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Invited Review

Sarcolemmal and mitochondrial K_{ATP} channels and myocardial ischemic preconditioning

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Abstract

Ischemic preconditioning (IPC) is the phenomenon whereby brief periods of ischemia have been shown to protect the myocardium against a sustained ischemic insult. The result of IPC may be manifest as a marked reduction in infarct size, myocardial stunning, or incidence of arrhythmias. While many substances and pathways have been proposed to play a role in the signal transduction mediating the cardioprotective effect of IPC, overwhelming evidence indicates an intimate involvement of the ATP-sensitive potassium channel (K_{ATP} channel) in this process. Initial hypotheses suggested that the surface or sarcolemmal K_{ATP} (sarcK_{ATP}) channel mediated the cardioprotective effects of IPC. However, much research has subsequently supported a major role for the mitochondrial K_{ATP} channel (mitoK_{ATP}) as the one involved in IPC-mediated cardioprotection. This review presents evidence to support a role for the sarcK_{ATP} or the mitoK_{ATP} channel as either triggers and/or downstream mediators in the phenomenon of IPC.

Keywords: ischemic preconditioning - ATP-sensitive K^+ channel - mitochondria - sarcolemma

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Introduction

Nearly twenty years have passed since the ATP-sensitive potassium channel (K_{ATP} channel) was discovered by Noma in isolated membrane patches prepared from guinea pig ventricular myocytes [1]. The K_{ATP} channel has also been shown to exist in other tissues, including brain, smooth muscle, skeletal muscle and the pancreas. It was originally hypothesized by Noma that this channel coupled myocardial metabolism to membrane electrical activity and he suggested that opening of the K_{ATP} channel may serve as an endogenous cardioprotective mechanism. Indeed, this has proved to be true, as a number of K_{ATP} channel openers have been shown to afford a marked protective effect on the myocardium in numerous models of ischemia. Furthermore, the K_{ATP} channel has been demonstrated to play a key role in the phenomenon termed ischemic preconditioning (IPC).

K_{ATP} channels are comprised of two different proteins, the inwardly-rectifying potassium channel (Kir) pore subunit, and the sulfonylurea receptor (SUR), which may have a regulatory role as well as a role in modulating the sensitivity of the channel to ATP and pharmacologic agents [2]. It is currently thought that the cardiac sarcolemmal K_{ATP} channel is comprised of an octomeric complex of 2 types of subunits, the Kir6.2 and the SUR2A subunit. There are also thought to be two K_{ATP} channels in the cell, a sarcolemmal channel (sarc K_{ATP} channel) in which the structure has been clearly delineated as mentioned above and a putative channel in the inner mitochondrial membrane (mito K_{ATP} channel). The mito K_{ATP} channel has been characterized pharmacologically but has not yet been cloned and its structure remains elusive.

IPC is the phenomenon whereby short bursts of sublethal ischemia either delay or reduce necrosis following a subsequent more prolonged episode of ischemia [3]. IPC has two distinct phases, an early phase that lasts for 1 to 3 hours following the IPC stimulus and a delayed phase (or second window) of protection that lasts for 24 to 96 hours. The molecular pathways underlying the two forms of protection most likely share common elements, but the former is thought to primarily involve post-translational modifications, whereas, the latter additionally involves changes in gene expression. The precise signaling cascade triggered by IPC is still unclear and under much debate, and there is still controversy as to whether IPC is protective against myocardial stunning. Regardless of this controversy, a number of triggers and mediators of IPC have been identified. Recent studies have implicated different protein kinases in the signalling cascade responsible for IPC, including Src tyrosine kinases, PI-3 kinase, p38 MAPK, and the JAK/STAT pathway [4]. A variety of "triggers" have also been identified which are activated and/or released during the preconditioning insult, including adenosine, catecholamines, angiotensin II, bradykinin, endothelin, and opioids [5]. In recent years, much research has also been focused on the central involvement of the sarc K_{ATP} or mito K_{ATP} channel as both a trigger and distal effector in IPC [6].

While considerable evidence supporting a role for the K_{ATP} channel in early IPC has been obtained, recent studies suggest that the K_{ATP} channel is also intimately involved as either a trigger or end-effector in delayed preconditioning. Therefore, the central theme of this review will be to discuss the evidence for a role of the sarcK_{ATP} versus the mitoK_{ATP} channel in both early and late IPC.

Role of sarcK_{ATP} in IPC

While it is has been shown that K_{ATP} channel antagonists block the infarct limiting effects of IPC, and agonists of the channel mimic the protective effect, the precise mechanism whereby opening of the K_{ATP} channel confers its cardioprotective effect is still controversial. It was initially hypothesized by Noma [1] that the opening of the surface or sarcK_{ATP} channel, induced by hypoxia, ischemia or pharmacologic K_{ATP} openers would enhance shortening of the cardiac action potential duration (APD) by accelerating phase 3 repolarization. An enhanced phase 3 repolarization would inhibit Ca^{2+} entry into the cell via L-type channels and prevent Ca^{2+} overload. Furthermore, the slowing of depolarization would also reduce Ca^{2+} entry and slow or prevent the reversal of the Na^{+}- Ca^{2+} exchanger. These

actions would potentially increase cell viability via a reduction in Ca^{2+} overload during ischemia and early reperfusion. This theory was supported by a number of early studies. Cole et al [7] demonstrated that glibenclamide, a non-selective K_{ATP} channel antagonist, attenuated the action potential shortening during ischemia in an isolated arterially perfused guinea pig right ventricular wall preparation, resulting in an impaired recovery of ventricular function following reperfusion. Cole and associates [7] also reported an acceleration of APD during ischemia, resulting in an improved recovery of ventricular function during reperfusion when the tissue was pretreated with the K_{ATP} channel opener, pinacidil. Furthermore, Tan et al [8] demonstrated that IPC or K_{ATP} channel openers increased the time to electrical uncoupling which was associated with an enhanced shortening of the APD. Similarly, Yao and Gross found that in the canine heart, IPC resulted in shortening of the APD, an effect inhibited by glibenclamide pretreatment [9]. The K_{ATP} channel opener, aprikalim, accelerated the rate and extent of APD shortening and improved segment function during reperfusion, suggesting that activation of K_{ATP} channels during ischemia, and the subsequent shortening of APD may be an endogenous mechanism affording myocardial protection during ischemia. In a later study, Yao and Gross further demonstrated that the threshold for IPC could be lowered by the K_{ATP} channel opener bimakalim, and that this occurred as a result of an enhanced rate of action potential shortening [9]. Schulz et al [10], also found that IPC resulted in an acceleration of APD shortening during ischemia in pigs, and that this was associated with a pronounced cardioprotective effect. However, it must be noted that the degree of shortening was not great (~10%) in the pig and was unlikely to account for the extent of protection observed.

Recent evidence supporting a protective role for sarcK_{ATP} has been provided by using K_{ATP} channel deficient COS-7 cells. Utilizing a method which allows cotransfection of Kir6.2/SUR2A genes, Jovanovic et al [11] demonstrated that gene delivery of Kir6.2 and SUR2A genes into COS-7 cells resulted in a K+ current in the presence of pinacidil. Furthermore, following cotransfection and treatment with pinacidil the cells became resistant to hypoxia-reoxygenation as a result of inhibition of intracellular Ca^{2+} loading [11,12]. Given that COS-7 cells are non-contracting, these data provide further credence to the hypothesis that sarcK_{ATP} channels may provide cardioprotection independent of APD shortening. A recent study by Suzuki et al [13]reported a failure of IPC-mediated protection in Kir6.2-deficient mice. However, as noted by the authors [13], the relative importance of the sarcK_{ATP} channel may have been exaggerated due to the rapid heart rate in the murine model. These results are supported by those of Rajashree et al [14] who also demonstrated that transgenic mice expressing a mutant Kir6.2, with a reduced ATP sensitivity, were insensitive to IPC. However, the study by Rajashree *et al.* used the measurement of post-ischemic contractile dysfunction as the endpoint of ischemic injury, rather than infarct size [14]. Reduction of infarct size is generally considered to be the "gold-standard" when examining IPC,

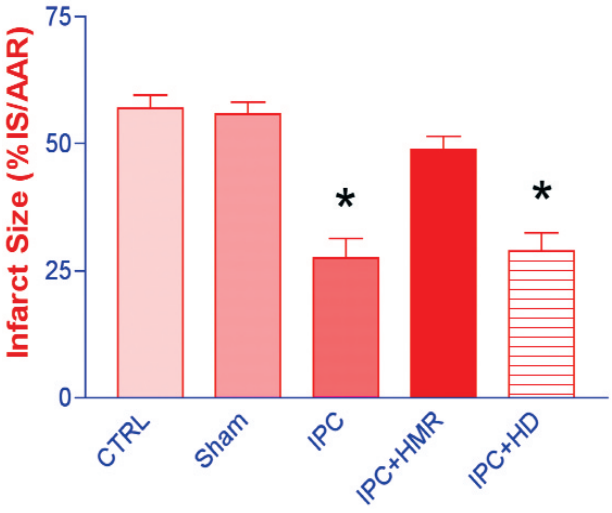

Fig. 1 Delayed preconditioning mediated via opening of sarcK_{ATP} channel. Ischemic preconditioning (IPC) was induced via three 5 min cycles of ischemia-reperfusion, 24 hours prior to sustained 30 min occlusion. IPC resulted in significant reduction in infarct size as assessed following 2 hour reperfusion (27.5±3.9 %IS/AAR as compared to 55.8±2.3% for sham-operated). 5-hydroxydecanote (HD, 10mg/kg) administered 5 min prior to 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.

and controversy still exists as to whether IPC attenuates acute myocardial stunning.

While it has been demonstrated that the sarcK_{ATP} channel may play a role in IPC, the mechanism by which this occurs remains elusive. The primary hypothesis involves APD shortening. Preliminary results obtained in the authors laboratory demonstrated that the sarcK_{ATP} is the trigger for delayed ischemic preconditioning (Fig. 1) in an *in vivo* rat model. In these studies cardioprotection was induced by an IPC protocol consisting of three 5 min cycles of ischemia interspersed with 5 minute reperfusion periods, 24 hours prior to the sustained ischemic insult. This cardioprotective effect was blocked by HMR 1098, a sarcK_{ATP} channel blocker, during the trigger phase rather than during the mediator phase. Furthermore, the results were duplicated by a 24 hour pretreatment with SNC 121, a selective delta-opioid receptor agonist [10]. Treatment with SNC 121 accelerated APD shortening, and this effect on infarct size and APD shortening was abolished by HMR 1098.

SarcK_{ATP} as a trigger of acute IPC

The mechanism by which the sarcK_{ATP} channel is activated during IPC and how it produces cardioprotection is unclear, however, a number of studies have shed some light on this topic. Liu et al [16] demonstrated that in isolated rabbit ventricular myocytes, adenosine and PKC activation increased I_{KATP} during metabolic inhibition, an effect abolished by treatment with the adenosine receptor antagonist, 8-SPT. Transient ischemia, as utilized for IPC, increases adenosine to levels which will activate adenosine A1 and A3 receptors [17]. It is possible that this increase in adenosine opens or primes the opening of the sarcK_{ATP} channel via adenosine receptor activation. Transient ischemia may open the sarcK_{ATP} channel following a synergistic action produced by PKC phosphorylation and adenosine receptor activation [16]. This effect of PKC has also been demonstrated by Light et al [18]. Nitric oxide has also been shown to activate the sarcK_{ATP} channel in both normoxic and hypoxic hearts [19], and indeed, the brief ischemic stress of IPC may lead to an increase in NO via eNOS [20]. However, the role of NO in early IPC remains equivocal although NO appears to be the primary mediator of delayed IPC as clearly shown by Bolli and his colleagues [21].

SarcK_{ATP} as a distal effector

Protein kinase C has been well established as a key intermediate in the pathway involved in IPC. SarcK_{ATP} channels have been previously linked with the activation of PKC. A recent study by Light *et al.* [18], demonstrated a functional coupling of PKC to the sarcK_{ATP} channel complex in the mediation of cardioprotection. However, some evidence suggests that PKC activation/translocation may be upstream of K_{ATP} channel activation.

IPC limits the inhibition of sarcolemmal Na^+-K^+ ATPase activity in the acute phase of ischemia and increases Na^+-Ca^+ exchange activity [22]. Haruna et al [23] recently reported that the infarct limiting effect of IPC may be modulated via an interaction between Na^+-K^+ ATPase and sarcK_{ATP} channels. In this study, the infarct-limiting effects of IPC in anesthetized rabbits were abolished by digoxin, an inhibitor of Na^+-K^+ ATPase [23]. By inhibiting Na^+-K^+ ATPase, digoxin would raise the amount of subsarcolemmal ATP, preventing the opening of the sarcK_{ATP} channel during IPC. However, digoxin did not alter the protective effects of the K_{ATP} opener, cromakalim, since K_{ATP} channel openers would be expected to act directly on the channels, and thus, have effects independent of ATP concentrations. Furthermore, diazoxide, a selective mitoK_{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 [23]. This is of special note, given that Garlid et al [24] demonstrated that cromakalim and diazoxide display a similar degree of potency for opening mitoK_{ATP} in reconstituted mitochondria and elicited a similar degree of cardioprotection following ischemia-reperfusion in the isolated rat heart. The lack of protection afforded by diazoxide in the rabbit heart, at a dose that would be expected to elicit cardioprotective effects via opening of the mitoK_{ATP}, suggest that the sarcK_{ATP} is intimately involved in the cardioprotective effects of IPC.

Another possible mechanism by which opening of sarcK_{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_{ATP} channel may lead to activation of the mitoK_{ATP} channel. Waring and colleges [25] demonstrated that hyperpolarization of rat hippocampal slices increased phospholipase D activity. Phospholiase D has been implicated in IPC [26]. Diacyl-glycerol produced by phospholipase D activates and translocates protein kinase C, which has been shown to potentiate the opening of the sarc and mitoK_{ATP}.

While it seems entirely plausible that activation of the sarcK_{ATP} channel may indeed lead to opening of the mitoK_{ATP} via cross talk, or vice versa, there have been a number of recent papers that have outlined differential roles of both the sarc and mitoK_{ATP} channels in IPC and cardioprotection. In a model of chronic hypoxia, in which rabbits were raised from birth in either a normoxic or hypoxic environment, Kong and colleges [27] recently demonstrated that either 5HD or HMR 1098 alone failed to completely abolish the protection afforded by chronic hypoxia and that only the combination of both 5-HD and HMR 1098 was successful in completely abrogating the protective effects of chronic hypoxia. Furthermore, a recent paper by Sanada *et al.* [28] demonstrated that glibenclamide, a non-specific K_{ATP} channel blocker completely abolished the protective effects of IPC, while 5-HD, a mitochondrial selective blocker, only partially blunted the anti-infarct effect. In a model of adenosine-enhanced IPC, Toyoda et al [29] detailed a temporal involvement of both sarc and mitoK_{ATP} channels. These investigators indicated that the infarct reducing effects of adenosine-enhanced preconditioning are mediated by mitoK_{ATP} channels during ischemia, while sarcK_{ATP} channels modulate functional recovery during both ischemia and reperfusion. This concept was also supported by the results of Light *et al.* [18], who reported that the protective effects of PMA (PKC activator) were partially inhibited by 5-HD during chemically-induced hypoxia but not at reoxygenation, while HMR 1098, acting in a PKC and adenosine-dependent manner, was effective in abolishing protection and intracellular Ca^{2+} overload during posthypoxic reoxygenation only.

Taken together, these data suggest that the sarc and mitoK_{ATP} channels are independently involved in producing ischemic tolerance provided by IPC and may work in concert to elicit cardioprotection.

MitoK_{ATP} and IPC

Since the first evidence for a role of the K_{ATP} channel in acute IPC was presented by Gross and Auchampach [30] in the canine heart there were a number of studies in many different models and species supporting a role of the sarcK_{ATP} channel as the end-effector in IPC. However, recent evidence has shifted our attention away from the sarcK_{ATP} channel to the mitoK_{ATP} channel as a trigger and end-effector in IPC.

The first report suggesting that an enhanced shortening of APD as a result of sarcK_{ATP} channel activation was not the mechanism responsible for cardioprotection provided by K_{ATP} openers was published by Yao and Gross in 1994 [31]. This study demonstrated that a low dose of the non-selective K_{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 be responsible for the efficacy of bimakalim to reduce infarct size independently of APD shortening. Grover *et al.* added further weight to this hypothesis [32]. They described a lack of correlation between APD shortening and cardioprotection following cromakalim treatment. The protective effects of IPC were not attenuated by dofetilide, a class III antiarrhythmic agent, which prevented APD shortening in preconditioned hearts [33]. Evidence for a role of K_{ATP} channel activation mediating the protective effects of IPC and K_{ATP} openers in the absence of a ventricular action potential has also been provided from studies in isolated nonbeating cardiac myocytes [34]. These data all suggest that the sarcK_{ATP} channel may not be accountable for the protective effects afforded by K_{ATP} openers and IPC in cardioprotection and imply a possible intracellular site of action.

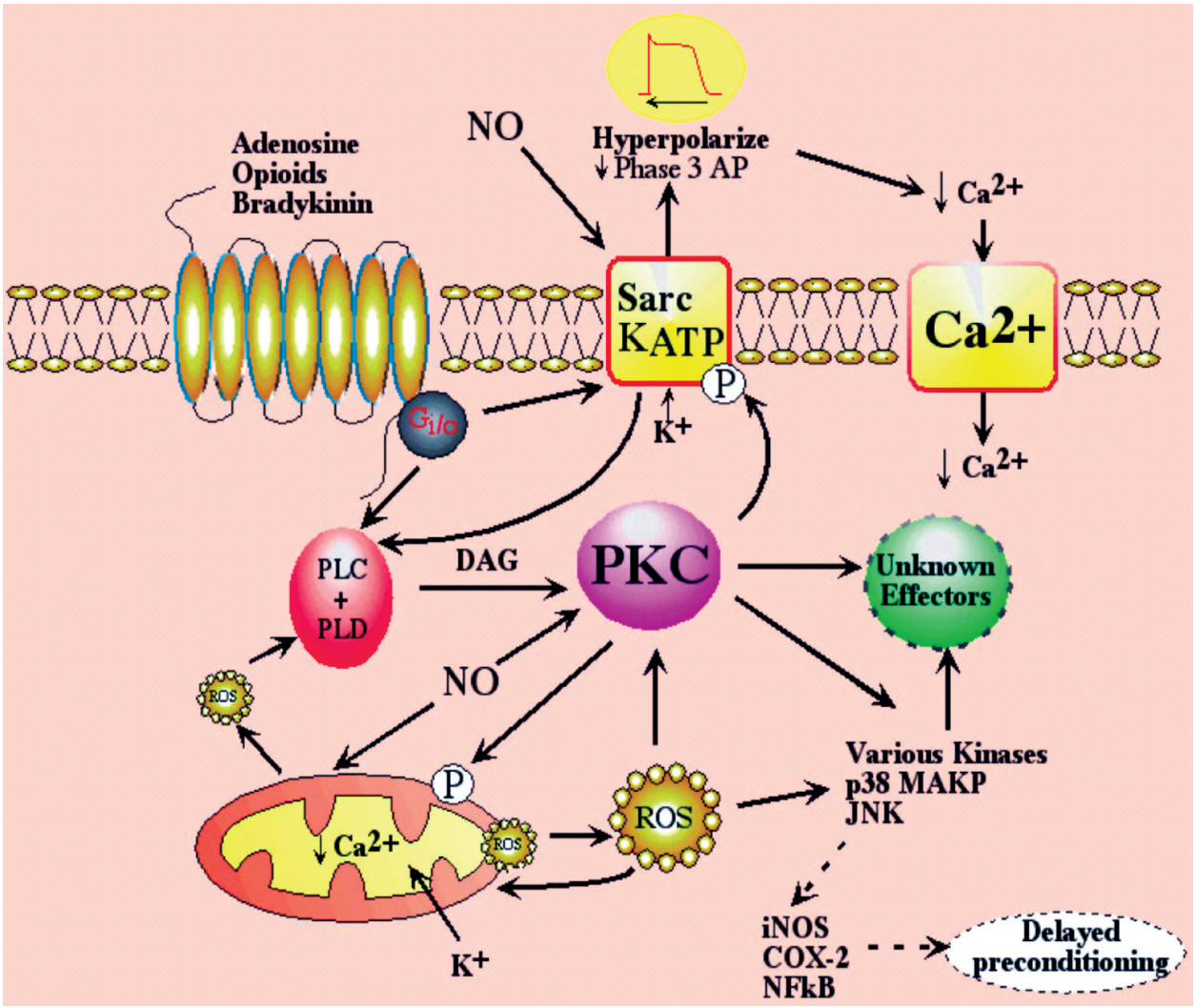

Fig. 2 Schematic diagram outlining proposed mechanisms whereby opening of the sarc or mito K_{ATP} channel may lead to cardioprotection. Sarc K_{ATP}, sarcolemmal K_{ATP} channel; NO, nitric oxide; DAG, diacylglycerol; PLC, phospholipase C; PLD, phospholipase D; PKC, protein kinase C; ROS, reactive oxygen species.

Garlid *et al.* [24] provided the first direct evidence to support a role for mitoK_{ATP} channels in cardioprotection. Utilizing reconstituted bovine heart mitochondria, these investigators found that diazoxide opened mitoK_{ATP} with a K1/2 of 0.8mmol/L whereas 800mmol/L was required to open the sarcK_{ATP} in the presence of diazoxide. Furthermore, diazoxide, at concentrations which did not activate the sarcK_{ATP} channel, produced a pronounced cardioprotective effect comparable to 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_{ATP} channel antagonists 5-HD and glibenclamide, suggesting that mitoK_{ATP} channels were responsible for these actions.

As with the sarcK_{ATP} channel, the role for mitoK_{ATP} channel activation in IPC remains controversial. While it is generally accepted that the mitoK_{ATP} is intimately involved in the cardioprotection afforded by IPC, it is still unclear as to whether its role is as a trigger/mediator or end

effector. Recent reports support a role for mitoK_{ATP} as both a trigger and end-effector [40,41,48,51].

MitoK_{ATP} channels as a trigger of IPC

The mechanisms by which mitoK_{ATP} are activated are essentially the same as those previously described for the sarcK_{ATP} channel. In isolated myocytes, anoxia induces a rapid opening of K_{ATP} channels [35]. Transient ischemia produces H_2O_2 and results in a loss of GABA receptor activity and activates mitoK_{ATP} channels via a PKC-e mediated pathway [36].

Activation and translocation of specific PKC isoforms, regardless of initiator (adenosine, etc) appears to be central to opening of mitoK_{ATP}, and these two phenomena may be co-dependent. Gaudette *et al.* [37] demonstrated in a sheep model that protection provided by direct K_{ATP} openers could be abolished by PKC antagonists and that protection mediated via activation of PKC could be removed with K_{ATP} blockers, implying that activation of PKC and K_{ATP} channels are both codependent and necessary for protection in the *in vivo* heart [37]. While these data are intriguing, much research has placed PKC-e upstream of mitoK_{ATP} activation. Studying embryonic chick ventricular myocytes, Liang [38] demonstrated that PKC activation with PMA was effective in preconditioning the myocyte, a response abolished by chelerythrine or calphostin C. Furthermore, pretreatment with either glibenclamide or 5-HD attenuated PMA-induced preconditioning, demonstrating that the K_{ATP} channel was a downstream effector of PKC-mediated preconditioning. In isolated rabbit hearts, Ohnuma et al [39] demonstrated that IPC leads to a dramatic elevation in PKC-e. Pretreatment with 5-HD abolished the protective effects of IPC without altering changes PKC-e activation. Furthermore, while infusion of diazoxide lead to a marked reduction in infarct size, diazoxide treatment failed to translocate PKC-e or accelerate its translocation.

In contrast, a recent study by Liu et al [40] demonstrated that PKC-e was downstream from mitoK_{ATP} activation in IPC's apoptosis limiting effects. Using ventricular myocytes from chick embryos, Liu and collegues reported that both IPC and diazoxide treatment reduced cardiocyte apoptosis and that both of these effects were abolished by pretreatment with specific PKC inhibitors. In addition, both IPC and diazoxide treatment activated PKC-e in the particulate fraction. These results were also supported by the data provided by Wang and Ashraf [41] who demonstrated a marked reduction in contractile function in hearts treated with both chelerythrine and diazoxide, (possibly via a Ca^{2+} dependent mechanism) and that pretreatment with diazoxide did indeed mediate translocation of specific PKC isoforms. These results indicate that mitoK_{ATP} activation may also serve as a trigger for delayed cardioprotection.

Diazoxide also induces early and late preconditioning via a NO-dependent pathway. In an *in vivo* rabbit heart model, Ockaili et al [42] demonstrated that diazoxide treatment induces both early and late preconditioning, an effect blocked by administration of 5HD, confirming that the anti-ischemic effect was due to opening of mitoK_{ATP} channels. Interestingly, the diazoxide-mediated protection was also abolished by L-NAME, an inhibitor of nitric oxide (NO) synthase, suggesting a NO dependency. NO has also been shown to modulate the sensitivity of the K_{ATP} channel to intracellular ATP [43]. Furthermore, NO has been demonstrated to activate certain PKC isoforms [44,45].

The general assumption given to mitoK_{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). MitoK_{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 [46] described that delayed protection in a cardiomyocyte-derived cell line involves p38MAPK and the opening of mitoK_{ATP} channels. In this model, protective effects of preconditioning were abolished when cells were pretreated with SB203580, a p38 MAPK inhibitor, prior to the preconditioning stimuli. Furthermore, protection was attenuated when cells were treated with 5-HD 30 min prior

to lethal simulated ischemia on the second day following preconditioning. These results suggest that mitoK$_{ATP}$ channel opening is downstream from p38 MAPK activation [46]. However, another study places MAPK activation downstream from mitoK$_{ATP}$ channel opening. Using THP-1 cells, Samavati *et al.* [47] demonstrate that diazoxide induces mitochondrial ROS production, as evidenced by an increased rate of dihydroethidium and dichlorofluorescein fluorescence. The increase in ROS resulted in an increase in the phosphorylation of ERK kinase, a member of the MAPK family. Thus, opening of mitoK$_{ATP}$ channels was associated with a downstream activation of ERK. The results of Wang and Ashraf, Samavati *et al.*, and Liu *et al.* suggest that mitoK$_{ATP}$ activation may also act as a trigger of the cardioprotection [40,41,47]. Further support for this hypothesis is given by Pain *et al.* [48]. They demonstrate that in isolated rabbit hearts, protection afforded by diazoxide could only be abolished when K$_{ATP}$ channel blockers were given during the diazoxide treatment, not following treatment. Free radical scavengers given during the trigger phase also abolished the protective effects of diazoxide [48]. Diazoxide has been previously shown to mediate cardioprotection in cells via a redox-sensitive mechanism whereby it initiates a "burst" of free radicals [49,50], a known trigger leading to a preconditioned state, possibly through activation of various/specific kinases.

The answer to the question "is the mitoK$_{ATP}$ the trigger or the mediator of IPC?" may be quite simple indeed, it may be both. A study by Wang et al [51] examined the mitoK$_{ATP}$ 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^{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 four-fold higher dose. The role of nifedipine during the mediation phase was not examined in this study. Thus, the trigger phase may be mediated by elevated intracellular Ca^{2+} and PKC activation, while mitoK$_{ATP}$ opening invokes protection during the mediation phase via unknown mechanisms, independently of PKC activation/ translocation.

Thus, there is considerable evidence that the mitoK$_{ATP}$ may have a role as the trigger and/or the mediator in IPC-mediated cardioprotection. However, the mechanism of protection as an end-effector is still unclear.

It appears that the mitochondria have an intimate role in cell survival through ATP synthesis and maintenance of Ca^{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 production of free radicals. In an isolated cell model, anoxia-reoxygenation may lead to a hyperpolarization of mitochondria. This hyperpolarization may indeed drive Ca^{2+} into the mitochondria, leading to Ca^{2+} overload. Xu and associates [52] demonstrated that treatment with diazoxide stabilized the mitochondrial membrane potential through limiting the loss of the membrane potential and inhibition of the high polarization observed during anoxia-reoxygenation [52]. Depolarization of the membrane potential may reduce the Ca^{2+} influx, limiting Ca^{2+} overload and myocyte injury. While having no effect on total intracellular Ca^{2+} levels, IPC inhibits the ischemia-induced elevation of the mitochondrial Ca^{2+} concentration, an effect attributed to mitoK$_{ATP}$ channel opening. Diazoxide reduced mitochondrial Ca^{2+} concentration and 5-HD inhibited the reduction in mitochondrial Ca^{2+} concentration provided by both IPC and diazoxide in isolated rat hearts [53]. The study by Xu and colleagues reported that diazoxide treatment also prevented ATP depletion [52]. McPherson et al [54] also describe this effect. In a model using arterially perfused guinea pig ventricular walls, McPherson et al reported that K$_{ATP}$ channel opening with pinacidil inhibited ischemia-induced depletion of high energy phosphates [54]. Pinacidil-induced preservation of creatine phosphate and ATP was abolished with glibenclamide and glibenclamide alone enhanced ischemic depletion of ATP. MitoK$_{ATP}$ channel opening may partially restore the membrane potential, allowing further extrusion of H^+, forming an electrochemical gradient [55] for ATP synthesis. Prevention of ATP depletion will maintain ATP-dependent ion

pumps such as the Na^+-K^+ pump and Ca^{2+} pump. 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 adenosine nucleotide pool during ischemia and the energy transfer during reperfusion [56]. Of interest are recent studies by Garlid *et al.* [57], who suggest 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 hypothesize that the only effect of opening the mitoK_{ATP} channels is in the regulation of mitochondrial volume [57]. However, that is not to say that regulation of volume has little effect, indeed, mitochondrial volume regulates the electron transport chain and preserves architecture of the intermembrane space, permitting 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 conclusion, recent data suggest that both the sarc and mitoK_{ATP} channels play complimentary roles in the protection afforded by IPC. Based on recent evidence, activation of the mitoK_{ATP} channel appears to limit cell death, while opening of the sarcK_{ATP} channel appears to limit stunning. Regardless, direct activation of either the sarc or mitoK_{ATP} channels provides significant cardioprotection and possible signaling pathways involved are schematically shown in Fig. 2. Activation, especially of the mitoK_{ATP}, may be involved in acute IPC as either a trigger/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 further/longer opening of the channel. As a potential end-effector, the role that the mitoK_{ATP} plays in preservation of 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}, or confer its own cardioprotection, via a PKC-dependent 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 sarc and mitoK_{ATP} have complimentary roles in the cardioprotection afforded by IPC, indeed, they may act in concert.

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