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Mitochondrial K_{ATP} channels in cell survival and death

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Abstract

Since the discovery of the mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) more than 13 years ago, it has been implicated in the processes of ischemic preconditioning (IPC), apoptosis and mitochondrial matrix swelling. Different approaches have been employed to characterize the pharmacological profile of the channel, and these studies strongly suggest that cellular protection well correlates with the opening of mitoK_{ATP}. However, there are many questions regarding mitoK_{ATP} that remain to be answered. These include the very existence of mitoK_{ATP} itself, its degree of importance in the process of IPC, its response to different pharmacological agents, and how its activation leads to the process of IPC and protection against cell death. Recent findings suggest that mitoK_{ATP} may be a complex of multiple mitochondrial proteins, including some which have been suggested to be components of the mitochondrial permeability transition pore. However, the identity of the pore-forming unit of the channel and the details of the interactions between these proteins remain unclear. In this review, we attempt to highlight the recent advances in the physiological role of mitoK_{ATP} and discuss the controversies and unanswered questions.

Keywords

Mitochondria; Potassium; Ischemic preconditioning; Diazoxide; Glibenclamide; Succinate dehydrogenase; Apoptosis; Reactive oxygen species

1. Introduction

Ischemic heart disease remains a major health problem in the world. Most of the research in this field has focused on finding ways to prevent ischemic injury by increasing blood supply to the myocardial area at risk. However, identification of an autoprotective mechanism known as ischemic preconditioning (IPC) has raised interest that endogenous pathways can be stimulated to protect the heart from ischemic injury [1]. IPC is a mechanism by which brief periods of ischemia produce protection against subsequent longer ischemic periods [1-3]. If specific cellular pathways or proteins can be targeted to stimulate the IPC protective process, the injury from an ischemic insult can be significantly attenuated. Thus, much effort over the past couple of decades has focused on identifying the underlying molecular basis of IPC. These studies have revealed multiple intracellular pathways to mediate IPC. These include the activation of protein kinase C, G-protein receptors, nitric oxide and generation of reactive oxygen species (ROS) [4-6]. The end-effector molecule(s) targeted by these pathways remain elusive.

Early studies suggested that adenosine triphosphate sensitive potassium channels (K_{ATP} channels) may be the common effector in IPC. Since opening of the surface K_{ATP} channels results in membrane hyperpolarization and shortens phase 3 of the cardiac action potential, the cardioprotective effects were initially attributed to surface K_{ATP} channels [7]. However, Yao and Gross [8] showed that a low dose of bimakalin, which did not shorten the action potential, had a similar cardioprotective effect as higher doses that enhanced action potential shortening. Similar lack of correlation between the extent of action potential shortening and the reduction of infarct size has also been shown with cromakalim and BMS-180448 [9,10]. Furthermore, Grover et al. [11] showed that prevention of action potential shortening by concomitant treatment of dofetilide did not reverse cardioprotective effects of cromakalim. These studies suggested that the opening of the surface K_{ATP} channel may not be necessary for the cardioprotection induced in IPC. An alternative mechanism involving the mitochondrial K_{ATP} channel (mito K_{ATP}) subsequently emerged as the possible effector of IPC [12,13].

Since early 1990s, K^+ selective transport has been widely observed in the mitochondria. A K^+ channel activity, with characteristics similar to those of surface K_{ATP} channel, was discovered in 1991 [14]. This mito K_{ATP} channel is modulated by a variety of K^+ channel openers and inhibitors [13]. Furthermore, these modulators significantly influence mitochondrial function and cell survival, suggesting a link between mito K_{ATP} and protection against ischemic injury. This protective effect of mito K_{ATP} has been demonstrated in several tissues besides the heart, including liver, brain, kidney and gut [15-18].

2. Pharmacology of mito K_{ATP}

Table 1 lists the known activators and inhibitors of mito K_{ATP} . Several of these chemicals are not specific for mito K_{ATP} and can also modulate the activity of the surface K_{ATP} channel. We will detail the studies on the role of these chemicals in mito K_{ATP} activity throughout this review.

3. Evidence for the existence of mito K_{ATP}

3.1. Studies on intact mitochondria

3.1.1. Patch-clamp of mitochondria—In 1991, Inoue et al. [14] identified the mito K_{ATP} channel in single channel patch-clamp recordings of rat liver mitochondrial inner membrane. Although the technique used is challenging and subject to the criticism that the mitochondrial preparation may have been contaminated with surface proteins, this study remains a compelling argument for the existence of mito K_{ATP} . These authors identified a single K^+ selective channel, which was inhibited by ATP with a K_i of ~ 0.8 mM when applied to the matrix face of the channel. The channel displayed a lower unitary conductance compared with the surface K_{ATP} channel (10 pS in 100 mM matrix K^+ and 33 mM cytosolic K^+). The channel was inhibited by the K^+ channel inhibitor 4-aminopyridine and a sulfonylurea, glibenclamide at 5 μ M concentrations. Since the surface K_{ATP} channel is sensitive to glibenclamide, this observation was used as additional evidence that the measured conductance was due to a K_{ATP} channel. This original report remains one of the only studies thus far directly demonstrating the presence of a mito K_{ATP} channel on intact mitochondrial inner membrane.

3.1.2. Measurement of mitochondrial volume— K^+ influx into the mitochondria, accompanied by the movement of water, leads to mitochondrial matrix swelling. The cation flux is tightly regulated to avoid rupture of the mitochondrial membranes. This is accomplished by movement of K^+ out of the matrix through the K^+/H^+ exchanger [19,20]. However, K^+ influx by mito K_{ATP} can transiently exceed the counterbalancing activity of the K^+/H^+ exchanger, resulting in mitochondrial matrix swelling [21]. Garlid's group has used the extent of light scattering at 520 nm to assess the steady-state matrix volume [22]. These studies have

supported the results obtained in other systems as far as the effect of different pharmacological agents on mitoK_{ATP}. There are limitations associated with this technique, including the use of supraphysiological ion gradients, nucleotide concentrations or metabolic substrates, hypo-osmotic conditions of the experiments to maintain linearity of the response, and the loss of normal cytoskeletal architecture [22,23]. Nevertheless, this approach is advantageous as compared to the reconstitution studies in that the mitochondrial channels and other proteins are intact, and that the pharmacological reagents can be applied directly to the mitochondria.

3.2. Studies on intact myocytes

3.2.1. Flavoprotein oxidation—The autofluorescence properties of the mitochondrial flavoproteins and NADH can be used to monitor mitochondrial redox state in intact cells and tissues [24-26]. The maximum and minimum oxidation states of the mitochondria can be calibrated using an uncoupler (i.e. 2,4-dinitrophenol) and an inhibitor of electron transport, such as cyanide [27]. Opening of mitoK_{ATP} results in mild uncoupling of oxidative phosphorylation, since the energy that would otherwise be used to produce ATP, gets dissipated. The partial uncoupling would then lead to an increase in flavoprotein fluorescence if the rate of NADH oxidation exceeds the rate of its production. Liu et al. [12] have used this technique to make a connection between mitoK_{ATP} activation and cardioprotection in isolated cardiomyocytes. This was the first demonstration of mitoK_{ATP} activation by a K⁺ channel opener (diazoxide) in intact cells. Diazoxide caused the reversible net oxidation of flavoproteins to ~40% of the completely uncoupled state, with a K_{1/2} of ~27 μM, similar to the protective efficacy of diazoxide in intact cells, whole hearts, and animal studies. In these experiments, diazoxide did not activate surface K_{ATP} channels, and 5-HD reversed its effects on redox state, providing important support that mitoK_{ATP} channels are involved.

Several studies have been reported since the initial report by Liu et al. on the pharmacology and regulation of mitoK_{ATP} using the flavoprotein oxidation technique [6,28-32]. These studies support the previous results from isolated mitochondria as far as the selectivity of K⁺ channel modulators for the mitoK_{ATP} vs. the surface isoform. One exception to this rule is the pinacidil derivative P-1075, which was found to be quite selective in isolated cells, but activates mitoK_{ATP} in mitochondrial preparations [29].

There have been reports by several groups discussing limitations associated with this technique. These issues have been reviewed recently, and will not be detailed here [33]. However, it should be mentioned that partial oxidation of the flavoprotein pool may not represent a significant change in the mitochondrial membrane potential under oxygenated conditions. In order to better understand the degree of mitochondrial uncoupling by the opening of mitoK_{ATP}, the change in oxygen consumption has been studied (see below).

3.2.2. Effects of mitoK_{ATP} on respiration and membrane potential—Induction of IPC or treatment with diazoxide or adenosine in C₂C₁₂ skeletal myoblast cells leads to stimulation of cellular oxygen consumption, a small decrease in ΔΨ_m and reduction of cellular ATP levels [34]. These changes, which are consistent with mild uncoupling, were reversed by 5-HD, suggesting a link between IPC and the uncoupling effects. A recent report on isolated rat skeletal muscle mitochondria and L6 myoblast cells, supports these results [35]. The rate of respiration in both systems was increased in response to diazoxide and nicorandil in a saturable and concentration dependent manner. These reagents caused a small change in K⁺ specific depolarization of ΔΨ_m (~8 mV with diazoxide and 4 mV with nicorandil), and this was completely reversed by glibenclamide and partially by 5-HD. Furthermore, both diazoxide and nicorandil caused a K⁺ specific oxidation of the NADH redox pool with K_{1/2}'s of less than 50 μM.

Why would the opening of mitoK_{ATP} channel cause a change in respiration and $\Delta\Psi_m$? In theory, the opening of the channel would contribute to an inward current and would lead to the utilization of a portion of the proton gradient to export K⁺ ions through the K⁺/H⁺ exchanger. These effects would cause a partial dissipation of the energy stored as the proton motive force. This uncoupling effect would then lead an increase in proton pumping and consumption of oxygen to maintain $\Delta\Psi_m$. However, since opening of mitoK_{ATP} causes only a small change in K⁺ concentrations in the mitochondria, other nonspecific effects of mitoK_{ATP} openers can mask their effects on respiration. Diazoxide has been shown to inhibit succinate-supported respiration by inhibiting succinate dehydrogenase (Complex II) without affecting NADH-supported respiration [36,37]. Thus, when NADH-linked substrates are used, mitochondrial oxygen consumption is increased by ~20% in the heart and liver [38] and ~50% in the brain [35,39] (due to higher levels of mitoK_{ATP} in the brain). These changes represent the uncoupling effects of mitoK_{ATP}. However, the nonspecific effects of diazoxide remain a controversial issue when it comes to studying the effects of this drug on metabolism as a whole.

3.3. Reconstitution studies on mitoK_{ATP}

3.3.1. Lipid bilayer studies—In the 1980s, several groups observed K⁺ channel activity of proteins purified from mitochondrial membranes and reconstituted into bilayer lipid membranes (BLM) [40,41]. In 1992, Paucek et al. [42] showed K⁺ channel activity with a conductance of ~30 pS in a highly purified mitochondrial fraction. However, a very high concentration of K⁺ (1 M KCl) was used in these studies. A subsequent study from the same lab suggested that modulation of the mitoK_{ATP} channel by ATP, GTP and palmitoyl coA was polarized, and the regulatory site was proposed to be on the cytosolic face of the channel [43].

The group of Mironova and Marinov have also studied mitoK_{ATP} activity in BLM. A 55 kDa protein was isolated using an ethanol/water method and was shown to confer mitoK_{ATP} activity in BLM [44-46]. This protein has not yet been identified. Nevertheless, the channel showed increased activity and reduced selectivity in the presence of the reducing agents, dithiothreitol at 1–3 mM. The channel is inhibited by ATP and is activated by GTP. A recent report demonstrated K⁺ channel activity in BLM with a conductance of 56 pS in 150 mM KCl [4]. The channel was modulated by ATP and GTP when they were added to the matrix side of the bilayer. Furthermore, diazoxide activated, while 5-HD and glibenclamide inhibited the channel. In contrast to the earlier report mentioned above [44], these authors observed activation of the channel by exogenously generated superoxide, and this activation was reversed by pretreatment with a thiol reducing agent or 5-HD.

3.3.2. Proteoliposome studies—Work from the laboratory of Garlid, using reconstituted proteoliposomes, has significantly contributed to our understanding of the regulation of mitoK_{ATP} [39,42,43,47,48]. The technique involves formation of proteoliposomes containing the protein(s) of interest with the fluorescent K⁺ indicator, PBFI, trapped inside the vesicles [49]. Upon transport of K⁺, the excitation peak of PBFI narrows, and the rate of fluorescence increase can be used as a measure of K⁺ transport into the proteoliposomes. A permeable anion such as SCN[−] or a pH gradient can be used to facilitate K⁺ transport and compensate for the charge gradient produced by the transport of the cation. The proteins that constitute the mitoK_{ATP} channel were affinity purified on an ATP-binding column, and were shown to have sizes of about 55 and 63 kDa [39,42]. The larger band was reported to specifically bind to fluorescent glibenclamide [39]. However, since the initial report of partial purification of these proteins in early 1990s, their identities remain elusive.

This method has been used extensively to study the regulation of the mitoK_{ATP} channel by different reagents. The channel was inhibited in a Mg²⁺-dependent fashion by ATP ($K_i \sim 40$

μM). ADP and glibenclamide inhibited the channel with K_i s of 280 and 3.1 μM , respectively [42]. The channel was also modulated by GTP, GDP, palmitoyl or oleyl-coA and the alkylating agent DCCD [48]. It was highly selective for K^+ over Na^+ and had a K_m for K^+ of 32 mM.

This system has allowed a direct comparison of mitoK_{ATP} and surface K_{ATP} channels. Diazoxide is about 2000-fold more potent in opening the mitoK_{ATP} channel compared with the surface K_{ATP} channel [47]. Furthermore, the effect of diazoxide is blocked by 5-HD (K_i of 45–85 μM) and glibenclamide (K_i of 1–6 μM). It should be noted that glibenclamide, but not 5-HD, can also inhibit mitoK_{ATP} activated by removing ATP or Mg^{2+} . Cromakalim and two of its derivatives, EMD60480 and EMD57970 were also demonstrated to activate the channel, however, they proved not to be selective for the mitochondrial isoform.

4. Controversies regarding mitoK_{ATP}

4.1. Lack of molecular identity

Despite the critical role mitoK_{ATP} plays in IPC and apoptosis, its molecular identity remains unclear. The surface K_{ATP} channels are thought to be composed of a tetramer of inward rectifier K^+ channels (Kir6.x) surrounded by four subunits of sulfonylurea receptors (SUR) [50–53]. The SUR subunits confer sensitivity to glibenclamide and many K_{ATP} channel modulators [54,55]. Based on similarities between mitoK_{ATP} and surface K_{ATP} channels, it has been proposed that mitoK_{ATP} may also consist of Kir and SUR subunits.

In support of this hypothesis, Garlid's group has purified a mitochondrial fraction containing a 63 kDa sulfonylurea binding protein and a putative pore-forming subunit of 55 kDa in size [39,42]. When reconstituted in proteoliposomes, the purified mitochondrial fraction confers ATP-sensitive potassium channel activity. This fraction was purified by applying mitochondrial extracts from the heart to a DEAE-cellulose column followed by further purification on an ATP column. Similar size proteins have been isolated from the brain and liver mitochondria [39]. The 63 kDa protein from brain was recently reported to bind to labeled glyburide, supporting that idea that this protein may be the sulfonylurea binding subunit of the mitoK_{ATP} channel [39].

Other groups have also attempted to identify a sulfonylurea binding protein in the mitochondria. Suzuki et al. [56] reported that a Kir6.1 antibody immunolocalizes to the mitochondria and binds to a 51 kDa protein in mitochondrial membrane preparations. In another study, a dominant negative Kir6.1 adenovirus, in which the pore signature sequence GFG was mutated to AFA, was constructed and infected into adult myocytes [57]. This construct had no effect on diazoxide activated mitochondrial redox state, indicating that the mitochondria probably do not contain a Kir6.1. However, the authors suggested that it is still possible that a mitochondrial Kir6.1 could exist, that does not heteromultimerize with the dominant negative form of Kir6.1, hence the lack of any effect by the adenovirus. Other recent studies also suggest that Kir6.1, Kir6.2 and SUR2A subunits are present in the mitochondria of the heart and brain [58,59]. The antibodies against Kir6.1 and Kir6.2 recognized proteins of 48 and 40 kDa in size in a mitochondrial preparation, but it remains to be proven if these antibodies are binding to a kin-like protein. Knockout of kir6.1 gene in intact mice, does not disrupt mitoK_{ATP} opening, but results in sudden death associated with Prinzmetal's angina and vasospasm [60].

As discussed above, diazoxide is also capable of inhibiting SDH [61]. Other SDH inhibitors, malonate and 3-nitropropionic acid (3-NPA), have also been shown to attenuate oxidant stress on the heart and have cardioprotective effects [62–64]. These observations have led to the alternative hypothesis that respiratory inhibition, rather than K^+ channel activation, underlies IPC [36,37]. The pharmacological overlap between SDH and mitoK_{ATP} led to the hypothesis that SDH and mitoK_{ATP} interact with each other, both physically and functionally [65]. Using

co-immunoprecipitation technique, Ardehali et al. [65] identified at least four mitochondrial proteins that interact with SDH (Fig. 1A). These proteins are the following: mitochondrial ATP-binding cassette protein-1 (mABC1), adenine nucleotide translocator (ANT), ATP synthase (ATPase) and inorganic phosphate carrier (PIC). A mitochondrial fraction containing these five proteins displayed K^+ channel activity when incorporated into liposomes and lipid bilayer (Fig. 1B). The channel activity also responded appropriately to mitoK_{ATP} modulators, ATP, glibenclamide, 5-HD and diazoxide (Fig. 1C). In order to better link the mitochondrial complex to the mitoK_{ATP} channel activity, pharmacological agents that target SDH (3-NPA and malonate) were also tested. The addition of these compounds resulted in a significant increase in the mitoK_{ATP} channel activity, suggesting that SDH, a member of the inner membrane multiprotein complex, influences mitoK_{ATP} activity. It was also shown that ANT modulators did not have an effect on mitoK_{ATP} activity, arguing against a role for ANT in the mitoK_{ATP} channel pore. These data reconcile the controversy over the basis of IPC by demonstrating that SDH may be a component of mitoK_{ATP}, as part of a novel macromolecular supercomplex, and provide the first tangible clue as to the structural basis of mitoK_{ATP} channels (Fig. 1D).

4.2. Nonselectivity for K^+ by diazoxide

A recent paper by Terzic's group suggested that diazoxide may not display K^+ selectivity in the mitochondria from rat hearts [62]. They found that the levels of ROS produced by isolated mitochondria are reduced by diazoxide and nicorandil in reperfused hearts after 5 min of anoxia. There was also better preservation of the mitochondrial structure and oxidative phosphorylation, and these effects were all reversed by 5-HD. These beneficial effects were also observed in a K^+ -free medium, suggesting that K^+ selective channels may not be required for the protective effects diazoxide or nicorandil.

4.3. Possible independence of protection and depolarization of $\Delta\Psi_m$

As explained above, K^+ influx through mitoK_{ATP} could lead to partial depolarization of $\Delta\Psi_m$ which could in turn decrease the driving force for the mitochondrial Ca^{2+} uptake during ischemia. However, Lawrence et al. [66] recently reported that no change in $\Delta\Psi_m$ or flavoprotein oxidation was observed when diazoxide was added under normoxic conditions. However, they observed protection by diazoxide against chemical cell death produced by iodoacetate and cyanide. However, it should be noted that the extent of $\Delta\Psi_m$ loss during metabolic inhibition was not examined in this study and it has been argued that diazoxide will induce a marked depolarization under ischemic conditions [67]. Moreover, IPC has been shown to enhance mitochondrial membrane depolarization during ischemia [68], which may contribute to the protective effect.

4.4. Is mitoK_{ATP} a trigger or an effector of IPC?

Despite the evidence that strongly suggests that mitoK_{ATP} plays a key role in IPC, it is unclear how this channel opens in response to ischemia. Some studies have tried to address the time frame for mitoK_{ATP} channel opening and IPC. Fryer et al. [69] administered 5-HD to rats either before or after the preconditioning phase or diazoxide application. In control animals, IPC reduced infarct size significantly. 5-HD applied before IPC reversed the protective effects of IPC, while it did not have any effects on the infarct size in the absence of preconditioning. Administration of diazoxide 15 min before the long ischemia reduced infarct size, and addition of 5-HD either before or after diazoxide administration completely reversed the protective effects. This study suggests that mitoK_{ATP} is both a trigger and an effector of IPC. This is in contrast to another study by Pain et al. [70] who showed that the addition of 5-HD or glibenclamide during diazoxide pre-treatment or the preconditioning period blocks the protective effects of IPC. They concluded that mitoK_{ATP} may only be a trigger of IPC.

4.5. Role of surface K_{ATP} channel

As discussed earlier, pharmacological studies argue against a role for the surface K_{ATP} channel in the process of IPC. This issue was recently readdressed in Kir6.2 knockout mice [71,72]. The knockout mice displayed a blunted response to IPC, and their hearts went into contracture within 5 min of exposure to ischemia and did not recover function during reperfusion. This ischemic response was similar to the effect of treating wild-type mice with HMR1098. In the absence of preconditioning, the knockout mice displayed more significant damage in response to ischemia than wild-type animals suggesting that the surface K_{ATP} channel may play a role in cardioprotection in mice. In contrast, inhibition of the surface K_{ATP} channels with HMR1098 does not seem to have an effect on ischemia or IPC in dogs, rats, rabbits or human myocardium [73-76], indicating a minimal role for sarcolemmal K_{ATP} activation in cardioprotection in large animals.

5. Mechanism of protection

Despite efforts by several groups, the mechanism of protection induced by the opening of mitoK_{ATP} remains unclear. There are three major hypotheses that have emerged as a result of investigation in this area. Although these hypotheses raise different mechanisms for IPC, they are probably not mutually exclusive, and they may all contribute to this process.

5.1. Changes in the mitochondrial Ca²⁺ levels

The hypothesis that mitoK_{ATP} opening leads to an attenuation of Ca²⁺ accumulation during IPC was first proposed by Liu et al. [12] in 1998. Subsequent pharmacological studies suggested that diazoxide and pinacidil reduced the magnitude of Ca²⁺ uptake, and it was reversed by 5-HD [77]. It was proposed that this effect could be through a partial depolarization (in the magnitude of ~10–24 mV) of $\Delta\Psi_m$ in response to mitoK_{ATP} openers. Wang et al. [78] has shown that mitoK_{ATP} opening attenuates the mitochondrial Ca²⁺ accumulation during ischemia in intact hearts. Similar observations were reported by Murata et al. [79] who showed that mitoK_{ATP} opening attenuated mitochondrial Ca²⁺ accumulation during ischemia. In this study, PTP opening was also shown to be attenuated by a reduction in Ca²⁺ accumulation in the mitochondria. A recent report found that diazoxide caused a depolarization of $\Delta\Psi_m$ under anoxic conditions, and a decrease in the mitochondrial Ca²⁺ loading, which resulted in protection of isolated mitochondria [67]. They also reported that this process prevented a mitochondrial permeability transition with reoxygenation, which could explain the antiapoptotic effects of mitoK_{ATP} opening.

5.2. Mitochondrial matrix swelling and changes in ATP synthesis

Matrix swelling of isolated mitochondria has long been used as an assay of mitoK_{ATP} opening [22]. Recently, Halestrap's group has used ³H₂O and [¹⁴C]sucrose to measure mitochondrial volume [36]. Their studies showed that 50 μ M diazoxide and two 5-min cycles of preconditioning increased matrix volume by 88% and 58%, respectively. 5-HD not only did not reverse this process, it also increased matrix volume when it was solely added. They also reported that in the mitochondria isolated prior to ischemia, IPC increased state 3 respiration by 2-oxyglutarate or succinate, but had no effect on ascorbate + TMPD, and that diazoxide inhibited state 3 respiration of 2-oxyglutarate by 22%, slightly inhibited that of succinate, but not that of ascorbate + TMPD. However, in mitochondria isolated after 30 min of ischemia, state 3 respiration was inhibited in all groups relative to preischemic mitochondria when oxyglutarate, succinate or ascorbate + TMPD were used as substrate. Diazoxide increased respiration in this group, but 5-HD did not reverse this effect, and actually resulted in an increase in respiration by itself. Respiration partially recovered in the mitochondria that were isolated after reperfusion when compared with the end-ischemic group, and diazoxide did not add any additional effect. This study suggests that protection may not correlate with changes

in matrix volume and respiration. However, it should be mentioned that the isolated mitochondria were studied in vitro, under conditions that differ from their native milieu, thus, it is difficult to extrapolate these results to the situation in the intact heart.

Kowaltowski et al. [38] proposed an alternative explanation for protection induced by mitochondrial swelling. According to their model, matrix swelling results in the inner and outer mitochondrial membranes coming closer to each other, which may lead to optimized contact between membrane and channel proteins on the inner and outer membranes. This may then result in an increase in ADP transport and ATP synthesis.

Isolated mitochondria from preconditioned or diazoxide treated hearts were also studied by Fryer et al. [75]. Ischemic reperfusion resulted in a decrease in ATP synthesis which was reversed by IPC. Diazoxide displayed protective effects but did not reverse the decline in ATP synthesis. Another study showed that mitochondria isolated from the chronically hypoxemic rabbit hearts displayed increased basal ATP synthesis rate, which was not changed by Bimkalim treatment, but was reversed by 5-HD and glibenclamide [80]. These observations suggest that mitoK_{ATP} channels may remain open in chronic hypoxemia, which could provide resistance to ischemia.

5.3. Changes in the ROS levels

ROS plays a double edge role in the process of IPC. The ROS that is produced during the preconditioning phase is thought to be protective. Several studies have shown that mitoK_{ATP} opening increases ROS production [70,81,82], which leads to PKC-dependent protection [83]. This process is reversed by the addition of ROS scavengers [81]. However, the ROS that is generated during reperfusion, which can be suppressed by mitoK_{ATP} openers, can cause cell death [62,84,85]. Thus, it is thought that mitoK_{ATP} opening increases production of protective ROS during preconditioning and decreases the levels of ROS produced during reperfusion [33].

6. mitoK_{ATP} and apoptosis

It is widely believed that apoptosis contributes to ischemia-mediated myocardial cell death and that IPC may decrease both apoptosis and necrosis. Takashi et al. [86] showed that diazoxide pretreatment decreased markers of apoptosis from ischemia/reperfusion injury, and that this effect was reversed by 5-HD. Akao et al. [87] recently showed that diazoxide inhibits H₂O₂-induced apoptosis in neonatal rat cardiac myocytes, and that 5-HD blocks this effect. In their study, they assessed apoptosis by measuring cytochrome *c* release, caspase activation, loss of mitochondrial membrane potential and cleavage of poly (ADP-ribose)polymerase. These authors later identified three distinct phases in H₂O₂-induced mitochondrial membrane potential loss and cell death in neonatal rat cardiac myocytes [88]. In the first phase (called the priming phase), there is a calcium-dependent morphological changes with preserved membrane potential, while in the second phase (called the depolarization phase), PTP opening leads to rapid depolarization of the membrane. These two phases are followed by cell fragmentation. Diazoxide decreased the probability of cells undergoing the priming phase, while the PTP opener (cyclosporine A) slowed the process of $\Delta\Psi_m$ depolarization and blunted its severity. These results suggest that: 1) mitoK_{ATP} plays a protective role in apoptosis, 2) apoptosis (at least in H₂O₂-induced form) is composed of three distinct phases, and 3) mitoK_{ATP} is involved in protection against early stages of apoptosis by preserving mitochondrial integrity.

7. Conclusions

Since its discovery more than 13 years ago, several studies have confirmed that mitoK_{ATP} is a key player in the process of IPC. Pharmacological evidence for mitoK_{ATP} is based on

multiple, and structurally distinct, modulators of the channel. Thus, these studies all support the notion that protection is well correlated with effects on the mitoK_{ATP}. However, the selectivity of the tested compounds for mitoK_{ATP} remains an issue to be resolved by further investigation.

A significant recent finding in this field is the discovery of a macromolecular complex in the inner membrane of the mitochondria with mitoK_{ATP} channel activity. This complex consists of (at least) five proteins in the mitochondrial inner membrane, and transports K⁺ with characteristics similar to those of native mitoK_{ATP}, providing a tangible clue regarding the molecular identity of this channel. These results also link SDH to mitoK_{ATP} activity for the first time. Nevertheless, the identity of the pore-forming unit of the channel still remains to be determined. In light of recent evidence linking mitoK_{ATP} activation to the inhibition of apoptosis and the permeability transition pore [87,89], the close proximity of mitoK_{ATP} and the putative components of the PTP suggest the possibility of a direct functional connection between these processes and new directions for future investigation.

References

1. Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E. Medical and cellular implications of stunning, hibernation, and preconditioning: an NHLBI workshop. *Circulation* 1998;97:1848–67. [PubMed: 9603540]
2. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124–36. [PubMed: 3769170]
3. Cohen MV, Baines CP, Downey JM. Ischemic preconditioning: from adenosine receptor of KATP channel. *Annu Rev Physiol* 2000;62:79–109. [PubMed: 10845085] In Process Citation
4. Zhang DX, Chen YF, Campbell WB, Zou AP, Gross GJ, Li PL. Characteristics and superoxide-induced activation of reconstituted myocardial mitochondrial ATP-sensitive potassium channels. *Circ Res* 2001;89:1177–83. [PubMed: 11739283]
5. Sato T, O'Rourke B, Marban E. Modulation of mitochondrial ATP-dependent K⁺ channels by protein kinase C. *Circ Res* 1998;83:110–4. [PubMed: 9670924]
6. Sasaki N, Sato T, Ohler A, O'Rourke B, Marban E. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. *Circulation* 2000;101:439–45. [PubMed: 10653837]
7. Noma A. ATP-regulated K⁺ channels in cardiac muscle. *Nature* 1983;305:147–8. [PubMed: 6310409]
8. Yao Z, Gross GJ. Effects of the KATP channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. *Circulation* 1994;89:1769–75. [PubMed: 8149542]
9. Grover GJ, D'Alonzo AJ, Parham CS, Darbenzio RB. Cardioprotection with the KATP opener cromakalim is not correlated with ischemic myocardial action potential duration. *J Cardiovasc Pharmacol* 1995;26:145–52. [PubMed: 7564356]
10. Grover GJ, D'Alonzo AJ, Hess T, Sleph PG, Darbenzio RB. Glyburide-reversible cardioprotective effect of BMS-180448 is independent of action potential shortening. *Cardiovasc Res* 1995;30:731–8. [PubMed: 8595620]
11. Grover GJ, D'Alonzo AJ, Dzwonczyk S, Parham CS, Darbenzio RB. Preconditioning is not abolished by the delayed rectifier K⁺ blocker dofetilide. *Am J Physiol* 1996;271:H1207–H1214. [PubMed: 8853361]
12. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998;97:2463–9. [PubMed: 9641699]
13. Grover GJ, Garlid KD. ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. *J Mol Cell Cardiol* 2000;32:677–95. [PubMed: 10756123]
14. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K⁺ channel in the mitochondrial inner membrane. *Nature* 1991;352:244–7. [PubMed: 1857420]
15. Oldenburg O, Cohen MV, Yellon DM, Downey JM. Mitochondrial K(ATP) channels: role in cardioprotection. *Cardiovasc Res* 2002;55:429–37. [PubMed: 12160940]

16. O'Rourke B. Myocardial K(ATP) channels in preconditioning. *Circ Res* 2000;87:845–55. [PubMed: 11073879]
17. Gross GJ, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K⁺ channels and myocardial preconditioning. *Circ Res* 1999;84:973–9. [PubMed: 10325234]
18. McCully JD, Levitsky S. The mitochondrial K(ATP) channel and cardioprotection. *Ann Thorac Surg* 2003;75:S667–73. [PubMed: 12607710]
19. Brierley GP, Jurkowitz M, Chavez E, Jung DW. Energy-dependent contraction of swollen heart mitochondria. *J Biol Chem* 1977;252:7932–9. [PubMed: 21185]
20. Garlid KD, Paucek P. The mitochondrial potassium cycle. *IUBMB Life* 2001;52:153–8. [PubMed: 11798027]
21. Szewczyk A, Mikolajek B, Pikula S, Nalecz MJ. Potassium channel openers induce mitochondrial matrix volume changes via activation of ATP-sensitive K⁺ channel. *Pol J Pharmacol* 1993;45:437–43. [PubMed: 8118486]
22. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxydecanoate. *J Biol Chem* 1998;273:13578–82. [PubMed: 9593694]
23. Garlid KD, Beavis AD. Evidence for the existence of an inner membrane anion channel in mitochondria. *Biochim Biophys Acta* 1986;853:187–204. [PubMed: 2441746]
24. Hassinen I, Chance B. Oxidation–reduction properties of the mitochondrial flavoprotein chain. *Biochem Biophys Res Commun* 1968;31:895–900. [PubMed: 4299232]
25. Chance B. The kinetics of flavoprotein and pyridine nucleotide oxidation in cardiac mitochondria in the presence of calcium. *FEBS Lett* 1972;26:315–9. [PubMed: 4344294]
26. Chance B, Salkovitz IA, Kovach AG. Kinetics of mitochondrial flavoprotein and pyridine nucleotide in perfused heart. *Am J Physiol* 1972;223:207–18. [PubMed: 4339003]
27. Romashko DN, Marban E, O'Rourke B. Subcellular metabolic transients and mitochondrial redox waves in heart cells. *Proc Natl Acad Sci USA* 1998;95:1618–23. [PubMed: 9465065]
28. Hu H, Sato T, Seharaseyon J, Liu Y, Johns DC, O'Rourke B, Marban E. Pharmacological and histochemical distinctions between molecularly defined sarcolemmal KATP channels and native cardiac mitochondrial KATP channels. *Mol Pharmacol* 1999;55:1000–5. [PubMed: 10347240]
29. Sato T, Sasaki N, Seharaseyon J, O'Rourke B, Marban E. Selective pharmacological agents implicate mitochondrial but not sarcolemmal K(ATP) channels in ischemic cardioprotection. *Circulation* 2000;101:2418–23. [PubMed: 10821820]
30. Seharaseyon J, Ohler A, Sasaki N, Fraser H, Sato T, Johns DC, O'Rourke B, Marban E. Molecular composition of mitochondrial ATP-sensitive potassium channels probed by viral Kir gene transfer. *J Mol Cell Cardiol* 2000;32:1923–30. [PubMed: 11185581]
31. Sato T, Sasaki N, O'Rourke B, Marban E. Adenosine primes the opening of mitochondrial ATP-sensitive potassium channels: a key step in ischemic preconditioning? *Circulation* 2000;102:800–5. [PubMed: 10942750]
32. Sasaki N, Murata M, Guo Y, Jo SH, Ohler A, Akao M, O'Rourke B, Xiao RP, Bolli R, Marban E. MCC-134, a single pharmacophore, opens surface ATP-sensitive potassium channels, blocks mitochondrial ATP-sensitive potassium channels, and suppresses preconditioning. *Circulation* 2003;107:1183–2118. [PubMed: 12615799]
33. O'Rourke B. Evidence for mitochondrial K⁺ channels and their role in cardioprotection. *Circ Res* 2004;94:420–32. [PubMed: 15001541]
34. Minners J, van den Bos EJ, Yellon DM, Schwalb H, Opie LH, Sack MN. Dinitrophenol, cyclosporin A, and trimetazidine modulate preconditioning in the isolated rat heart: support for a mitochondrial role in cardioprotection. *Cardiovasc Res* 2000;47:68–73. [PubMed: 10869531]
35. Debska G, Kicinska A, Skalska J, Szewczyk A, May R, Elger CE, Kunz WS. Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. *Biochim Biophys Acta* 2002;1556:97–105. [PubMed: 12460666]
36. Lim KH, Javadov SA, Das M, Clarke SJ, Suleiman MS, Halestrap AP. The effects of ischaemic preconditioning, diazoxide and 5-hydroxydecanoate on rat heart mitochondrial volume and respiration. *J Physiol* 2002;545:961–74. [PubMed: 12482899]

37. Hanley PJ, Mickel M, Loffler M, Brandt U, Daut J. K(ATP) channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. *J Physiol* 2002;542:735–41. [PubMed: 12154175]
38. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria. *Am J Physiol Heart Circ Physiol* 2001;280:H649–H657. [PubMed: 11158963]
39. Bajgar R, Seetharaman S, Kowaltowski AJ, Garlid KD, Paucek P. Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. *J Biol Chem* 2001;276:33369–74. [PubMed: 11441006]
40. Mironova GD, Fedotcheva NI, Makarov PR, Pronevich LA, Mironov GP. Protein from beef heart mitochondria inducing the potassium channel conductivity of bilayer lipid membrane. *Biofizika* 1981;26:451–7. [PubMed: 6266510]
41. Diwan JJ, Haley T, Sanadi DR. Reconstitution of transmembrane K⁺ transport with a 53 kDa mitochondrial protein. *Biochem Biophys Res Commun* 1988;153:224–30. [PubMed: 3377787]
42. Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K⁺ channel from rat liver and beef heart mitochondria. *J Biol Chem* 1992;267:26062–9. [PubMed: 1464617]
43. Yarov-Yarovoy V, Paucek P, Jaburek M, Garlid KD. The nucleotide regulatory sites on the mitochondrial KATP channel face the cytosol. *Biochim Biophys Acta* 1997;1321:128–36. [PubMed: 9332501]
44. Grigoriev SM, Skarga YY, Mironova GD, Marinov BS. Regulation of mitochondrial KATP channel by redox agents. *Biochim Biophys Acta* 1999;1410:91–6. [PubMed: 10076019]
45. Marinov BS, Grigoriev SM, Skarga Y, Olovjanishnikova GD, Mironova GD. Effects of pelargonidine and a benzocaine analogue *p*-diethylaminoethyl benzoate on mitochondrial K(ATP) channel. *Membr Cell Biol* 2001;14:663–71. [PubMed: 11699869]
46. Mironova GD, Skarga YY, Grigoriev SM, Negoda AE, Kolomytkin OV, Marinov BS. Reconstitution of the mitochondrial ATP-dependent potassium channel into bilayer lipid membrane. *J Bioenerg Biomembr* 1999;31:159–63. [PubMed: 10449243]
47. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K⁺ channels. Possible mechanism of cardioprotection. *Circ Res* 1997;81:1072–82. [PubMed: 9400389]
48. Paucek P, Yarov-Yarovoy V, Sun X, Garlid KD. Inhibition of the mitochondrial KATP channel by long-chain acyl-CoA esters and activation by guanine nucleotides. *J Biol Chem* 1996;271:32084–8. [PubMed: 8943260]
49. Jezek P, Orosz DE, Garlid KD. Reconstitution of the uncoupling protein of brown adipose tissue mitochondria. Demonstration of GDP-sensitive halide anion uniport. *J Biol Chem* 1990;265:19296–302. [PubMed: 2229076]
50. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JPt, Boyd AE 3rd, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 1995;268:423–6. [PubMed: 7716547]
51. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K⁺ channels. *Neuron* 1996;16:1011–7. [PubMed: 8630239]
52. Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M, Seino Y, Mizuta M, Seino S. Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J Biol Chem* 1995;270:5691–4. [PubMed: 7890693]
53. Inagaki N, Gonoi T, Clement JPt, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995;270:1166–70. [PubMed: 7502040]
54. Babenko AP, Aguilar-Bryan L, Bryan J. A view of sur/KIR6.X, KATP channels. *Annu Rev Physiol* 1998;60:667–87. [PubMed: 9558481]
55. Seino S. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 1999;61:337–62. [PubMed: 10099692]

56. Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gonoi T, Seino S, Takata K. Kir6.1: a possible subunit of ATP-sensitive K⁺ channels in mitochondria. *Biochem Biophys Res Commun* 1997;241:693–7. [PubMed: 9434770]
57. Seharaseyon J, Sasaki N, Ohler A, Sato T, Fraser H, Johns DC, O'Rourke B, Marban E. Evidence against functional heteromultimerization of the KATP channel subunits Kir6.1 and Kir6.2. *J Biol Chem* 2000;275:17561–5. [PubMed: 10837494]
58. Lacza Z, Snipes JA, Kis B, Szabo C, Grover G, Busija DW. Investigation of the subunit composition and the pharmacology of the mitochondrial ATP-dependent K⁺ channel in the brain. *Brain Res* 2003;994:27–36. [PubMed: 14642445]
59. Lacza Z, Snipes JA, Miller AW, Szabo C, Grover G, Busija DW. Heart mitochondria contain functional ATP-dependent K⁺ channels. *J Mol Cell Cardiol* 2003;35:1339–47. [PubMed: 14596790]
60. Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K, Koseki H, Iwanaga T, Nakaya H, Seino S. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* 2002;8:466–72. [PubMed: 11984590]
61. Schafer G, Wegener C, Portenhaus R, Bojanovski D. Diazoxide, an inhibitor of succinate oxidation. *Biochem Pharmacol* 1969;18:2678–81. [PubMed: 4327387]
62. Ozcan C, Bienengraeber M, Dzeja PP, Terzic A. Potassium channel openers protect cardiac mitochondria by attenuating oxidant stress at reoxygenation. *Am J Physiol Heart Circ Physiol* 2002;282:H531–H539. [PubMed: 11788400]
63. Ockaili RA, Bhargava P, Kukreja RC. Chemical preconditioning with 3-nitropropionic acid in hearts: role of mitochondrial K(ATP) channel. *Am J Physiol Heart Circ Physiol* 2001;280:H2406–H2411. [PubMed: 11299248]
64. Horiguchi T, Kis B, Rajapakse N, Shimizu K, Busija DW. Opening of mitochondrial ATP-sensitive potassium channels is a trigger of 3-nitropropionic acid-induced tolerance to transient focal cerebral ischemia in rats. *Stroke* 2003;34:1015–20. [PubMed: 12649508]
65. Ardehali, H. AHA Scientific conference on molecular mechanisms of growth, death, and regeneration in the myocardium: basic biology and insights into ischemic heart disease and heart failure. Snowbird; Utah: 2003. Succinate dehydrogenase modulates mitochondrial ATP-sensitive K⁺ channel activity as part of a macromolecular complex.
66. Lawrence CL, Billups B, Rodrigo GC, Standen NB. The KATP channel opener diazoxide protects cardiac myocytes during metabolic inhibition without causing mitochondrial depolarization or flavoprotein oxidation. *Br J Pharmacol* 2001;134:535–42. [PubMed: 11588107]
67. Korge P, Honda HM, Weiss JN. Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. *Proc Natl Acad Sci USA* 2002;99:3312–7. [PubMed: 11867760]
68. Ylitalo KV, Ala-Rami A, Liimatta EV, Peuhkurinen KJ, Hassinen IE. Intracellular free calcium and mitochondrial membrane potential in ischemia/reperfusion and preconditioning. *J Mol Cell Cardiol* 2000;32:1223–38. [PubMed: 10860765]
69. Fryer RM, Hsu AK, Gross GJ. Mitochondrial K(ATP) channel opening is important during index ischemia and following myocardial reperfusion in ischemic preconditioned rat hearts. *J Mol Cell Cardiol* 2001;33:831–4. [PubMed: 11273735]
70. Pain T, Yang XM, Critz SD, Yue Y, Nakano A, Liu GS, Heusch G, Cohen MV, Downey JM. Opening of mitochondrial K(ATP) channels triggers the preconditioned state by generating free radicals. *Circ Res* 2000;87:460–6. [PubMed: 10988237]
71. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Seino S, Marban E, Nakaya H. Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest* 2002;109:509–5016. [PubMed: 11854323]
72. Suzuki M, Saito T, Sato T, Tamagawa M, Miki T, Seino S, Nakaya H. Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. *Circulation* 2003;107:682–5. [PubMed: 12578868]
73. Billman GE, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part II: effects on susceptibility to ventricular fibrillation induced by myocardial ischemia in conscious dogs. *J Pharmacol Exp Ther* 1998;286:1465–73. [PubMed: 9732412]

74. Ghosh S, Standen NB, Galinanes M. Evidence for mitochondrial K ATP channels as effectors of human myocardial preconditioning. *Cardiovasc Res* 2000;45:934–40. [PubMed: 10728419]
75. Fryer RM, Eells JT, Hsu AK, Henry MM, Gross GJ. Ischemic preconditioning in rats: role of mitochondrial K(ATP) channel in preservation of mitochondrial function. *Am J Physiol Heart Circ Physiol* 2000;278:H305–H312. [PubMed: 10644614]
76. Jung O, Englert HC, Jung W, Gogelein H, Scholkens BA, Busch AE, Linz W. The K(ATP) channel blocker HMR 1883 does not abolish the benefit of ischemic preconditioning on myocardial infarct mass in anesthetized rabbits. *Naunyn Schmiedebergs Arch Pharmacol* 2000;361:445–51. [PubMed: 10763861]
77. Holmuhamedov EL, Wang L, Terzic A. ATP-sensitive K⁺ channel openers prevent Ca²⁺ overload in rat cardiac mitochondria. *J Physiol* 1999;519(Pt 2):347–60. [PubMed: 10457054]
78. Wang L, Cherednichenko G, Hernandez L, Halow J, Camacho SA, Figueredo V, Schaefer S. Preconditioning limits mitochondrial Ca(2+) during ischemia in rat hearts: role of K(ATP) channels. *Am J Physiol Heart Circ Physiol* 2001;280:H2321–H2328. [PubMed: 11299237]
79. Murata M, Akao M, O'Rourke B, Marban E. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca(2+) overload during simulated ischemia and reperfusion: possible mechanism of cardio-protection. *Circ Res* 2001;89:891–8. [PubMed: 11701616]
80. Eells JT, Henry MM, Gross GJ, Baker JE. Increased mitochondrial K(ATP) channel activity during chronic myocardial hypoxia: is cardioprotection mediated by improved bioenergetics? *Circ Res* 2000;87:915–21. [PubMed: 11073888]
81. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. *Circ Res* 2001;88:802–9. [PubMed: 11325872]
82. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 1998;273:18092–8. [PubMed: 9660766]
83. Oldenburg O, Cohen MV, Downey JM. Mitochondrial K(ATP) channels in preconditioning. *J Mol Cell Cardiol* 2003;35:569–75. [PubMed: 12788373]
84. Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci USA* 1987;84:1404–7. [PubMed: 3029779]
85. Vanden Hoek TL, Becker LB, Shao ZH, Li CQ, Schumacker PT. Preconditioning in cardiomyocytes protects by attenuating oxidant stress at reperfusion. *Circ Res* 2000;86:541–8. [PubMed: 10720416]
86. Takashi E, Wang Y, Ashraf M. Activation of mitochondrial K(ATP) channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. *Circ Res* 1999;85:1146–53. [PubMed: 10590241]see comments
87. Akao M, Ohler A, O'Rourke B, Marban E. Mitochondrial ATP-sensitive potassium channels inhibit apoptosis induced by oxidative stress in cardiac cells. *Circ Res* 2001;88:1267–75. [PubMed: 11420303]
88. Akao M, O'Rourke B, Kusuoka H, Teshima Y, Jones SP, Marban E. Differential actions of cardioprotective agents on the mitochondrial death pathway. *Circ Res* 2003;92:195–202. [PubMed: 12574147]
89. Hausenloy DJ, Yellon DM, Mani-Babu S, Duchon MR. Preconditioning protects by inhibiting the mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol*. 2004
90. Sato T, Sasaki N, O'Rourke B, Marban E. Nicorandil, a potent cardio-protective agent, acts by opening mitochondrial ATP-dependent potassium channels. *J Am Coll Cardiol* 2000;35:514–8. [PubMed: 10676702]
91. D'Hahan N, Moreau C, Prost AL, Jacquet H, Alekseev AE, Terzic A, Vivaudou M. Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. *Proc Natl Acad Sci USA* 1999;96:12162–7. [PubMed: 10518593]
92. Notsu T, Tanaka I, Mizota M, Yanagibashi K, Fukutake K. A cAMP-dependent protein kinase inhibitor modulates the blocking action of ATP and 5-hydroxydecanoate on the ATP-sensitive K⁺ channel. *Life Sci* 1992;51:1851–6. [PubMed: 1333026]
93. Jilkina O, Kuzio B, Grover GJ, Folmes CD, Kong HJ, Kupriyanov VV. Sarcolemmal and mitochondrial effects of a KATP opener, P-1075, in “polarized” and “depolarized” Langendorff-perfused rat hearts. *Biochim Biophys Acta* 2003;1618:39–50. [PubMed: 14643932]

94. Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM, et al. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. *Circulation* 2001;103:3111–6. [PubMed: 11425777]

Fig. 1.

Evidence for physical and functional interaction between SDH and mitoK_{ATP}. (A) Co-immunoprecipitation studies revealed that the 30- and 70-kDa components of SDH interact with four mitochondrial proteins: mABC1, ANT, ATPase and PIC. (B) Unitary K⁺ currents were recorded in lipid planar bilayers after fusion of native microsomes from a partially purified mitochondrial fraction containing the mABC1-SDH-PIC-ANT-ATPase protein complex. (C) The channel activity was increased by diazoxide, and the diazoxide activated channel activity was inhibited by 5-HD, glibenclamide and ATP. (D) Schematic presentation of the proteins associated with mitoK_{ATP} activity. The complex containing SDH-PIC-mABC1-ATPase-ANT is capable of transporting K⁺ with characteristics similar to those of mitoK_{ATP}. The pore-

forming unit of the channel has not been characterized yet. Gli, glibenclamide; IMS, intermembrane space; Mito IM, mitochondrial inner membrane.

Table 1Modulators of K_{ATP} channels and their selectivity toward mitochondrial and surface channels

	mitoK _{ATP}	mitoK _{ATP} and surface K _{ATP}	Surface K _{ATP}
Openers	Diazoxide	Cromakalim	P-1075 ^a
	Nicorandil	Pinacidil	MCC-134 ^b
	BMS 191095	P-1060	
		Sildenafil	
		Isoflurane	
		EMD60480	
		Aprikalim	
		EMD60480	
		Minoxidil Sulfate	
		KRN2391	
		Levosimendan	
Blockers	5-Hydroxydecanoate	Glibenclamide	HMR1098 (1833)
	MCC-134	Glipizide	Glimepiride ^c

Diazoxide and nicorandil can activate surface K_{ATP} channel at high concentrations [90,91]. 5-Hydroxydecanoate can inhibit surface K_{ATP} channel at low pH [92].

^aP-1075 is selective for surface K_{ATP} channel in rabbit myocytes [29]. In isolated rat mitochondria, it activated mitoK_{ATP} [93].

^bMCC-134 selectively activates surface K_{ATP} and inhibits mitoK_{ATP} [32].

^cGlimepiride inhibits surface K_{ATP} without any effect on cardioprotection [94].