Mitochondrial ATP-Dependent Potassium Channels Novel Effectors of Cardioprotection?

Yongge Liu, PhD; Toshiaki Sato, MD, PhD; Brian O’Rourke, PhD; Eduardo Marban, MD, PhD

**Background**—Brief interruptions of coronary blood flow paradoxically protect the heart from subsequent prolonged ischemia. The basis of such endogenous cardioprotection, known as “ischemic preconditioning,” remains uncertain. Pharmacological evidence has implicated ATP-dependent potassium (K\(_{\text{ATP}}\)) channels in the mechanism of preconditioning; however, the effects of sarcolemmal K\(_{\text{ATP}}\) channels on excitability cannot account for the protection.

**Methods and Results**—We simultaneously measured flavoprotein fluorescence, an index of mitochondrial redox state, and sarcolemmal K\(_{\text{ATP}}\) currents in intact rabbit ventricular myocytes. Our results show that diazoxide, a K\(_{\text{ATP}}\) channel opener, selectively activates mitochondrial K\(_{\text{ATP}}\) channels. Diazoxide induced reversible oxidation of flavoproteins with an EC\(_{50}\) of 27 \(\mu\text{mol/L}\) but did not activate sarcolemmal K\(_{\text{ATP}}\) channels. The subcellular site of diazoxide action is further localized to mitochondria by confocal imaging of fluorescence arising from flavoproteins and tetramethylrhodamine ethyl ester. In a cellular model of simulated ischemia, inclusion of diazoxide decreased the rate of cell death to about half of that in controls. Both the redox changes and protection are inhibited by the K\(_{\text{ATP}}\) channel blocker 5-hydroxydecanoic acid.

**Conclusions**—Our results demonstrate that diazoxide targets mitochondrial but not sarcolemmal K\(_{\text{ATP}}\) channels and imply that mitochondrial K\(_{\text{ATP}}\) channels may mediate the protection from K\(_{\text{ATP}}\) channel openers. *(Circulation. 1998;97:2463-2469.)*

**Key Words:** ischemia ■ potassium channels ■ mitochondria

Lethal injury to the heart can be dramatically blunted by brief conditioning periods of ischemia. Such ischemic preconditioning\(^1\) exists in all species examined, including humans.\(^2\) Despite intensive investigation, the mechanism of preconditioning remains poorly understood. K\(_{\text{ATP}}\) channel openers mimic and K\(_{\text{ATP}}\) channel inhibitors block ischemic preconditioning.\(^3\) Opening of sarcolemmal K\(_{\text{ATP}}\) channels shortens the action potential and thus depresses contractility;\(^4\) this has been proposed as the mechanism for protection of ischemic myocardium.\(^1\) However, recent evidence contradicts this hypothesis. The degree of action potential shortening can be divorced from the extent of protection.\(^5,6\) Furthermore, K\(_{\text{ATP}}\) channel openers and ischemic preconditioning are protective even in unstimulated cardiac myocytes,\(^7,8\) in which action potential abbreviation cannot be a factor.

Cardiac myocytes have another type of K\(_{\text{ATP}}\) channel, in the inner mitochondrial membrane, which responds to many of the same openers and blockers as the sarcolemmal channels (albeit with different potencies).\(^9-12\) Although the physiological roles of mitochondrial K\(_{\text{ATP}}\) channels in cardiac myocytes remain unclear, opening of any potassium-selective ion channels in the inner mitochondrial membrane would tend to dissipate the membrane potential established by the proton pump.\(^13\) Such dissipation accelerates electron transfer by the respiratory chain and leads to net oxidation of the mitochondrial matrix. The fluorescence of FAD-linked enzymes can be used to index mitochondrial redox state.\(^14,15\) Low concentrations (1 to 100 \(\mu\text{mol/L}\)) of the K\(_{\text{ATP}}\) channel opener diazoxide have been reported to activate mitochondrial K\(_{\text{ATP}}\) channels,\(^12\) whereas cardiac sarcolemmal K\(_{\text{ATP}}\) channels are quite resistant to this drug.\(^12,16\) To determine whether diazoxide can selectively open mitochondrial K\(_{\text{ATP}}\) channels in intact living cells, we simultaneously measured flavoprotein fluorescence and sarcolemmal K\(_{\text{ATP}}\) currents (\(I_{\text{K}_{\text{ATP}}}\)) in intact rabbit ventricular myocytes. Our results show that diazoxide selectively activates mitochondrial K\(_{\text{ATP}}\) channels. Diazoxide also protects myocytes against simulated ischemia. We propose that mitochondrial K\(_{\text{ATP}}\) channels may be the elusive effectors of preconditioning. Recognition of this role for mitochondrial K\(_{\text{ATP}}\) channels identifies a promising new target for the development of cardioprotective drugs and implicates the mitochondria in the process of lethal ischemic injury.
Selected Abbreviations and Acronyms

- CN = sodium cyanide
- DNP = dinitrophenol
- 5-HD = 5-hydroxydecanoic acid sodium
- K$_{ATP}$ = ATP-dependent potassium
- TMRE = tetramethylrhodamine ethyl ester

Methods

The investigation conforms with The Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85–23, revised 1985).

Chemicals

Collagenase (type II) was purchased from Worthington. Diazoxide was obtained from Sigma Chemical Co. Pinacidil and 5-HD were purchased from Research Biochemical Int. TMRE was obtained from Molecular Probes. Diazoxide, pinacidil, and TMRE were dissolved in DMSO before being added into experimental solutions. The final concentration of DMSO was <0.1%.

Electrophysiology and Flavoprotein Fluorescence Measurement

Ventricular myocytes were isolated from adult rabbit hearts by conventional enzymatic dissociation, then washed several times with calcium-free solution. Calcium concentration was gradually brought back to 1 mmol/L. Cells were then cultured on laminin-coated coverslips in M199 culture medium with 5% fetal bovine serum at 37°C. Experiments were performed over the next 2 days. For whole-cell patch recordings, the internal pipette solution contained (in mmol/L): potassium glutamate 120, KCl 25, MgCl$_2$ 0.5, K-EGTA 10, HEPES 10, and MgATP 1 (pH 7.2 with KOH). The external solution included (in mmol/L): NaCl 140, KCl 5, CaCl$_2$ 1, MgCl$_2$ 1, and HEPES 10 (pH 7.4 with NaOH). Whole-cell currents were elicited every 6 seconds from a holding potential of −80 mV by two consecutive steps to −40 mV (for 100 ms) and 0 mV (for 380 ms). Currents at 0 mV were measured 200 ms into the pulse. Endogenous flavoprotein fluorescence was excited with a xenon arc lamp with a band-pass filter centered at 480 nm, but only during the 100-ms step to −40 mV to minimize photobleaching. Emitted fluorescence was recorded at 530 nm by a photomultiplier tube and digitized (Digidata 1200, Axon Instruments). Relative fluorescence was averaged during the excitation window and calibrated with the values after DNP and CN exposure. In some cells, contracture averaging 8 to 10 sequential images having stable mean fluorescence intensities during the exposure to each agent. To localize mitochondria, cells were loaded with 100 nmol/L TMRE, which distributes into negatively charged cellular compartments, for 10 minutes. TMRE fluorescence was excited with the 535-nm line of a helium neon laser and recorded at >605 nm. A pseudocolor palette was applied to visualize the relative increase in mitochondrial flavoprotein oxidation state. Images were analyzed on a personal computer with the software program ImageTool (University of Texas Health Sciences Center in San Antonio). All the recordings were performed at room temperature (21°C to 22°C).

Simulated Ischemia and Cellular Injury

The procedure to determine cell injury was modified from Vander Heide et al. After cell isolation, cells were washed with incubation buffer (in mmol/L): NaCl 119, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.2, KCl 4.8, MgSO$_4$ 1.2, HEPES 10, and glucose 11 and supplemented with creatine, taurine, and amino acids (pH 7.4). Calcium was added into the buffer stepwise (0.25 mmol/L every 5 minutes) to a final concentration of 1 mmol/L. An aliquot of each cell suspension (0.5 mL) was placed into a 0.5-mL microcentrifuge tube and centrifuged for 20 seconds into a pellet. Each pellet occupied a volume of ~0.2 mL. Approximately 0.25 mL of excess supernatant was removed to leave a thin fluid layer above the pellet, and 0.2 mL of mineral oil was layered on the top of the pellet to exclude gaseous diffusion. After 60 minutes and 120 minutes of pelleting, 5 µL of cell pellet was sampled through the oil layer and mixed with 75 µL of 85 mosm hypotonic staining solution (in mmol/L): NaHCO$_3$ 11.9, KH$_2$PO$_4$ 0.4, KCl 2.7, MgSO$_4$ 0.8, and CaCl$_2$ 1 and 0.5% glutaraldehyde, 0.5% trypan blue. Microscopic examination was performed 2 to 5 minutes after mixing to determine the permeability of the cells to trypan blue. Cells permeable to trypan blue were counted as dead and expressed as a percentage of the total cells counted (>200 for each sample). The killing of cells by ischemia was quantified as percentage of the vital cells at the beginning of each experiment (78% to 90% of total, mean=82±1%, n=24). The small percentage of cells (~18%) that were nonviable at the beginning of the experiment were mostly rounded and had been damaged as a known consequence of the enzymatic isolation process. Individual experiments in each group were performed on cells isolated from different hearts. Four groups of experiments were performed. In the control group (Cont), cells were pelleted and sampled at 60 minutes and 120 minutes. For the diazoxide-treated group (DIAZO), 50 µmol/L of diazoxide was added to the solution 15 minutes before the pelleting. In the third group (5-HD), 100 µmol/L of 5-HD was added to the cell suspension 20 minutes before pelleting. Cells in the DIAZO+5-HD group were treated the same as in the third group, except that 50 µmol/L of diazoxide was added to the cell suspension 15 minutes before pelleting. Once applied, drugs were not washed out and thus were present throughout the period of simulated ischemia.

Statistical Analysis

Data are presented as mean±SEM, and the number of cells or experiments is shown as n. ANOVA combined with Tukey’s honestly significant difference post hoc test was used to test for differences among groups for electrophysiological and fluorescence data. Cell pelleting data were analyzed by two-way ANOVA combined with Tukey’s highly significant difference post hoc test. P<0.05 was considered significant.

Results

Figure 1 shows results from simultaneous measurements of flavoprotein fluorescence and membrane K$_{ATP}$ in cells exposed to diazoxide. The periods of drug treatment are marked with horizontal bars. Diazoxide (100 µmol/L) induced irreversible oxidation of the flavoproteins (Figure 1A) but did not activate K$_{ATP}$ (Figure 1B). The redox signal was calibrated by exposing the cells to DNP followed by CN at the end of the experiments. DNP, a protonophore that uncouples respiration from ATP synthesis and collapses the mitochondrial potential, induced maximal oxidation, whereas CN, which inhibits the cytochrome oxidase and thus stops electron transfer, caused complete reduction of the flavoproteins (Figure 1A and 1C). Although membrane currents were unchanged by diazoxide, K$_{ATP}$ eventually turned on after prolonged exposure to DNP (Figure 1B and 1D), indicating that these channels are operable under our experimental conditions despite the inability of diazoxide to open them. Diazoxide 100 µmol/L [DIAZO(1)] reversibly increased mitochondrial...
oxidation to 48±3% of the DNP value (Figure 1E). This oxidation was reproducible, because after washout of the response, a second exposure to diazoxide [DIAZO(2)] in the same cells increased flavoprotein oxidation to 43±5%. 5-HD 100 μmol/L attenuated the oxidative effect of diazoxide by about half [DIAZO+5-HD(100)], whereas 500 μmol/L 5-HD further reduced oxidation to 8±3% [DIAZO+5-HD(500); *P<.01 versus DIAZO(1), DIAZO(2), and DIAZO+5-HD(100) groups]. Treatment with diazoxide and 5-HD did not activate $I_{K_{ATP}}$, whereas prolonged exposure (>6 minutes) to DNP did turn on $I_{K_{ATP}}$ (Figure 1F). The EC$_{50}$ for diazoxide to induce mitochondrial oxidation is 27 μmol/L, as shown in Figure 1G.

We also examined another $K_{ATP}$ channel opener, pinacidil, which opens sarcolemmal $K_{ATP}$ channels and is known to induce pharmacological preconditioning. As shown in Figure 2, pinacidil 100 μmol/L induced 35±8% mitochondrial oxidation, comparable to the effect of diazoxide exposure in the same cell (41±5%). Unlike diazoxide, pinacidil activated sarcolemmal $I_{K_{ATP}}$ (0.74±0.54 nA measured at 0 mV) in addition to inducing flavoprotein oxidation, suggesting that pinacidil activates both mitochondrial and sarcolemmal $K_{ATP}$ channels.

We further localized the subcellular site of diazoxide action by imaging flavoprotein fluorescence (Figure 3A). Fluorescence is low under control conditions, but exposure to diazoxide increased fluorescence in strips parallel to the myofibril orientation. Subsequent exposure to DNP increased fluorescence even further, in a pattern similar to that revealed by diazoxide. CN reduced the fluorescence to the basal level.

**Figure 1.** Diazoxide effect on flavoprotein fluorescence and $I_{K_{ATP}}$. A, Diazoxide (DIAZO) 100 μmol/L induced a reversible increase of mitochondrial oxidation. B, Diazoxide did not activate $I_{K_{ATP}}$. C, 5-HD 500 μmol/L completely blocked oxidative effect of diazoxide. D, Diazoxide and 5-HD did not activate $I_{K_{ATP}}$. E and F, Pooled data for fluorescence and $I_{K_{ATP}}$. DIAZO(1) indicates first exposure to diazoxide; DIAZO+5-HD(100), diazoxide in presence of 100 μmol/L 5-HD; DIAZO+5-HD(500), diazoxide in presence of 500 μmol/L 5-HD; DIAZO(2), second exposure to diazoxide; and DNP, exposure to DNP. Bar indicates periods when cells were exposed to drug. G, Dose-response curve for diazoxide. Each point constitutes measurements from 5 to 6 cells. *$P<.01$ vs DIAZO(1), DIAZO(2), and DNP groups.
Mitochondrial K\(_{\text{ATP}}\) Channels and Preconditioning

![Graph A: Effect of pinacidil and diazoxide on flavoprotein fluorescence and K\(_{\text{ATP}}\) channel activity](image)

The distribution of fluorescence induced by diazoxide and DNP is as expected for mitochondria, which occupy \(\approx 35\%\) of cardiomyocyte volume and are clustered longitudinally between myofibrils. This correspondence was further confirmed by use of TMRE (Figure 3B), which distributes into negatively charged cellular compartments, to localize mitochondrial vesicles or isolated mitochondria and measure potassium flux, Garlid et al. The pattern of TMRE fluorescence was virtually identical to that of the flavoprotein fluorescence induced by diazoxide.

Figure 4 plots the fraction of cells killed by 60 or 120 minutes of ischemia as a percentage of the total number of viable cells before ischemia. Pelleting for 60 minutes and 120 minutes killed \(35\pm2\%\) and \(46\pm4\%\) of cells, respectively, in the controls. However, inclusion of 50 \(\mu\)mol/L diazoxide significantly decreased cell death during simulated ischemia to about half of that in the controls (18\% after 60 minutes and 23\% after 120 minutes, \(P<0.01\) versus control). The protection by diazoxide was completely blocked by 100 \(\mu\)mol/L 5-HD (31\% after 60 minutes and 41\% after 120 minutes). 5-HD alone did not significantly alter the percentage of cells killed by simulated ischemia: 31\% after 60 minutes and 47\% after 120 minutes. Glibenclamide 1 \(\mu\)mol/L also blocked the protection from diazoxide (data not shown). Diazoxide at 100 \(\mu\)mol/L had a similar protective effect (data not shown). For each experiment, there was always an isochronal nonischemic group in which cells were not pelleted. In these groups, \(<5\%\) of trypan blue-resistant cells became permeable to trypan blue during the 2-hour experiments.

**Discussion**

Although much evidence demonstrates the cardioprotective effects of K\(_{\text{ATP}}\) channels and their involvement in ischemic preconditioning, the underlying mechanisms for such protection are poorly understood. One of the early hypotheses proposed that opening of sarcolemmal K\(_{\text{ATP}}\) channels shortens the action potential duration. By a cardioplegic effect, energy consumption and calcium overload would be attenuated during ischemia. Although preconditioning has been shown to accelerate action potential shortening slightly during lethal ischemia, several recent studies indicate that abbreviation of action potential duration may not be necessary for the protection from preconditioning and K\(_{\text{ATP}}\) channel openers. Grover et al. showed that doxifluridine, a class III antiarrhythmic agent, abolished the action potential shortening during ischemia but did not abolish ischemic preconditioning in dogs. Yao and Gross found that bimakalim, a K\(_{\text{ATP}}\) channel opener, had minimal effect on action potential duration but still reduced infarction. Such dissociation has also been shown in several other studies. Furthermore, K\(_{\text{ATP}}\) channel openers and ischemic preconditioning are protective even in models using unstimulated cardiac myocytes. Because adult ventricular myocytes are electrically quiescent in these models, action potential duration shortening should not be a factor. These experimental results challenge the idea that the protective effect of K\(_{\text{ATP}}\) channels is targeted to sarcolemmal K\(_{\text{ATP}}\) channels.

Cardiac myocytes and other cells have another type of K\(_{\text{ATP}}\) channel. Inoue et al. were the first to demonstrate the existence of K\(_{\text{ATP}}\) channels in the inner mitochondrial membrane by patch clamping mitoplasts prepared from rat liver mitochondria. Later on, a fraction containing mitochondrial K\(_{\text{ATP}}\) channel activity was purified from the inner membranes of rat liver and beef heart mitochondria. Using reconstituted mitochondrial vesicles or isolated mitochondria and measuring potassium flux, Garlid et al. demonstrated that heart and liver mitochondrial K\(_{\text{ATP}}\) channels share some pharmacological properties with the channels found in sarcolemma while possessing a distinct profile. The outstanding pharmacological signature of mitochondrial channels is their high sensitivity to opening by diazoxide, which exceeds the sensitivity of sarcolemmal channels 2000-fold. To study the selectivity of diazoxide in intact cells, we simultaneously measured endogenous flavoprotein fluorescence and sarcolemmal K\(_{\text{ATP}}\) currents by whole-cell patch clamp. Although the physiological and pathophysiological roles of the mitochondrial K\(_{\text{ATP}}\) channel are not yet very clear, opening of mitochondrial K\(_{\text{ATP}}\) channels dissipates the inner mitochondrial membrane potential established by the proton pump. This dissipation accelerates electron transfer by the respiratory chain and if uncompensated by increased production of electron donors (such as NADH), leads to net oxidation of the mitochondria.
Mitochondrial redox state can be monitored by recording the fluorescence of FAD-linked enzymes in the mitochondria. Our data show that diazoxide reversibly oxidizes the mitochondrial matrix, as would be expected if it opens the mitochondrial K<sub>ATP</sub> channel. Diazoxide had no effect on sarcolemmal K<sub>ATP</sub> channels. This insensitivity is consistent with the phenotype of the cardiac sarcolemmal isoform of K<sub>ATP</sub> channels. Considering the diffusion barriers between extracellularly applied diazoxide and the mitochondria and other differences in the experimental conditions, our value of 27 µmol/L for the EC<sub>50</sub> of diazoxide induction of mitochondrial oxidation is not inconsistent with the EC<sub>50</sub> of ≈3 µmol/L for enhanced potassium flux in isolated mitochondria. We found that pinacidil, another K<sub>ATP</sub> channel opener, is also capable of causing mitochondrial oxidation. This indicates that the ability of K<sub>ATP</sub> channel openers to induce mitochondrial oxidation may be a general property of such drugs. Nevertheless, pinacidil in addition activates K<sub>ATP</sub> channels in the surface membrane, whereas diazoxide can specifically open K<sub>ATP</sub> channels in mitochondria without turning on sarcolemmal I<sub>K,ATP</sub>. This finding is consistent with the known pharmacology of various K<sub>ATP</sub> channel agonists, which indicates that diazoxide is unique in its selectivity for mitochondrial K<sub>ATP</sub> channels.

The specificity of diazoxide for mitochondrial K<sub>ATP</sub> channels is further supported by the fact that 5-HD, which has been shown to inhibit K<sub>ATP</sub> channels in sarcolemma and isolated mitochondria, reversibly blocked the flavoprotein oxidation induced by diazoxide (Figure 1C). Although Notsu et al showed that 5-HD blocked action potential shortening and K<sub>ATP</sub> channel openings induced by metabolic inhibition in guinea pig ventricular myocytes, McCullough et al did not resolve any effect of 5-HD on cromakalim-activated sarcolemmal I<sub>K,ATP</sub>. 5-HD is widely used to block ischemic preconditioning and cardioprotection induced by K<sub>ATP</sub> chan-
nel openers. Results from this study as well as others show that 5-HD is an effective blocker of mitochondrial K<sub>ATP</sub> channels. The possibility that 5-HD is selective for mitochondrial K<sub>ATP</sub> channels merits further investigation. We also tested another K<sub>ATP</sub> channel inhibitor, glibenclamide. We did not observe consistent blockade of mitochondrial oxidation, probably because glibenclamide alone caused oxidation of the flavoproteins especially at concentrations >1 μmol/L (data not shown). This is consistent with the finding that glibenclamide uncouples mitochondria with a K<sub>d</sub> of 4 μmol/L. Similarly, high concentrations of glibenclamide have been shown to affect the function of isolated mitochondria nonspecifically. Therefore, we caution against the interpretation at face value of studies using glibenclamide to test the involvement of K<sub>ATP</sub> channels in cardioprotection.

To test the idea that mitochondrial K<sub>ATP</sub> channels may play a role in cardioprotection, we examined the effect of diazoxide in a cellular ischemia model. Cells were centrifuged into a pellet to simulate the restricted extracellular space and reduced oxygen supply during ischemia, sampled at designated time points and stained with a hypotonic (85 mosm) trypan blue solution to test the osmotic fragility of the membrane. Previous studies have shown that simulated ischemia preconditions myocytes in this model and that the underlying mechanisms for the protection are similar to those in intact hearts. Our results demonstrated that diazoxide treatment protects rabbit ventricular myocytes to the same extent as preconditioning in our previously published results. Interestingly, a cardioprotective EC<sub>50</sub> of 11 μmol/L diazoxide has been reported in intact hearts. This concentration corresponds closely to that which we observed to induce flavoprotein oxidation (Figure 1G). Using the same cellular model as in the present study, Armstrong et al showed that pinacidil afforded protection. Notably, if pinacidil was only added into the cell pellet without preincubation, there was no protection. In the present study, diazoxide was added before pelleting and was present during the simulated ischemia. Further studies are required to dissect the time course of the cardioprotective effect of diazoxide.

We have previously shown that adenosine and protein kinase C can synergistically activate sarcolemmal I<sub>K<sub>ATP</sub></sub>. It will be very interesting to investigate whether adenosine and protein kinase C have similar effects on mitochondrial K<sub>ATP</sub> channels. Our preliminary unpublished results suggest that protein kinase C activation can indeed augment diazoxide-induced flavoprotein fluorescence. A detailed study in this area is currently in progress in the laboratory.

Because of the lack of a single-cell model of ischemic preconditioning, we were unable to investigate the involvement of mitochondrial K<sub>ATP</sub> channels in ischemic preconditioning in the present experimental system. Important differences are known to exist between ischemic preconditioning and the cardioprotective effects of K<sub>ATP</sub> channel openers in terms of efficacy and memory. Nevertheless, the protection from ischemic preconditioning and K<sub>ATP</sub> channel openers is blocked by glibenclamide and 5-HD. Thus, although it is reasonable to propose that mitochondrial K<sub>ATP</sub> is the target for both, further studies are warranted to bolster the links between pharmacological and genuine ischemic preconditioning.

Our results demonstrate that diazoxide targets only mitochondrial K<sub>ATP</sub> channels but not sarcolemmal K<sub>ATP</sub> channels and suggest that mitochondrial K<sub>ATP</sub> channels may serve as effectors of cardioprotection by K<sub>ATP</sub> channel openers. The question remains as to how opening of mitochondrial K<sub>ATP</sub> channels might protect myocytes against ischemic damage. One possibility is that dissipation of mitochondrial membrane potential decreases the driving force for calcium influx through the calcium uniporter. Inhibition of the mitochondrial calcium uniporter by ruthenium red protects hearts against ischemia and reperfusion injury. Consistent with this hypothesis. Another possibility is that opening of mitochondrial K<sub>ATP</sub> channels, by decreasing the membrane potential, could promote the binding of the endogenous mitochondrial ATPase inhibitor I<sub>P</sub> and thus conserve ATP during ischemia. Finally, a change of mitochondrial membrane potential could alter glycolytic pathways during ischemia in favor of myocyte survival. Further studies on mitochondrial K<sub>ATP</sub> channels will help us not only to dissect the mechanism of cardioprotection from K<sub>ATP</sub> channels and ischemic preconditioning but also to understand the pathogenesis of ischemic and reperfusion injury.

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