharmacology of the mitochondrial permeability transition pore. Cardiovasc Res. 2009; 83: 213-225.

92. Halestrap AP, Pasdois P. The role of the mitochondrial permeability transition pore in heart disease. Biochim Biophys Acta. 2009; 1787: 1402-1415.

93. Hunter DR, Haworth RA, Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. J Biol Chem. 1976; 251: 5069-5077.

94. Crompton M, Virji S, Ward JM. Cyclophilin-D binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore. Eur J Biochem. 1998; 258: 729-735.

95. Javadov S, Kuznetsov A. Mitochondrial permeability transition and cell death: the role of cyclophilin d. Front Physiol. 2013; 4: 76.

96. Woodfield K, Ruck A, Brdiczka D, Halestrap AP. Direct demonstration of a specific interaction between cyclophilin-D and the adenine nucleotide translocase confirms their role in the mitochondrial permeability transition. Biochem J. 1998; 336:287-290.

97. Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM. p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell. 2012; 49: 1536-1548.

98. Karch J, Molkentin JD. Is p53 the long-sought molecular trigger for cyclophilin D-regulated mitochondrial permeability transition pore formation and necrosis? Circ Res. 2012; 111: 1258-1260.

99. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat Cell Biol. 2007; 9: 550-555.

100. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. J Biol Chem. 2005; 280: 18558-18556.

101. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature. 2005; 434: 658-662.

102. Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, et al. Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A. 2013; 110: 5887-5892.

103. Sileikyte J, Blachly-Dyson E, Sewell R, Carpi A, Menabo R, Di Lisa F, et al. Regulation of the Mitochondrial Permeability Transition Pore by the Outer Membrane does not Involve the Peripheral Benzodiazepine Receptor (TSPO). J Biol Chem. 2014; 289:13769-13781.

104. Nederlof R, Eerbeek O, Hollmann MW, Southworth R, Zuurbier CJ. Targeting hexokinase II to mitochondria to modulate energy metabolism and reduce ischaemia-reperfusion injury in heart. Br J Pharmacol. 2014; 171: 2067-2079.

105. Gavish M, Bachman I, Shoukrun R, Katz Y, Veenman L, Weisinger G, et al. Enigma of the peripheral benzodiazepine receptor. Pharmacol Rev. 1999; 51: 629-650.

106. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P, et al. Translocator protein (8kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci. 2006; 27: 402-409.

107. Xu Z, Lee S, Han J. Dual role of cyclic GMP in cardiac cell survival. Int J Biochem Cell Biol. 2013; 45: 1577-1584.

108. Förster K, Paul I, Solenkova N, Staudt A, Cohen MV, Downey JM, et al. NECA at reperfusion limits infarction and inhibits formation of the mitochondrial permeability transition pore by activating p70S6 kinase. Basic Res Cardiol. 2006; 101: 319-326.

109. Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol Rev. 1999; 79: 1127-1155.

110. Halestrap AP, Kerr PM, Javadov S, Woodfield KY. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. Biochim Biophys Acta. 1998; 1366: 79-94.

111. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, et al. Superoxide activates mitochondrial uncoupling proteins. Nature. 2002; 415: 96-99.

112. Weiss JN, Korge P, Honda HM, Ping P. Role of the mitochondrial permeability transition in myocardial disease. Circ Res. 2003; 93: 292-301.

113. Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? Cardiovasc Res. 2002; 55: 534-543.

114. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. Circulation. 2005; 111: 194-197.

115. Petronilli V, Penzo D, Scorrano L, Bernardi P, Di Lisa F. The

mitochondrial permeability transition, release of cytochrome c and cell death. Correlation with the duration of pore openings in situ. J Biol Chem. 2001; 276: 12030-12034.

116. Lim SY, Davidson SM, Hausenloy DJ, Yellon DM. Preconditioning and postconditioning: the essential role of the mitochondrial permeability transition pore. Cardiovasc Res. 2007; 75: 530-535.

117. Giorgio M, Trinei M, Migliaccio E, Pelicci PG. Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? Nat Rev Mol Cell Biol. 2007; 8: 722-728.

118. Migliaccio E, Giorgio M, Pelicci PG. Apoptosis and aging: role of p66Shc redox protein. Antioxid Redox Signal. 2006; 8: 600-608.

119. Pelicci G, Lanfrancone L, Grignani F, McGlade J, Cavallo F, Forni G, et al. A novel transforming protein (SHC) with an SH2 domain is implicated in mitogenic signal transduction. Cell. 1992; 70: 93-104.

120. Carpi A, Menabò R, Kaludercic N, Pelicci P, Di Lisa F, Giorgio M. The cardioprotective effects elicited by p66(Shc) ablation demonstrate the crucial role of mitochondrial ROS formation in ischemia/reperfusion injury. Biochim Biophys Acta. 2009; 1787: 774-780.

121. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell. 2005; 122:221-233.

122. Khanday FA, Yamamori T, Mattagajasingh I, Zhang Z, Bugayenko A, Naqvi A, et al. Rac leads to phosphorylation-dependent increase in stability of the p66shc adaptor protein: role in Rac-induced oxidative stress. Mol Biol Cell. 2006; 17:122-129.

123. Zaccagnini G, Martelli F, Fasanaro P, Magenta A, Gaetano C, Di Carlo A, et al. p66ShcA modulates tissue response to hindlimb ischemia. Circulation. 2004; 109: 2917-2923.

124. Menini S, Amadio L, Oddi G, Ricci C, Pesce C, Pugliese F, et al. Deletion of p66Shc longevity gene protects against experimental diabetic glomerulopathy by preventing diabetes-induced oxidative stress. Diabetes. 2006; 55: 1642-1650.

125. Nemoto S, Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. Science. 2002; 295: 2450-2452.

126. Di Lisa F, Kaludercic N, Carpi A, Menabò R, Giorgio M. Mitochondria and vascular pathology. Pharmacol Rep. 2009; 61: 123-130.

127. Cosentino F, Francia P, Camici GG, Pelicci PG, Lüscher TF, Volpe M. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. Arterioscler Thromb Vasc Biol. 2008; 28: 622-628.

128. Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, et al. Protein kinase C beta and prolyl isomerase regulate mitochondrial effects of the life-span determinant p66Shc. Science. 2007; 315: 659-663.

129. Graiani G, Lagrasta C, Migliaccio E, Spillmann F, Meloni M, Madeddu P, et al. Genetic deletion of the p66Shc adaptor protein protects from angiotensin II-induced myocardial damage. Hypertension. 2005; 46: 433-440.

130. Rota M, LeCapitaine N, Hosoda T, Boni A, De Angelis A, Padin-Iruegas ME, et al. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. Circ Res. 2006; 99: 42-52.

131. Bach AW, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan SW, et al. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proc Natl Acad Sci U S A. 1988; 85: 4934-4938.

132. Edmondson DE, Mattevi A, Binda C, Li M, Hubálek F. Structure and mechanism of monoamine oxidase. Curr Med Chem. 2004; 11: 1983-1993.

133. Abell CW, Kwan SW. Molecular characterization of monoamine oxidases A and B. Prog Nucleic Acid Res Mol Biol. 2001; 65: 129-156.

134. Edmondson DE, Binda C, Mattevi A. The FAD binding sites of human monoamine oxidases A and B. Neurotoxicology. 2004; 25: 63-72.

135. Huang YH, Jiang M, Fu BY. Immunocytochemical localization of monoamine oxidase type B in rat liver. Eur J Histochem. 2008; 52: 11-18.

136. Wang J, Edmondson DE. Topological probes of monoamine oxidases A and B in rat liver mitochondria: inhibition by TEMPO-substituted pargyline analogues and inactivation by proteolysis. Biochemistry. 2011; 50: 2499-2505.

137. Kaludercic N, Carpi A, Menabò R, Di Lisa F, Paolocci N. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. Biochim Biophys Acta. 20; 1813: 1323-1332.

138. Kaludercic N, Mialet-Perez J, Paolocci N, Parini A, Di Lisa F. Monoamine oxidases as sources of oxidants in the heart. J Mol Cell Cardiol. 2014; 73: 34-42.

139. Sivasubramaniam SD, Finch CC, Rodriguez MJ, Mahy N, Billett EE. A comparative study of the expression of monoamine oxidase-A and -B mRNA and protein in non-CNS human tissues. Cell Tissue Res. 2003 Sep;33(3):29-300.

140. Mialet-Perez J, Bianchi P, Kunduzova O, Parini A. New insights on receptor-dependent and monoamine oxidase-dependent effects of serotonin in the heart. J Neural Transm (Vienna). 2007; 114: 823-827.

141. Bianchi P, Pimentel DR, Murphy MP, Colucci WS, Parini A. A new hypertrophic mechanism of serotonin in cardiac myocytes: receptor-independent ROS generation. FASEB J. 2005; 19: 641-643.

142. Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja D, et al. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. Circ Res. 2010;106: 193-202.

143. Lairez O, Calise D, Bianchi P, Ordener C, Spreux-Varoquaux O, Guilbeau-Frugier C, et al. Genetic deletion of MAO-A promotes serotonin-dependent ventricular hypertrophy by pressure overload. J Mol Cell Cardiol. 2009; 46: 587-595.

144. Ojaimi C, Qanud K, Hintze TH, Recchia FA. Altered expression of a limited number of genes contributes to cardiac decompensation during chronic ventricular tachypacing in dogs. Physiol Genomics. 2007; 29: 76-83.

145. Bianchi P, Kunduzova O, Masini E, Cambon C, Bani D, Raimondi L, et al. Oxidative stress by monoamine oxidase mediates receptor-independent cardiomyocyte apoptosis by serotonin and postischemic myocardial injury. Circulation. 2005; 112: 3297-3305.

146. Sturza A, Leisegang MS, Babelova A, Schröder K, Benkhoff S, Loot AE, et al. Monoamine oxidases are mediators of endothelial dysfunction in the mouse aorta. Hypertension. 2013; 62: 140-146.

147. Anderson EJ, Efird JT, Davies SW, O'Neal WT, Darden TM, Thayne KA, et al. Monoamine oxidase is a major determinant of redox balance in human atrial myocardium and is associated with postoperative atrial fibrillation. J Am Heart Assoc. 2014; 3:00073.

148. Villeneuve C, Guilbeau-Frugier C, Sicard P, Lairez O, Ordener C, Duparc T, et al. p53-PGC-alpha pathway mediates oxidative mitochondrial damage and cardiomyocyte necrosis induced by

monoamine oxidase-A upregulation: role in chronic left ventricular dysfunction in mice. Antioxid Redox Signal. 2013; 18: 5-18.

149. Carnicer R, Crabtree MJ, Sivakumaran V, Casadei B, Kass DA. Nitric oxide synthases in heart failure. Antioxid Redox Signal. 2013; 8: 1078-1099.

150. Shabeeh H, Melikian N, Dworakowski R, Casadei B, Chowienczyk P, Shah AM. Differential role of endothelial versus neuronal nitric oxide synthase in the regulation of coronary blood flow during pacing-induced increases in cardiac workload. Am J Physiol Heart Circ Physiol. 2013; 304: 1277-1282.

151. Dedkova EN, Ji X, Lipsius SL, Blatter LA. Mitochondrial calcium uptake stimulates nitric oxide production in mitochondria of bovine vascular endothelial cells. Am J Physiol Cell Physiol. 2004; 286: 406-415.

152. Zaobornyj T, Ghafourifar P. Strategic localization of heart mitochondrial NOS: a review of the evidence. Am J Physiol Heart Circ Physiol. 2012; 303: 1283-1293.

153. Zaobornyj T, Valdez LB2. Heart mitochondrial nitric oxide synthase: a strategic enzyme in the regulation of cellular bioenergetics. Vitam Horm. 2014; 96: 29-58.

154. Penna C, Angotti C, Pagliaro P. Protein S-nitrosylation in preconditioning and postconditioning. Exp Biol Med (Maywood). 2014; 239: 647-662.

155. Xu W, Charles IG, Moncada S. Nitric oxide: orchestrating hypoxia regulation through mitochondrial respiration and the endoplasmic reticulum stress response. Cell Res. 2005; 15: 63-65.

Cite this article

Tullio F, Perrelli MG, Femminò S, Penna C, Pagliaro P (2016) Mitochondrial Sources of ROS in Cardio Protection and Ischemia/Reperfusion Injury. Ann Cardiovasc Dis 1(2): 1006.