Diazoxide Opens the Mitochondrial Permeability Transition Pore and Alters Ca^{2+} Transients in Rat Ventricular Myocytes

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Background—The mitochondrial K\textsubscript{ATP} channel (mitoK\textsubscript{ATP}) has been implicated as an end effector or trigger of ischemic preconditioning (IP). Although a mitoK\textsubscript{ATP} opener, diazoxide, mimics IP, mechanisms for the cardioprotective action remain unclear.

Methods and Results—We measured Ca\textsuperscript{2+} transients (CaTs) and mitochondrial inner membrane potential (\(\Delta\psi\textsubscript{im}\)) with confocal microscopy and the fluorescent probes fluo-4 and tetramethylrhodamine ethyl ester perchlorate in rat ventricular myocytes. Diazoxide increased the amplitudes and diastolic levels of CaTs dose dependently. The effects of diazoxide on CaTs were inhibited by the mitoK\textsubscript{ATP} antagonist sodium 5-hydroxydecanoic acid (100 \(\mu\text{mol/L}\)), whereas application of diazoxide caused little change in \(\Delta\psi\textsubscript{im}\). After sarcoplasmic reticulum function was disabled with ryanodine and thapsigargin, the effects of diazoxide on CaTs were still observed. The opening of the mitochondrial permeability transition pore was monitored with fluorescent calcein. Diazoxide accelerated the leakage of calcein from mitochondrial matrix (16% of control; \(P<0.05\)), and this effect was inhibited by cyclosporin A (2 \(\mu\text{mol/L}\)). Cyclosporin A also abolished the effects of diazoxide on CaTs. Diazoxide oxidized flavoprotein fluorescence reversibly, and this effect was partially blunted by cyclosporin A (by 24%; \(P<0.05\)).

Conclusions—We conclude that in rat ventricular myocytes, diazoxide modulates the opening of the mitochondrial permeability transition pore, resulting in an increase in CaTs independent of the changes in \(\Delta\psi\textsubscript{im}\). The action of diazoxide on the mitochondrial permeability transition pore also affects the mitochondrial redox state. (Circulation. 2002;105:2666-2671.)

Key Words: myocytes ■ ischemia ■ calcium

Ischemic preconditioning (IP) is a phenomenon in which transient nonlethal periods of ischemia increase the resistance to a subsequent prolonged ischemic period.\textsuperscript{1} Various substances and signaling pathways could be involved in IP, including sarcoplasmal and mitochondrial ATP-sensitive potassium channels (sarcoK\textsubscript{ATP} and mitoK\textsubscript{ATP}).\textsuperscript{2} Emerging evidence has suggested the contribution of mitoK\textsubscript{ATP} as an end effector or trigger of IP.\textsuperscript{3-6} Although it has been demonstrated that diazoxide, a selective mitoK\textsubscript{ATP} opener,\textsuperscript{5,7} mimicked the effects of IP and that sodium 5-hydroxydecanoic acid (5-HD), a mitoK\textsubscript{ATP} channel inhibitor, abolished the protective action of IP and diazoxide,\textsuperscript{2,3,5} the precise mechanisms for the cardioprotective action of diazoxide remain elusive. It has been speculated that the opening of mitoK\textsubscript{ATP} dissipates the mitochondrial inner membrane potential (\(\Delta\psi\textsubscript{im}\)), leading to a reduction in mitochondrial Ca\textsuperscript{2+} uptake.\textsuperscript{5-8}

Mitochondria play pivotal roles in the maintenance of cellular Ca\textsuperscript{2+} homeostasis.\textsuperscript{9} Ca\textsuperscript{2+} uptake occurs through the Ca\textsuperscript{2+} uniporter driven by \(\Delta\psi\textsubscript{im}\). The mechanisms for Ca\textsuperscript{2+} efflux are Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (NCX) and Ca\textsuperscript{2+}/H\textsuperscript{+} exchange.\textsuperscript{10} The opening of the proteinaceous pore in the mitochondrial membrane, ie, the mitochondrial permeability transition pore (mPTP), causes permeabilization of the mitochondrial membrane and provides another pathway for Ca\textsuperscript{2+} efflux.\textsuperscript{11} Cyclosporin A (CsA) is known as a specific inhibitor of mPTP. Classically, irreversible opening of mPTP has been implicated as an early event in lethal cell damage (ie, apoptosis).\textsuperscript{12,13} Recent studies, however, suggested that mPTP could contribute to Ca\textsuperscript{2+} homeostasis under physiological conditions.\textsuperscript{14}

In this study, we aimed to investigate the changes in \(\Delta\psi\textsubscript{im}\) and Ca\textsuperscript{2+} transients (CaTs) by diazoxide in intact myocytes and showed that diazoxide modulated mPTP and increased Ca\textsuperscript{2+} transients via a CsA-sensitive pathway.

Methods

Cells and Solutions
Rat ventricular myocytes were prepared as described previously.\textsuperscript{15} Myocytes were placed in a chamber and superfused with standard Tyrode solution. All experiments were conducted at 22°C. Standard Tyrode solution contained (mmol/L) 140 NaCl, 6 KCl, 1 MgCl\textsubscript{2}, 5 HEPES, 5.8 glucose, and 1 CaCl\textsubscript{2} (pH 7.4 with NaOH). Cells were field stimulated (at 1 Hz) with 2-ms voltage pulses of 1.5 times the threshold amplitude.
Measurement of CaTs and Ca\(^{2+}\) Sparks

Cells were loaded with 20 \(\mu\)mol/L fluo-4-AM for 20 minutes. Fluorescence imaging was performed with a laser scanning confocal microscope (LSCM; Oz, Noran) coupled to an inverted microscope (Axiovert S100, Zeiss) with a 63\(\times\) water immersion objective (numerical aperture [NA] = 1.3; Zeiss), excitation wavelength of 488 nm, and emission at 510 nm.

Image acquisition for quantitative analysis of CaTs was made in the line-scan mode (scanned at 250 lines/s), and CaTs were derived from averaged fluorescence intensities along the scanned line. In the measurement of \(\text{Ca}^{2+}\) sparks, \([\text{Ca}^{2+}]_i\) was calculated, and visually identified \(\text{Ca}^{2+}\) sparks were accepted if local \(\text{Ca}^{2+}\) changes exceeded 60 nmol/L with duration at half-amplitude >8 ms.\(^{15}\)

Measurement of Membrane Potential in Mitochondria

To measure \(\Delta\Psi_m\), cells were loaded with 100 nmol/L tetramethylrhodamine ethyl ester perchlorate (TMRE) for 20 minutes. Images of TMRE fluorescence at 514-nm excitation and 590-nm emission were obtained. TMRE is distributed between cellular compartments according to Nernst’s equation.\(^{16}\) For the measurement of time-dependent TMRE fluorescent changes, images were recorded every 30 seconds (illumination time was 480 ms/image). Fluorescence was integrated over regions of interest (60\(\times\)60 pixels), placed over the bright portion of myocytes.

Imaging of mPTP Opening With Calcein

Cells were loaded with 1 \(\mu\)mol/L calcein-AM for 20 minutes, and the quenching of cytosolic calcein was achieved by addition of 5 mmol/L CoCl\(_2\) to the solution.\(^{17}\) Images of calcein fluorescence (excitation at 488 nm and emission at 505 to 550 nm) were recorded, and fluorescent intensity of the region of interest was integrated.

Measurement of Flavoprotein Fluorescence

Mitochondrial redox state was assessed by measurement of fluorescence of FAD-linked enzymes.\(^{5}\) Endogenous flavoprotein fluorescence images were recorded (excitation wavelength at 488 nm and emission at >505 to 550 nm).

Statistical Analyses

Results are expressed as mean±SEM for the indicated number of myocytes from at least 3 animals. Statistical significance was determined by paired \(t\) test or ANOVA. Values of \(P<0.05\) were considered significant.

Results

Diazoxide Increases CaTs and Ca\(^{2+}\) Sparks

Figure 1A demonstrates that perfusion of diazoxide (200 \(\mu\)mol/L) for 10 minutes increased diastolic level and the amplitudes of CaTs. Dose-dependent effects of diazoxide on CaTs are summarized in Figure 1B, which shows that concentrations of diazoxide \(>200\ \mu\)mol/L altered CaTs. Thus, subsequent experiments were conducted with 200 \(\mu\)mol/L diazoxide, unless otherwise stated. Diazoxide also increased resting \(\text{Ca}^{2+}\) spark frequency from 37.9±12.8 sparks \(\cdot\) pL\(^{-1}\) \cdot\) s\(^{-1}\) to 56.9±13.8 sparks \(\cdot\) pL\(^{-1}\) \cdot\) s\(^{-1}\) (\(P<0.05\); \(n=5\); Figures 1C and 1D).
Diazoxide increased SR Ca\textsuperscript{2+} content but did not alter sarcolemmal NCX activity. A, CafTs (caffeine transients). After cessation of field stimulation, caffeine (10 mmol/L) was applied rapidly to evoke CafTs. B, Pooled data of amplitude of CafTs (as index of SR Ca\textsuperscript{2+} content). C, Pooled data of time constant of decay of CafTs (as index of Ca\textsuperscript{2+} efflux via sarcolemmal NCX). Data are mean±SEM from 8 experiments. ctl indicates control; Dz, diazoxide; and wo, washout. *P<0.05 vs control (paired t-test).

Next, we investigated whether SR Ca\textsuperscript{2+} content and the activity of sarcolemmal NCX were affected by diazoxide. After field stimulation was omitted, caffeine (10 mmol/L) was applied rapidly (Figure 2A), and the amplitude of caffeine transients (CafTs) was measured as an estimate of SR Ca\textsuperscript{2+} content. Figure 2B demonstrated that diazoxide increased the amplitude of CafTs slightly but significantly (by 5.0±0.2% versus control; *P<0.05; n=8). The declining phase of CafTs was fitted to single exponential decay to index Ca\textsuperscript{2+} efflux via NCX. There were no effects of diazoxide on Ca\textsuperscript{2+} efflux via NCX, because the time constants of the CafT decline were not changed by diazoxide (Figure 2C).

To address whether increased CafTs could be attributed to the direct effects of diazoxide on SR, we applied diazoxide under conditions such that SR function was inhibited. Cells were pretreated with ryanodine (5 μmol/L) and thapsigargin (5 μmol/L) for 15 minutes, and then diazoxide was applied. The effect of diazoxide on CafTs was not attenuated under these conditions (Figures 3A and 3B), which indicates that the effects of diazoxide on CafTs were not mediated directly via SR.

**Diazoxide Does Not Alter Mitochondrial Membrane Potential**

It has been speculated that the opening of mitoK\textsubscript{ATP} would dissipate Δψ\textsubscript{m}, leading to decreased driving force for the Ca\textsuperscript{2+} uniporter.\textsuperscript{8} The reduction of mitochondrial Ca\textsuperscript{2+} uptake could increase cytosolic Ca\textsuperscript{2+}. Thus, we monitored the changes in Δψ\textsubscript{m} before and after application of diazoxide. As shown in Figure 4A, diazoxide (500 μmol/L) did not alter Δψ\textsubscript{m}. Application of FCCP (2 μmol/L) after washout of diazoxide decreased the TMRE signal remarkably (the same results were observed in 3 other cells). We next investigated whether 5-HD could inhibit the effect of diazoxide on CafTs. In control experiments, reaplication of diazoxide after the first exposure and washout caused the same increase in CafTs as the initial application (data not shown). When cells were treated with 5-HD (100 μmol/L) for 10 minutes before the second application, subsequent application of diazoxide in the presence of 5-HD did not increase CafTs (Figures 4B and 4C). These results indicated that although opening of mitoK\textsubscript{ATP} could be involved, depolarization of Δψ\textsubscript{m} was not correlated with the observed increase in CafTs.

**CsA-Sensitive Pathway and CafTs**

A previous study with isolated mitochondria showed that diazoxide released Ca\textsuperscript{2+} from mitochondria via a CsA-sensitive mechanism.\textsuperscript{8} Thus, we examined whether CsA inhibits the effects of diazoxide on CafTs. After exposure and washout of diazoxide, cells were treated with 2 μmol/L CsA for 15 minutes. Reapplication of diazoxide with CsA did not increase CafTs, which indicates that a CsA-sensitive mechanism was responsible for the increase in CafTs (Figures 5A and 5B). Because CsA is a potent inhibitor of mPTP, we investigated the effects of diazoxide on mPTP by measuring calcine signal as an index for mPTP opening.\textsuperscript{17} Perfusion of Tyrode solution for 10 minutes decreased the calcine signal by 6.3%, and perfusion of Tyrode plus CsA (2 μmol/L) caused only a slight decrease in signal intensity (by 1.9%; data not shown). Diazoxide decreased the calcine signal by 16% (*P<0.05), and there was little change when diazoxide was applied with CsA (2.0%; Figure 5C). These results suggested that there was a CsA-sensitive leakage of calcine from mitochondria and that diazoxide accelerated this leakage significantly.
CsA-Sensitive Pathway and Flavoprotein Oxidation

To investigate the possible link between diazoxide and the mitochondrial metabolic state, we measured flavoprotein fluorescence to index the mitochondrial redox state. The redox signal was calibrated by exposure of the cells to 2,4-DNP (100 μmol/L) followed by sodium cyanide (2 mmol/L). As shown in Figure 6A, diazoxide reversibly induced oxidation of flavoprotein to 37% of the DNP value, and CsA (2 μmol/L) partially attenuated the oxidative effect of diazoxide by 24% (P < 0.05, n = 4; Figure 6B).

Discussion

In this study, we demonstrated that diazoxide increased CaTs, CaTs, and Ca2+ spark frequency without causing significant changes in ΔΨm, and CsA attenuated the effects of diazoxide on CaTs. Diazoxide caused flavoprotein oxidation, which was partially inhibited by CsA. These results indicate the possible involvement of a CsA-sensitive mechanism for the effects of diazoxide on cytosolic Ca2+ homeostasis and the mitochondrial redox state.

Figure 4. Diazoxide did not alter mitochondrial membrane potential, but effects of diazoxide on CaTs were inhibited by 5-HD. A. Representative recording of TMRE signal after application of diazoxide (500 μmol/L) and FCCP (2 μmol/L). TMRE signal was expressed as % of initial intensity. Drugs were added as indicated in bars. B. After application and washout of diazoxide, cells were treated with 5-HD (100 μmol/L) for 10 minutes, and diazoxide was reapplied with 5-HD. C. Pooled data from 4 cells. F/F0 indicates normalized fluorescent intensity, where F0 indicates basal fluorescence; ctl, control; and Dz, diazoxide. Data represent mean±SEM. *P<0.05 vs control (paired t test).

Figure 5. CsA inhibits effects of diazoxide on Ca2+ transients. A. After application and washout of diazoxide, cells were incubated with CsA (2 μmol/L) for 10 minutes. Subsequent application of diazoxide with CsA did not increase CaTs. B. Pooled data from 4 experiments. Data represent mean±SEM. P<0.05 vs control (paired t test). C. Time courses of changes in calcine signal during perfusion of control Tyrode solution (○) and during perfusion of diazoxide in the presence (●) and absence (●) of CsA (2 μmol/L). n=4. *P<0.05 vs diazoxide plus CsA (●) and †P<0.05 vs Tyrode’s ○ (ANOVA). F/F0 indicates normalized fluorescent intensity, where F0 indicates basal fluorescence; ctl, control; and Dz, diazoxide.

Diazoxide and CaTs

Here, we showed that diazoxide increased CaTs dose dependently and that >200 μmol/L was required, which was close to the reported concentration range of diazoxide for cardio-

Figure 6. Oxidative effect of diazoxide (Dz) is partially inhibited by CsA (2 μmol/L). A. Representative recording of changes in flavoprotein signal. Signal was normalized as 100% for 2,4-DNP (100 μmol/L)-induced maximum oxidation and 0% for sodium cyanide (CN; 2 mmol/L)-induced complete reduction. B. Pooled data from 4 experiments (mean±SEM). *P<0.05 vs CsA(−) (paired t test).
protection (10 to 100 μmol/L). However, a higher concentration of diazoxide could have unexpected toxic effects on mitochondrial function as a metabolic inhibitor and a protophore.\(^{18}\) We have observed that even at 10 μmol/L, diazoxide has effects in skinned myocytes (unpublished data). Therefore, the reduced accessibility of diazoxide to mitochondria in intact myocytes might account for the relatively higher concentration. An increase in [Ca\(^{2+}\)], by diazoxide has been reported previously.\(^6,19\)

Several mechanisms could be involved in the increase in CaTs, such as (1) increased Ca\(^{2+}\) entry via sarcolemmal Ca\(^{2+}\) channels, (2) decreased Ca\(^{2+}\) efflux via NCX and Ca\(^{2+}\) pump, and (3) increased Ca\(^{2+}\) release from SR. Here, we demonstrated that diazoxide did not alter Ca\(^{2+}\) efflux via NCX (Figure 2C). In rat myocytes, SR dominates for the regulation of [Ca\(^{2+}\)], during twitch, and only small portions of Ca\(^{2+}\) were extruded via the Ca\(^{2+}\) pump.\(^{20}\) It has been shown that diazoxide does not affect I\(_{\text{Ca}}\).\(^9\) Therefore, neither the changes in Ca\(^{2+}\) influx or Ca\(^{2+}\) efflux via sarcolemma could contribute to the increased CaTs. In addition, there was no direct action of diazoxide on SR (Figure 3). Thus, diazoxide does not likely modulate these Ca\(^{2+}\) handling systems. Wang et al\(^{6}\) reported that blockade of CaTs by verapamil in diazoxide-pretreated hearts abolished cardiac protection and suggested the possible involvement of sarcolemmal Ca\(^{2+}\) channels for activation of mitoK\(_{\text{ATP}}\) by diazoxide. Because we showed that Ca\(^{2+}\) release from SR was unlikely to be involved as a source of elevated Ca\(^{2+}\), the changes in I\(_{\text{Ca}}\) (as a trigger of Ca\(^{2+}\)-induced Ca\(^{2+}\) release) by diazoxide might not alter CaTs significantly. The different experimental conditions (myocytes or Langendorff-perfused hearts and the existence of ischemia during protocols) may explain these differences.

### Diazoxide and Mitochondrial Membrane Potential

Previous reports suggested that opening mitoK\(_{\text{ATP}}\) depolarized Δψ\(_{\text{m}}\) and reduced mitochondrial Ca\(^{2+}\) uptake.\(^5,8\) Our results, however, did not support this hypothesis, because there were no detectable changes in Δψ\(_{\text{m}}\) by diazoxide. There are possible explanations for undetected changes in Δψ\(_{\text{m}}\). First, the K\(^{+}\) influx through mitoK\(_{\text{ATP}}\) could be compensated rapidly enough that we could not follow the changes in Δψ\(_{\text{m}}\)\(^{21}\) Second, depolarization of Δψ\(_{\text{m}}\) was too small to be detected by TMRE. Lawrence et al\(^{22}\) reported that similar concentrations of diazoxide as used in this study (100 to 200 μmol/L) did not change Δψ\(_{\text{m}}\) but protected myocytes from metabolic inhibition. Another study, however, demonstrated that cardioprotective effects of diazoxide during ischemia/reperfusion have been associated with mild depolarization of Δψ\(_{\text{m}}\)\(^{23}\). In the experiments with isolated mitochondria and neonatal cardiac myocytes, diazoxide depolarized Δψ\(_{\text{m}}\) by 15 mV,\(^9\) whereas Kowalski et al\(^{24}\) reported that estimated changes of Δψ\(_{\text{m}}\) from calculated K\(^{+}\) influx through mitoK\(_{\text{ATP}}\) were only 1 to 2 mV. Different experimental conditions and different methods could account for these discrepancies.

### Diazoxide and mPTP Opening

The loading of cells with calcine-AM plus cobalt to assess mPTP opening was initially reported in hepatocytes\(^{17}\) but not in cardiac myocytes. To confirm that the calcine signal originated from mitochondria, we compared the fluorescent intensities before and after saponin permeabilization of the sarcolemma and found that reduction of the calcine signal was <10%. We have also observed that calcine fluorescence decreased when cells were coloaded with TMRE, in agreement with an original report\(^{17}\) (data not shown). Taken together, these results indicate it is likely that the calcine signal mainly originated from mitochondria. We showed that diazoxide accelerated the calcine leakage from mitochondria, and these effects were attenuated by CsA, which suggests the possible action of diazoxide on mPTP.

In our experimental conditions, CsA alone did not alter CaTs (data not shown), and CsA per se had few effects on SR and NCX in rat myocytes.\(^{24}\) Thus, our results that CsA inhibited the effect of diazoxide on CaTs support the idea that mPTP might contribute to the increase in CaTs.

The opening of mPTP has been associated with dissipation of Δψ\(_{\text{m}}\) matrix swelling, and uncoupling of oxidative phosphorylation and plays a key role in apoptosis by releasing cytochrome c.\(^{13,25}\) It is recognized that in ischemia/reperfusion injury, some cells undergo apoptotic cell death as opposed to necrosis and that mPTP may act as the trigger for apoptosis. In this regard, closing but not opening the mPTP is ideal to prevent cell death. This might be the case in irreversible opening of the mPTP. Diazoxide was reported to inhibit the release of cytochrome c and prevented apoptosis in myocytes exposed to hydrogen peroxide.\(^{26}\) However, isolated mitochondria have been reported to release cytochrome c in response to K\(_{\text{ATP}}\) openers.\(^{27}\)

Evidence has revealed the existence of the flickering of mPTP (low-conductance state).\(^{12,28–30}\) A brief and reversible mPTP opening does not cause mitochondrial swelling, is not related to the release of cytochrome c, and might serve as a physiological means of ridding mitochondria of excess metabolites or ions, in particular Ca\(^{2+}\).\(^7\) Our results indicate that diazoxide could induce a low-conductance state of mPTP and release Ca\(^{2+}\) from mitochondria, which is a different phenomenon from irreversible mPTP opening. Previous studies demonstrated that a mPTP-related Ca\(^{2+}\) release occurred in conditions in which mitochondria were relatively Ca\(^{2+}\) overloaded.\(^8\) However, it is not likely that mitochondrial Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{\text{m}}\)) were heavily loaded in the present study. Although Ca\(^{2+}\) efflux pathways with low-conductance mPTP remain unresolved, both mPTP per se and the reversed mode of the uniporter might be involved.\(^{10,28}\)

Investigation of [Ca\(^{2+}\)]\(_{\text{m}}\) is essential to elucidate the action of diazoxide on mitochondria. However, [Ca\(^{2+}\)]\(_{\text{m}}\) measurement with confocal microscopy and a Ca\(^{2+}\) indicator (eg, Rhod-2) in intact myocytes has not been established.\(^{31}\)

The mechanism for the action of diazoxide on mPTP is poorly understood. It has been suggested\(^{14,28}\) that low-conductance mPTP is principally operated by matrix pH (elevated pH opens mPTP) rather than Ca\(^{2+}\). In relation to this, it has been postulated that opening of mitoK\(_{\text{ATP}}\) causes K\(^{+}\) influx, and this charge could be compensated for by proton extrusion, leading to raised matrix pH, which triggers low-conductance mPTP. Additional studies are necessary to clarify the precise mechanisms involved.
Regardless of the duration of opening, the mPTP opening must be accompanied by the depolarization of $\Delta \psi_m$. However, Petronilli et al. reported that a short mPTP opening was detected only by the calcine-plus-cobalt method, whereas TMRM distribution required longer mPTP opening. Another problem is the production of reactive oxygen species, which are potent mPTP-inducing agents, reported by exposure to the laser. Thus, we shortened laser excitation duration, and the intervals for each recording were prolonged to 30 seconds. The released $\text{Ca}^{2+}$ from mitochondria could fill the SR and provide sufficient changes of $[\text{Ca}^{2+}]_i$ in a tiny localized space to activate ryanodine receptors. This could explain the increased SR $\text{Ca}^{2+}$ contents and $\text{Ca}^{2+}$ spark frequency after the addition of diazoxide. Iachas et al. suggested the interaction between mitochondria and SR within restricted space as the mechanism termed mitochondrial $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release.

**MPTP and Mitochondrial Redox State**

We demonstrated that diazoxide increased flavoprotein oxidation and that CsA partially inhibited this effect of diazoxide (Figures 6A and 6B). Thus, although the exact process is unknown, the effects of diazoxide on mPTP could be related to the mitochondrial redox state. Because $[\text{Ca}^{2+}]_i$ stimulates key enzymes for NADH regulation, the reduced $[\text{Ca}^{2+}]_i$ may inhibit NADH oxidation. Thus, the diazoxide-induced low-conductance mPTP could serve as an endogenous uncoupler of oxidative phosphorylation.

In summary, we provide evidence that diazoxide modifies the low-conductance state of mPTP and that there are possible links between mPTP, $[\text{Ca}^{2+}]_i$, and the mitochondrial redox state in intact myocytes. Additional studies are required to elucidate the underlying mechanisms between the diazoxide-induced mPTP opening and cardioprotective actions.

**References**

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