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Forum Review

Role of Reactive Oxygen Species in Ischemic Preconditioning of Subcellular Organelles in the Heart

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ABSTRACT

Ischemic preconditioning (IPC) is an endogenous adaptive mechanism and is manifested by early and delayed phases of cardioprotection. Brief episodes of ischemia–reperfusion during IPC cause some subtle functional and structural alterations in sarcolemma, mitochondria, sarcoplasmic reticulum, myofibrils, glycocalyx, as well as nucleus, which render these subcellular organelles resistant to subsequent sustained ischemia–reperfusion insult. These changes occur in functional groups of various receptors, cation transporters, cation channels, and contractile and other proteins, and may explain the initial effects of IPC. On the other hand, induction of various transcriptional factors occurs to alter gene expression and structural changes in subcellular organelles and may be responsible for the delayed effects of IPC. Reactive oxygen species (ROS), which are formed during the IPC period, may cause these changes directly and indirectly and act as a trigger of IPC-induced cardioprotection. As ROS may be one of the several triggers proposed for IPC, this discussion is focused on the current knowledge of both ROS-dependent and ROS-independent mechanisms of IPC. Furthermore, some events, which are related to functional preservation of subcellular organelles, are described for a better understanding of the IPC phenomenon. *Antioxid. Redox Signal.* 6, 393–404.

INTRODUCTION

MYOCARDIAL INFARCTION is associated with blockade of the coronary artery and development of a defined area of myocardial necrosis due to ischemia (1) and is considered to be the major cardiovascular problem of global concern (34, 108). Although several clinical trials have indicated that mortality and cardiac pump dysfunction due to acute myocardial infarction are attenuated upon establishing reflow to the infarcted myocardium (50, 66, 132, 134), reperfusion, if not carried out within a certain period of ischemia, is known to produce detrimental morphologic and functional effects in the heart, and this phenomenon is termed as reperfusion injury (14, 95). Biochemically, reperfusion injury has been described as a complex interaction between substances that accumulate during ischemia and those that are delivered as a result of reperfusion (106). The reperfusion injury was found to be reduced upon subjecting the heart to brief periods of ischemia–reperfusion before inducing sustained ischemia (92), and this intervention was termed ischemic preconditioning (IPC). IPC not only serves as an endogenous adaptation for delaying myocardial necrosis (92, 147), but also provides cardioprotection by (a) abolishing ischemia–reperfusion-induced ventricular arrhythmias (13), (b) preserving postischemic endothelial function (67, 133), (c) attenuating neutrophil-mediated inflammation response in myocardium (93, 133), (d) improving contractile function after ischemia (20), and (e) inhibiting ischemia–reperfusion-induced apoptotic cell death to minimize myocyte loss (151).

IPC is manifested by two critically time-dependent phases of cardioprotection (Fig. 1). The first early phase of classic preconditioning or the first window of protection occurs within minutes and disappears after 2–4 h. The delayed phase of cardioprotection termed the second window of protection ap-

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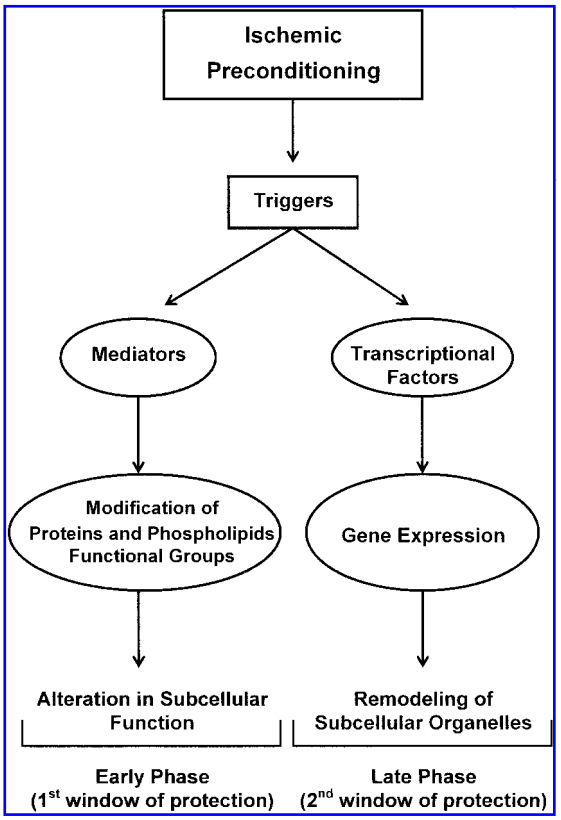

FIG. 1. Proposed mechanisms for the early phase and delayed phase of IPC effects in the ischemic–reperfused hearts.

pears 24 h after IPC and lasts for 48–72 h (11, 104, 147). Several investigators (38, 119, 148) have proposed various triggers, mediators, and end effectors of IPC, and these are given in Table 1. All these signal transduction mechanisms affect various subcellular organelles, including sarcolemma, mitochondria, sarcoplasmic reticulum, myofibrils, nucleus, as well as glycocalyx (extracellular matrix) (Fig. 2). Although extensive research has been carried out to find the subcellular targets of IPC, the mechanisms underlying IPC are not fully elucidated because of complex interplay of various subcellular processes. Reactive oxygen species (ROS), which include superoxide anion ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^\bullet OH$), and peroxynitrite ($ONOO^{-\bullet}$), are generated during myocardial ischemia–reperfusion (27) mainly from mitochondria in cardiomyocytes, activated neutrophils, and xanthine oxidase of vascular endothelium (100). The excessive formation of ROS is known to result in cardiac cell damage and postischemic contractile dysfunction (29); however, subthreshold amounts of ROS generated during brief periods of ischemia–reperfusion have been reported to be responsible for the cardioprotective effect of IPC (101, 129). Moreover, preconditioned hearts (28) and mitochondria isolated from preconditioned hearts (107) have been shown to generate less amount of malondialdehyde as well as $O_2^{-\bullet}$ after sustained ischemia–reperfusion. Therefore, on the one hand, ROS are reported to cause structural changes in various subcellular organelles (122, 149), but on the other, subthreshold amounts of ROS produce functional changes in these organelles by activating various mediators of IPC to maintain a redox balance in cardiomyocytes (Fig. 3). Although different receptor-dependent and receptor-independent triggers as well as various mediators have been shown to participate in IPC (Table 1), this discussion is focused on the interaction of ROS with various cellular organelles to understand the acute beneficial effects of IPC on the ischemia–reperfusion-induced cardiac dysfunction.

TABLE 1. COMPONENTS OF SUBCELLULAR MECHANISMS OF PRECONDITIONING

A. Triggers	
(a) Receptor-dependent	Adenosine, bradykinin, opioids, angiotensin II, calcitonin gene-related peptide, prostaglandins, endothelin, norepinephrine
(b) Receptor-independent	Reactive oxygen species, nitric oxide, calcium
B. Mediators	
Protein kinase C, tyrosine protein kinase, mitogen-activated protein kinase	
C. End effectors	
Mitochondrial ATP-sensitive potassium channel, nuclear factor-κB, heat shock protein 27, cytokines, inducible nitric oxide synthase, cyclooxygenase-2, aldose reductase, manganese superoxide dismutase, Cu,Zn-superoxide dismutase, catalase, glutathione peroxidase, Na^+/H^+ exchanger, tumor necrosis factor-α	

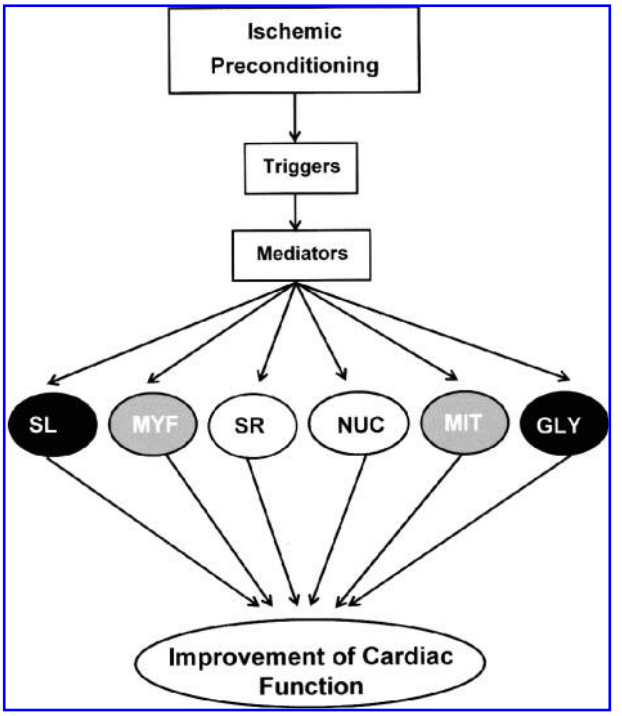

FIG. 2. Role of subcellular organelles in improving cardiac function due to IPC. SL, sarcolemma; MYF, myofibrils; SR, sarcoplasmic reticulum; NUC, nucleus; MIT, mitochondria; GLY, glycocalyx.

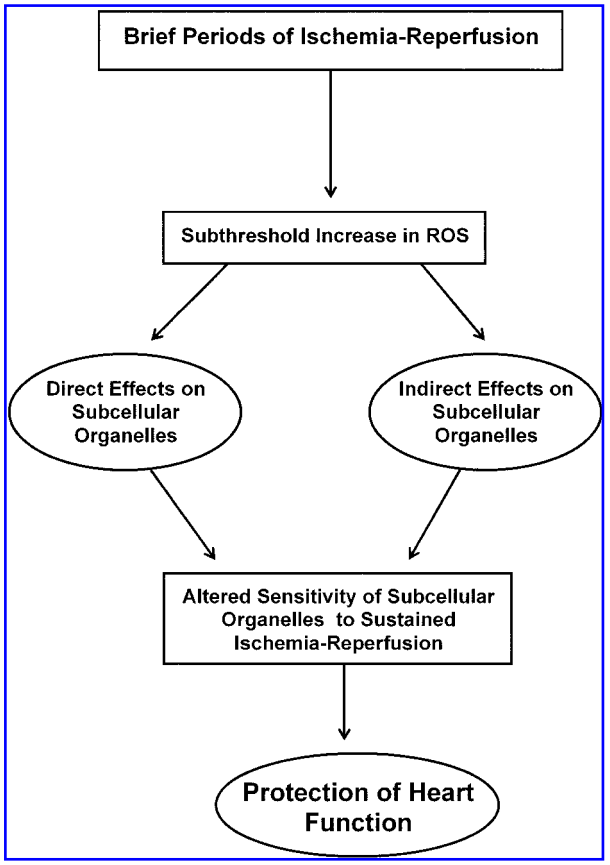

FIG. 3. Role of subthreshold concentrations of ROS generation in the IPC-induced improvement of heart function due to ischemia–reperfusion injury.

CARDIAC SARCOLEMMA

Sarcolemmal membrane is a thin phospholipid bilayer in which various types of receptors, cation pumps, cation exchangers, and cation channels are embedded to form the outer boundary of cardiomyocyte. Modulation of the functional groups of these sarcolemmal proteins due to the formation of ROS, activation of various mediators, and end effectors plays an important role in the cardioprotective effect of IPC.

Receptors in sarcolemma

Previous studies have established that adenosine (10), bradykinin (85), angiotensin II (97), norepinephrine (118), calcitonin gene-related peptide (CGRP) (68), and endogenous opioids (120) are released during ischemia. Activation of various sarcolemmal receptors, including adenosine A_1 and A_3 receptors (39), bradykinin B_2 receptor (47), angiotensin II AT_1 receptor (31), adrenergic α_{1b} receptor (136), CGRP receptor (82), and opioid δ_1 and κ receptors (63, 141), have been shown to participate in triggering the IPC-induced cardioprotective mechanisms. Receptor coupled G protein-mediated activation of phospholipase C–diacylglycerol and/or phospholipase D–diacylglycerol leads to protein kinase C (PKC) translocation to sarcolemma. This event is followed by opening of mitochondrial ATP-sensitive potassium channels (mito K_{ATP}) and is the common pathway of protection due to activation of adenosine (80), bradykinin (15), angiotensin (111), adrenergic (88), and opioid (63) receptors. On the other hand, inhibition of tumor necrosis factor α production, which is downstream from PKC activation, without the involvement of mito K_{ATP} is the end effector of CGRP-induced cardioprotection (79). Additionally, opioid receptor activation has been suggested to cause instigation of tyrosine kinases (37), which may act downstream (6), upstream, or in parallel (60) to PKC. Synthesis of nitric oxide (NO) (128), activation of 38-kDa mitogen-activated protein kinase (p38 MAPK) (58), and phosphorylation of low-molecular-weight heat shock protein 27 (HSP27) (23) are the specific effectors of adenosine receptor activation mediated cardioprotection. However, Armstrong *et al.* (2) have demonstrated that increase in p38 MAPK activity and HSP27 phosphorylation in early ischemia does not correlate with the cardioprotective effect of IPC. Moreover, several recent studies have shown that inhibition of p38 MAPK activation is associated with the cardioprotective effect of IPC (55, 84, 117). Use of diverse experimental models, variation in efficacy of p38 MAPK inhibitors, and presence of different isoforms of p38 MAPK are the major reasons for these conflicting results. Nonetheless, ROS generated during brief episodes of ischemia–reperfusion (5) are interconnected with receptor-coupled cardioprotection. Stimulation of adrenergic, bradykinin, and opioid receptors via activation of PKC followed by opening of mito K_{ATP} channels during IPC leads to generation of free radicals (21), which subsequently turn on the p38 MAPK-mediated protective pathway to provide adaptation to ischemia–reperfusion insult (150).

Cation exchangers, cation pumps, and cation channels in sarcolemma

Sarcolemmal Na^+/H^+ exchanger (NHE) extrudes protons (139), which accumulate during ischemia and are exchanged with Na^+ (91). An increase in the intracellular concentration of Na^+ causes an increase in Ca^{2+} via reverse mode of Na^+/Ca^{2+} exchanger, and this may contribute to ischemia–reperfusion injury (130). Inhibition of NHE diminishes the reperfusion-induced myocardial injury by reducing the cardiomyocyte Ca^{2+} uptake through the reverse mode of Na^+/Ca^{2+} exchanger (45). However, the contribution NHE inhibition in IPC is controversial. Bugge and Ytrehus (16) have shown that NHE inhibition-mediated cardioprotection is additive to the cardioprotection afforded by IPC. On the other hand, Xiao and Allen (145) have demonstrated that that NHE may act as the end effector of IPC as IPC mediated inhibition of its reactivation, which occurs upon reperfusion after sustained ischemia provides cardioprotection. Adenosine is also reported to block NHE (4), but the protective effect is not mediated by opening of mito K_{ATP} channels (53). Moreover, PKC activates rather than blocks NHE (69). Nonetheless, the role of ROS, produced during the brief periods of ischemia–reperfusion, in modifying NHE directly cannot be ruled out.

Na^+,K^+-ATPase in sarcolemma. The Na^+,K^+-ATPase activity is preserved in the early phase of sustained ischemia–reperfusion in preconditioned hearts with subsequent increase in Na^+-Ca^{2+} exchange activity (35, 94). This reduces

the intracellular Ca^{2+} overload, which can activate sarcolemmal phospholipases and proteases that degrade membrane phospholipids and proteins leading to impairment of the sarcolemmal integrity (3, 121). Infarct size limiting the effect of IPC through preservation of Na^+,K^+-ATPase is mediated by opening of the sarcolemmal ATP-sensitive potassium (sarc K_{ATP}) channels (59), which cause shortening of action potential duration (APD) and subsequent reduction of Ca^{2+} influx into cardiomyocytes through sarcolemmal L-type Ca^{2+} channels (96). In contrast to this study, Imahashi *et al.* (65) have proposed that Na^+, K^+-ATPase-induced cardioprotection during IPC is mediated by mito K_{ATP} channel opening. Opening of mito K_{ATP} channels produces free radicals (138) that may act as second messengers in IPC. The effect of IPC on Na^+,K^+-ATPase activity seems to be independent of PKC as chelerythrine, a selective PKC antagonist, increases the Na^+,K^+-ATPase activity and limits the ischemia–reperfusion injury in the isolated rat heart (83).

Sarcolemmal K_{ATP} channels. The role of sarc K_{ATP} channels in IPC was described previously by using two K_{ATP} channel antagonists, namely, glibenclamide and 5-hydroxydecanoate, in canine hearts (48). Shortening of APD by opening of sarc K_{ATP} channels is followed by reduction in the intracellular Ca^{2+} overload in cardiomyocytes during ischemia–reperfusion (8, 22); this was proposed to be the mechanism for IPC-induced cardioprotection. However, several investigators have seriously questioned the relation between shortening of APD and cardioprotection (36, 49, 57). Nonetheless, the recent introduction of the transgenic animal has rejuvenated the overlooked role of sarc K_{ATP} channels in IPC. Suzuki *et al.* (127) have shown that knockout of Kir 6.2 (the pore subunit of sarc K_{ATP} channels) eradicates the cardioprotective effect of IPC. Similarly, no cardioprotection was shown by IPC in Kir 6.2-deficient mice lacking metabolism–sensing sarc K_{ATP} channels (Kir 6.2-KO) (54); this further supports the role of intact sarc K_{ATP} channels in IPC. Oxygen free radicals can open sarc K_{ATP} channels (64) and can act as a trigger to initiate sarc K_{ATP}-mediated cardioprotection in IPC. It appears that, in addition to sarc K_{ATP} channels, several receptors, different cation pumps, and cation transporters are altered by subthreshold amounts of ROS generated during IPC, and these may attenuate the occurrence of intracellular Ca^{2+} overload and oxidative stress due to sustained ischemia–reperfusion injury.

CARDIAC MITOCHONDRIA

The inner mitochondrial membrane contains mito K_{ATP} channels where they regulate inner mitochondrial volume and energetics (109). Several studies have demonstrated that mito K_{ATP} channels act as a trigger as well as an effector of IPC-induced cardioprotection (36, 43, 105). Garlid *et al.* (42) demonstrated that K^+ influx by opening of mito K_{ATP} channels followed by activation of K^+/H^+ antiporter leads to matrix swelling. This matrix swelling may activate fatty acid oxidation and ATP production with improved rate of oxidative phosphorylation (56). Moreover, improvement in ATP production in mitochondria isolated from preconditioned hearts as compared with nonpreconditioned hearts was also observed by Fryer *et al.* (36). Another mechanism is based on the fact that the occurrence of intracellular Ca^{2+} overload during reperfusion causes mitochondrial dysfunction in terms of depolarization of mitochondrial membrane and uncoupling of oxidative phosphorylation (32). Opening of mito K_{ATP} channels may decrease mitochondrial Ca^{2+} overload by dissipation of the inner mitochondrial membrane potential and release of Ca^{2+} from mitochondria to cytoplasm (43, 142). NO production by activation of Ca^{2+}/calmodulin–dependent NO synthase in mitochondria during IPC by opening of mito K_{ATP} channels also provides cardioprotection in the early phase of IPC (44, 81). Besides this, opening of mito K_{ATP} channels before inducing sustained ischemia–reperfusion generates free radicals that cause cardioprotection by activation of various kinases, including PKC and p38 MAPK (105). ROS and PKC act both upstream and downstream from mito K_{ATP} channels as these can modulate mito K_{ATP} channels (115, 116). Therefore, mitochondria set a redox balance and opening of mito K_{ATP} channels, which can enhance or attenuate mitochondrial ROS production depending on the phase of IPC, sustained ischemia or reperfusion. Besides this, ROS generated during IPC cause the expression of nucleus-encoded and mitochondrial localized manganese superoxide dismutase (Mn-SOD) via the formation of nuclear factor κB (NFκB) and activator protein-1 (AP-1). These transcriptional factors play an important role in delayed IPC and protect the mitochondria from $O_2^{-\bullet}$-mediated damage (62). It is thus likely that oxyradicals generated by mitochondria during IPC may open mito K_{ATP} channels and thus prevent the occurrence of mitochondrial Ca^{2+} overload, defect in energy production, and impairment of cardiac function due to sustained ischemia–reperfusion. Besides Mn-SOD, ROS-mediated activation of other antioxidant enzymes, including Cu, Zn-superoxide dismutase (Cu, Zn-SOD), glutathione peroxidase, and catalase, also seems to play an important role in IPC-mediated cardioprotection (9, 24, 28).

CARDIAC SARCOPLASMIC RETICULUM

Sarcoplasmic reticulum (SR) plays a predominant role in cardiac excitation–contraction coupling by virtue of its ability to regulate the intracellular concentration of Ca^{2+} in cardiomyocytes. Cytosolic Ca^{2+} overload due to SR dysfunction is one of the major determinants of ischemia–reperfusion-induced myocardial injury (26). Although Cave and Garlick (18) have proposed that functional SR is not necessary for the cardioprotective effect of IPC in terms of preservation of post-ischemic contractile function, other investigators have emphasized that IPC may attenuate the intracellular Ca^{2+} overload by preservation of SR function (102, 152). Moreover, ischemia–reperfusion-induced depressed expression of mRNA levels of SR Ca^{2+}-cycling proteins, such as ryanodine receptors, Ca^{2+} pump ATPase, phospholamban, and calsequestrin, is improved by IPC (131). Similar improvement in SR Ca^{2+}-cycling protein expression was also observed in hearts subjected to Ca^{2+} paradox (70), a phenomenon where a brief period of Ca^{2+} depletion followed by Ca^{2+} repletion results in marked contractile dysfunction. Additionally, a transient increase in intracellular Ca^{2+} during IPC may activate and maintain the activity of Ca^{2+}/calmodulin protein kinase II (103), which causes phosphorylation of SR Ca^{2+} pump and phos-

pholamban to enhance the Ca^{2+} uptake activity in SR vesicles (135) during sustained ischemia–reperfusion. Oxidation of highly reactive sulfhydryl groups of SR ryanodine receptors during transient IPC protects the heart from subsequent prolonged ischemia–reperfusion-induced intracellular Ca^{2+} overload-mediated injury. ROS generated during IPC may cause this oxidation directly or indirectly by activating different mediators of IPC (153). PKC to some extent is also activated by ROS (46), and oxidative stress can induce translocation of PKC from cytoplasm to sarcolemma (140). PKC activation during IPC due to an increase in the intracellular Ca^{2+} also causes cardioprotection by increasing SR Ca^{2+} uptake via phosphorylation of phospholamban (90). PKC increases ecto-5′-nucleotidase, an enzyme that releases adenosine from ADP and mediates the cardioprotection via enhancement of adenosine production in IPC (73). Cardioprotection by attenuation of depression in SR function in hearts undergoing intracellular Ca^{2+} overload may be partially mediated by adenosine as pretreatment of hearts with 8-(*p*-sulfophenyl)theophylline (an adenosine receptor antagonist) abolished the beneficial effects of IPC on SR function (71). Taken together, it is evident that different Ca^{2+}-cycling and regulatory proteins in the SR membrane are altered by oxyradicals generated during IPC in such a manner that the reperfusion injury to SR is attenuated, and this may explain the beneficial effects of IPC on cardiac performance.

CARDIAC MYOFILAMENTS

IPC can mediate cardioprotection by functional alterations in contractile elements composed of thick myosin filaments and thin actin filaments. It is now well established that cytosolic Ca^{2+} overload during ischemia–reperfusion damages the contractile apparatus and impairs the normal physiologic response to Ca^{2+} (40, 74). IPC preserves the myofilament Ca^{2+} responsiveness; the resultant cardioprotection (110) may be by PKC activation, which is a known mediator of IPC (125). Moreover, ROS-mediated activation of p38 MAPK during IPC may phosphorylate mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 (25), which in turn phosphorylates HSP27 (112, 126). HSP27 appears to increase the tolerance of cytoskeleton to sustained ischemic insult by polymerization of actin filament and thus increases the stability of contractile apparatus (51, 77), leading to the delayed phase of cardioprotection in IPC (114). IPC also causes the direct translocation of HSP27 to sarcomere of myofilament (113), and the role of this event cannot be overlooked. Besides the preservation of actin filaments during IPC, activation of the p38 MAPK–HSP27 pathway may open mito K_{ATP} channels, which ultimately salvage the myocardium (7) via ROS. Accordingly, it is apparent that oxyradicals generated during IPC modify contractile elements both directly and indirectly and thus make them more resistant to the sustained ischemia–reperfusion insult.

CARDIAC NUCLEUS

Redox-sensitive NFκB is an inducible DNA binding transcriptional factor that exists as an inactive form in the cytosol (99) in association with its inhibitory protein IκB. NFκB is activated during myocardial ischemia–reperfusion (19, 123) and plays an important role in ischemia–reperfusion-induced myocardial injury. In fact, several genes induced by NFκB, such as those for cytokines and leukocyte adhesion molecules, are implicated in ischemia–reperfusion-induced myocardial injury (17, 52, 123). On the other hand, IPC is also reported to induce translocation of NFκB to the nucleus (86) and reduce the activation of NFκB after sustained ischemia–reperfusion in the heart (89). Various studies have provided indirect evidence that PKC, tyrosine kinases, and p38 MAPK, which are important mediators of IPC, can activate NFκB (33, 86, 146). ROS generated during IPC trigger a tyrosine kinase signal transduction pathway followed by activation of p38 MAPK and MAPKAP kinase 2, leading to NFκB activation (25). Bolli (12) has reported that NO and ROS production during IPC directly or indirectly by $ONOO^{-\bullet}$ formation translocates PKC, which activates mainly Src and/or Lck tyrosine kinase; these mediators lead to the translocation of NFκB to nucleus. Once translocated to nucleus, NFκB causes production of NO by inducible NO synthase up-regulation in the late phase of IPC (146), which further protects the heart by cyclooxygenase-2 activation, and subsequent production of cytoprotective prostaglandins PGE_2 and PGI_2 (124). ROS-mediated NFκB translocation also causes delayed phase of protection by induction of Mn-SOD, which can scavenge $O_2^{-\bullet}$ generated during sustained ischemia–reperfusion (62). Additionally, NFκB translocation during IPC may cause the expression of antiapoptotic bcl-2 gene and produces cardioprotection by decreasing apoptotic cell death during sustained ischemia–reperfusion (87). Thus, in addition to altering the apoptotic effect of ischemia–reperfusion injury, IPC has been shown to attenuate changes in cardiac gene expression (71, 131). These observations provide evidence that the nucleus is involved in both the early and delayed phases of IPC.

CARDIAC GLYCOCALYX

Although glycocalyx or extracellular matrix plays a critical role in maintaining heart function, very little work has been carried out to examine the effects of IPC or ischemia–reperfusion on glycocalyx. Recently, Lalu *et al.* (75) have shown that IPC decreases ischemia–reperfusion-induced release and activation of matrix metalloproteinase-2 (MMP-2), which cleaves myofilament troponin I (144), and hence protects the contractile machinery. $ONOO^{-\bullet}$ accumulated after sustained ischemia–reperfusion also activates MMP-2 (143) by oxidizing the sulfhydryl bond between the cysteine residue of prodomain and Zn^{2+} catalytic center, resulting in an active enzyme (98). IPC attenuates the accumulation of $ONOO^{-\bullet}$ and thus provides cardioprotection by preserving cardiac mechanical function. Subthreshold amounts of $ONOO^{-\bullet}$ are released during IPC, but these are insufficient to activate MMP-2 as no significant release of MMP-2 has been observed from hearts during IPC (75). As the MMP enzymes are known to remodel the extracellular matrix, it is possible that the activation of MMP enzymes may affect glycocalyx during ischemia–reperfusion as this effect is attenuated by IPC for improving cardiac function. It is also pointed out that gap junctions are highly specialized regions of plasma membrane;

however, these act as connections between cardiomyocytes (30) and thus can be seen to represent as components of the glycocalyx. Cardiac gap junctions have channel proteins, mainly connexin 43 (137), which is phosphorylated by different protein kinases and reduces junctional communication (72, 76). Although the exact mechanism of cardioprotection by modulating gap junction-mediated communication during IPC is not clear, Garcia-Dorado *et al.* (41) have hypothesized that activation of different kinases and production of ROS during IPC tend to reduce the intercellular communication and limit cell-to-cell spreading of the reperfusion-induced hypercontracture in addition to preventing cardiomyocyte death in preconditioned hearts. Such findings are interpreted to indicate that the improvement of the ischemia–reperfused hearts by IPC may be partly due to the involvement of alterations at the glycocalyx level.

CONCLUSIONS

From the foregoing discussion, it is evident that a wide variety of triggers, including ROS, have been documented to explain the beneficial effects of IPC in the ischemic–reperfused hearts. As different mediators, such as PKC, have also been implicated in the IPC phenomenon, the exact mechanisms for the improvement of cardiac function due to IPC in the ischemic– reperfused hearts are poorly understood. Various end effectors as well as triggers and mediators are proposed to be involved in IPC and considered to affect directly or indirectly different subcellular organelles, such as sarcolemma, SR, myofibrils, mitochondria, nucleus, and glycocalyx, in the heart. Accordingly, it appears that brief periods of ischemia–reperfusion modify the activities of subcellular proteins, such as sarcolemmal receptors, cation pumps, cation transporters, and cation channels, as well as myofibrillar, mitochondrial, nuclear, SR, and glycocalyx proteins. Such changes may occur as a consequence of ROS formation (Fig. 4) and seem to make these subcellular organelles more resistant to the ischemia–reperfusion injury. Thus, attenuation of these changes, including apoptosis by IPC, may be associated with the early phase of IPC, whereas attenuation of changes in gene expression due to reperfusion injury to the nucleus may represent the delayed phase of IPC. As intracellular Ca^{2+} overload as seen in Ca^{2+} paradox hearts has been suggested to produce oxidative stress (61, 78), it is possible that the beneficial effects of IPC in Ca^{2+}-paradoxic hearts may be mediated through a reduction of ROS levels. Nonetheless, in the absence of clear-cut evidence, we consider that IPC produces its beneficial effects in the ischemic–reperfused hearts by reducing the degree of both oxidative stress and intracellular Ca^{2+} overload. In addition, it also decreases the sensitivity of subcellular organelles to sustained ischemia–reperfusion injury. In spite of the fact that extensive studies are required to understand the IPC phenomenon, the current information helps in identifying several targets for drug development for the treatment of ischemic heart disease.

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ABBREVIATIONS

APD, action potential duration; CGRP, calcitonin gene-related peptide; Cu, Zn-SOD, Cu, Zn-superoxide dismutase; HSP27, heat shock protein 27; IPC, ischemic preconditioning; MAPKAP kinase 2, mitogen-activated protein kinase-

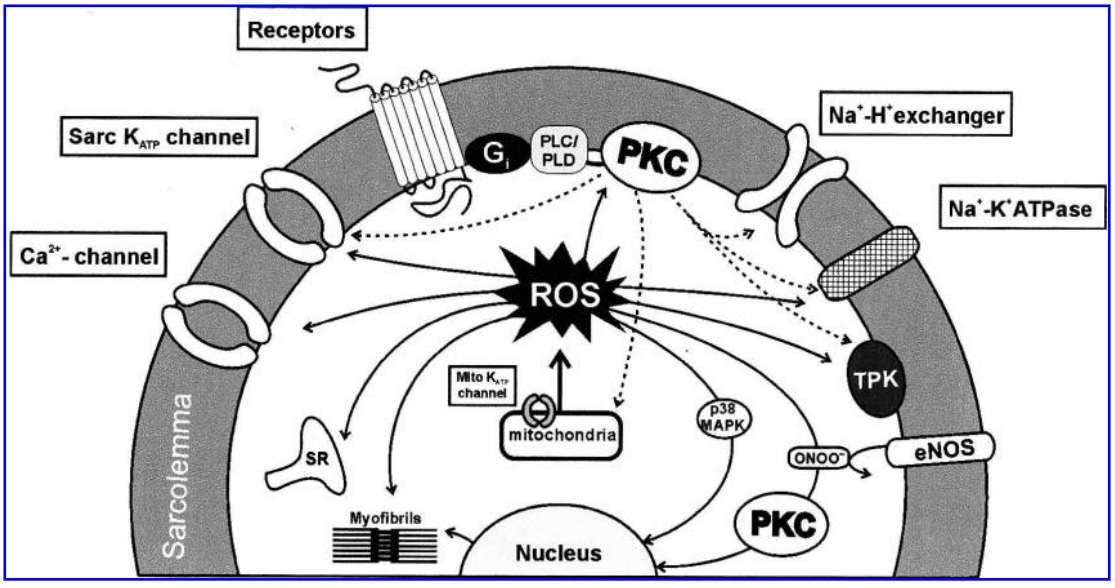

FIG. 4. Schematic representation of the effects of ROS on some mediators and end effectors in cardiomyocytes. eNOS, endothelial nitric oxide synthase; PLC, phospholipase C; PLD, phospholipase D; and TPK, tyrosine protein kinase.

activated protein kinase 2; mito K_{ATP} channels, mitochondrial ATP-sensitive potassium channels; MMP-2, matrix metalloproteinase-2; Mn-SOD, manganese superoxide dismutase; NFκB, nuclear factor-κB; NHE, Na^+/H^+ exchanger; NO, nitric oxide; $O_2^{-\bullet}$, superoxide anion; $ONOO^{-\bullet}$, peroxynitrite; p38 MAPK, 38-kDa mitogen-activated protein kinase; PKC, protein kinase C; ROS, reactive oxygen species; sarc K_{ATP} channels, sarcolemmal ATP-sensitive potassium channels; SR, sarcoplasmic reticulum.

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