Mitochondria in Postconditioning

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Abstract

Several signal transduction pathways are activated by cardioprotective stimuli, including ischemic or pharmacological postconditioning. These pathways converge on a common target, the mitochondria, and cardioprotection by postconditioning is associated with preserved mitochondrial function after ischemia/reperfusion. The present review discusses the role of mitochondria in cardioprotection, especially the involvement of ATP-dependent potassium channels, reactive oxygen species, and the mitochondrial permeability transition pore, and focuses on the effects of postconditioning on mitochondrial function (i.e., their oxygen consumption and calcium retention capacity). The contribution of mitochondria to loss of protection by postconditioning in diseased or aged myocardium is also addressed. *Antioxid. Redox Signal.* 14, 863–880.

Cardioprotection and Its Signaling: Background and Conceptual Framework

THE PROGNOSIS OF PATIENTS with acute myocardial infarction is determined by the amount of irreversible cardiomyocyte loss (i.e., infarct size) and the severity of left ventricular dysfunction (23). Over the last decades, protective phenomena have been recognized and confirmed in humans; they share certain signaling elements in experimental analyses (56). Ischemic preconditioning is the reduction of infarct size, which results from prolonged ischemia and reperfusion, by one or several cycles of brief ischemia and reperfusion preceding the prolonged ischemia (92). Ischemic postconditioning is the protection of the heart from infarction by one or several cycles of brief ischemia and reperfusion following a prolonged episode of myocardial ischemia (155); the recognition of the ischemic postconditioning phenomenon terminated all discussion on the existence of reperfusion injury (54).

The signal transduction of ischemic preconditioning has traditionally been classified into initiating triggers, intracellular mediators, and end-effectors. Triggers were considered active during the preconditioning brief episode(s) of ischemia/reperfusion and antagonized by inhibitors bracketing the preconditioning ischemia/reperfusion, whereas mediators and effectors were active during the prolonged ischemia. However, with the recognition of the postconditioning phenomenon, it became apparent that pre- and postconditioning share a number of signaling elements and that preconditioning, like postconditioning, induces activation of signaling elements during the early reperfusion following the prolonged ischemia (37). Thus, an easy temporal distinction of triggers, mediators, and end-effectors was no longer possible. Mitochondria are involved in three different ways: a) as triggers

or signal amplifiers (in terms of activation of mitochondrial K_{ATP} channels and the resulting formation of small amounts of reactive oxygen species (ROS), b) as end-effectors (in terms of inhibition of mitochondrial permeability transition pore (MPTP) opening and of release of pro-apoptotic factors into the cytosol), and c) as targets of damage and protection from it (in terms of their functional and morphological integrity). Also, ROS are involved in two different ways: a) as signaling elements when formed in small amounts, and b) as damaging elements when formed in excessive amounts.

The present review attempts to characterize in detail the multiple roles of mitochondria in cardioprotection, notably in postconditioning.

Role of Mitochondria in Cardiomyocyte Function After Ischemia/Reperfusion Injury

The most important role of mitochondria is the generation of adenosine triphosphate (ATP). Mitochondria also contribute to the generation and scavenging of ROS, are involved in cellular ion homeostasis, notably calcium homeostasis, and contribute to cardiomyocyte apoptosis and necrosis. Mitochondria consume large amounts of oxygen during oxidative phosphorylation. During ischemia, the lack of oxygen inhibits electron flow, and myocardial ATP utilization becomes inefficient. The F_0F_1ATP synthase, which normally produces ATP, switches into reverse mode and consumes ATP to pump protons from the matrix into the intermembrane space.

Depending on the species, 50%–80% of ATP is consumed by the F_0F_1ATPase during ischemia (49). The ATP, which is otherwise required for ion pumps to maintain the cellular ion homeostasis, is used to maintain the mitochondrial membrane potential (90). At reperfusion, an increased ATP concentration

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via restored oxidative phosphorylation may contribute to the reestablishment of cellular ion homeostasis; however, increased ATP content paradoxically contributes to reperfusion injury; in rapidly re-energized cells, Ca^{2+} oscillations between the sarcoplasmic reticulum, and the cytosol contribute to hypercontracture of cardiomyocytes, membrane disruption, and subsequent necrosis (114).

Two populations of mitochondria exist within cardiomyocytes: subsarcolemmal mitochondria (SSM) are located directly beneath the plasma membrane, and interfibrillar mitochondria (IFM) are located between the myofibrils (100). SSM and IFM differ in morphology, and IFM have a higher respiratory and calcium handling capacity than SSM (100, 119). Following ischemia/reperfusion mitochondrial oxygen consumption is reduced (17, 101, 110, 111), and this reduction in oxygen consumption is accompanied by decreased activities of protein complexes of the electron transport chain [e.g., complex I (101), complex II (24), and complex III (110, 111)]. Oxidative phosphorylation after 45 min ischemia is specifically reduced in SSM and not in IFM (81).

ROS are generated in different cellular compartments and by several enzymes, including NADPH oxidases at the plasma membrane (3, 80) and cytosolic xanthine oxidases (14). However, clearly mitochondria are the most important cellular source of ROS (11, 38, 91). Within mitochondria, the largest amount of oxygen is reduced to water at respiratory complex IV. However, at respiratory complexes I or III, electrons from the electron transport chain can be transferred to oxygen, resulting in the generation of partially reduced oxygen, especially superoxide anions. Superoxide dismutases then rapidly convert superoxide anions to hydrogen peroxide (43). Apart from the electron transport chain, ROS can also be produced by monoamine oxidases in the outer mitochondrial membrane, which transfer electrons from amine compounds to oxygen and thereby generate hydrogen peroxide. Within mitochondria, p66[Shc] oxidizes reduced cytochrome c, which induces the partial reduction of oxygen to peroxide (45). p66[Shc] is present in the cytosol under physiological conditions, becomes phosphorylated by protein kinase C β under stress conditions, and subsequently translocates into the mitochondrial intermembrane space (97, 112).

Detrimental effects of excessive ROS formation include protein and lipid damage, which leads to cellular dysfunction and cell death. The proximity of mitochondrial DNA to the production site of ROS, the lack of protection of mitochondrial DNA by histones, and the limited capacity of repair mechanisms render the mitochondrial DNA highly susceptible to increased oxidative stress (27).

Excessive oxidative stress contributes to reversible and irreversible myocardial injury after ischemia/reperfusion (2, 13, 77, 120, 139, 141). The reversible contractile dysfunction following myocardial ischemia and reperfusion ("stunning") is clearly a manifestation of excessive oxidative stress (22, 50). ROS scavenging by administration of phenanthroline and MPG (mercaptopropionylglycine) at reperfusion reduces cell death of embryonic chick cardiomyocytes, confirming that the burst of ROS at the onset of reperfusion is causal for cell death (140). In contrast, ROS scavenging by a combination of superoxide dismutase and catalase during reperfusion did not reduce infarct size in dog hearts *in vivo*; however, microvascular injury and the low-reflow phenomenon were attenuated (115).

The burst of ROS at the onset of reperfusion facilitates opening of the MPTP, a large conductance pore in the inner mitochondrial membrane. MPTP opening at reperfusion enhances inner mitochondrial membrane permeability to solutes with molecular weights up to 1.5 kDa and leads to mitochondrial depolarization, followed by ATP depletion (35). Mitochondrial matrix volume increases and induces rupture of the outer mitochondrial membrane, leading to loss of pyridine nucleotides and release of pro-apoptotic factors such as cytochrome c and thus also inhibiting electron flow via the electron transport chain. Not only ROS, but also increased concentrations of calcium ions (Ca^{2+}), inorganic phosphate and mitochondrial depolarization—conditions occurring during ischemia and reperfusion—favor MPTP opening (34, 57). These factors are counteracted by physiological MPTP antagonists, such as increased mitochondrial membrane potential, high concentrations of protons, magnesium ions, adenine nucleotides, notably ADP, as well as nitric oxide (34, 142). Whereas the pathophysiological conditions favoring MPTP opening and consequences resulting from MPTP opening are well established, the molecular identity of the protein(s) forming this pore is still unknown. Originally, it had been suggested that the MPTP is formed by the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide transporter (ANT) in the inner membrane, and cyclophilin D in the matrix. However, experiments with VDAC and ANT knockout mice demonstrated efficient permeability transition upon Ca^{2+}-stimulation, indicating that VDAC and ANT are dispensable for permeability transition (10, 74). Whereas in fibroblasts and in liver mitochondria of cyclophilin D knockout mice Ca^{2+}-induced MPTP opening is clearly delayed (9, 12), the sensitivity of MPTP opening in response to adenine nucleotides or oxidative stress is similar in cyclophilin D-deficient and in wild-type mitochondria, indicating that cyclophilin D is important for MPTP opening but that permeability transition can also occur in the absence of cyclophilin D (12).

Functional mitochondria participate in fusion and fission, whereas nonfunctional and damaged mitochondria are removed by autophagy. Therefore, removal of mitochondria, which trigger apoptosis or necrosis, contributes to cardioprotection (for review, see (47)).

Taken together, mitochondria are damaged during myocardial ischemia and reperfusion. Mitochondria not only suffer from but also contribute to myocardial injury in that MPTP opening induces cell death. Thus, preservation of mitochondrial integrity appears an attractive target to reduce damage, notably infarct size after ischemia/reperfusion injury (56, 57).

Mitochondria in the Cardioprotection by Ischemic and Pharmacological Preconditioning

The activation of endogenous cardioprotective mechanisms attenuates irreversible myocardial injury following ischemia/reperfusion. One of these mechanisms is ischemic preconditioning (IP), that is, the infarct size reduction by brief, nonlethal episodes of ischemia/reperfusion preceding a period of sustained ischemia/reperfusion (92). The major signal transduction pathways involved in IP cardioprotection seem to converge at the level of mitochondria and impact on mitochondrial function (for review see (56, 88, 89)). Activated

protein kinase C and protein kinase G, which are part of the intracellular mediator signaling program (56), project onto mitochondrial ATP-dependent potassium channels and facilitate their opening (29, 30, 144). Opening of ATP-dependent potassium channels (mitoK$_{ATP}$ channels) in the inner mitochondrial membrane is essential for cardioprotection (5). Evidence for the involvement of mitoK$_{ATP}$ channels in the inner mitochondrial membrane in IP is mainly derived from pharmacological modification of mitoK$_{ATP}$ channel opening by substances such as diazoxide that opens mitoK$_{ATP}$ channels, and 5-hydroxydecanoate (5-HD) that closes mitoK$_{ATP}$ channels. Recently, increased potassium fluxes in response to diazoxide have been measured in intact mitochondria using the potassium sensitive dye PBFI (potassium-binding benzofuran isophthalate) or directly by patch-clamping of mitochondria devoid of the outer membrane (Fig. 1) (86, 122). The exact molecular structure of the mitoK$_{ATP}$ channels has not been identified up to now, although immunoreactivity for the sarcolemmal K$_{ATP}$ channel subunits Kir6.1 and Kir6.2 has been detected in heart mitochondria (79). Pharmacological opening of mitoK$_{ATP}$ channels by diazoxide contributes to the formation of small amounts of ROS (53), mainly superoxide anions derived from complex I of the electron transport chain (4). Also, mitochondrial uncoupling (i.e., proton influx into the mitochondrial matrix without phosphorylation of ADP) contributes to ROS formation (123).

Small amounts of ROS function as trigger molecules of IP, presumably by activating protein kinases such as protein kinase C (8) or the mitogen-activated protein kinase (MAP kinase) p38 (31). Accordingly, scavenging of ROS with ascorbic acid attenuates infarct size reduction by IP in pig myocardium (127) and by pharmacological preconditioning with diazoxide in isolated rabbit hearts (99).

Within cardiomyocytes, connexin 43 (Cx43) is predominantly localized at gap junctions; however, the protein is also present at the inner membrane of cardiomyocyte subsarcolemmal mitochondria (16, 21, 121) where it regulates mitochondrial potassium fluxes (86, 122). Cx43 is a target of several protein kinases, and mitochondrial Cx43 is highly phosphorylated under physiological conditions (137). A decrease of the mitochondrial Cx43 content is sufficient to abolish the cardioprotection by pharmacological preconditioning with diazoxide (53, 121). Recent studies demonstrated that pharmacological inhibition or genetic ablation of mitochondrial Cx43 confers resistance to mitoK$_{ATP}$ channel opening in response to diazoxide in patch-clamped mitoplasts (mitochondria devoid of the outer membrane). However, since the open-probability of these channels was not affected under baseline conditions, Cx43 rather regulates mitoK$_{ATP}$ channel activity than constituting the pore forming unit of the mitoK$_{ATP}$ channel (122).

In isolated cardiomyocytes, hypoxic preconditioning attenuates oxidative stress at the beginning of reoxygenation (140). Also, in mitochondria isolated from rat hearts *in vitro*, IP reduces the burst of ROS production at reperfusion (102). Large amounts of ROS favor MPTP opening, and IP and pharmacological preconditioning confer cardioprotection by inhibiting MPTP opening at the onset of reperfusion (52, 65). Vice versa, the preservation of mitochondrial function by IP results in an attenuation of the ischemia/reperfusion-induced decrease in ADP-stimulated respiration (150). In preconditioned hearts, tissue levels of ATP decline more slowly than in
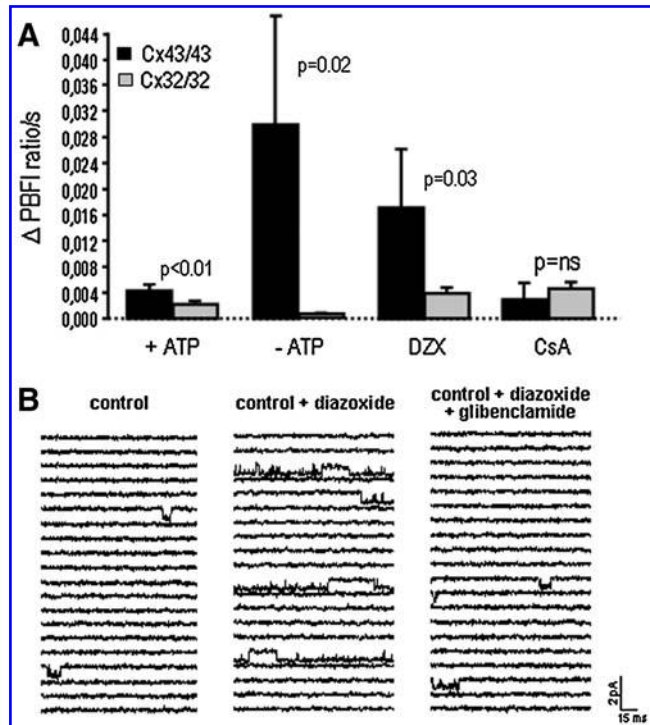

FIG. 1. Mitochondrial potassium uptake via the mito-K$_{ATP}$ channel. (A) Cardiomyocytes were isolated from wild-type mice (Cx43/43) or from mice, in which Cx43 was replaced by Cx32 (Cx32/32, Cx32 forms hemichannels with lower conductivity for K$^+$ ions than Cx43), and permeabilized with digitonin. K$^+$ was substituted by TEA$^+$ (tetraethylammonium), and permeabilized cells were incubated in K$^+$-free buffer. Mitochondria were stained with the potassium-sensitive dye PBFI. 20 mM KCl was added and the rate of PBFI ratio-fluorescence change during the initial 15 s after the KCl pulse was measured in the presence of 2 mM ATP (+ATP), in the absence of ATP (-ATP), in the presence of 200 μM diazoxide (DZX), and in the presence of 1 μM CsA. From (86). (B) Mitoplasts (mitochondria devoid of the outer membrane) were prepared from wild-type mice and analyzed by patch-clamping in mitoplast-attached configuration at −60 mV. In K$^+$ solution (150 mM), voltage-dependent single-channel currents were obtained under control conditions (*left*). Diazoxide (100 μM) activated these currents (*middle*), and glibenclamide (10 μM) blocked the diazoxide-induced currents. From (122).

control hearts, resulting in diminished stimulation of anaerobic glycolysis, decreased reduction of intracellular pH, and attenuation of the ionic alterations occurring during ischemia (131, 132). Following an ischemic preconditioning stimulus, F$_0$F$_1$ATP synthase activity is reduced via binding of the inhibitor protein IF$_1$ (36, 106). The inhibition of the F$_0$F$_1$ATP synthase may contribute to preservation of ATP content during ischemia, when the F$_0$F$_1$ATP synthase works in the reverse mode. Details on the role of the F$_0$F$_1$ATP synthase in cardioprotection are reviewed in detail elsewhere (84). As in preconditioned hearts, ATP hydrolysis during ischemia is also reduced by overexpression of bcl-2 (62). In addition, inhibition of glycogen synthase kinase 3 β (GSK3β) may slow adenine nucleotide transport through the outer mitochondrial membrane via dephosphorylation of the voltage-dependent

anion channel (32, 131). The adenine nucleotide transporter (ANT) in the inner mitochondrial membrane exchanges ATP/ADP by an antiport mechanism. The ischemia-induced dephosphorylation of ANT is prevented by pharmacological preconditioning, and the preserved ANT phosphorylation may enhance ATP production during ischemia (40).

Taken together, the common target of the signal transduction pathways activated by ischemic and pharmacological preconditioning are the mitochondria. Within mitochondria, the generation of small amounts of ROS during the preconditioning ischemia/reperfusion phase through activation of mitoK$_{ATP}$ channels reduces the excessive oxidative stress resulting from the subsequent prolonged phase of ischemia/reperfusion. The inhibition of MPTP opening contributes to cell survival.

Mitochondria in the Cardioprotection by Ischemic and Pharmacological Postconditioning

In contrast to IP, where nonlethal periods of ischemia/reperfusion before the sustained ischemia/reperfusion reduce infarct size, ischemic postconditioning induces cardioprotection by short cycles of ischemia and reperfusion at the immediate onset of reperfusion (155). Obviously, ischemic postconditioning specifically reduces reperfusion injury (54). Delaying the first re-occlusion of the postconditioning maneuver for several minutes abolishes cardioprotection (73, 151). Ischemic postconditioning can also be mimicked pharmacologically [e.g., by various anaesthetics (26, 118)]. Postconditioning activates signaling cascades [e.g., the RISK (reperfusion injury salvage kinase) and the SAFE (survivor activating factor enhancement) pathway, which are reviewed in detail elsewhere (53a). However, while established in small animals, mainly rodents, the causal involvement of the RISK pathway in ischemic postconditioning was not confirmed in pigs (126, 129).

Cx43 is clearly essential for the cardioprotection by ischemic preconditioning (125); however, data on the role of Cx43 in ischemic postconditioning are limited. Although the mitochondrial Cx43 content decreases with ischemic postconditioning (107), such reduction of Cx43 does not interfere with protection. In fact, infarct size in heterozygous Cx43-deficient mice is reduced by ischemic postconditioning to a similar extent as in wild-type mice (58). Whether a further decrease of the Cx43 protein content, which can be achieved in conditional knockout mice, abolishes the cardioprotection by ischemic postconditioning, remains to be established. Despite striking similarities between the signal transduction pathways of ischemic pre- and postconditioning, some elements such as mitochondrial Cx43 might be specific for either pre- or postconditioning.

STAT3 (signal transducer and activator of transcription 3) is part of the janus kinase (JAK)/STAT signal transduction pathway and of the SAFE pathway (78), and transduces stress signals from the plasma membrane to the nucleus, leading to changes in gene transcription. Apart from its role in cardiomyocyte hypertrophy and apoptosis, STAT3 is involved in the infarct size development following ischemia/reperfusion (for review, see (18)). In mice with a cardiomyocyte-specific deletion of STAT3, infarct size reduction by a postconditioning stimulus of 3 cycles 10 s ischemia and reperfusion each was abolished (15). Also, postconditioning with exogenous tumor necrosis factor α did not protect isolated hearts from cardiomyocyte-specific STAT3 knockout mice (78). Recently, STAT3 has been identified by Western blot analysis in cardiomyocyte mitochondria (145). We confirmed the presence of STAT3 in isolated mitochondria by confocal laser scan microscopy (Fig. 2). However, whether specifically mitochondrial STAT3 contributes to the cardioprotection by postconditioning must be addressed in further studies.

Similar to IP, the signal transduction pathways of postconditioning converge at the level of mitochondria. The subsequent review will focus on the ischemic or pharmacological postconditioning maneuvers that confer cardioprotection by interaction with mitochondrial function.

Role of mitochondrial ATP-dependent potassium channels in the cardioprotection by ischemic and pharmacological postconditioning

As in ischemic preconditioning, activated protein kinase C and G facilitate mitoK$_{ATP}$ channel opening also in ischemic postconditioning (30, 108). In isolated rat hearts subjected to 30 min ischemia and 2 h reperfusion, ischemic postconditioning by 5 cycles of 10 s ischemia/reperfusion each reduced infarct size. Inhibition of mitoK$_{ATP}$ channel opening by 5-HD during the entire reperfusion or during the postconditioning maneuver only abolished the protection by ischemic postconditioning (105); protection by postconditioning was also lost by 5-HD administered following the postconditioning maneuver for the remaining 117 min of reperfusion (105). These data suggest that mitoK$_{ATP}$ channel activation persists during reperfusion. The loss of protection after mitoK$_{ATP}$ channel inhibition by 5-HD was confirmed in isolated guinea pig hearts (39) and in rabbit and dog hearts *in vivo* (94, 151).

The mitoK$_{ATP}$ channel opener pinacidil (10 μM) for the first hour of reperfusion reduced cell death after 1 h ischemia and 3 h reperfusion in embryonic chick cardiomyocytes (140). Intermittent mitoK$_{ATP}$ channel opening in isolated rat hearts with 5 cycles 10 s oxygenated buffer and 10 s oxygenated buffer supplemented with 30 μM diazoxide at reperfusion reduced infarct size following 30 min ischemia and 2 h reperfusion (105).

The calcium sensitizer levosimendan impacts on both sarcolemmal (154) and mitochondrial (75) K$_{ATP}$ channels. In isolated guinea pig hearts, pharmacological postconditioning with 3 cycles of 30 s perfusion with 0.1 μM levosimendan reduced infarct size (39); addition of 5-HD during the postconditioning maneuver abolished the protection by levosimendan. Protection by levosimendan postconditioning was confirmed in rat hearts *in vivo*, where an intravenous bolus of 24 μg/kg levosimendan 5 min before reperfusion reduced infarct size reduction following 30 min ischemia and 30 min reperfusion (59). Again, the beneficial effect of levosimendan was abolished by 5-HD. These data support a role for opening of mitoK$_{ATP}$ channels in pharmacological postconditioning by levosimendan.

Morphine at 3 μM for the first 10 min of reperfusion reduced infarct size in isolated rat hearts, and the protection was abrogated by simultaneous 5-HD (25). Also, protection by isoflurane (1 minimum alveolar concentration (MAC)) in rabbit hearts *in vivo* for 5 min starting 3 min before reperfusion was abrogated by 5-HD (76).

FIG. 2. STAT3 is present in cardiomyocyte mitochondria. Mitochondria were isolated from left ventricular rat myocardium, stained with antibodies against STAT3 (*red*) and the mitochondrial matrix marker protein cyclophilin D (*green*), and analyzed by confocal laser scan microscopy. Overlay pixels are shown in *yellow*.
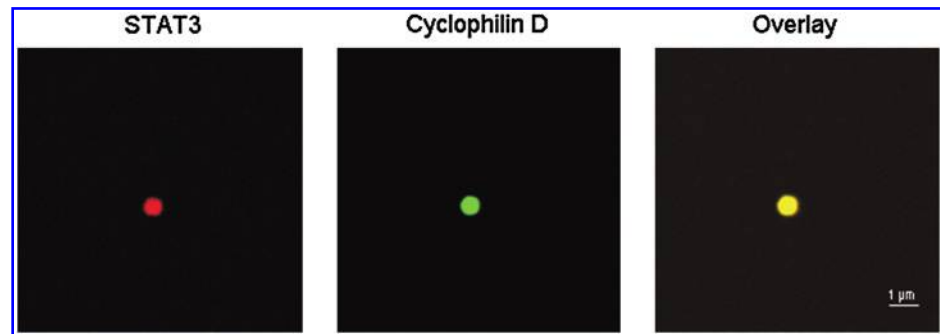
Taken together, ischemic pre- and postconditioning share mitoK$_{ATP}$ channels as an important signaling element for cardioprotection.

The studies on the role of mitoK$_{ATP}$ channels in postconditioning have been summarized in Table 1.

Role of reactive oxygen species for the cardioprotection by ischemic and pharmacological postconditioning

Ischemic postconditioning by 3 cycles of 5 min hypoxia and reoxygenation reduced superoxide anion formation in neonatal rat cardiomyocytes subjected to 3 h hypoxia and 30 min reoxygenation, demonstrating that postconditioning indeed impacts on ROS formation (133). The reduced superoxide production was paralleled by decreased cytosolic and mitochondrial calcium concentrations (133). Postconditioning also decreased superoxide anion formation in neonatal rat cardiomyocytes after longer periods (3 and 6 h, respectively) of hypoxia and reoxygenation (134), and in rat hearts undergoing 30 min ischemia and 3 h reperfusion *in vivo* (71).

Isolated rat hearts were subjected to 30 min ischemia and 2 h reperfusion, and postconditioning by 5 cycles of 10 s ischemia and reperfusion each significantly reduced infarct size. Whereas the protection by ischemic postconditioning was lost in hearts perfused with NAC (N-acetylcysteine, 4 mM) for the entire 2 h reperfusion period, infarct size was still reduced when the perfusion with NAC was initiated after the first 3 min of reperfusion, demonstrating an essential role of ROS formation during **early** reperfusion in protection by postconditioning (108). Infarct size reduction by ischemic postconditioning in mouse hearts *in vivo* was attenuated by the ROS scavenger MPG when administered 10 min before but not 10 min after the postconditioning stimulus (138).

Reperfusion of isolated rabbit hearts with acidic buffer for the first 2 min of reperfusion significantly reduces infarct size. This cardioprotection is blocked by MPG applied for 20 min starting 5 min before reperfusion (28), suggesting the involvement of ROS signaling.

In mice, pharmacological postconditioning by either 1.4 % isoflurane or 10 mg/kg of the delta-opioid receptor agonist SNC-121 was also abolished by MPG when applied 10 min before, but not 10 min after reperfusion (138). NAC for the first 15 min of reperfusion also abolished postconditioning by sevoflurane in isolated rat hearts (152). These data support a central role for ROS signaling during early reperfusion in the protection by ischemic postconditioning.

ROS signaling is downstream of mitoK$_{ATP}$ channel opening, as evidenced from isolated rat hearts subjected to ischemia/reperfusion with an intermittent infusion of diazoxide or diazoxide + MPG at the onset of reperfusion, since MPG attenuated diazoxide-induced protection.

While ROS scavenging attenuates the infarct size reduction by postconditioning, increasing ROS formation at the onset of reperfusion does not confer protection. Increasing ROS by reperfusion with purine/xanthine oxidase for the first 3 min or with 5 cycles of 10 s oxygenated buffer and 10 s oxygenated buffer + purine/xanthine oxidase failed to reduce infarct size (105).

In summary, whereas excessive ROS formation during reperfusion enhances cell death, ROS signaling during **early** reperfusion is essential for the protection by ischemic and pharmacological postconditioning. Opening of mitoK$_{ATP}$ channels is upstream of ROS signaling. Studies on the contribution of ROS to ischemic and pharmacological postconditioning's protection are listed in Table 2.

Ischemic and pharmacological inhibition of MPTP opening at reperfusion

In ischemic preconditioning, the program of activated mediator kinases converges on glycogen synthase kinase-3β (GSK-3β) which, when phosphorylated, inhibits MPTP opening (68). In ischemic postconditioning, the obligatory role of GSK-3β for protection is still controversial and possibly species specific (46, 55, 95, 129). Despite the unresolved molecular identity of the MPTP, mitochondrial permeability transition can be modified pharmacologically [e.g., by cyclosporine A (CsA), which delays MPTP opening by preventing binding of cyclophilin D to the inner mitochondrial membrane, or by atractyloside, which opens the MPTP via inhibition of adenine nucleotide transport]. Both agents have been used to study the contribution of the MPTP to protection by ischemic or pharmacological postconditioning in isolated mitochondria, isolated cardiomyocytes, mammalian hearts *in vitro* and *in vivo*, notably including patients with acute myocardial infarction.

Calcium retention capacity (i.e., the amount of calcium uptake into the mitochondrial matrix in response to pulses of exogenous calcium before MPTP opens) has been determined in isolated mitochondria from hearts following different durations of ischemia and reperfusion without and with postconditioning maneuvers as a measure of their MPTP function (57). In mitochondria isolated from rabbit hearts undergoing 30 min ischemia and 1 h reperfusion with a postconditioning maneuver of 4 cycles of 1 min ischemia and reperfusion each calcium retention capacity was increased (6). Similar results were obtained when the reperfusion period was shortened to 10 min (98) or prolonged to 4 h (7). CsA (5 mg/kg) 1 min

TABLE 1. ISCHEMIC AND PHARMACOLOGICAL POSTCONDITIONING TARGET THE MITOCHONDRIAL K_{ATP} CHANNEL.

Model	Postconditioning stimulus	Duration Isch/Rep	Endpoint	Reference
Embryonic chick cardiomyocytes	10 μM pinacidil for the first hour of rep	1 h isch, 3 h rep	Cell death ↓	(140)
H9C2 cells	3 cycles of 5 min hypoxia and reoxygenation each 100 μM 5-HD, 5 min before 3 cycles of 5 min hypoxia and reoxygenation each	8 h hypoxia, 3 h Reoxygenation	MPTP opening ↓ MPTP opening ↔	(94)
	50 μM diazoxide, 5 min before reoxygenation		MPTP opening ↓	(105)
Isolated rat hearts	5 cycles of 10 s oxygenated buffer and 10 s oxygenated buffer + 30 μM diazoxide each 5 cycles of 10 s isch and rep each 5 cycles of 10 s isch + 100 μM 5-HD and 10 s rep + 100 μM 5-HD each	30 min isch, 2 h rep	Infarct size ↓ Infarct size ↓ Infarct size ↔	
Isolated rat hearts	5 cycles of 10 s isch and rep each 5 cycles of 10 s isch and rep each + 100 μM 5-HD during rep	30 min isch, 2 h rep	Infarct size ↓ Infarct size ↔	(108)
Isolated rat hearts	3 μM morphine for 10 min at rep 3 μM morphine + 10 μM 5-HD for min 10 at rep	45 min isch, 1 h rep	Infarct size ↓ Infarct size ↔	(25)
Isolated guinea pig hearts	3 cycles of 30 s isch and rep each 3 cycles of 30 s isch and rep + 200 μM 5-HD each 3 cycles of 30 s 0.1 μM levosimendan and vehicle each 3 cycles of 30 s 200 μM 5-HD and 200 μM 5-HD + 0.1 μM levosimendan each	40 min isch, 30 min rep	Infarct size ↓ Infarct size ↔ Infarct size ↓ Infarct size ↔	(39)
Rat hearts *in vivo*	24 μg/kg levosimendan iv bolus 5 min before rep 5 mg/kg iv 5-HD 10 min before rep + 24 μg/kg iv levosimendan 5 min before rep	30 min isch, 30 min rep	Infarct size ↓ Infarct size ↔	(59)
Rabbits hearts *in vivo*	4 cycles of 30 s isch and rep each 5 mg/kg 5-HD iv bolus 5 min before 4 cycles of 30 s isch and rep each	30 min isch, 3 h rep	Infarct size ↓ Infarct size ↔	(151)
Rabbit hearts *in vivo*	1 MAC isoflurane for 5 min starting 3 min before rep 1 MAC isoflurane for 5 min starting 3 min before rep + 10 mg/kg 5-HD iv bolus 10 min before rep	30 min isch, 3 h rep	Infarct size ↓ Infarct size ↔	(76)
Dog hearts *in vivo*	3 cycles of 30 s isch and rep each 10 mg/kg 5-HD iv 5 min before 3 cycles of 30 s isch and rep each	1 h isch, 24 h rep	Infarct size ↓ Infarct size ↔	(94)

5-HD, 5-hydroxydecanoate; isch, ischemia; iv, intravenously; MAC, minimum alveolar concentration; rep, reperfusion; ↓, reduction; ↔, no effect.

TABLE 2. EFFECT OF ROS ON ISCHEMIC AND PHARMACOLOGICAL POSTCONDITIONING

Model	Postconditioning stimulus	Duration isch/rep	Endpoint	Reference
Embryonic chick cardiomyocytes	10 μM 10-phenanthroline + 400 μM MPG during rep	1 h hypoxia, 3 h reoxygenation	Cell death ↓	(140)
Neonatal rat cardiomyocytes	3 cycles of 5 min hypoxia and reoxygenation each	3 h hypoxia, 30 min reoxygenation	Superoxide anion production ↓	(133)
		3 h hypoxia, 1 h reoxygenation	Mitochondrial calcium concentration ↓	
Neonatal rat cardiomyocytes	3 cycles of 5 min hypoxia and reoxygenation each	3 h hypoxia, 6 h reoxygenation	Superoxide anion production ↓	(134)
Isolated rat hearts	5 cycles of 10 s isch and rep each	30 min isch, 2 hrep	Infarct size ↓	(108)
	Ischemic postconditioning + 4 mM NAC during entire rep		Infarct size ↔	
	Ischemic postconditioning + 4 mM NAC starting after 3 min rep		Infarct size ↓	
Isolated rat hearts	3% sevoflurane during the first 15 min of rep	30 min isch, 1 h rep	Infarct size ↓	(152)
	3% sevoflurane and 4 mM NAC during the first 15 min of rep		Infarct size ↔	
Isolated rat hearts	5 cycles of 10 s oxygenated buffer and 10 s oxygenated buffer + 30 μM diazoxide each	30 min isch, 2 h rep	Infarct size ↓	(105)
	5 cycles of 10 s isch and 10 s rep + 30 μM diazoxide + 300 μM MPG each		Infarct size ↔	
	5 cycles of 10 s isch and rep each		Infarct size ↓	
	5 cycles of 10 s isch and 10 s rep each + 4 mM NAC		Infarct size ↔	
	5 cycles of 10 s oxygenated buffer and 10 s oxygenated buffer + 1 mM purine/xanthine oxidase		Infarct size ↔	
	1 mM purine/xanthine oxidase for first 3 min rep		Infarct size ↔	
Mouse hearts *in vivo*	3 cycles of 20 s isch and rep each	30 min isch, 2 h rep	Infarct size ↔	(138)
	20 mg/kg MPG bolus 10 min before ischemic postconditioning		Infarct size ↓	
	20 mg/kg MPG bolus 10 min after ischemic postconditioning		Infarct size ↔	
	1.4% isoflurane for 3 min before and 2 min after rep		Infarct size ↓	
	20 mg/kg MPG bolus 10 min before isoflurane postconditioning		Infarct size ↓	
	20 mg/kg MPG bolus 10 min after isoflurane postconditioning		Infarct size ↔	
	10 mg/kg SNC-121 for 3 min before and 2 min after rep		Infarct size ↓	
	20 mg/kg MPG bolus 10 min before SNC-121 postconditioning		Infarct size ↔	
	20 mg/kg MPG bolus 10 min after SNC-121 postconditioning		Infarct size ↓	
Rat hearts *in vivo*	3 cycles of 10 s isch and rep each	30 min isch, 3 h rep	Superoxide anion production ↓	(71)
Dog hearts *in vivo*	5 mg/kg SOD + 5 mg/kg catalase intra-atrial bolus 1 min before rep, followed by continuous infusion of 5 mg/kg SOD + catalase for entire rep	2 h isch, 4 h rep	Infarct size ↔	(115)
			Microvascular injury ↓, low reflow ↓	

Isch, ischemia; MPG, mercaptopropionylglycine; NAC, N-acetylcysteine; rep, reperfusion; SNC-121, 4-[(aR)-a-((2S,5R)-4-propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide; SOD, superoxide dismutase; ↓, decrease; ↔, no effect.

before reperfusion enhanced calcium retention capacity of rabbit mitochondria following 30 min ischemia and 10 min reperfusion (98). The nonimmunosuppressive CsA derivative NIM811 5 mg/kg 1 min before reperfusion also increased calcium retention capacity of isolated rabbit mitochondria (7). Still at 24 h reperfusion following 1 h ischemia in mouse hearts *in vivo*, isolated mitochondria had increased calcium retention capacity when hearts were postconditioning by 3 cycles of 1 min ischemia and reperfusion each (46); again, calcium retention capacity was also increased by CsA 5 min before reperfusion.

Pharmacological postconditioning with different—chemically unrelated—substances also increases mitochondrial calcium retention capacity. In isolated mouse hearts undergoing 30 min ischemia and 30 min reperfusion and anaesthetic postconditioning by 1 MAC isoflurane from 5 min before to 3 min after reperfusion, mitochondrial calcium retention capacity was increased (44). Likewise, postconditioning by 0.3 mg/kg morphine for 3 min starting 5 min before reperfusion in rat hearts subjected to 35 min ischemia and 10 min or 2 h reperfusion increased mitochondrial calcium retention capacity (96).

Postconditioning by activation of adenosine receptors reduced infarct size (66, 72), and the adenosine A1/A2 receptor agonist NECA (5′-(N-ethylcarboxamido) adenosine) reduced swelling of mitochondria isolated from rat hearts undergoing 30 min ischemia and 10 min reperfusion *in vitro* (148), suggesting delayed/reduced MPTP opening.

Resveratrol, a polyphenole present in grapes and red wines, reduced infarct size of isolated rat hearts following 30 min ischemia and 2 h reperfusion when administered for 35 min starting 5 min before reperfusion, associated with reduced mitochondrial swelling (149). The phytoestrogen genistein administered 5 min before reperfusion reduced infarct size of rabbit hearts following 20 min ischemia and 4 or 72 h reperfusion, respectively; the protection by genistein was associated with reduced calcium-induced mitochondrial swelling, suggesting that genistein reduces MPTP opening (136).

In H9C2 cells undergoing 8 h hypoxia and 3 h reoxygenation, postconditiong by 3 cycles of 5 min hypoxia and reoxygenation each preserved mitochondrial membrane potential, as did CsA at reperfusion, suggesting delayed MPTP opening (94).

The protection by ischemic postconditioning was abrogated in rat hearts *in vivo*, which were treated with 5 mg/kg of the MPTP opener atractyloside 5 min before the postconditioning maneuver (64). Also, the infarct size reduction by 1.5 MAC isoflurane for the first 15 min of reperfusion was abolished, when isolated rat hearts simultaneously received 10 μM atractyloside (41). The infarct size reduction by 1 μM morphine, when given for 20 min starting 5 min before reperfusion to isolated rat hearts, was also abolished by 60 μM atractyloside (64). Atractyloside also attenuated infarct size reduction by postconditioning with the adenosine A3 receptor agonist IB-MECA (N^6-(3-iodobenzyl)-adenosine-5′-N-methylcarboxamide) (103) and with bradykinin (104) in isolated rat hearts.

NIM811 (5 mg/kg, 1 min before reperfusion) reduced infarct size in rabbit hearts *in vivo* undergoing 30 min ischemia and 4 h reperfusion (7). When the reperfusion period was extended to 24 h, CsA (10 mg/kg, 5 min before reperfusion) still reduced infarct size in mouse hearts *in vivo* (46). In rabbit

hearts *in vivo*, infarct size reduction by CsA for 2 min starting 5 min before reperfusion was dependent on dose, whereas 5 mg/kg CsA did not reduce infarct size: significant protection was achieved by 10 mg/kg CsA (76). Inhibition of MPTP opening by CsA was not only cardioprotective in small animals, but also reduced infarct size in pig hearts *in vivo* equally well as ischemic postconditioning (Fig. 3) (128).

In patients with acute myocardial infarction undergoing percutaneous coronary interventional reperfusion, postconditioning by 4 cycles of 1 min inflation and 1 min deflation of the angioplasty balloon reduced infarct size (130). The protective effect of postconditioning improved contractile function up to 1 year after myocardial infarction (135). In a recent study, an intravenous bolus of 2.5 mg/kg CsA immediately before reperfusion also provided protection (113). Creatine-kinase release and the area of hyperenhancement on MRI imaging were reduced in CsA-treated patients, reflecting reduced infarct size and suggesting that inhibition of MPTP opening is also beneficial in patients with myocardial infarction.

Studies on the contribution of MPTP opening to postconditioning are summarized in Table 3.

In conclusion, reduced MPTP opening at reperfusion is achieved by ischemic and pharmacological postconditioning stimuli and associated with protection; the cause–effect relationship between reduced MPTP opening and protection is suggestive but not proven (57). Nevertheless, MPTP is certainly an attractive target to achieve protection, notably also in patients with acute myocardial infarction.

Mitochondrial function and morphology in response to ischemic and pharmacological postconditioning

In mitochondria isolated from rabbit hearts subjected to 30 min ischemia and 1 h reperfusion, neither ischemic postconditioning nor the MPTP inhibitor NIM811 affected basal state 4 or ADP-stimulated state 3 respiration, excluding uncoupling or inhibition of the respiratory chain as a mechanism
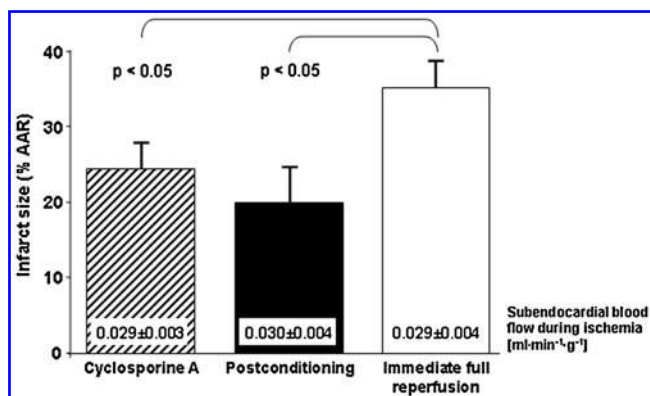

FIG. 3. CsA reduces infarct size in pig myocardium *in vivo*. CsA (5 mg/kg intravenously) was administered 5 min before reperfusion to Göttinger minipigs undergoing 1.5 h ischemia and 2 h reperfusion. Postconditioning was performed by 6 cycles of 20 s ischemia and reperfusion each at the beginning of reperfusion. Control pigs underwent immediate full reperfusion. Subendocardial blood flow was matched between groups. Infarct size (in % of the area at risk, AAR) was reduced to a similar extent by CsA and by ischemic postconditioning. From (128).

of MPTP inhibition (6). Postconditioning did also not influence mitochondrial respiration, neither in SSM nor in IFM from rabbit hearts subjected to 30 min ischemia and 10 min reperfusion (98). In contrast, ADP-stimulated respiration was increased after pharmacological postconditioning with morphine in mitochondria isolated from rat hearts subjected to 35 min ischemia and 10 min or 2 h reperfusion whereas basal respiration was not affected (96). Ultrastructural alterations in mitochondria isolated from rat hearts undergoing 30 min ischemia and 2 h reperfusion were characterized by disruption of membranes and broken christae, and the mitochondrial damage was reduced by ischemic postconditioning (107).

Studies on the effect of postconditioning on mitochondrial integrity and respiration are summarized in Table 4.

Taken together, ischemic and pharmacological postconditioning clearly prevent the loss of mitochondrial membrane potential, inhibit MPTP opening, and preserve mitochondrial morphology; it is less established whether postconditioning also results in alterations of mitochondrial oxygen consumption. Methodologically, the measurement of mitochondrial respiration is dependent on the quality of the isolation procedure, since the normalization of oxygen consumption to total protein content does not account for different amounts of functionally active or inactive mitochondria in the respective preparations.

Reduction of apoptosis by ischemic and pharmacological postconditioning

Cardiomyoctye death after ischemia/reperfusion is primarily necrotic, but apoptosis also contributes. Anti-apoptotic substances when given during early reperfusion reduce infarct size (87, 156), and ischemic postconditioning is associated with reduced apoptosis (107, 134, 143).

Pro-apoptotic signaling pathways also involve mitochondria, for example, the release of cytochrome c or AIF (apoptosis-inducing factor) from the mitochondrial intermembrane space into the cytosol. In neonatal rat cardiomyocytes subjected to 3 h hypoxia and reoxygenation each, ischemic postconditioning reduces the release of cytochrome c from the mitochondrial intermembrane space to the cytosol (83).

Cytochrome c release is also decreased by ischemic postconditioning in isolated rat hearts (107) and by morphine postconditioning in rabbit hearts *in vivo* (146). The release of AIF from the mitochondria to the cytosol is also reduced by ischemic postconditioning in rat hearts *in vivo* (143).

Role of mitochondria in remote ischemic postconditioning

The heart is not only protected by short cycles of ischemia and reperfusion at the onset of reperfusion in the heart itself, but also by ischemia and reperfusion in remote organs or tissues, a phenomenon termed remote ischemic postconditioning (70). Remote ischemic postconditioning elicits cardioprotection originating from the kidney (85), skeletal muscle (82), or brain (48). The signal transduction of remote ischemic postconditioning may involve the mitoK$_{ATP}$ channel (124). Reduced irreversible injury involves mitochondrial anti-apoptotic pathways; decreased apoptosis by renal postconditioning in rabbit hearts is associated with increased expression of the anti-apoptotic protein bcl-2 (69).

Potential Loss of Protection in Diseased and/or Aged Myocardium

Most studies analyzing ischemic or pharmacological postconditioning have been performed in young and healthy animals. However, myocardial infarction is a disease of older age and affects patients with co-morbidities and under pharmacological treatment that may result in loss of cardioprotection (42).

With aging, the myocardial tolerance for ischemia/reperfusion injury is reduced (1) and the expression of proteins important for cardioprotection is altered (19, 20). Aging also interferes with the protection by ischemic postconditioning. In fact, postconditioning by either 3 or 6 cycles of 10 s ischemia and reperfusion each failed to reduce infarct size in 20–24-month-old mouse hearts (116). In middle-aged mouse hearts (>13 months), postconditioning by 5 cycles of 5 s ischemia and reperfusion each reduced infarct size, whereas 3 cycles of 10 s ischemia and reperfusion did not (15). Four cycles of 10 s ischemia and reperfusion were sufficient to protect aged (16–18 months) rat hearts (153). Apparently, stimulus strength is critical for ischemic postconditioning in the aged heart.

The loss of protection in aged hearts could relate to impaired mitochondrial function. ROS are considered as causal for the process of aging (51) their amount increases with aging in the cardiovascular system (67, 93, 147), and they may favor MPTP opening. Calcium retention capacity was decreased in aged mitochondria of different origin (brain, liver, and lymphocytes) (33). Calcium retention capacity was decreased in mitochondria isolated from 24 months as compared to 6-month-old rat hearts (63). We also studied MPTP opening in aged mitochondria and quantified the calcium-induced loss of mitochondrial membrane potential (rhodamine 123 fluorescence); however, mitochondria from young (4 months) and aged (18 months) mice tolerated similar amounts of calcium until loss of mitochondrial membrane potential and MPTP opening occurred (Fig. 4).

In isolated hearts from spontaneously hypertensive rats, ischemic postconditioning elicited only a slight but nonsignificant infarct size reduction, whereas infarct size was significantly reduced in normotensive rat hearts (109). Likewise, isoflurane reduced infarct size in rabbit hearts undergoing 40 min myocardial ischemia and 3 h reperfusion, but the protection was abrogated by hyperglycemia (117). In obese Zucker rats *in vivo*, the infarct size reduction by postconditioning with 70% helium for the first 15 min of reperfusion (61) or with sevoflurane (60) were abolished. CsA 5 min before reperfusion did not restore protection, suggesting that cyclophilin D-mediated MPTP inhibition is impaired in these prediabetic obese Zucker rats.

Further studies are needed to demonstrate the specific contribution of mitochondria to loss of protection by ischemic and pharmacological postconditioning in aged and/or diseased hearts.

Conclusion

Mitochondria and ROS are essential signaling elements of cardioprotection, including protection by postconditioning. Mitochondria serve a signal amplifier function insofar activation of mitoK$_{ATP}$ channels induces the formation of small amounts of ROS. Mitochondria also serve as end-effectors of

TABLE 3. ISCHEMIC AND PHARMACOLOGICAL POSTCONDITIONING INHIBIT MPTP OPENING

Model	Postconditioning stimulus	Duration isch/rep	Endpoint	Reference
Isolated mouse mitochondria	3 cycles of 1 min isch and rep each	1h isch, 24h rep, *in vivo*	Calcium retention capacity ↑	(46)
Isolated mouse mitochondria	10 mg/kg CsA iv, 5 min before rep	30 min isch, 30 min rep, *in vitro*	Calcium retention capacity ↑	(44)
	1 MAC isoflurane from 5 min before to 3 min after rep	35 min isch, 10 min rep, *in vivo*	Calcium retention capacity ↑	
Isolated rat mitochondria	0.3 mg/kg morphine iv for 3 min, starting 5 min before rep	35 min isch, 2h rep, *in vivo*	Calcium retention capacity ↑	(96)
Isolated rat mitochondria	10 µM resveratrol, starting 5 min before rep	30 min isch, 10 min rep, *in vitro*	Mitochondrial swelling ↓	(149)
Isolated rat mitochondria	100 nM NECA during reperfusion	30 min isch, 10 min rep, *in vitro*	Mitochondrial swelling ↓	(148)
Isolated rabbit mitochondria	4 cycles 1 min isch, 1 min rep	30 min isch, 10 min rep, *in vivo*	Calcium retention capacity ↑; Mitochondrial depolarization ↔	(98)
	5 mg/kg CsA iv, 1 min before rep		Calcium retention capacity ↑; Mitochondrial depolarization ↔	
Isolated rabbit mitochondria	4 cycles 1 min isch, 1 min rep	30 min isch, 1h rep, *in vivo*	Calcium retention capacity ↑	(6)
	5 mg/kg NIM811 iv before rep		Calcium retention capacity ↑	
Isolated rabbit mitochondria	4 cycles of 1 min isch and 1 min rep each	30 min isch, 4h rep, *in vivo*	Calcium retention capacity ↑	(7)
	5 mg/kg NIM811 iv 1 min before rep		Calcium retention capacity ↑	
Isolated rabbit mitochondria	100 µg/kg genistein iv 5 min before reperfusion	20 min isch, 10 min rep, *in vivo*	Mitochondrial swelling ↓	(136)
H9C2 cells	3 cycles of 5 min hypoxia and reoxygenation each	8h hypoxia, 3h reoxygenation	Mitochondrial membrane potential ↑	(94)
	100 nM CsA, 5 min before reoxygenation		Mitochondrial membrane potential ↑	
Neonatal rat cardiomyocytes	3 cycles of 5 min hypoxia and reoxygenation each	3h hypoxia, 3h reoxygenation	Mitochondrial membrane potential ↑	(83)
Isolated rat hearts	3% sevoflurane during the first 15 min of rep	30 min isch,1h rep	NAD$^+$ content ↑	(152)
	3% sevoflurane and 4 mM NAC during the first 15 min of rep		NAD$^+$ content ↔	

Preparation	Intervention	Protocol	Outcome	Ref.
Isolated rat hearts	1.5 % MAC isoflurane for first 15 min of rep 1.5 % MAC isoflurane + 10 μM atractyloside for first 15 min of rep	40 min isch, 1 h rep	Infarct size ↓ Infarct size ↔	(41)
Isolated rat hearts	1 μM morphine, 5 min before rep until 15 min rep Morphine postconditioning + 60 μM atractyloside 5 min before rep until 15 min rep	30 min isch, 2 h rep	Iinfarct size ↓ Iinfarct size ↔	(64)
Isolated rat hearts	1 μM IB-MECA, starting 5 min before rep, for 75 min total 1 μM IB-MECA + 20 μM atractyloside, starting 5 min before rep, for 75 min total	30 min isch, 2 h rep	Iinfarct size ↓ Infarct size ↔	(103)
Isolated rat hearts	100 nM bradykinin, 5 min before rep, for 75 min total 100 nM bradykinin + 20 μM atractyloside, 5 min before rep, for 75 min total	30 min isch, 2 h rep	Infarct size ↓ Infarct size ↔	(104)
Mouse hearts *in vivo*	10 mg/kg CsA iv 5 min before rep	1 h isch, 24 h rep	Infarct size ↓	(46)
Rat hearts *in vivo*	6 cycles of 10 s isch and rep each 5 mg/kg atractyloside 5 min before ischemic postconditioning	30 min isch, 2 h rep	Infarct size ↓ Infarct size ↔	(64)
Rabbit hearts *in vivo*	5 mg/kg NIM811 iv 1 min before rep	30 min isch, 4 h rep	Infarct size ↓	(7)
Rabbit hearts *in vivo*	5 mg/kg CsA iv for 2 min starting 5 min before rep 10 mg/kg CsA iv for 2 min starting 5 min before rep	30 min isch, 3 h rep	Infarct size ↔ Infarct size ↓	(76)
Pig hearts *in vivo*	5 mg/kg CsA iv 5 min before rep	90 min isch, 2 h rep	Infarct size ↓	(128)
Patients with acute myocardial infarction	2.5 mg/kg CsA iv immediately before percutaneous coronary intervention	not determined	Infarct size ↓ (creatine kinase release) ↓	(113)

CsA, cyclosporine A; IB-MECA, N6-(3-iodobenzyl)-adenosine-5′-N-methylcarboxamide; isch, ischemia; iv, intravenously; NAC, N-acetylcysteine; NAD+, nicotineamide adenine dinucleotide; NECA, 5′-(N-ethylcarboxamido) adenosine; NIM811, N-methyl-4-isoleucine cyclosporin; MAC, minimum alveolar concentration; rep, reperfusion; ↑, increase; ↓, decrease; ↔, no effect.

Table 4. Ischemic and Pharmacological Postconditioning: Effects on Mitochondrial Integrity and Respiration

Model	Postconditioning stimulus	Duration isch/rep	Endpoint	Reference
Isolated rat mitochondria	5 cycles of 10 s isch and rep each	30 min isch, 2 h rep, *in vitro*	Integrity of mitochondria ↑	(107)
Isolated rat mitochondria	0.3 mg/kg morphine iv for 3 min, starting 5 min before rep	35 min isch, 10 min rep, *in vivo* 35 min isch, 2 h rep, *in vivo*	State 3 respiration ↑	(96)
Isolated rabbit mitochondria, SSM and IFM	4 cycles of 1 min isch and rep each	30 min isch, 10 min rep, *in vivo*	State3 and state 4 respiration ↔	(98)
	5 mg/kg CsA iv, 1 min before rep		State3 and state 4 respiration ↔	
Isolated rabbit mitochondria	4 cycles of 1 min isch and rep each	30 min sich, 1 h rep, *in vivo*	State3 and state 4 respiration ↔	(6)
	5 mg/kg NIM811 iv at rep		State3 and state 4 respiration ↔	

CsA, cyclosporine A; IFM, interfibrillar mitochondria; isch, ischemia; iv, intravenously; NIM811, N-methyl-4-isoleucine cyclosporin; rep, reperfusion; SSM, subsarcolemmal mitochondria; ↑, increase; ↔, no effect.

protection since inhibition of MPTP opening and of release of apoptotic signals into the cytosol contributes to cardiomyocyte survival. Finally, mitochondria are targets of functional and structural damage by ischemia and reperfusion, notably by excessive amounts of ROS. Clearly, mitochondria and

ROS are attractive mechanistic targets for pharmacological cardioprotection. Indeed, a first proof-of-concept study demonstrated a beneficial effect of the MPTP inhibitor CsA during early reperfusion in patients with acute myocardial infarction. However, experimental studies suggest that the
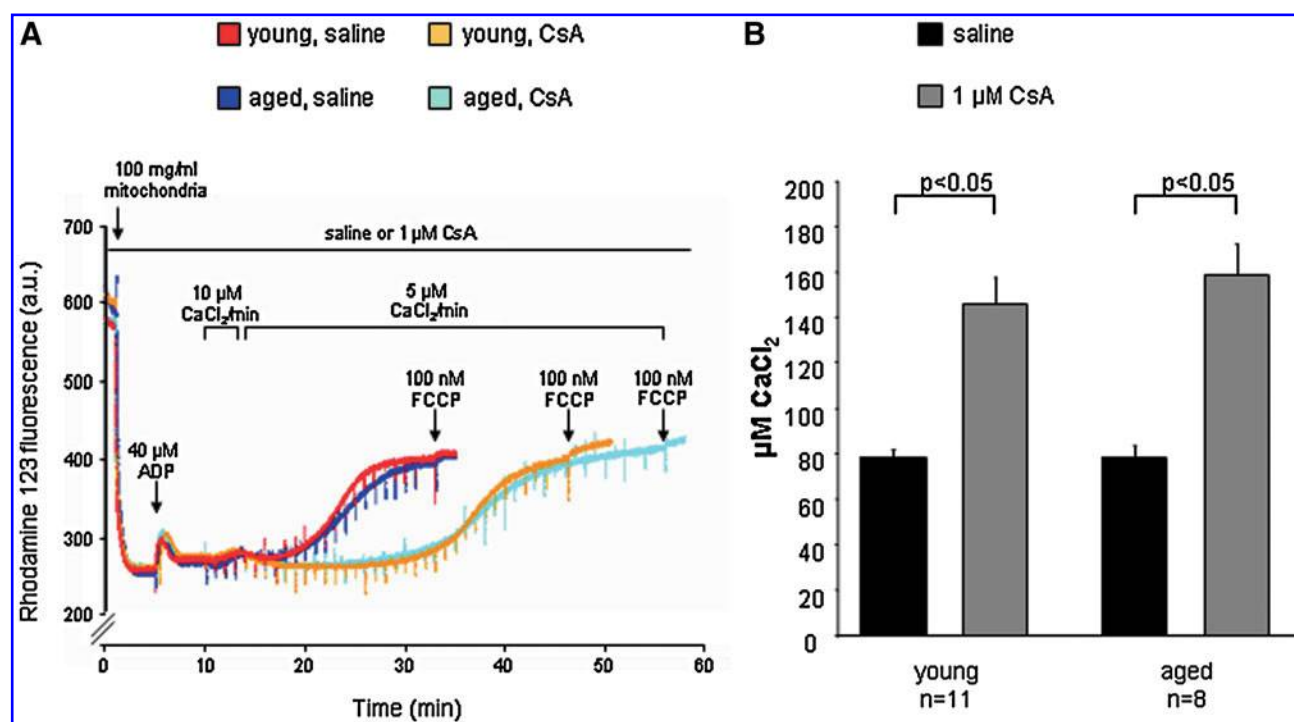

FIG. 4. Calcium retention capacity of mitochondria isolated from young and aged mouse hearts. (A) Rhodamine 123 fluorescence was measured in a buffer with glutamate/malate as substrates for complex I in the presence or absence of 1 μM cyclosporine A (CsA). Mitochondria were added at minute 1, and ADP at minute 4. 10 μM $CaCl_2$ pulses were given per minute from minutes 10 to 13, and 5 μM $CaCl_2$ pulses from minute 14 until the end of the experiment. 100 nM FCCP was administered at the end of the experiment to ensure that the membrane potential was totally lost. (B) The $CaCl_2$ concentration at which loss of membrane potential occurred (increase in Rhodamine 123 fluorescence) was calculated in the presence or absence of CsA. *$p < 0.05$.

cardioprotection by postconditioning may be lost in aged and/or diseased myocardium.

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Abbreviations Used

5-HD = 5-hydroxydecanoate
AAR = area at risk
AIF = apoptosis-inducing factor
ANT = adenine nucleotide transporter
CsA = cyclosporine A
Cx43 = connexin 43
GSK3β = glycogen synthase kinase 3β
IB-MECA = N^6-(3-iodobenzyl)-adenosine-5′-N-methylcarboxamide
IFM = interfibrillar mitochondria
IP = ischemic preconditioning
JAK = janus kinase
MAC = minimum alveolar concentration
mitoK$_{ATP}$ channel = mitochondrial ATP-dependent potassium channel
MPG = mercaptopropionylglycine
MPTP = mitochondrial permeability transition pore
NAC = N-acetylcysteine
NECA = 5′-(N-ethylcarboxamido) adenosine
NIM811 = N-methyl-4-isoleucine cyclosporine
PBFI = potassium-binding benzofuran isophthalate
RISK = reperfusion injury salvage kinase
ROS = reactive oxygen species
SAFE = survivor activating factor enhancement
SNC-121 = 4-[(aR)-a-((2S,5R)-4-propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide
SOD = superoxide dismutase
SSM = subsarcolemmal mitochondria
STAT3 = signal transducer and activator of transcription 3
TEA = tetraethylammonium
VDAC = voltage-dependent anion channel

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