CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

DIAZOXIDE PROTECTS MITOCHONDRIA FROM ANOXIC INJURY: IMPLICATIONS FOR MYOPRESERVATION

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Background: Heart muscle primarily relies on adenosine triphosphate produced by oxidative phosphorylation and is highly vulnerable to anoxic insult. Although a number of strategies aimed at improving myopreservation are available, no effective means of preserving mitochondrial energetics under conditions of anoxic injury have been developed. Openers of mitochondrial adenosine triphosphate-sensitive potassium channels have emerged as powerful cardioprotective agents presumably capable of maintaining mitochondrial function under metabolic stress. Here, we evaluated the ability of a prototype mitochondrial adenosine triphosphate-sensitive potassium channel opener, diazoxide, to preserve oxidative phosphorylation in mitochondria subjected to anoxia and reoxygenation.

Methods: Mitochondria were isolated from rat hearts and subjected to 20 minutes of anoxia, followed by reoxygenation. Mitochondrial respiration and oxidative phosphorylation, as well as mitochondrial integrity, were assessed by means of ion-selective minielectrodes, high-performance liquid chromatography, fluorometry, and electron microscopy.

Results: Anoxia-reoxygenation decreased the rate of adenosine diphosphate-stimulated oxygen consumption, inhibited adenosine triphosphate production, and disrupted mitochondrial integrity. On average, anoxic stress reduced adenosine diphosphate-stimulated respiration from 291 ± 14 to 141 ± 15 ng-atoms $O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein and decreased the rate of adenosine triphosphate production from 752 ± 14 to 414 ± 34 nmol adenosine triphosphate $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. After anoxia, the majority (88%) of mitochondria was damaged or swollen and released adenylate kinase, a marker of mitochondrial integrity. Diazoxide (100 $\mu\text{mol/L}$), present throughout anoxia, preserved adenosine diphosphate-stimulated respiration at 255 ± 7 ng-atoms $O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein and adenosine triphosphate production at 640 ± 39 nmol adenosine triphosphate $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. Diazoxide also protected mitochondrial structure from anoxia-mediated damage, so that after anoxic stress, 67% of mitochondria remained intact and adenylate kinase was confined to the mitochondria.

Conclusions: The present study demonstrates that diazoxide diminishes anoxia-induced functional and structural deterioration of cardiac mitochondria.

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dria. By protecting mitochondria and preserving myocardial energetics, diazoxide may be useful under conditions of reduced oxygen availability, including global surgical ischemia or storage of donor heart. (J Thorac Cardiovasc Surg 2001;121:298-306)

The inherent vulnerability of cardiomyocytes to oxygen deprivation and metabolic stress contributes to myocardial dysfunction in cardiac operations and cardiac transplantation.¹ Optimal myocardial function requires adequate supply of intracellular adenosine triphosphate (ATP), which is generated primarily by mitochondria through oxidative phosphorylation.² Conditions associated with decreased oxygen supply, as observed in global surgical ischemia or ex vivo storage of donor heart, can severely compromise mitochondrial energetics and precipitate cell injury.³⁻⁵ Although a number of strategies aimed at improving cardiopreservation have been proposed,^{1,6-10} no effective means of preserving mitochondrial ATP production under conditions of anoxic injury have been developed.

Pretreatment with mitochondrial ATP-sensitive potassium (K_{ATP}) channel openers has recently emerged as a promising approach in preserving myocardial function in the surgically relevant setting of prolonged ischemia.¹¹ The prototype mitochondrial K_{ATP} channel opener diazoxide has been found to reduce infarct size in the whole heart, protect right atrial myocardium, and enhance survival of single cardiomyocytes.¹²⁻¹⁴ However, it remains controversial whether potassium channel openers can maintain mitochondrial ATP synthesis under conditions of metabolic stress.¹⁵⁻¹⁹

Here we provide direct evidence that diazoxide maintains the functional and structural integrity of isolated cardiac mitochondria exposed to anoxia-reoxygenation. By targeting mitochondria and preserving oxidative ATP synthesis, diazoxide-like agents would preserve bioenergetics and thereby provide added protection in cardiac operations.

Methods

The investigation conformed to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996, and was approved by the Institutional Animal Care and Use Committee.

Isolation of mitochondria. Mitochondria were isolated from hearts of sodium pentobarbital-anesthetized (100 mg/kg administered intraperitoneally) rats (Harlan Sprague-Dawley, Indianapolis, Ind), as previously described.^{15,20} In brief, ventricles were removed into an ice-cold buffer (sucrose, 50

mmol/L; mannitol, 200 mmol/L; KH_2PO_4 , 5 mmol/L; ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid, 1 mmol/L; 3(N-Morpholino)propanesulfonic acid (MOPS), 5 mmol/L [pH 7.3]; and 0.2% bovine serum albumin) and homogenized (PT 10/35 Polytron; Brinkman Instruments, Westbury, NY), and mitochondria were separated by means of centrifugation (Sorvall RCSC; Kendro Laboratory Products, Newtown, Conn). To increase mitochondrial yield, the obtained pellet was rehomogenized (Potter-Elvehjem homogenizer) and centrifuged. Resulting supernatants were centrifuged, and mitochondria were washed in isolation buffer (without ethyleneglycol-bis-[β -aminoethylether]-N,N,N',N'-tetraacetic acid and bovine serum albumin) and kept on ice. Protein concentration was determined with a DC protein kit (Bio-Rad, Hercules, Calif).

Anoxia-reoxygenation protocol. Mitochondria (2 mg of protein) were added into an air-tight, closed, multichannel chamber "ESON-6CH" filled with 1 mL of incubation medium (KCl , 110 mmol/L; KH_2PO_4 , 5 mmol/L; pyruvate, 2.5 mmol/L; malate, 2.5 mmol/L; and MOPS, 10 mmol/L [pH 7.3]) and continuously stirred at 30°C.^{15,20} Anoxia was reached within 5 minutes as mitochondria consumed all available oxygen in the chamber. Throughout the anoxic interval (20 minutes), the oxygen level was at zero within the closed chamber containing the mitochondrial suspension. After the 20-minute anoxic period, mitochondrial suspension was exposed to room air to achieve reoxygenation.²¹

Mitochondrial oxygen consumption and membrane potential. Mitochondrial respiration and membrane potential were continuously monitored by means of oxygen and tetraphenylphosphonium-selective minielectrodes,^{15,20} and data were acquired and processed by use of the Bioquest software.²² Mitochondrial respiration was determined in the absence (state 2, V_2) or presence (state 3, V_3) of 250 μ mol/L adenosine diphosphate (ADP). The maximal respiratory capacity of mitochondria was determined after a challenge with 50 μ mol/L 2,4-dinitrophenol (DNP), a mitochondrial uncoupler. Mitochondrial membrane potential was calculated according to the following equation:

$$\Delta\Psi = 59\log(v/V) - 59\log(10^{(E-E_0)/59} - 1)$$

in which $\Delta\Psi$ is membrane potential (in millivolts), v is mitochondrial matrix volume (1.6 μ L/mg mitochondrial protein), V is volume of incubation medium (1 mL), and E_0 and E are electrode potentials before and after addition of mitochondria, respectively.

ATP levels. ATP levels were measured in K_2CO_3 -MOPS-neutralized $HClO_4$ -soluble mitochondrial extracts by means of high-pressure liquid chromatography (Hewlett

Fig 1. Anoxia impairs ADP-initiated oxidative phosphorylation in isolated cardiac mitochondria. Mitochondria (2 mg of protein) were stirred in an airtight close chamber (1 mL), and the concentration of oxygen within the suspension and the membrane potential of mitochondria were monitored simultaneously in control conditions (*left panel*) or after 20 minutes of anoxia in reoxygenation (*right panel*). In control mitochondria addition of 250 $\mu\text{mol/L}$ ADP vigorously activated respiration, which induced a rapid decline in oxygen concentration (*upper left*). In reoxygenation, after anoxia, 250 $\mu\text{mol/L}$ ADP produced only marginal acceleration of oxygen consumption (*upper right*). ADP-stimulated membrane depolarization was significantly attenuated in anoxic-reoxygenated mitochondria (*lower right*) compared with control mitochondria (*lower left*).

Packard, Waldbronn, Germany), as previously described.²³ In addition, the kinetics of ADP to ATP conversion were monitored in mitochondrial suspension as changes in reduced nicotinamide adenine dinucleotide phosphate fluorescence in a coupled hexokinase/glucose-6-phosphate dehydrogenase ATP assay by a multiplate reader (Ascent FL; Scientific Resources, St Paul, Minn).

Enzyme activity. Mitochondrial suspension was centrifuged, and release of adenylate kinase was monitored in the supernatant and pellet. Adenylate kinase activity was measured with a multiplate reader (Ascent FL) in 100 mmol/L potassium acetate, 20 mmol/L α -hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH 7.5), 20 mmol/L glucose, 4 mmol/L MgCl_2 , 2 mmol/L nicotinamide adenine dinucleotide phosphate, 1 mmol/L ethylenediamine tetraacetic acid, 1 mmol/L dithiothreitol, 4.5 U/mL hexokinase, and 2 U/mL glucose-6-phosphate dehydrogenase, and the reaction was initiated with 2 mmol/L ADP.²³

Electron microscopy. Mitochondria were fixed with Trump buffer (1% glutaraldehyde, 4% formaldehyde, and 0.1 mol/L phosphate buffer [pH 7.2]), rinsed with phosphate-buffered sucrose, and postfixed in phosphate-buffered 1% osmium tetroxide. Samples were en bloc stained with 2% uranyl acetate for 30 minutes at 60°C, rinsed, dehydrated, and embedded in Spurr resin. Twelve thin sections for each sample were cut on an Ultracut E ultramicrotome (Reichert-Jung, Wien, Austria), placed on copper grids, and stained with lead citrate. Isolated mitochondria were micrographed with a JEOL EM-1200 EX II electron microscope (JEOL USA, Inc, Peabody, Mass), and greater than 500 mitochondria were examined under each experimental condition. Mitochondrial structure was defined as intact,

swollen, or damaged. Intact mitochondria were defined as those with uninterrupted outer and inner membranes, thin intermembrane space, and regular cristae enfolding into a compact matrix. Swollen mitochondria were defined as distended mitochondria with increased intermembrane space and swollen cristae. Damaged mitochondria were defined as those with damaged outer and inner membranes, disrupted matrices, and amorphous cristae.

Drugs. Diazoxide was purchased from Research Biochemicals International (Natick, Mass) and dissolved as a concentrated stock solution in dimethyl sulfoxide (DMSO). The maximal concentration of DMSO within the incubation medium was kept under 0.5%, and control experiments were performed with corresponding DMSO concentrations. All other chemicals were from Sigma Chemical Company (St Louis, Mo).

Statistical analysis. Data are presented as means $\pm$ SEM, and n represents the number of mitochondrial isolations used for functional study or the actual number of imaged mitochondria in electron micrographs. Group comparisons were performed by analysis of variance with the Student-Newman-Keuls post hoc correction procedure.

Results

Anoxia reduces oxidative phosphorylation. Oxidative phosphorylation was assessed by monitoring ADP-stimulated changes in the rate of mitochondrial oxygen consumption and membrane potential before and after anoxia-reoxygenation (Fig 1). Under normoxic conditions (control), the rate of oxygen con-

Fig 2. Diazoxide preserves ADP-stimulated respiration in postanoxic mitochondria. **A**, Changes in oxygen concentration induced by 250 $\mu\text{mol/L}$ ADP added after 20 minutes of anoxia in the absence ($- \text{Diaz}$) or presence ($+ \text{Diaz}$) of 100 $\mu\text{mol/L}$ diazoxide and compared with control values. **B**, Diazoxide (100 $\mu\text{mol/L}$) maintained the rate of ADP- and DNP-stimulated respiration in postanoxic mitochondria to levels similar to those measured before anoxia. **C**, ADP-induced mitochondrial membrane depolarization in postanoxic mitochondria in the absence ($- \text{Diaz}$) and presence ($+ \text{Diaz}$) of 100 $\mu\text{mol/L}$ diazoxide. Mitochondrial depolarization was blunted and more prolonged in the absence than in the presence of diazoxide. **D**, Diazoxide (100 $\mu\text{mol/L}$) maintained the amplitude and duration of ADP-induced mitochondrial membrane depolarization in postanoxic mitochondria to levels similar to those measured before anoxia. In **B** and **D**, asterisks indicate significant difference from values obtained in control conditions ($P < .05$), whereas **C** indicates control (normoxic) conditions.

sumption (state 2 respiration) was 31 ± 3 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein (Fig 1, left panel). Addition of 250 $\mu\text{mol/L}$ ADP, the substrate of oxidative phosphorylation, increased oxygen consumption (state 3 respiration) to 291 ± 14 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein ($n = 10$; Fig 1, left panel). In normoxia mitochondrial membrane potential at steady-state was -192 ± 2 mV and reversibly depolarized (by up to 8 ± 3 mV) on addition of ADP (250 $\mu\text{mol/L}$), reflecting ADP to ATP conversion (Fig 1, left panel). On average, ADP stimulated the rate of mitochondrial respiration by 838% and caused transient depolarization lasting 36 ± 2 seconds ($n = 10$). This indicates a vigorous capacity of normoxic mitochondria to phosphorylate added ADP.

After 20 minutes of anoxia followed by reoxygenation, the rate of oxygen consumption was 57 ± 4 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein and increased to 141 ± 15 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ on addition of 250 $\mu\text{mol/L}$ ADP (Fig 1, right panel). Thus anoxia-reoxygenation induces a 52% decrease ($P < .05$) in ADP-stimulated respiration when compared with normoxic mitochondria ($n = 10$). At the end of the 20-minute period of anoxia, the mitochondrial membrane potential was -170 ± 1 mV and returned to -190 ± 3 mV on reoxygenation ($n = 10$; Fig 1, right panel). ADP-stimulated membrane depolarization was significantly attenuated in anoxic-reoxygenated mitochondria (3 ± 1 mV, $P < .05$), and the duration of depolarization was significantly prolonged (128 ± 11 seconds,

Fig 3. Diazoxide preserves ATP production in anoxic mitochondria. **A** and **B**, Kinetics of ATP generation in control, anoxic, and diazoxide-treated anoxic mitochondria. **C**, Chromatograms showing nucleotide profiles in anoxic mitochondria in the absence (*- Diaz*) or presence (*+ Diaz*) of 100 $\mu\text{mol/L}$ diazoxide. ATP was higher and ADP was lower in diazoxide-treated anoxic mitochondria compared with untreated anoxic mitochondria. **D**, Average rates of ATP production. ATP production was measured from changes in reduced nicotinamide adenine dinucleotide phosphate (NADPH) fluorescence in a coupled enzyme assay (**A** and **B**) or with high-performance liquid chromatography (**C** and **D**). In **B** and **D**, asterisks indicate significant difference from values obtained in control conditions ($P < .05$), whereas **C** indicates control (normoxic) conditions.

$P < .05$) compared with that of normoxic mitochondria (Fig 1). This indicates an impaired capacity of mitochondria to phosphorylate added ADP after an anoxia-reoxygenation insult.

Diazoxide preserves oxidative phosphorylation in anoxic mitochondria. In contrast to mitochondria exposed to anoxic stress in the absence of a potassium channel opener, those treated with diazoxide throughout the anoxic insult maintained a vigorous respiratory response to ADP (250 $\mu\text{mol/L}$) after reoxygenation (Fig

2, A). Under these experimental conditions, the apparent median effective concentration was 42 $\mu\text{mol/L}$, with the maximal effect observed at 100 $\mu\text{mol/L}$ diazoxide. In 100 $\mu\text{mol/L}$ diazoxide-treated mitochondria, ADP-stimulated respiration after anoxia was 255 ± 7 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein ($n = 10$), a value significantly higher than that obtained in the absence of diazoxide ($P < .05$) and similar to that of normoxic mitochondria ($P > .05$; Fig 2, B). Similarly, mitochondria treated with diazoxide (100 $\mu\text{mol/L}$) also preserved

Fig 4. Diazoxide preserves the structural integrity of anoxic mitochondria. Transmission electron micrographs of preanoxic (A), postanoxic (B), and diazoxide-treated postanoxic (C) mitochondria. (Original magnification 10,000x.) Mitochondria were defined as intact (I), swollen (S), or damaged (D). The majority of preanoxic mitochondria were intact, which was in contrast to damaged postanoxic mitochondria. Diazoxide (100 $\mu\text{mol/L}$) preserved the structural integrity of postanoxic mitochondria.

their maximal rate of respiration after anoxia-reoxygenation, as determined by the rate of uncoupled oxygen consumption in the presence of 50 $\mu\text{mol/L}$ DNP (Fig 2, B). DNP-stimulated respiration was 343 ± 8 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein in diazoxide-treated mitochondria compared with 359 ± 27 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein for normoxic mitochondria ($P > .05$) and 190 ± 19 ng-atoms $\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein for anoxic mitochondria in the absence of diazoxide ($P < .05$, $n = 10$; Fig 2, B). Treatment with diazoxide (100 $\mu\text{mol/L}$) also preserved ADP-stimulated membrane depolarization of anoxic mitochondria both in terms of amplitude (Fig 2, C) and duration (Fig 2, D). Thus pretreatment with diazoxide reduced the decline in ADP-stimulated respiration and associated membrane depolarization in postanoxic mitochondria, suggesting opener-mediated preservation of the oxidative phosphorylation capacity from anoxic insult.

Diazoxide preserves ATP production in anoxic mitochondria. Monitoring of the kinetics of ATP generation indicated that anoxia produced significant depression in the rate of oxidative phosphorylation (Fig 3, A). On average, anoxic stress halved the rate of mitochondrial ATP generation from 197 ± 11 U $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ before to 97 ± 11 U $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein after anoxia ($n = 9$, $P < .05$; Fig 3, B). Diazoxide (100 $\mu\text{mol/L}$) attenuated anoxia-induced decrease in the rate of oxidative phosphorylation and maintained mitochon-

Fig 5. Diazoxide decreases leakage of a mitochondrial protein from anoxic mitochondria. Adenylate kinase activity was measured in mitochondria (Pellet) and in the extramitochondrial milieu (Super). Anoxia releases adenylate kinase from mitochondria when compared with control values. Diazoxide (Diaz; 100 $\mu\text{mol/L}$) prevents redistribution of adenylate kinase, maintaining adenylate kinase in mitochondria.

drial ATP generation at 139 ± 9 U $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein ($n = 9$; Fig 3, A and B). Consequently, the nucleotide profile of anoxic mitochondrial preparations was shifted from high ADP and low ATP levels in the absence of diazoxide (Fig 3, C, upper trace) to low ADP and high ATP levels in the presence of 100 $\mu\text{mol/L}$ diazoxide (Fig 3, C, lower trace). Accordingly, the rate of ATP

synthesis was 640 ± 39 nmol ATP $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein in diazoxide-treated anoxic mitochondria compared with 752 ± 14 nmol ATP $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein for normoxic mitochondria ($P > .05$) and 414 ± 34 nmol ATP $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein for anoxic ($P < .05$) mitochondria in the absence of diazoxide, respectively ($n = 5$; Fig 3, D). Thus diazoxide prevents the decline in ATP synthesis in postanoxic mitochondria.

Diazoxide preserves the structural integrity of anoxic mitochondria. In normoxia 83% of isolated mitochondria displayed intact morphology, with 17% being swollen or damaged ($n = 504$; Fig 4, A). On reoxygenation and after 20 minutes of anoxia, 88% of mitochondria were damaged or swollen, with only 12% remaining intact ($n = 506$; Fig 4, B). Diazoxide (100 μ mol/L) preserved mitochondrial structure, and after anoxia-reoxygenation, 67% of mitochondria remained intact ($n = 509$; Fig 4, C). Under normoxia, adenylate kinase activity, a marker of mitochondrial integrity, was predominantly confined within mitochondria (1.23 ± 0.001 and 0.74 ± 0.008 mU $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein in the mitochondrial pellet and supernatant, respectively). After 20 minutes of anoxia, on reoxygenation, adenylate kinase was released from mitochondria (0.65 ± 0.003 and 1.38 ± 0.005 mU $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein in the mitochondrial pellet and supernatant, respectively), indicating anoxia-induced damage to mitochondria. Diazoxide (100 μ mol/L) prevented release of adenylate kinase from mitochondria (1.38 ± 0.005 and 0.74 ± 0.006 mU $\cdot$ min⁻¹ $\cdot$ mg⁻¹ protein in the mitochondrial pellet and supernatant, respectively; Fig 5). Thus, diazoxide preserves mitochondrial structural integrity and prevents release of mitochondrial proteins after anoxic stress.

Discussion

The present study demonstrates that cardiac mitochondria can be protected from anoxic injury by the mitochondrial potassium channel opener diazoxide. Anoxic stress had a profound disruptive effect on mitochondrial structure and severely impaired oxidative phosphorylation. However, treatment of mitochondria with diazoxide during the anoxic period effectively preserved the structural and functional integrity of mitochondria, maintaining ATP generation after anoxic insult. By protecting myocardial energetics under conditions of reduced oxygen availability, diazoxide may prove useful for improved intraoperative myocardial protection and general myopreservation.

Mitochondrial dysfunction during global surgical ischemia or cardiac storage for transplantation has been associated with compromised myocardial viability and impaired contractility.^{1,6,11,24,25} In this regard, mito-

chondrial structural and functional parameters have been used to assess the protective efficacy of cardioplegic solutions,²⁶⁻²⁹ hypothermia,³⁰⁻³² and/or assist devices for hemodynamic support.³³ Here we evaluated the protective efficacy of a prototype mitochondrial potassium channel opener on isolated mitochondria by using a multiparametric approach that included measurements of ADP-stimulated respiration, membrane potential, ATP generation, adenylate kinase release, and morphologic integrity. This is of importance because mitochondrial ATP synthesis requires an intact inner mitochondrial membrane and a maintained electrochemical gradient supported by coupled respiration.² Dissipation of the electrochemical gradient and uncoupling of respiration caused by disruption of the inner mitochondrial membrane precipitates mitochondrial dysfunction with arrest of ATP production.^{2,3} Diazoxide was consistently effective in reducing anoxia-induced structural damage of cardiac mitochondria and preserving mitochondrial oxidative phosphorylation, indicating a generalized protection of the organelle from anoxic injury.

Diazoxide is an established member of the potassium channel opener family.¹² This class of agents has been recognized for its powerful cardioprotective properties and potential beneficial role as adjuncts to cardioplegia.^{1,6,7,9,10,34,35} The primary target of potassium channel opener action is believed to be the K_{ATP} channel, a major metabolic sensor responsible for coupling cellular energetics with membrane excitability.³⁶ Overexpression of recombinant K_{ATP} channel subunits or pharmacologic stimulation promotes cytoprotection,^{37,38} whereas genetic disruption or inhibition of this ion conductance precipitates injury during metabolic stress.^{39,40} In addition to the plasmalemmal K_{ATP} channel, a subcellular target site for potassium channel openers has more recently been recognized and associated with the inner mitochondrial membrane.⁴¹ This putative mitochondrial K_{ATP} channel is particularly sensitive to stimulation by diazoxide and has been implicated in cardioprotection and ischemic preconditioning.^{11-14,20,42,43} Although the protective effect of diazoxide during ischemia and hypoxia has been so far primarily evaluated in the whole heart,¹² atrial trabeculae,¹³ or single cardiomyocytes,¹⁴ the present study provides direct evidence for a beneficial property of this agent at the mitochondrial level itself. This is of significance because it identifies mitochondria as the target for diazoxide action without the confounding contribution of cytoplasmic factors, such as nucleotides and protein kinase C, which have been implicated in modulating the outcome of opener action.^{17,44,45}

The molecular mechanism responsible for the protective effect of diazoxide on oxidative phosphorylation and the integrity of anoxic mitochondria remains unknown. In normal mitochondria it has been assumed that opening of mitochondrial potassium channels promotes K^+ influx dissipating the electrochemical H^+ gradient and reducing the driving force for ATP synthesis.¹⁵ However, on anoxia and reoxygenation, mitochondrial Ca^{2+} overload and excessive generation of superoxide anions induces mitochondrial deterioration disrupting the inner membrane and reducing oxidative phosphorylation.^{46,47} By preventing mitochondrial Ca^{2+} loading²⁰ and reducing the generation of reactive oxygen species,⁴⁸ potassium channel openers could maintain the structural and functional integrity of cardiac mitochondria under anoxic conditions. In this way diazoxide-mediated protection of anoxic mitochondria may contribute to improved cardiac bioenergetics and increased myocardial resistance under conditions of reduced oxygen supply.

In conclusion, this is a first report identifying the use of a mitochondrial potassium channel opener as an effective means in protecting mitochondrial function and sustaining ATP production after anoxic stress. It should be pointed out that the present study was conducted under conditions of simulated anoxia in isolated mitochondria suspended in experimental medium. In view of the potential implications of the present findings toward a safer cardioplegia and improved myopreservation, the therapeutic profile of potassium channel openers needs to be further tested in the whole heart in the setting of global surgical ischemia, heart storage, or both. For a potassium channel opener to be considered as an adjunct to cardioplegia, rigorous comparative studies with other cardioprotective agents are warranted.

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