The Mitochondrial K_{ATP} Channel and Cardioprotection

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Adenosine triphosphate (ATP)–sensitive potassium (K_{ATP}) channels allow coupling of membrane potential to cellular metabolic status. Two K_{ATP} channel subtypes coexist in the myocardium, with one subtype located in the sarcolemma (sarcK_{ATP}) membrane and the other in the inner membrane of the mitochondria (mitoK_{ATP}). The K_{ATP} channels can be pharmacologically modulated by a family of structurally diverse agents of varied potency and selectivity, collectively known as potassium channel openers and blockers. Sufficient evidence exists to indicate that the K_{ATP} channels and, in particular, the mitoK_{ATP} channels play an important role both as a trigger and an effector in surgical cardioprotection. In this review, the biochemistry and surgical specificity of the K_{Atp} channels are examined.

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Myocardial ischemia/reperfusion injury continues to occur after cardiac operations that have been performed in a technically adequate manner. This injury contributes significantly to postoperative morbidity and mortality, despite meticulous adherence to presently known principles of myocardial protection. Traditionally, cardioplegia has been used as a myoprotective agent for the alleviation of surgically induced ischemia/reperfusion injury incurred during cardiac operative procedures, to allow functional preservation of the myocardium. These solutions permit rapid electromechanical arrest of the myocardium through alteration of cellular electrochemical gradients [1–3]. Most cardioplegia solutions use high potassium, which maintains the heart in a depolarized state [3]. The advantages of cardioplegic arrest in providing the surgeon a bloodless field are tempered in that depolarization also leads to the alteration of ion flux across the sarcolemmal membrane and is associated with both increased cytosolic calcium accumulation and the significant depletion of cellular high-energy adenosine triphosphate (ATP) reserves [4–10]. In previous investigations we have shown that magnesium-supplemented potassium cardioplegia (K/Mg, DSA) provides superior cardioprotection as compared with high potassium cardioplegia [4–7, 11–17]. In a series of studies, using the isolated perfused rabbit heart [4–7, 9–13] and in situ, blood-perfused sheep [16] and pig [17] heart models, we have shown that K/Mg cardioplegia partially modifies the biochemical changes that lead to lethal myocardial ischemia/reperfusion injury. We have also shown that the mechanisms by which magnesium-supplemented potassium cardioplegia affords enhanced cardioprotection involve the amelioration of cytosolic, mitochondrial, and nuclear calcium overload, enhanced preservation and resynthesis of high energy phosphates, and modulation of nuclear and mitochondrial function [4–7, 18]. The end effector of these mechanisms remains to be elucidated; however, recent investigations have suggested that the mitochondrial ATP-sensitive potassium channels play an important role in the cardioprotection afforded by K/Mg cardioplegia [14, 17].

ATP-Sensitive Potassium Channel Structure

The ATP-sensitive potassium ($K_{ATP)}$ channels belong to the ATP-binding cassette transporter superfamily and are comprised of two subunits: (1) a pore-forming, inward-rectifying potassium channel subunit (Kir), and (2) a regulatory sulfonylurea receptor (SUR). The Kir and SUR subunits coassemble with a 4:4 stoichiometry to form a hetero-octameric K_{ATP} channel with the Kir subunits forming the K^+ ion permeation pathway (Fig 1). Both Kir and SUR subunits are required to form fully functional channels with the SUR subunit cooperating with the Kir subunit to act as ATP-dependent potassium channel complex.

The SUR subunit contains two transmembrane domains with 13 to 17 transmembrane segments and two nucleotide binding folds (Fig 1). The two nucleotide binding folds (NBD-1, NBD-2) are located toward the carboxy terminus of the SUR subunit and contain Walker A and Walker B motifs, essential in providing membrane sensitivity to ATP and Mg^{2+}-ADP. The Walker A and B motifs are conserved protein sequences. The Walker A motif (also referred to as the ATP/GTP binding site) contains the characteristic glycine-rich amino acid sequence $GlyX_6GlyXXGlyXGlyLys(Ser/Thr)$ allowing binding of ATP or GTP by means of the terminal phosphate group. The Walker B motif contains the characteristic

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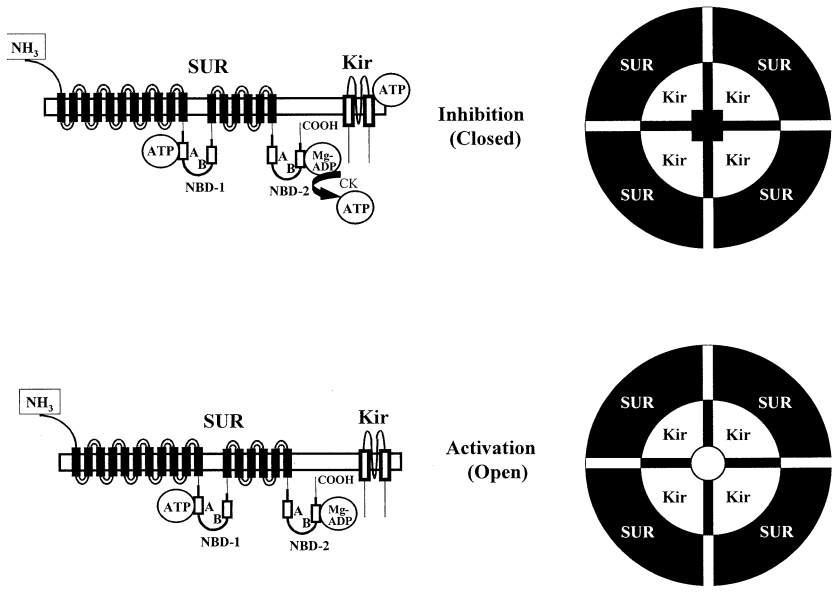

*Fig 1. Schematic representation of K_{ATP} channel. Two-dimensional representations of pore-forming inward rectifying potassium channel subunit (*Kir*) and a regulatory sulfonylurea receptor (*SUR*) during closed and open states. Nucleotide binding folds (*NBD-1, NBD-2*) containing Walker A and Walker B motifs are shown. Under homeostatic conditions K_{ATP} channels are closed. High concentrations of ATP allow binding of ATP to Kir closing the pore-forming unit. Binding of ATP to NBD-1 and hydrolysis of Mg^{2+}-ATP at NBD-2 allows limited resident binding of Mg^{2+}-ADP to NBD-2 due to catalytic actions of creatine kinase (*CK*), thus preventing channel opening. Under conditions of ischemia ATP concentrations are decreased and ADP concentrations are increased and the catalytic actions of creatine kinase are reduced or inhibited and resident binding of Mg^{2+}-ADP to NBD-2 is increased allowing conformational stabilization and K_{ATP} channel opening and K^+ flux across the inner membrane.*

amino acid sequence $ArgX_{11}AspX_6Asp$ and is involved in ATP hydrolysis and binds the Mg^{2+} moiety of Mg^{2+}-ATP. The nucleotide binding fold NBD-1 has limited catalytic activity, whereas NBD-2 hydrolyzes ATP and has been shown to play an essential role in K_{ATP} channel gating [19]. Both NBD-1 and NBD-2 are required for K_{ATP} channel function.

ATP-Sensitive Potassium Channel Regulation

The K_{ATP} channels allow coupling of membrane potential to cellular metabolic status, and it has been hypothesized that K_{ATP} channel gating occurs as a result of the accumulation of nucleotide diphosphates as a consequence of elevated ischemia-induced, metabolic demands [20–23]. Under normal conditions the K_{ATP} channels are inhibited (ie, closed) by direct ATP binding to the Kir pore-forming subunit, preventing K^+ flux across the membrane. This inhibition occurs by free $[ATP]_i$ and $[Mg^{2+}\text{-}ATP]_i$ at levels greater than 1 mmol/L and is responsive to changes in [ATP] produced by glycolysis but not by increases through application of exogenous ATP [24, 25].

The K_{ATP} channels are stimulated (ie, opened) through ATP hydrolysis at the SUR subunit, primarily at NBD-2. Evidence from photolabeling studies suggests that ATP binds to NBD-1 with high affinity, whereas ATP hydrolysis occurs at NBD-2 [26]. These studies also show that Mg^{2+}-ADP antagonizes ATP binding at NBD-1 [26]. Current hypothetical models suggest that during homeostasis ATP concentration exceeds Mg^{2+}-ADP, thus favoring high-affinity binding of ATP to NBD-1, whereas ATP hydrolysis at NBD-2 allows binding of Mg^{2+}-ADP at NBD-2 (Fig 1). Binding of ATP to NBD-1 and Mg^{2+}-ADP to NBD-2 permits conformational stabilization and opening of the K_{ATP} channel. Under homeostatsis, the catalytic actions of creatine kinase limit the resident binding duration of the Mg^{2+}-ADP to NBD-2, preventing conformational stabilization and channel opening (Fig 1). However, during ischemia/reperfusion, creatine kinase activity is decreased and Mg^{2+}-ADP concentration is increased. These events extend the resident binding time of Mg^{2+}-ADP at NBD-2 and the binding of ATP at NBD-1, resulting in SUR subunit conformational stabilization. The stabilization of the SUR subunit overcomes ATP-induced Kir subunit pore closure, resulting in K_{ATP} channel opening and K^+ flux across the inner membrane [19].

ATP-Sensitive Potassium Channel Subtypes in the Myocardium

Two ATP-sensitive potassium (K_{ATP}) channel subtypes coexist in the myocardium, with one subtype located in the sarcolemma (sarcK_{ATP}) membrane and the other in the inner membrane of the mitochondria (mitoK_{ATP}). The cardiac sarcK_{ATP} channels, first reported in guinea pig ventricular myocytes and then later shown to exist in a variety of tissues, have been molecularly characterized as SUR2A/Kir6.2 [27–30]. The Kir 6.2 subunit has a molecular mass of 51 kD, whereas the SUR2A subunit has a molecular mass of 140 kD [28–30].

The mitoK_{ATP} channels were first identified in the liver and then in the heart, and have been shown to be located in the inner membrane of the mitochondria [31, 32]. The mitoK_{ATP} channels have yet to be molecularly fully characterized; however, there is sufficient evidence to indicate that neither Kir6.1 nor Kir6.2 is a constituent member [33, 34]. High-affinity azido-[^{125}I]glyburide and fluorescent BODIPY-FL-glyburide studies have allowed

isolation of a mitochondrial Kir subunit with a molecular mass of 55 kD and a mitochondrial SUR subunit with a molecular mass of 63 kD [35]. Garlid and Paucek [36] have predicted that the mitoSUR subunit will be shown to be a half-molecule ATP-binding cassette transporter protein.

Pharmacologic Modulation of K_{ATP} Channels

The K_{ATP} channels can be pharmacologically modulated by a family of structurally diverse agents collectively known as potassium channel openers and blockers [37]. In general, most potassium channel openers are nonselective and act with varied potency through binding to the SUR subunits of the K_{ATP} channels to modulate activation (for review see [37, 38]). The potassium channel openers are thought to act by interaction or active competition at regulatory ATP binding sites in the K_{ATP} channel, allowing conformational stabilization through interaction with a conserved amino acid motif [38–40]. In contrast, potassium channel blockers act to inhibit conformational stabilization. The best-known potassium channel blocker is glibenclamide (glyburide), which binds to a high-affinity site localized on the SUR subunit and a low-affinity site on the Kir subunit of the K_{ATP} channel. Glibenclamide is a nonspecific K_{ATP} channel blocker and acts on both sarc- and mitoK$_{ATP}$ channels.

The selectivity of sarc- and mitoK$_{ATP}$ channel openers and blockers is primarily dependent upon SUR phenotype (SUR1 vs SUR 2) and SUR subtype sensitivity [36–40]. Pinacidil, cromakalim, nicorandil, and related analogues are potassium channel openers that have been shown to act preferentially on SUR2A/B subunit-K_{ATP} channels and have relatively little effect on SUR1 subunit-K_{ATP} channels. Potassium channel blockers (glibenclamide, 5-hydroxy decanoate, and analogs) seem to act in a similar manner. The potassium channel blockers also bind at the SUR2A/B subunit but at alternative sites. Recombinant and in vitro studies have shown that the mitoK$_{ATP}$ channels can be selectively blocked with 5-hydroxy decanoate [36, 38], whereas sarcK$_{ATP}$ channels can be inhibited by HMR 1883 or HMR 1098 [40, 41]. There is, at present, no selective sarcK$_{ATP}$ channel opener; however, mitoK$_{ATP}$ channels can be selectively opened with diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide), a nondiuretic benzothiadiazine analogue shown to be 2,000-fold more selective for cardiac mitoK$_{ATP}$ channels as compared with cardiac sarcK$_{ATP}$ channels [36–38].

The mitoK$_{ATP}$ Channels in Cardiac Surgery

There is sufficient evidence to support the role of both the sarcK$_{ATP}$ and the mitoK$_{ATP}$ channels in cardioprotection; however, evidence is accumulating to indicate that the mitoK$_{ATP}$ channels play the predominant role in this mechanism and that specific mitoK$_{ATP}$ channel modulation is required to allow enhanced cardioprotection. Support for this cardioprotective mechanism comes from previous investigations in isolated crystalloid-perfused, in situ blood–perfused animal models and in vitro studies using human trabeculae [14, 42–47]. These studies have demonstrated that nonspecific potassium channel openers such as nicorandil and pinacidil, which permit unmodulated opening of both sarc- and mitoK$_{ATP}$ channels, significantly enhance cardioprotection when used alone; however, these cardioprotective effects, when used in conjunction with cardioplegia, are limited or are inhibited altogether [14, 42–47]. In a series of studies using both the isolated perfused rabbit heart and the in situ blood–perfused pig heart, we have examined the role of mitoK$_{ATP}$ and sarcK$_{ATP}$ channels in the cardioprotection afforded by magnesium-supplemented potassium (K/Mg, crystalloid cardioplegia; DSA, blood cardioplegia, 1:4, v:v) cardioplegia [14, 17, 41]. Our results indicated that selective blockade of mitoK$_{ATP}$ channels with 5-hydroxy deconoate (5HD) before ischemia completely abolished K/Mg infarct size reduction, such that no significant difference as compared with unprotected global ischemia hearts was observed [16]. The mitoK$_{ATP}$ channel blockade with 5-hydroxy decanoate during reperfusion did not significantly affect K/Mg infarct size reduction. However, mitoK$_{ATP}$ channel blockade both before ischemia and during reperfusion completely abolished K/Mg enhanced myocardial infarct size reduction [14]. In contrast, specific blockade of sarcK$_{ATP}$ channels with HMR 1883, either before ischemia or during reperfusion, had no effect on the infarct size reduction afforded by K/Mg cardioplegia and had no effect on LVPDP or $+dP/dt$ [14]. These results indicate that the mitoK$_{ATP}$ channels play an important role in the cardioprotection allowed by cardioplegia, and that the effects of this cardioprotection are modulated before surgically-induced ischemia and not during reperfusion.

Opening of mitoK$_{ATP}$ Channels With Cardioplegia Is Required to Provide Surgical Cardioprotection

It would be reasonable, based on these investigations, to hypothesize that the selective opening of mitoK$_{ATP}$ channels with diazoxide alone would provide surgical cardioprotection, thus eliminating the need for cardioplegia. However, our data from studies in the in situ blood–perfused pig heart and more recent studies using a model of acute myocardial infarction would suggest that the opening of mitoK$_{ATP}$ channels alone does not afford enhanced cardioprotection [14, 17, 41]. Our results indicate that diazoxide significantly decreases infarct size as compared with global ischemia hearts, but that this decrease is significantly less than that observed with magnesium-supplemented potassium (K/Mg) cardioplegia (Fig 2). In addition, diazoxide alone had no effect on functional recovery (Fig 3). These results are in agreement with our previous data indicating that the selective opening of mitoK$_{ATP}$ channels with diazoxide provides infarct-limiting effects and is not associated with antistunning effects.

Of specific relevance to cardiac surgery, we have shown that the addition of diazoxide (50 μmol/L) to K/Mg cardioplegia significantly decreased ($p < 0.05$) infarct size as compared to K/Mg cardioplegia, with no significant

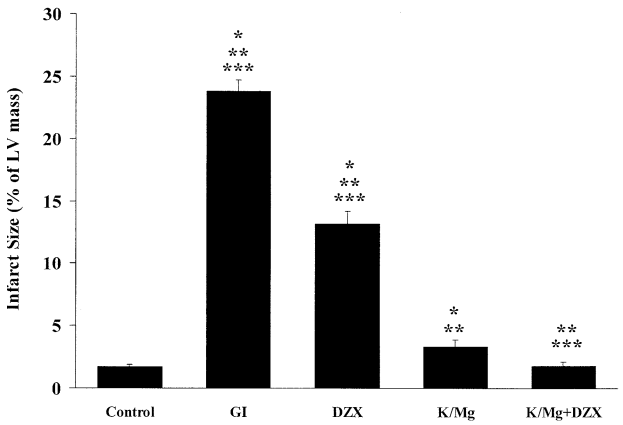

*Fig 2. Effects of diazoxide and diazoxide + K/Mg cardioplegia on infarct size. Infarct size expressed as percentage of left ventricular (LV) volume after 30 minutes of equilibrium, 30 minutes of normothermic global ischemia, and 120 minutes of reperfusion in isolated Langendorff-perfused rabbit heart. Control hearts received 180 minutes of normothermic perfusion without ischemia. Global ischemia (GI) hearts received 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Diazoxide hearts (DZX) received 5 minutes of perfusion of 50 μmol/L diazoxide before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Magnesium-supplemented potassium cardioplegia hearts (K/Mg) received 5 minutes of K/Mg cardioplegia before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. K/Mg +DZX hearts received 5 minutes of K/Mg cardioplegia + 50 μmol/L diazoxide before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Results are shown as mean ± SE; n = 7 to 8 for each group. *Significant differences at p < 0.05 vs control hearts. **Significant differences at p < 0.05 vs GI hearts. ***Significant differences at p < 0.05 vs K/Mg hearts. Results indicate that K/Mg +DZX significantly decreases infarct size as compared to K/Mg. No significant difference between control and K/Mg +DZX was observed.*

difference being observed between K/Mg +diazoxide and control hearts that received no ischemia (Fig 2). We have also investigated the effects of K_{ATP} channel opener specificity using pinacidil, a nonselective K_{ATP} channel opener [14]. Our results show that pinacidil, when added to K/Mg cardioplegia, induced irreversible ventricular fibrillation immediately upon reperfusion in all hearts and significantly increased infarct size ($p < 0.05$ vs K/Mg), in agreement with previous investigations by others [42, 43]. These results indicate that the specific pharmacologic opening of mitoK$_{ATP}$ channels, independent of cardioplegia, provides only minimal infarct size reduction and has no effect on postischemic functional recovery (NS vs global ischemia) [14]. However, pharmacologic opening of mitoK$_{ATP}$ channels, when used with cardioplegia, provides enhanced infarct-limiting effects.

Recently, we have investigated selective opening of mitochondrial ATP–sensitive potassium channels with diazoxide (50 μmol/L) when used in conjunction with cold blood, magnesium-supplemented potassium cardioplegia (DSA; 4:1; v:v) using a clinically relevant model of acute myocardial infarction in the in situ blood–perfused pig model [17]. In this model, a region of the left ventricle is made ischemic by snaring a portion of the left anterior descending coronary artery, after a 30-minute period of regional ischemia. Cardioplegic arrest after cross-clamping occurs, and the heart is subjected to global arrest. After a 30-minute period to mimic the placement of a coronary artery bypass graft, the snare and the cross-clamp are released and the ischemic zones reperfused. This protocol was used to mimic surgical events in an experimental model that would allow the most relevant comparison. Our results indicate that pharmacologic opening of mitoK$_{ATP}$ channels with diazoxide (50 μmol/L) with DSA cardioplegia significantly decreased infarct size ($p < 0.05$ vs DSA) as compared with the effect of DSA cardioplegia alone.

Hemodynamic Effects of Diazoxide in Cardioprotection

Diazoxide is highly bound to albumin (predominately hydrophobic and, to a lesser extent, hydrogen bonding) and has a plasma half-life of 22 hours [48]. Diazoxide is used as antihypertensive drug and has vasodilatory properties; however, at concentrations of 50 μmol/L, we and others have shown that the infarct-limiting effects of diazoxide are independent of vasodilatation [14, 17, 37–39]. In our investigations we have used 50 μmol/L diazoxide in both Langendorff- and in situ blood– perfused models to investigate the role of mitoK$_{ATP}$ channels in cardioprotection. In in situ blood–perfused models, diazoxide (50 μmol/L) was added to DSA cardioplegia (600 mL) only during the initial administration of cardioplegia, and was not included when DSA was readministered after 15 minutes. of global ischemia. This protocol was based on preliminary results showing that readministration of diazoxide resulted in a significant decrease in mean arterial pressure upon reperfusion. Using only a single dose in the initial administration of cardioplegia and using cardioplegia without diazoxide in all subsequent administrations, no difference in heart rate, mean arterial pressure, or coronary flow was observed between hearts receiving DSA and those receiving DSA + diazoxide.

No definitive study has been performed to indicate the exact diazoxide concentration required for in vivo cardioprotection; however, our studies suggest that based on a total blood volume of 3 to 4 L in the pig, a circulating diazoxide concentration of approximately 7.5 to 10 μmol/L is adequate. This is in agreement with the investigations of Garlid and colleagues [39], who have shown that diazoxide decreases cell injury in a dose-dependent manner at concentrations between 1 and 30 μmol/L.

Cardioprotective Actions of K_{ATP} Channels

The cardioprotective actions of the sarc- and mitoK$_{ATP}$ channels are the focus of active current research by many groups and remain to be fully elucidated. At present, consensus opinion suggests that the opening of the sarcK$_{ATP}$ channels probably acts to reduce cytosolic Ca^{2+} influx through the L-type calcium channels and to enhance Ca^{2+} efflux by means of the Na^{2+}–Ca^{2+} exchanger,

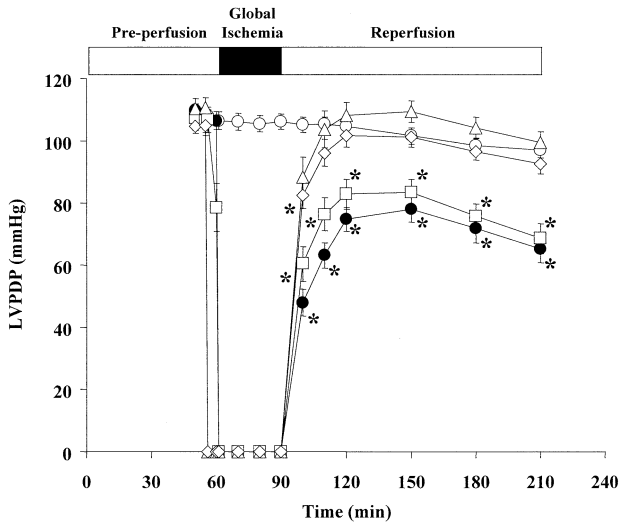

Fig 3. Left ventricular peak developed pressure (LVPDP; mm Hg) *after 30 minutes of equilibrium, 30 minutes of normothermic global ischemia, and 120 minutes of reperfusion in isolated Langendorff-perfused rabbit heart. Control* (open circles) *hearts received 180 minutes of normothermic perfusion without ischemia. Global ischemia (GI)* (black circles) *hearts received 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Diazoxide hearts (DZX)* (squares) *received 5 minutes of perfusion with 50 μmol/L diazoxide before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Magnesium-supplemented potassium cardioplegia hearts (K/Mg)*(triangles) *received 5 minutes of K/Mg cardioplegia before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. K/Mg +DZX* (diamonds) *hearts received 5 minutes of K/Mg cardioplegia + 50 μmol/L diazoxide before 30 minutes of normothermic global ischemia and 120 minutes of reperfusion. Results are shown as mean ± SE; n = 7 to 8 for each group. *Significant differences at* $p < 0.05$ *vs control hearts. Results indicate that DZX has no effect on functional recovery. No significant difference was observed between DZX and GI hearts. No significant difference was observed between control hearts and K/Mg and K/Mg+DZX hearts.*

thereby reducing the Ca^{2+}-related energy cost of contraction and providing protection from cellular injury and the effects of stunning [49]. In our studies we have not observed a role for the sarcK$_{ATP}$ channels in the cardioprotection afforded by magnesium-supplemented potassium cardioplegia; however, the actions of the sarcK$_{ATP}$ channels may be masked by the cardioprotective properties of magnesium-supplemented potassium cardioplegia. It is also possible that the antistunning effects of sarcK$_{ATP}$ channel activation may be delayed and may represent a reperfusion event beyond the investigative scope of our experimental models.

We and others have shown that pharmacologic or endogenous induction allowing early opening or greater absolute activation of mitoK$_{ATP}$ channels before lethal ischemia acts to reduce postischemic infarct size and cell death [20–22, 38–40]. Several possible cardioprotective mechanisms have been associated with opening of the mitoK$_{ATP}$ channels, which include the following: alterations in mitochondrial calcium [50, 51]; alterations occurring at the mitochondrial permeability transition pore [52]; changes in mitochondrial membrane depolarization [53]; modulation of reactive oxygen species formation [54, 55]; alterations in mitochondrial volume [38, 39]; and alterations modulating the release of cytochrome C [56]. The involvement of these mechanisms in cardioplegic cardioprotection is unknown.

At present, the most appropriate and relative cardioprotective mechanism associated with opening of the mitoK$_{ATP}$ channels has been proposed by Garlid and associates who have suggested that the regulation of mitochondrial volume, and electron transport are the preeminent mechanisms in maintaining mitochondrial function in the intact myocardium [36, 38, 39, 57–61]. In an elaborate series of experiments examining mitochondrial respiration, enzyme kinetics, membrane integrity, and nucleotide synthesis and transport, this group has shown that diazoxide acts to preserve the low mitochondrial outer membrane permeability to nucleotides and impermeability to cytochrome c, and that these beneficial effects allow enhanced preservation of adenine nucleotides during ischemia and efficient energy transfer during reperfusion [38, 61].

Although these experimental findings have been garnered from investigations in the isolated perfused heart model and from isolated mitochondrial and permeabilized cardiac fiber studies they suggest that the cardioprotection afforded by the opening of mitoK$_{ATP}$ channels may act to inhibit ATP wastage during ischemia and thus provide for the maintenance of efficient energy resources to allow resumption of essential energy dependent mechanism during the initial reperfusion required for cellular maintenance. This mechanism, is consistent with present experimental data indicating that mitoK$_{ATP}$ openers significantly decrease myocardial infarct without effecting cardiac function [14, 17, 37, 39].

Current Application and Future Directions for Use of mitoK$_{ATP}$ Channel Openers

At present, use of diazoxide and other United States Food and Drug Administration–approved potassium channel openers in cardioplegia and cardioprotection has been limited to animal models; however, sufficient evidence is currently available to permit clinical trials in a variety of areas. There is overwhelming data from ischemic preconditioning studies to indicate that specific mitoK$_{ATP}$ channel openers provide enhanced infarct limitation [38, 40, 41, 49, 53, 54, 57, 59]. Opening of mitoK$_{ATP}$ channels has also been shown to enhance tissue preservation in both lung and heart transplant models [62, 63]. Kevelatitis and colleagues [63] have shown that rat hearts perfused with diazoxide before arrest and storage in Celsior (IMITIX, Amstelveen, The Netherlands) for 10 hours showed significantly better preserved left ventricular compliance upon reperfusion than control hearts. The use of diazoxide has also been shown to be efficacious when used with St. Thomas'cardioplegia [42, 64]. In our studies we have used diazoxide as the preferred selective mitoK$_{ATP}$ channel opener and have found that the protective effects of

diazoxide act independent of vasodilatation. Recently BMS-191095 (Bristol-Myers Squibb, Princeton, NJ) has also been shown to provide infarct-limiting effects independent of vasodilatation or action potential shortening [65–67].

In conclusion, our results demonstrate that mitoK$_{ATP}$ channels act as both a trigger and an effector in the myoprotection provided by cardioplegia, and that the addition of diazoxide, a specific mitoK$_{ATP}$ channel opener, significantly enhances the infarct-limiting effects of magnesium-supplemented potassium (K/Mg, DSA) cardioplegia. Our results indicate that addition of diazoxide significantly enhances the inherent calcium-ameliorating cardioprotection afforded by magnesium-supplemented potassium cardioplegia by significantly decreasing myocardial infarct and represents an additional modality for enhancing myocardial protection.

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