K
\textsubscript{ATP} channels and myocardial preconditioning: an update

Garrett J. Gross and Jason N. Peart
Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Gross, Garrett J. and Jason N. Peart. K
\textsubscript{ATP} channels and myocardial preconditioning: an update. Am J Physiol Heart Circ Physiol 285: H921–H930, 2003; 10.1152/ajpheart.00421.2003.—Ischemic or myocardial preconditioning (IPC) is a phenomenon whereby brief periods of ischemia have been shown to protect the myocardium against a more sustained ischemic insult. The result of IPC may be manifest as a marked reduction in infarct size, myocardial stunning, or incidence of cardiac arrhythmias. Whereas many endogenous neurotransmitters, peptides, and hormones have been proposed to play a role in the signal transduction pathways mediating the cardioprotective effect of IPC, nearly universal evidence indicates the involvement of the ATP-sensitive potassium (K
\textsubscript{ATP}) channel. Initial evidence suggested that the surface or sarcolemmal K
\textsubscript{ATP} (sarcK
\textsubscript{ATP}) channel triggered or mediated the cardioprotective effects of IPC; however, more recent findings have suggested a major role for a mitochondrial site or possibly a mitochondrial K
\textsubscript{ATP} channel (mitoK
\textsubscript{ATP}). This review presents evidence that supports a role for these two channels as a trigger and/or downstream mediator in the phenomenon of IPC or pharmacologically induced PC as well as recent evidence that suggests the involvement of a mitochondrial calcium-activated potassium (mitoK
\textsubscript{Ca}) channel or the electron transport chain in mediating the beneficial effects of IPC or pharmacologically induced PC.

NOMA (35) identified, in 1983, an ATP-sensitive potassium channel (K
\textsubscript{ATP} channel) in membrane patches prepared from isolated guinea pig ventricular myocyte. After this landmark finding, K
\textsubscript{ATP} channels have also been shown to exist in other tissues, including the brain, smooth muscle, skeletal muscle, intestine, kidney, and pancreas and appears to consist of various subtypes depending on the tissue or organelle studied. It was originally suggested that this channel coupled myocardial metabolism to membrane electrical activity, and it was suggested that opening of the myocardial K
\textsubscript{ATP} channel may serve a cardioprotective function against various stresses, including ischemia and hypoxia. Indeed, this hypothesis has been borne out based on published data, which demonstrated that a number of direct openers of the K
\textsubscript{ATP} channel have been shown to afford a marked protective effect on the myocardium in numerous models of reversible or irreversible ischemia-reperfusion injury. Furthermore, the K
\textsubscript{ATP} channel has been demonstrated to play a central role in the phenomenon termed ischemic preconditioning (IPC).

Structurally, K
\textsubscript{ATP} channels are composed of two distinct proteins, an inwardly rectifying potassium channel (Kir) pore subunit and the sulfonylurea receptor (SUR), which may have a regulatory role as well as a function in modulating the sensitivity of the channel to ATP, other nucleotides, and pharmacological agonists or antagonists (69). It is currently known that the cardiac sarcolemmal K
\textsubscript{ATP} channel is composed of an octomeric complex of two types of subunits, the Kir6.2 and the SUR2A subunit. There is also evidence to suggest that there are two K
\textsubscript{ATP} channels in the cell, a sarcolemmal channel (sarcK
\textsubscript{ATP} channel) in which the structure has been clearly delineated and a putative channel in the inner mitochondrial membrane (mitoK
\textsubscript{ATP} channel). The mitoK
\textsubscript{ATP} channel has been characterized pharmacologically in cells and in isolated lipid bilayers; however, it has not been cloned and its molecular structure remains unknown. In fact, its very existence has been questioned in several recent publications (8, 29) and is currently an area of considerable controversy.

IPC is a phenomenon whereby brief intervals of sublethal ischemia either delay or reduce the extent of necrosis following a subsequent more prolonged episode of ischemia (23). IPC has two distinct phases, an early phase that lasts for 1–3 h following the IPC stimulus and a delayed phase (or second window) of protection that reappears at 18–24 h and persists for 24–72 h. The signaling pathways underlying the two forms of protection most likely share common elements, but the former is thought to primarily involve

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posttranslational modifications, whereas the latter also involves changes in gene expression and amounts of cardioprotective proteins. The signaling cascade triggered by IPC is still under debate, and there is evidence that the pathways involved in the protection mediated by acute IPC may depend on the stimulus used to elicit IPC and the end point of injury used to demonstrate the protective effect, i.e., infarct size, stunning, and arrhythmias (2). Infarct size reduction has been considered the gold standard when studying the efficacy of a preconditioning stimulus to protect the heart and is always attenuated following IPC. On the other hand, myocardial stunning is most likely not a good index of the efficacy of acute IPC, although it appears to be a more reliable indicator of IPC when studying the second window of protection, particularly in conscious animal models. Protection of IPC against arrhythmias is somewhat controversial and is very species dependent. A variety of “triggers” have also been identified that are activated and/or released during the preconditioning stimulus, including adenosine, catecholamines, bradykinin, opioids, and nitric oxide (NO) (49). These triggers may be species dependent and also dependent on the severity and length of the preconditioning stimulus (15). As an example, Schulz et al. (51) found that bradykinin was the dominant trigger released during a less severe preconditioning stimulus, whereas adenosine was only released during a more intense preconditioning stimulus. In rabbits, opioids, adenosine, and bradykinin all seem to play an equal triggering role. Regardless of these triggers, a number of mediators of IPC have been identified. Recent studies have implicated different protein kinases in the signaling cascade responsible for IPC, including Src tyrosine kinases, protein kinase C (PKC), phosphatidylinositol-3 kinase, p38 mitogen-activated protein kinase (MAPK), and the JAK/STAT pathway (2). In recent years, much research has focused on the central involvement of the sarcoKATP or mitoKATP channel as both a trigger and distal effector in IPC with equivocal results (26, 49).

Although considerable evidence supporting a role for the KATP channel in acute IPC has been obtained, recent studies suggest that the KATP channel is also intimately involved as either a trigger or end effector in delayed IPC. Therefore, the central theme of this review will be to discuss the evidence for the involvement of the sarcoKATP versus the mitoKATP channel or a mitochondrial site of action in both early and late IPC and potential mechanisms responsible for the cardioprotection observed. In addition, we will review recent evidence that supports the possibility of alternate sites of action of IPC and KATP channel openers within the mitochondria.

Evidence Supporting Involvement of SarcoKATP Channel in IPC

Although it is well known that KATP channel antagonists block the infarct-limiting effects of IPC and agonists of the channel mimic the protective effect, the subtype of KATP channel and the cellular mechanism whereby opening of this KATP channel confers its cardioprotective effects is still controversial. It was initially hypothesized by Noma (35) that opening of the sarcoKATP channel, induced by hypoxia, ischemia, or pharmacological KATP openers would enhance shortening of the cardiac action potential duration (APD) by accelerating phase 3 repolarization. An enhanced phase 3 repolarization would inhibit Ca2+ entry into the cell via L-type channels and prevent Ca2+ overload. Furthermore, the slowing of depolarization would also reduce Ca2+ entry and slow or prevent the reversal of the Na+/Ca2+ exchanger. These actions may increase cell viability via a reduction in Ca2+ overload during ischemia and early reperfusion. In this regard, Cole et al. (7) first demonstrated that glibenclamide, a non-selective KATP channel antagonist, attenuated the APD shortening, which occurs during ischemia in an isolated arterially perfused guinea pig right ventricular wall preparation, resulting in an impaired recovery of ventricular function following reperfusion. Moreover, this group (7) also showed an acceleration of APD shortening during ischemia, which resulted in an improved recovery of ventricular function during reperfusion when the tissue was pretreated with the KATP channel opener pinacidil. Furthermore, Tan et al. (57) demonstrated that IPC or KATP channel openers increased the time to electrical uncoupling, which was associated with an enhanced APD shortening. Similarly, Yao and Gross (67) found in the canine heart that IPC resulted in shortening of the APD, both effects being inhibited by glibenclamide. Furthermore, the KATP channel opener aprakalim accelerated the rate and extent of APD shortening and improved segment function during reperfusion, suggesting that activation of KATP channels during ischemia and the subsequent shortening of the APD may be a mechanism affording myocardial protection during ischemia. Subsequently, Yao and Gross (68) further demonstrated that the threshold for IPC could be lowered by the KATP channel opener bimakalim and that this occurred as a result of an enhanced rate of APD shortening. Schulz et al. (52) also found that IPC resulted in an acceleration of APD shortening during ischemia in pigs and that this was associated with a pronounced reduction in infarct size. Finally, Suzuki et al. (55) recently demonstrated in wild-type and Kir6.2 knockout mice that diazoxide had no cardioprotective effect to enhance contractile recovery in knockout mice, and the protection it produced in the wild-type mice was associated with an enhanced APD shortening. This effect was blocked by HMR-1098, a putative sarcoKATP channel blocker but not by 5-hydroxydecanoate (5-HD). These results strongly support a major involvement of the sarcoKATP channel in IPC in the mouse heart and no role for a mitoKATP channel or mitochondrial site of action.

Additional evidence supporting a protective role for the sarcoKATP channel has been provided by using KATP-deficient COS-7 cells. By cotransfection of Kir6.2/SUR2A genes, Jovanovic et al. (23) demonstrated that delivery of Kir6.2 and SUR2A genes into COS-7 cells...
resulted in a \( K^+ \) current in the presence of pinacidil. Furthermore, after cotransfection and treatment with pinacidil, the cells became resistant to hypoxia-reoxygenation as a result of inhibition of intracellular \( Ca^{2+} \) loading (23, 24). Given that COS-7 cells are noncontracting, these data provide further credence to the hypothesis that sarc\( K_{ATP} \) channels may provide cardioprotection independent of APD shortening. A recent study by Suzuki et al. (55) reported a failure of IPC to reduce infarct size in Kir6.2-deficient mice. However, as noted by these authors (55), the relative importance of the sarc\( K_{ATP} \) channel may have been exaggerated due to the rapid heart rate in the murine model. Nevertheless, these results were supported by those of Rajashree et al. (44) who also demonstrated that transgenic mice expressing a mutant Kir6.2 subunit, with a reduced ATP sensitivity, were insensitive to IPC. However, the study of Rajashree et al. (44) performed in isolated hearts, used the measurement of postischemic contractile dysfunction as the end point of ischemic injury rather than infarct size. Because there is not always a good correlation between the extent of stunning and infarct size in isolated hearts (22), it is possible that these investigators may have missed a reduction in infarct size in these mutant mice subjected to IPC.

Although recent results demonstrate that the sarc\( K_{ATP} \) channel may trigger IPC, at least in mice, recent preliminary results obtained in the authors' laboratory demonstrated that the sarc\( K_{ATP} \) channel is the trigger for delayed IPC (see Fig. 1) in rats. In these studies, cardioprotection was induced by an IPC protocol consisting of three 5-min cycles of ischemia-reperfusion with 5 min of reperfusion, 24 h before the more sustained ischemic insult. The cardioprotective effect observed was blocked by HMR-1098 during the trigger phase rather than during the mediator phase (unpublished results). Furthermore, the results were duplicated by a 24-h pretreatment with SNC-121, a selective \( \delta \)-opioid receptor agonist (42). Treatment with SNC-121 accelerated APD shortening, and its effect to reduce infarct size and APD shortening was abolished by HMR-1098.

**Fig. 1.** Delayed preconditioning mediated via opening of sarcosomal \( Na\text{-}K\text{-}ATPase-sensitive \( K \) (sarc\( K_{ATP} \)) channel. Ischemic preconditioning (IPC) was instigated via three 5-min cycles of ischemia-reperfusion, 24 h before sustained 30 min of occlusion. IPC resulted in significant reduction in infarct size (IS) as assessed following 2 h of reperfusion (27.5 ± 3.9 %IS/AAR, compared with 55.8± 2.3% for sham operated, where AAR is area at risk). 5-Hydroxydecanote (HD, 10 mg/kg) administered 5 min before IPC protocol failed to attenuate IPC-mediated protection (28.8 ± 3.7% IS/AAR); however, HMR-1098 (HMR) abolished IPC-mediated protection (48.8 ± 2.7% IS/AAR).

\*\( P < 0.001 \).
cromakalim, because K\textsubscript{ATP} channel openers would be expected to act directly on the channel, thus, having effects independent of intracellular ATP concentrations. Furthermore, diazoxide, a selective mitoK\textsubscript{ATP} channel opener, failed to reduce infarct size when administered at a similar or 10-fold higher dose than that needed for cromakalim to produce its infarct-limiting effects (19). This finding is puzzling, however, because Garlid et al. (12) demonstrated that cromakalim and diazoxide had a similar degree of potency for opening mitoK\textsubscript{ATP} in reconstituted mitochondria in lipid bilayers and elicited a similar degree of cardioprotection against stunning following ischemia-reperfusion in the isolated rat heart. The lack of protection afforded by diazoxide at a dose that would be expected to elicit cardioprotection via opening of the mitoK\textsubscript{ATP} channel is surprising and suggests that the sarcK\textsubscript{ATP} channel is intimately involved in the cardioprotective effects of IPC in this particular model.

Another possible mechanism by which opening of sarcK\textsubscript{ATP} channels confers cardioprotection may be the result of a channel-induced change in a specific intracellular signaling pathway. Hyperpolarization following the activation of the sarcK\textsubscript{ATP} channel may lead to activation of the mitoK\textsubscript{ATP} channel. Waring and colleagues (65) demonstrated that hyperpolarization of rat hippocampal slices increases phospholipase D activity. Phospholipase D has been implicated in IPC (58), and diacylglycerol produced by phospholipase D has been demonstrated to activate and translocate PKC, which has been suggested to potentiate the opening of the sarcK\textsubscript{ATP} and/or mitoK\textsubscript{ATP} channel.

Although it seems possible that activation of the sarcK\textsubscript{ATP} channel may lead to opening of the mitoK\textsubscript{ATP} channel via cross talk or vice versa there is no direct evidence to support this hypothesis, and there have been a number of recent papers that have presented different roles for the sarcK\textsubscript{ATP} and mitoK\textsubscript{ATP} channel in IPC and cardioprotection. In a model of chronic hypoxia, where rabbits were raised from birth in either a normoxic or hypoxic environment, Kong and colleagues (26) demonstrated that either 5-HD, a selective mitoK\textsubscript{ATP}, antagonist, or HMR-1098, a putative selective sarcK\textsubscript{ATP}, antagonist, failed to completely abolish the protection afforded by chronic hypoxia and that only the combination of 5-HD and HMR-1098 was successful in completely abolishing the protective effects of chronic hypoxia. Furthermore, a recent paper by Sanada et al. (47) demonstrated that glibenclamide, a nonselective K\textsubscript{ATP} channel blocker, completely abolished the protective effects of IPC, whereas 5-HD, a mitochondrial selective blocker, only partially blunted the infarct size-reducing effect in dogs. In a model of adenosine-enhanced IPC, Toyoda et al. (59) presented a temporal involvement of both sarcK\textsubscript{ATP} and mitoK\textsubscript{ATP} channels. These investigators indicated that the infarct size-reducing effects of adenosine-enhanced preconditioning are mediated by mitoK\textsubscript{ATP} channels during ischemia, whereas sarcK\textsubscript{ATP} channels modulate functional recovery during both ischemia and reperfusion. This concept is also supported by Light et al. (28), who reported that the protective effects of phorbol 12-myristate 13-acetate (PMA), a PKC activator, were partially inhibited by 5-HD during chemically induced hypoxia but not at reoxygenation, whereas HMR-1098, acting in a PKC and adenosine-dependent manner, was only effective in abolishing protection and the reduction in intracellular Ca\textsuperscript{2+} overload during reoxygenation. In contrast, recent work of Downey and Cohen’s group (39) suggest that adenosine may signal independently of the K\textsubscript{ATP} channel and act directly via a kinase pathway. Taken together, these data suggest that the sarcK\textsubscript{ATP} and mitoK\textsubscript{ATP} channels are independently involved in producing ischemic tolerance provided by IPC and may produce additive effects resulting in cardioprotection. Of course, a caveat in many of these studies is related to the selectivity of the pharmacological agents used as selective blockers of the sarcK\textsubscript{ATP} and mitoK\textsubscript{ATP} channels 5-HD and HMR-1098. There are studies to suggest that both of these so-called selective agents may have effects unrelated to K\textsubscript{ATP} channel blockade or lack selectivity for the channel in question (29).

Evidence for Involvement of MitoK\textsubscript{ATP} channel in Acute IPC

Since the first evidence for a role of the K\textsubscript{ATP} channel in acute IPC was presented by Gross and Auchampach (14) in the canine heart, results obtained in a number of studies in different models and species supported a role for the sarcK\textsubscript{ATP} channel as the end effector in IPC. However, recent evidence suggests an alternate site from the sarcK\textsubscript{ATP} channel to a mitochondrial site of action or to a putative mitoK\textsubscript{ATP} channel as a trigger and end effector in IPC or pharmacologically induced PC.

The first report that suggested that an enhanced shortening of the APD as a result of sarcK\textsubscript{ATP} channel activation was not the mechanism responsible for cardioprotection provided by K\textsubscript{ATP} openers was published by Yao and Gross in 1994 (68). This study demonstrated that a low dose of the nonselective K\textsubscript{ATP} channel opener bimakalim, which did not effect APD shortening, still produced a cardioprotective effect comparable to that of two higher doses of bimakalim, which produced a significant shortening of APD. It was hypothesized that an intracellular site of action may have been responsible for the efficacy of bimakalim to reduce infarct size independent of APD shortening. Moreover, Grover et al. (17) added further weight to this hypothesis when they described a lack of correlation between APD shortening and cardioprotection following cromakalim and the protective effects of IPC were not attenuated by dofetilide, a class III antiarrhythmic, which prevented APD shortening in preconditioned hearts (16). Evidence for a role of K\textsubscript{ATP} channel activation mediating the protective effects of IPC and K\textsubscript{ATP} openers in the absence of a ventricular action potential has also been provided from studies in isolated nonbeating cardiac myocytes (1). These data all suggested that the sarcK\textsubscript{ATP} channel may not be totally account-
able for the protective effects afforded by $K_{\text{ATP}}$ openers and IPC and suggested a possible intracellular site of action.

Garlid et al. (12) provided the first direct evidence to support a role for the mito$K_{\text{ATP}}$ channel in cardioprotection. Utilizing reconstituted bovine heart mitochondria, these investigators found that diazoxide opened mito$K_{\text{ATP}}$ with a concentration of drug to produce a 50% increase in $K_{\text{ATP}}$ channel opening in lipid bilayers of 0.8 μmol/l, whereas 800 μmol/l was required by diazoxide to open the sarc$K_{\text{ATP}}$ channel. Furthermore, diazoxide, at concentrations that did not activate the sarc$K_{\text{ATP}}$ channel, produced a pronounced cardioprotective effect comparable to that of cromakalim at similar doses, as evidenced by an increase in time to ischemic contracture and enhanced functional recovery following global ischemia and reperfusion in isolated rat hearts. The effects of diazoxide and cromakalim were abolished by the $K_{\text{ATP}}$ channel antagonists 5-HD and glibenclamide, suggesting that mito$K_{\text{ATP}}$ channels may be responsible for these effects. In a more recent paper, Sasaki et al. (48) identified a novel pharmacological agent, MCC-134, which opens sarc$K_{\text{ATP}}$ channels and blocks mito$K_{\text{ATP}}$ channels in the same molecule. In rabbit ventricular myocytes, MCC-134 blocked diazoxide-induced flavoprotein oxidation and activated sarc$K_{\text{ATP}}$ channels. Similar results were also observed in mouse ventricular myocytes, and MCC-134 attenuated the effects of IPC in a rabbit myocyte model and in intact mouse hearts. All of these results are consistent with a primary role for the mito$K_{\text{ATP}}$ channel or a mitochondrial site of action in IPC and diazoxide-induced cardioprotection and a lesser role for the sarc$K_{\text{ATP}}$ channel. In contrast, in one of the few studies performed in a large animal species, Schwartz et al. (53) was not able to block IPC in swine hearts subjected to multiple preconditioning cycles with 5-HD pretreatment.

Whereas it is generally accepted that the mito$K_{\text{ATP}}$ channel, or at least a mitochondrial site of action, is involved in the cardioprotection afforded by IPC and diazoxide, it is still unclear as to whether its role is as a trigger or distal effector or both. Indeed, several reports support an involvement for the mito$K_{\text{ATP}}$ channel as both a trigger and end effector of IPC and $K_{\text{ATP}}$ openers such as diazoxide (15, 49).

Mechanisms by Which Mito$K_{\text{ATP}}$ Channels Serve as a Trigger or Distal Effector of IPC

Mechanisms by which mito$K_{\text{ATP}}$ channels are activated are similar to many of those previously described for the sarc$K_{\text{ATP}}$ channel. In isolated myocytes, anoxia induces a rapid opening of mito$K_{\text{ATP}}$ channels (25). Transient ischemia produces $H_2O_2$, and this leads to the activation of mito$K_{\text{ATP}}$ channels via a PKC-ε-mediated pathway (71).

Activation and translocation of specific PKC isoforms, regardless of initiator (adenosine, etc.) appears to be central to opening of mito$K_{\text{ATP}}$ channels, and indeed, these two phenomena may be codependent.
The general assumption given to mitoK\textsubscript{ATP} activation is that during transient ischemia a preconditioned state is triggered via various mechanisms. These “triggers” then lead to activation/translocation of PKC and other downstream kinases (p38MAPK, JUNK, and ERK1/2). MitoK\textsubscript{ATP} channels are then subsequently phosphorylated and opened earlier to provide protection via unknown mechanisms during the sustained ischemic insult. A recent study by Carroll and Yellon (6) described that delayed protection in a cardiomyocyte-derived cell line involves p38 MAPK and the opening of mitoK\textsubscript{ATP} channels. In this model, the protective effects of IPC were abolished when cells were pretreated with SB-203580, a p38 MAPK inhibitor, before the preconditioning stimuli. Furthermore, protection was attenuated when cells were treated with 5-HD 30 min before lethal simulated ischemia on the second day following preconditioning. These results suggest that mitoK\textsubscript{ATP} channel opening is downstream from p38 MAPK activation (6). However, another study places MAPK activation downstream from mitoK\textsubscript{ATP} channel opening. Using THP-1 cells, Samavati et al. (46) demonstrated that diazoxide induces mitochondrial reactive oxygen species (ROS) production, as evidenced by an increased rate of dihydroethidium and dichlorofluorescein fluorescence. Moreover, the increase in ROS resulted in an increase in the phosphorylation of ERK, a member of the MAPK family. Thus, opening of mitoK\textsubscript{ATP} channels was associated with the downstream activation of ERK. The results of Wang and Ashraf (63), Samavati et al. (46), and Liu et al. (31) suggest that mitoK\textsubscript{ATP} activation may also act as a trigger of cardioprotection. In agreement, Pain et al. (41) demonstrated that in isolated rabbit hearts, protection afforded by diazoxide could only be abolished when K\textsubscript{ATP} channel blockers were given during diazoxide treatment, not after treatment. Furthermore, free radical scavengers given during the trigger phase also abolished the protective effects of diazoxide (10). Diazoxide has been previously shown to mediate cardioprotection in cells via a redox-sensitive mechanism whereby it initiates a burst of free radicals (5, 10), a known trigger leading to a preconditioned state, possibly through activation of specific kinases. Vanden Hoek et al. (60) presented evidence in isolated chick myocytes that a brief period of hypoxia generated ROS, which triggered a preconditioning-like response and attenuated a more marked release of ROS following a more prolonged period of ischemia and reoxygenation (61). They also found that PKC and the mitoK\textsubscript{ATP} channel appeared to be an important part of the trigger phase of this response (60).

The answer to the question “is the mitoK\textsubscript{ATP} channel the trigger or the mediator of IPC?” may be quite simple indeed, it may be both. A study by Wang et al. (64) examined the mitoK\textsubscript{ATP} channel as both a trigger and a mediator. In an isolated rabbit heart model, the protective effects of diazoxide pretreatment (with a washout period) were eliminated by coadministration of either 5-HD, the L-type Ca\textsuperscript{2+} channel blocker nifedipine or by the PKC inhibitor chelerythrine. In contrast, when given following diazoxide treatment, chelerythrine was unsuccessful and 5-HD was only able to block the protection at a fourfold higher dose. Thus it was proposed that the trigger phase may be mediated by the elevation of intracellular Ca\textsuperscript{2+} and PKC activation, whereas mitoK\textsubscript{ATP} opening invokes protection during the mediator phase via unknown mechanisms, which are independent of PKC activation/translocation. Thus there is considerable evidence that the mitoK\textsubscript{ATP} may have a role as a trigger and/or distal effector in IPC-mediated cardioprotection. However, recently Schulz et al. (50) presented data in anesthetized pigs to suggest that the K\textsubscript{ATP} channel only served a trigger role in this species because glibenclamide only blocked IPC when given before the brief ischemic stimulus and not 10 min after IPC. This study did not use 5-HD to determine whether the mitoK\textsubscript{ATP} or sarc-K\textsubscript{ATP} channel was involved in the trigger phase (50).

It appears that the mitochondria have an intimate role in cell survival through maintaining or enhancing ATP synthesis and maintenance of Ca\textsuperscript{2+} homeostasis as well as regulation of mitochondrial volume. Ischemia-reperfusion impairs mitochondrial function through an alteration of membrane potential, imbalance of cytosolic ions, electron transport, and an increased production of free radicals. In an isolated cell model, anoxia-reoxygenation may lead to a hyperpolarization of mitochondria. This hyperpolarization may indeed drive Ca\textsuperscript{2+} into the mitochondria, leading to Ca\textsuperscript{2+} overload. Xu and associates (66) demonstrated that treatment with diazoxide stabilized the mitochondrial membrane potential through limiting the decrease of membrane potential and by inhibition of the high polarization observed during anoxia-reoxygenation. Depolarization of the membrane potential may reduce Ca\textsuperscript{2+} influx, limiting Ca\textsuperscript{2+} overload and myocyte injury. Although having no effect on total intracellular Ca\textsuperscript{2+} levels, IPC has been shown to inhibit the ischemia-induced elevation of mitochondrial Ca\textsuperscript{2+} concentrations, an effect attributed to mitoK\textsubscript{ATP} channel opening. Diazoxide reduced mitochondrial Ca\textsuperscript{2+} concentration and 5-HD inhibited the reduction in mitochondrial Ca\textsuperscript{2+} concentration provided by IPC and diazoxide in isolated rat hearts (63). The study by Xu and colleagues (66) reported that diazoxide treatment also prevented ATP depletion. In a model using arterially perfused guinea pig right ventricular walls, McPherson et al. (32) reported that K\textsubscript{ATP} channel opening with pinacidil inhibited ischemia-induced depletion of high-energy phosphates. This pinacidil-induced preservation of creatine phosphate and ATP was abolished by glibenclamide pretreatment and glibenclamide alone enhanced the ischemia-induced depletion of ATP. MitoK\textsubscript{ATP} channel opening may partially restore the membrane potential, allowing further extrusion of H\textsuperscript{+}, forming a more favorable electrochemical gradient (56) for ATP synthesis. In this regard, diazoxide has recently been found to exert a unique protective action on mitochondrial membrane potential and membrane integrity (9). In cultured cardiac myocytes exposed to H\textsubscript{2}O\textsubscript{2}, diazoxide decreased the number of cells undergoing membrane

AJP-Heart Circ Physiol • VOL 285 • SEPTEMBER 2003 • www.ajpheart.org
depolarization and delayed the loss in membrane potential. In combination with cyclosporin A, diazoxide also exhibited an additive effect to inhibit the progression to cell death. These effects were selectively blocked by 5-HD, whereas other cardioprotective mechanisms, such as those produced by the adenine nucleotide translocase inhibitor bongkrekic acid were not blocked by 5-HD. Although not measured in this study, these effects would reduce ATP depletion, which will maintain ATP-dependent ion pumps such as the Na-K pump and Ca\(^{2+}\) pumps. Thus maintenance of ATP levels may further support Ca\(^{2+}\) homeostasis. Diazoxide increases the half-saturation constant for ADP stimulation of respiration and limits ATP hydrolysis, thus effectively preserving the adenine nucleotide pool during ischemia and the energy transfer during reper-
fission (56). Recent studies by Garlid et al. (11), suggested that the reported changes in mitochondrial membrane potential and Ca\(^{2+}\) accumulation are merely “epiphenomena” produced by high concentrations of mitoK\(_{ATP}\) openers. Garlid et al. (11) hypothesized that the critical effect resulting from opening mitoK\(_{ATP}\) channels is in the regulation of mitochondrial matrix volume. Mitochondrial volume has been shown to regulate the electron transport chain and preserve the architecture of the intermembrane space, permitting a more efficient energy transfer between mitochondria and cellular ATPases. Indeed, opening of the mitoK\(_{ATP}\) channel may maintain the structure of the intermembrane space during ischemia, preserving the low permeability to ADP and ATP. In contrast, Lim et al. (29) and Das et al. (8) both recently suggested that changes in mitochondrial volume could not explain the beneficial effects produced by IPC and diazoxide treatment.

**Evidence for Involvement of Electron Transport Chain in IPC and Pharmacological PC**

Although it is well known that diazoxide is an effective inhibitor of succinate oxidation in mitochondria at higher concentrations (40), it has not been recognized until recently that this effect could also translate into a myocardial protective effect and give an alternate mechanism to opening of a mitoK\(_{ATP}\) channel whose existence has still not been validated by molecular cloning techniques. Recently, Hanley and co-workers (18) have shown in submitochondrial particles isolated from pig hearts that diazoxide concentration dependently (10–100 μM) decreased succinate oxidation without any effect on NADH oxidation. Furthermore, these authors also showed that pinacidil, another K\(_{ATP}\) opener, inhibited NADH oxidation without any effect on succinate oxidation. They showed that these effects produced by diazoxide and pinacidil occurred in the absence of any changes in mitochondrial membrane potential. Using HPLC and electron spray ionization, these investigators also showed that the selective mitoK\(_{ATP}\) blocker 5-HD was converted to 5-HD CoA, which may exert effects on electron transport that would inhibit the actions of diazoxide and pinacidil. These results suggest that there may be an alternate mechanism occurring within mitochondria that may produce cardioprotection independent of a mitoK\(_{ATP}\) channel (Fig. 2). Lim et al. (29) have recently presented new evidence to support this view. These investigators showed that changes in matrix volume that were produced by diazoxide and IPC were similar; however, the changes produced by IPC were accompanied by increases in ADP-stimulated and uncoupled 2-oxoglutarate and succinate oxidation, whereas diazoxide produced decreases in succinate and oxoglutarate oxidation. Treatment of hearts with 100 or 300 μM of 5-HD also increased mitochondrial volume and inhibited respiration. These authors also demonstrated that 5-HD was rapidly converted to 5-HD CoA by mitochondrial fatty acyl CoA synthetase in isolated mitochondria, and they further showed that this metabolite of 5-HD was either a weak substrate or inhibitor of mitochondrial respiration. These results strongly suggest that 5-HD and diazoxide may not be selective modulators of mitoK\(_{ATP}\) channels in the heart and that changes in mitochondrial volume are most likely not responsible for the cardioprotective effects of IPC or K\(_{ATP}\) openers. Further studies are necessary to resolve these conflicting theories.

In conclusion, recent data suggest that both the sarcK\(_{ATP}\) and mitoK\(_{ATP}\) channels play complimentary roles in the protection afforded by IPC. On the basis of recent evidence, activation of the mitoK\(_{ATP}\) channel appears to limit cell death, whereas opening of the sarcK\(_{ATP}\) channel appears to limit stunning. Regardless, direct activation of either the sarcK\(_{ATP}\) or mitoK\(_{ATP}\) channels provides significant cardioprotection, and the possible signaling pathways involved are schematically shown in Fig. 3. Activation, especially of the mitoK\(_{ATP}\), may be involved in acute IPC as either a trigger and mediator, end effector, or both. Opening of the mitoK\(_{ATP}\) channel may result in a ROS burst, which may in itself have a preconditioning effect. Translocation/activation of PKC and other kinases may lie both upstream and downstream of the mitoK\(_{ATP}\) channel, perhaps acting as a positive feedback to elicit a more robust opening of the channel. As a potential end effector, the role that opening of the mitoK\(_{ATP}\) plays in increasing mitochondrial cell volume may be intimately involved in the protective effects of both IPC and direct activation of the channel. Following that, the sarcK\(_{ATP}\) channel may indeed act as a trigger for the opening of the mitoK\(_{ATP}\) channel or confer its own cardioprotective effect via a PKC-dependant mechanism, which ultimately leads to a reduction in Ca\(^{2+}\) overload during ischemia. Moreover, Kir6.2-deficient mice are insensitive to IPC. Thus it appears that both the sarcK\(_{ATP}\) and mitoK\(_{ATP}\) have complimentary roles in the cardioprotection afforded by IPC and certain K\(_{ATP}\) openers, indeed, they may act together to elicit beneficial effects on the myocardium.

**REFERENCES**


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