Mitochondrial ATP-Sensitive Potassium Channels Inhibit Apoptosis Induced by Oxidative Stress in Cardiac Cells

Masaharu Akao, Andreas Ohler, Brian O’Rourke, Eduardo Marbán

Abstract—Mitochondria can either enhance or suppress cell death. Cytochrome c release from mitochondria and depolarization of the mitochondrial membrane potential (ΔΨ) are crucial events in triggering apoptosis. In contrast, activation of mitochondrial ATP-sensitive potassium (mitoK\textsubscript{ATP}) channels prevents lethal ischemic injury in vivo, implicating these channels as key players in the process of ischemic preconditioning. We probed the relationship between mitoK\textsubscript{ATP} channels and apoptosis in cultured neonatal rat cardiac ventricular myocytes. Incubation with 200 μmol/L hydrogen peroxide induced TUNEL positivity, cytochrome c translocation, caspase-3 activation, poly(ADP-ribose) polymerase cleavage, and dissipation of ΔΨ. Pharmacological opening of mitoK\textsubscript{ATP} channels by diazoxide (100 μmol/L) preserved mitochondrial integrity and suppressed all markers of apoptosis. Diazoxide prevented ΔΨ depolarization in a concentration-dependent manner (EC\textsubscript{50} ≈ 40 μmol/L, with saturation by 100 μmol/L), as shown by both flow cytometry and quantitative image analysis of cells stained with fluorescent ΔΨ indicators. These cytoprotective effects of diazoxide were reproduced by pinacidil, another mitoK\textsubscript{ATP} agonist, and blocked by the mitoK\textsubscript{ATP} channel antagonist 5-hydroxydecanoate (500 μmol/L). Our findings identify a novel mitochondrial pathway that is protective against apoptosis. The results also pinpoint mitoK\textsubscript{ATP} channels as logical therapeutic targets in diseases of enhanced apoptosis and oxidative stress. (Circ Res. 2001;88:1267-1275.)

Key Words: apoptosis ■ ischemia ■ oxidative stress

Acute coronary syndromes remain the leading causes of death in developed countries. The classical notion that interruption of blood flow kills cells solely by necrosis (catastrophic cell rupture) has been challenged by evidence that apoptosis contributes to ischemic injury in the heart.1,2 Apoptosis is a genetically encoded, highly orchestrated mode of cell death in which mitochondria play a key role.3,4 Release of the electron transport protein cytochrome c into the cytosol activates caspases, culminating in DNA fragmentation and cytolysis.5 In contrast, another mitochondrial pathway promotes cell survival rather than cell death in “ischemic preconditioning.”6,7 This endogenous process, well-documented to be operative in vivo in diverse species and tissues, refers to the paradoxical protection against lethal ischemia by brief episodes of prior “conditioning” ischemia. Similar cardioprotection can be recruited by drugs such as diazoxide that open mitochondrial ATP-sensitive potassium (mitoK\textsubscript{ATP}) channels; conversely, mitoK\textsubscript{ATP} channel blockers (5-hydroxydecanoate [5-HD] or glibenclamide) prevent both preconditioning and pharmacological cardioprotection.7-9 Thus, mitochondria are key determinants both of cell death and of cell survival.

These observations prompted us to investigate whether the cytoprotective effect of mitoK\textsubscript{ATP} channel activation is related to inhibition of apoptosis. Oxidative stress induces cardiac myocyte apoptosis in vitro,10,11 and contributes to tissue injury in ischemic syndromes12,13 and congestive heart failure.14 To test our hypothesis, we have investigated apoptosis induced by oxidative stress in cardiac myocytes. Our findings reveal that activation of mitoK\textsubscript{ATP} channels suppresses programmed cell death.

Materials and Methods

Primary Culture of Neonatal Rat Cardiac Ventricular Myocytes

Cardiac ventricular myocytes were prepared from 1- to 2-day-old Sprague-Dawley rats and cultured as described.15 In brief, the hearts were removed, and the ventricles were minced in calcium- and bicarbonate-free Hanks’ buffer with HEPES. These tissue fragments were digested by stepwise trypsin dissociation. The dissociated cells were preplated for 1 hour to enrich the culture with myocytes. The nonadherent myocytes were then plated at a density of 1200 cells/mm² in plating medium consisting of DMEM (Mediatech) supplemented with 5% FBS, penicillin (100 U/mL), streptomycin (100 mg/mL), and 2 μg/mL vitamin B₁₂. The final myocyte cultures contained >90% cardiac myocytes at partial confluence, as detected by immunostaining with α-sarcomeric actin antibody (Sigma). The cells were maintained at 37°C in the presence of 5% CO₂ in a humidified incubator. Bromodeoxyuridine (0.1 mmol/L) was in-
cluded in the medium for the first 3 days after plating to inhibit fibroblast growth. Cultures were then placed in serum-free DMEM containing 3.8 g/L glucose, vitamin B<sub>12</sub>, transferrin, and insulin 24 hours before the drug treatment.

**Experimental Protocol**

Neonatal rat cardiac myocytes in primary culture were randomly assigned to one of four experimental groups, as follows: (1) control; (2) incubation with 200 μmol/L hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>); (3) 100 μmol/L diazoxide, applied together with 200 μmol/L H<sub>2</sub>O<sub>2</sub>; and (4) 100 μmol/L diazoxide and 500 μmol/L 5-HD, applied together with 200 μmol/L H<sub>2</sub>O<sub>2</sub>. At the beginning of the experiment, culture media were replaced with fresh serum-free DMEM containing those drugs, and cells were exposed to those drugs during the entire experimental period.

**Terminal Deoxynucleotidyl Transferase (TdT)–Mediated dUTP Nick End-Labeling (TUNEL) Staining**

TUNEL staining was performed according to the manufacturer’s protocol (Roche). Fluorescein labels incorporated in nucleotide polymers were detected by laser scanning confocal microscopy at an excitation wavelength of 488 nm (argon laser). Cells were identified as apoptotic if they showed positive TUNEL staining in the nucleus.

**Immunofluorescence Staining**

For immunofluorescence, cells were plated on glass coverslips, treated with drugs as indicated, fixed in an ice-cold 1:1 mixture of methanol/acetone, blocked with 10% normal goat serum and 0.075% saponin in PBS, and incubated with primary antibody dissolved in blocking solution at a dilution of 1:100. For cytochrome c staining, mouse monoclonal anti–cytochrome c antibody (Pharmingen; 6H2.Ba4) was used; for caspase-3 staining, rabbit polyclonal anti–caspase-3 (Pharmingen), which recognizes only the processed 20-kDa subunit of cleaved caspase-3, was used; and for poly(ADP-ribose) polymerase (PARP) staining, rabbit polyclonal antisemur raised against the activated form of caspase-3 (Pharmingen), and were viewed using an enhanced chemiluminescence detection system (Amersham). Quantitative immunoreactivity was determined by densitometry of the developed film.

**Assessment of Mitochondrial Membrane Potential (∆Ψ)**

Loss of ∆Ψ was assessed using either a laser scanning confocal microscope or flow cytometry analysis of cells stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazole-carboxylic acid iodine (JC-1, Molecular Probes). Cells were incubated with 2 μg/mL JC-1 for 10 minutes at 37°C. After applying the dye, cells were scanned with a confocal microscope using a 10× objective lens. Fluorescence was excited by the 488-nm line of an argon laser and the 543-nm line of a helium/argon laser. The red emission of the dye is due to a potential-dependent aggregation in the mitochondria, reflecting ∆Ψ. Green fluorescence reflects the monomeric form of JC-1, appearing in the cytosol after mitochondrial membrane depolarization. For flow cytometry, cells were harvested by trypsinization after loading of the dye and analyzed by FACScan (10 000 cells/sample). The excitation wavelength was 488 nm, and the emission fluorescence for JC-1 was monitored at 530 nm (FL-1) and 582 nm (FL-2). The flow cytometry data were analyzed using Cell Quest (Becton Dickinson). In this protocol, because green fluorescence intensity did not change significantly among groups, we simply plotted the changes in red fluorescence intensity.

**Quantitative Image Analysis**

Quantitative image analysis was performed for caspase-3 activation and JC-1 staining using image analysis software (ImageJ). Images were thresholded and masked to exclude background, and red- and/or green-field fluorescence was measured.

**Time Course of ∆Ψ Loss**

For time-lapse analysis of ∆Ψ, we used another fluorescent indicator, tetramethylrhodamine ethyl ester (TMRE, Molecular Probes). Cardiac myocytes plated on 35-mm dishes were loaded with 100 nmol/L TMRE for 15 minutes. Throughout the assay, cells were maintained at 37°C using a heater platform (Warner Instrument) installed on a microscope stage and were placed in phenol red–free and CO<sub>2</sub>-independent Leibovitz’s L-15 medium (Life-Tech) supplemented with 50 nmol/L TMRE, to avoid pH change in a non–CO<sub>2</sub>-equilibrated environment. After the desired temperature was reached, time-lapse confocal microscopy was started with a 5-minute interval using a 20× objective lens. TMRE was excited using a 543-nm line of a helium/neon laser. Fifty cells were randomly selected in each scan by drawing regions around individual cells, and red fluorescence intensity was sequentially monitored every 5 minutes.

**Statistical Analysis**

All quantitative data are presented as mean±SEM. Statistical analysis of apoptotic nuclei among four experimental groups, and of changes in protein levels for PARP holoenzyme among groups, were performed using 1-way ANOVA with Fisher’s least significant difference as the post hoc test. Differences in the mean values of caspase-3 activation among different groups as a function of time were tested using 2-way ANOVA. A level of P<0.05 was accepted as statistically significant.

**Results**

As an indicator of DNA fragmentation, Figures 1A through 1D demonstrate TUNEL staining in each experimental group. Control cells showed few TUNEL-positive nuclei (Figure 1A), but exposure to 200 μmol/L H<sub>2</sub>O<sub>2</sub> for 16 hours increased the number of TUNEL-positive nuclei (Figure 1B). Diazoxide decreased the frequency of H<sub>2</sub>O<sub>2</sub>-induced TUNEL-positive nuclei (Figure 1C), indicating a protective effect of the mitoK<sub>ATP</sub> channel agonist. This effect was blocked by the mitoK<sub>ATP</sub> channel antagonist.
5-HD (Figure 1D). Figure 1E shows a quantitative determination of apoptotic nuclei in each experimental group. Cells incubated for 16 hours were stained by DAPI, and the apoptotic cells were identified by the characteristic condensed, fragmented nuclei. An average of 300 to 500 nuclei from random fields were analyzed in each sample. The data indicate that diazoxide has a significant protective effect on the preservation of nuclear morphology, confirming the TUNEL results.

Figures 2A through 2D demonstrate cytochrome c immunofluorescence. In control, the distribution of cytochrome c is reticular and punctate (Figure 2A), indicative of a normal mitochondrial pattern. We have confirmed that the cytochrome c signals in control cells colocalize with the specific mitochondrial marker dye, MitoTracker (Molecular Probes) (data not shown). Incubation with 200 μmol/L H₂O₂ for 16 hours induced cytochrome c translocation to the cytoplasm, resulting in a homogeneous distribution (Figure 2B). Diazoxide inhibited the translocation of cytochrome c (Figure 2C), whereas the addition of 500 μmol/L 5-HD abrogated the effect of diazoxide (Figure 2D).

Figures 3A through 3D demonstrate the immunofluorescent staining for caspase-3, visible as red, using polyclonal antiserum that recognizes only the active form of the enzyme. In control, cells showed little cytosolic fluorescence (Figure 3A), but 200 μmol/L H₂O₂ markedly increased the red signals (Figure 3B). Incubation with 100 μmol/L diazoxide inhibited the H₂O₂-induced activation of caspase-3 (Figure 3C), whereas addition of 500 μmol/L 5-HD negated the effect of diazoxide (Figure 3D). From images such as these, mean red fluorescence intensity was measured as an index of caspase-3 activity (Figure 3E). Two-way ANOVA revealed that the treatment group and the exposure duration were both independent and significant factors. Additional analysis with the Bonferroni method indicates that the activities in the H₂O₂ and 5-HD groups (category 1) are significantly higher than those in control and diazoxide groups (category 2), but there are no significant differences between groups within each category. The activity at 8 hours is significantly higher than that at 4 hours and does not decrease at 16 hours. Furthermore, diazoxide delayed the onset of caspase-3 activation and also blunted the overall response. Similar results were obtained for activated caspase-7 (data not shown).
Next, we examined PARP, one of the main targets of caspase-3 or -7 in vivo, using a polyclonal antibody that detects only the cleaved form (Figures 4A through 4D). In parallel with the activation of caspase-3 or -7, PARP cleavage was augmented in H2O2-treated cells (Figure 4B). Diazoxide inhibited the H2O2-induced PARP cleavage (Figure 4C), and addition of 500 μmol/L 5-HD prevented the diazoxide effect (Figure 4D). To confirm these findings using complementary methods, we examined PARP protein levels by immunoblot analysis. As shown in Figure 4E, the PARP holoenzyme was detected as a 117-kDa band. H2O2 exposure enhanced the cleavage of PARP, resulting in a loss of the holoenzyme band in a time-dependent manner. Diazoxide attenuated the loss of PARP holoenzyme, and 5-HD abolished the protective effect of diazoxide. Densitometry was performed on PARP holoenzyme immunoreactivity (Figure 4F). Although the effect of diazoxide on preservation of PARP holoenzyme did not reach statistical significance at 8 hours (26.5±3.4% in the H2O2 group, 43.2±9.5% in the diazoxide group, and 27.2±5.3% in the 5-HD group; P=0.068 between the H2O2 and diazoxide groups and P=0.078 between the 5-HD and diazoxide groups; n=3), it became statistically significant at 16 hours (6.9±1.4% in the H2O2 group, 18.7±5.1% in the diazoxide group, and 9.7±2.0% in the 5-HD group; P=0.017 between the H2O2 and diazoxide groups and P=0.050 between the 5-HD and diazoxide groups; n=3). All of these features indicate that oxidative stress induces apoptosis, which is inhibited by mitoKATP channel activation.

Reduction of cytochrome c has been linked to the loss of mitochondrial membrane potential (ΔΨ) and the opening of the mitochondrial permeability transition pore.17 We assessed ΔΨ in H2O2-stimulated myocytes using a fluorescent probe, JC-1. Control cells exhibit punctate red staining (Figure 5A), indicative of normal mitochondrial uptake driven by maintained ΔΨ. Cells exposed to 200 μmol/L H2O2 for 2 hours lose punctate red staining (Figure 5B), in favor of a diffuse green cytosolic signal indicative of the loss of ΔΨ. Incubation with 100 μmol/L diazoxide protects against H2O2-induced loss of mitochondrial integrity (Figure 5C). The protective effect of diazoxide is blocked by 500 μmol/L 5-HD (Figure 5D). These observations were rendered quantitative by image analysis. Figure 5E shows the representative data for red fluorescence, an indicator of ΔΨ. Exposure to 200 μmol/L H2O2 for 2 hours resulted in mitochondrial depolarization, whereas diazoxide prevented loss of ΔΨ in a concentration-dependent manner; the EC50 of ≈40 μmol/L is close to the value of 27 μmol/L for mitoKATP channel activation in intact heart cells.8 Addition of 500 μmol/L 5-HD antagonized the salutary effect of diazoxide.

To further confirm the central role of mitoKATP channels, we examined another mitoKATP channel opener, pinacidil.18 As shown in Figure 5E, incubation with 100 μmol/L pinacidil was equi-effective to diazoxide in preventing the loss of ΔΨ induced by 200 μmol/L H2O2. Moreover, the classical KATP blocker glibenclamide (10 μmol/L), which blocks both the surface KATP and the mitoKATP channel, reversed the salutary effect of diazoxide. To rule out the possibility that diazoxide cancels the effect of H2O2 by a direct chemical interaction, we measured the activity of H2O2 in diazoxide-containing medium using a commercially available fluorometric assay kit (Amplex Red Hydrogen Peroxide Assay Kit; Molecular Probes). The content of H2O2 was not affected by the presence of diazoxide (data not shown). Furthermore, addition of diazoxide did not affect the transient p38 MAPK activation induced by H2O2 (authors’ unpublished observation, 2000). Therefore, the protective effect of diazoxide against H2O2-
induced cell death reflects a genuine and specific biological response, not a radical-scavenging effect of diazoxide.

We further quantified the effects of mitoK<sub>ATP</sub> channel activation by flow cytometry (Figure 5F through 5J). Incubation with 200 μmol/L H<sub>2</sub>O<sub>2</sub> for 2 hours decreased the red fluorescence and shifted the distribution curve leftward. In agreement with the confocal image analysis, diazoxide prevented the H<sub>2</sub>O<sub>2</sub>-induced dissipation of ΔΨ in a concentration-dependent manner, as shown by the progressive rightward shift of the distribution curve with increasing concentrations of diazoxide (Figures 5F through 5H; in μmol/L, F 20, G 50, and H 100). 5-HD (500 μmol/L) abolished the effect of diazoxide and reverted the distribution to that of the H<sub>2</sub>O<sub>2</sub> group (Figure 5I). Importantly, 5-HD alone had no effect on ΔΨ in the absence of diazoxide (Figure 5J). 5-HD alone did not promote any of the apoptotic markers examined here compared with the control group (data not shown), and its incubation together with H<sub>2</sub>O<sub>2</sub> did not further aggravate those markers compared with the H<sub>2</sub>O<sub>2</sub>-treated drug-free group.

To examine the time-dependent changes of ΔΨ on a single-cell basis, we used time-lapse confocal analysis of cardiac myocytes loaded with another ΔΨ indicator dye, TMRE, at 5-minute intervals. Throughout the period of observation, TMRE fluorescence intensities of control cells remained unchanged (Figure 6A). In contrast, cells treated with 200 μmol/L H<sub>2</sub>O<sub>2</sub> progressively lost red fluorescence intensity, indicating irreversible dissipation of ΔΨ (Figure 6B). Diazoxide remarkably inhibited this catastrophic loss of ΔΨ (Figure 6C), and this protective effect was abrogated by 5-HD (Figure 6D). Fifty cells were randomly and prospectively selected in each group, and the mean values of red fluorescence intensity from each cell are plotted in Figures 6F through 6I. Diazoxide prevented the loss of ΔΨ induced by 200 μmol/L H<sub>2</sub>O<sub>2</sub> in the majority of cells, and 5-HD inhibited the protective effect of diazoxide. The duration of ΔΨ loss of each cell was abrupt (5 to 10 minutes) and complete, regardless of the time elapsed since the oxidative stress was applied. Thus, although the averages of the individual cell data appear as a progressive decline in signals (Figure 6E), a more rigorous analysis of the cumulative first latency to ΔΨ loss for individual cells reveals that the majority of cells lost ΔΨ within 1 hour (Figure 6D). Diazoxide not only decreased the number of cells undergoing dissipation of ΔΨ, but also delayed the onset of ΔΨ loss. However, it did not change the duration required for ΔΨ loss in those cells that did lose their
inner membrane potential. Movie files reconstituted from time-lapse confocal images are available in the online data supplement (http://circres.ahajournals.org/).

Discussion

Here we have identified a mechanistic link between mitoK<sub>ATP</sub> channels and the mitochondrial apoptotic pathway. The principal findings are as follows. (1) In isolated cardiac myocytes, the mitoK<sub>ATP</sub> channel opener diazoxide inhibits activation of the mitochondrial apoptotic pathway induced by oxidative stress in a concentration-dependent manner. (2) The channel blocker 5-HD (as well as glibenclamide) abolishes the antiapoptotic effect of diazoxide. These observations support the hypothesis that activation of mitoK<sub>ATP</sub> channels inhibits apoptosis, thereby contributing, at least in part, to the infarct size-limiting effect of this agent.

Besides the activation of mitoK<sub>ATP</sub> channels, diazoxide has been argued to have other pharmacological actions, such as succinate dehydrogenase (SDH) inhibition. 19 However, we believe that the antiapoptotic properties of diazoxide are exclusively attributable to mitoK<sub>ATP</sub> activation and not to SDH inhibition, for the following reasons. (1) The concentration needed to achieve SDH inhibition is much higher (≈400 μmol/L), 19,20 (2) SDH inhibition is insensitive to K<sub>ATP</sub> channel blockers, 21 (3) Pinacidil, which is equi-effective in cardioprotection, is not known to inhibit SDH. The concentration range of diazoxide for cardioprotection is 10 to 100 μmol/L, 8,9 which is quite consistent with the antiapoptotic range observed in this study. Although this range is higher than that for mitoK<sub>ATP</sub> channel opening in reconstituted liposomes or isolated mitochondria, 22 the accessibility of diazoxide to mitochondria may differ in isolated mitochondria and in intact myocytes, given the net negative charge of this agent at physiological pH. 19 Finally and most importantly, the present results show that diazoxide protects cells against DC<sub>i</sub> loss and cell death induced by oxidative stress, a property that cannot be written off as a toxic side effect of the drug. 23 Furthermore, diazoxide treatment alone had no ill effects on cell viability compared with controls.

The mechanisms by which mitoK<sub>ATP</sub> channel opening protects against apoptosis are still unknown. However, as evidenced in this study, the opening of mitoK<sub>ATP</sub> channels may act quite early in the apoptotic cascade by inhibiting cytochrome c release and ΔΨ depolarization; these are the
earliest alterations in the cascade, and the two events are closely associated. In good agreement with a previous report, we observed the dissipation of $\Delta \Psi$ within 1 hour in the majority of cells that were exposed to $H_2O_2$ (Figure 6); once initiated, $\Delta \Psi$ dissipation is rapid, complete, and irreversible. Diazoxide not only decreased the number of cells undergoing dissipation of $\Delta \Psi$, but also delayed the onset of $\Delta \Psi$ loss, whereas it did not change the duration of $\Delta \Psi$ loss in each cell. These observations suggest that diazoxide could modulate the initiation process of $\Delta \Psi$ loss but not the dissipating process itself. In other words, diazoxide appears to delay or block the entry into the point

Figure 6. Time-lapse analysis of loss of mitochondrial membrane potential ($\Delta \Psi$) in neonatal rat cardiac myocytes. A, Control cells maintain constant level of a $\Delta \Psi$ indicator dye, TMRE, over the time course. B, Cells exposed to 200 $\mu$mol/L $H_2O_2$ show progressive loss of TMRE fluorescence. C, Diazoxide 100 $\mu$mol/L protects against $H_2O_2$-induced loss of $\Delta \Psi$, as shown by the preservation of red signals and intact morphology. D, 5-HD 500 $\mu$mol/L abrogates the protective effect of diazoxide. E, Mean fluorescence intensity from 50 cells randomly and prospectively selected in each group. Concentration of $H_2O_2$ was 200 $\mu$mol/L for all groups. Results are normalized red fluorescence intensity. Similar results were obtained in 3 independent experiments. F through I, Time course of red fluorescence in each individual cell. F, Control cells; G, $H_2O_2$ 200 $\mu$mol/L; H, $H_2O_2$ 200 $\mu$mol/L+diazoxide 100 $\mu$mol/L; I, $H_2O_2$ 200 $\mu$mol/L+diazoxide 100 $\mu$mol/L+5-HD 500 $\mu$mol/L. All of 50 cells are shown. J, Cumulative first latency plot of number of cells that have undergone $\Delta \Psi$ dissipation. Results are mean±SEM from 3 independent experiments. E and J, Abbreviations and symbols as in Figure 3E.
of no return. Such inhibition could result from a decrease in mitochondrial calcium overload, which is a reported consequence of diazoxide.\textsuperscript{25,26} However, further investigation is needed to elucidate the precise mechanism. Along with significant advances in our understanding of the biochemical and molecular basis of apoptosis, the development of novel therapeutic strategies against apoptosis-related diseases has drawn considerable attention.\textsuperscript{27} Caspases were among the first obvious therapeutic targets for modulating apoptosis. In various models of ischemia-reperfusion injury, including heart and brain, caspase inhibition has shown remarkable efficacy, demonstrated by the reduction of infarct size or preservation of organ function.\textsuperscript{28–30} However, it has also been reported that caspase inhibitors do not inhibit cytochrome c release or $\Delta \Psi$ dissipation,\textsuperscript{31,32} giving reason to wonder whether such drugs effectively preserve mitochondrial function. In this context, diazoxide has the potential advantage that it blocks the apoptotic pathway upstream of cytochrome c release or $\Delta \Psi$ dissipation, thus preserving mitochondrial integrity and minimizing functional loss. Paradoxically, isolated mitochondria have been reported to release cytochrome c in response to diazoxide;\textsuperscript{33} however, this effect was observed in highly calcium-loaded isolated mitochondria and was not blocked by mitoK\textsubscript{ATP} channel inhibitors, suggesting that this permeability transition may not have been due to specific mitoK\textsubscript{ATP} opening.

Our findings have various conceptual and therapeutic implications. First, the fact that mitoK\textsubscript{ATP} channel activation inhibitors apoptosis provides further evidence for the central role of these organelles in programmed cell death. Second, the potent cytoprotection by mitoK\textsubscript{ATP} channel recruitment suggests that ischemic preconditioning reflects inhibition of apoptosis in addition to necrosis.\textsuperscript{34} Finally, and most importantly, mitoK\textsubscript{ATP} channel opening represents a novel therapeutic strategy against apoptosis induced by oxidative stress. A lead compound, diazoxide, already exists and has been used safely for decades to treat hypertension. The spectrum of diseases potentially susceptible to such pharmacological intervention is staggering,\textsuperscript{35,36} given the implication of accelerated apoptosis in pathologies ranging from heart failure to autoimmunity or neurodegeneration.

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