Diazoxide triggers cardioprotection against apoptosis induced by oxidative stress

Masashi Ichinose, Hidetoshi Yonemochi, Toshiaki Sato, and Tetsunori Saikawa. Diazoxide triggers cardioprotection against apoptosis induced by oxidative stress. Am J Physiol Heart Circ Physiol 284: H2235–H2241, 2003. First published March 6, 2003; 10.1152/ajpheart.01073.2002.—Although mitochondrial ATP-sensitive potassium (mitoKATP) channels have been reported to reduce the extent of apoptosis, the critical timing of mitoKATP channel opening required to protect myocytes against apoptosis remains unclear. In the present study, we examined whether the mitoKATP channel serves as a trigger of cardioprotection against apoptosis induced by oxidative stress. Apoptosis of cultured neonatal rat cardiomyocytes was determined by flow cytometry (light scatter and propidium iodide/annexin V-FITC fluorescence) and by nuclear staining with Hoechst 33342. Mitochondrial membrane potential (ΔΨ) was measured by flow cytometry of cells stained with rhodamine-123 (Rh-123). Exposure to H2O2 (500 μM) induced apoptosis, and the percentage of apoptotic cells increased progressively and peaked at 2 h. This H2O2-induced apoptosis was associated with the loss of ΔΨ, and the time course of decrease in Rh-123 fluorescence paralleled that of apoptosis. Pretreatment of cardiomyocytes with diazoxide (100 μM), a putative mitoKATP channel opener, for 30 min before exposure to H2O2 elicited transient and mild depolarization of ΔΨ and consequently suppressed both apoptosis and ΔΨ loss after 2-h exposure to H2O2. These protective effects of diazoxide were abrogated by the mitoKATP channel blocker 5-hydroxydecanoate (500 μM) but not by the sarcolemmal KATP channel blocker HMR-1098 (30 μM). Our results suggest for the first time that opening of mitoKATP channels triggers cardioprotection against apoptosis induced by oxidative stress in rat cardiomyocytes. 

ICHO TAKEMOTO, Morita, and Tetsunori Saikawa.

MATERIALS AND METHODS
Preparation of cultured neonatal rat cardiomyocytes. The experimental protocol was approved in advance by the Ethics Review Committee for Animal Experimentation of Oita Medical University. Neonatal cardiomyocytes were prepared from 3- to 5-day-old Wistar rats as described previously (35). Cardiomyocytes were then plated onto 30-mm culture dishes at a density of 5 × 10^4 per dish and cultured in DMEM supplemented with 5% fetal bovine serum at 37°C under 5% CO2. On day 4, cardiomyocytes beating synchronously were used for experiments.

Assessment of apoptosis and mitochondrial membrane potential by flow cytometry. Flow cytometric analysis was performed with an EPICS (Beckman Coulter Instruments) on a minimum of 1 × 10^4 unfixed cells per sample. Cardiomyocytes were trypsinized with trypsin-EDTA, resuspended in PBS, and loaded with 10 μM propidium iodide (PI; Wako Pure Chemical Industries, Osaka, Japan) and 1 μM annexin V-FITC (Immunotech, Marseille, France) at 4°C for 10 min. In separate experiments, before being resuspended by trypsinization, cardiomyocytes were incubated with 10 μM rhodamine-123 (Rh-123; Molecular Probes, Eugene, OR) at

Address for reprint requests and other correspondence: H. Yonemochi, Dept. of Laboratory Medicine, Oita Medical Univ. 1-1 Idaigaoka, Hasama, Oita 879-5593, Japan (E-mail: yonemo@oita-med.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
37°C for 10 min and then loaded with PI at room temperature for 5 min. Apoptosis was identified as cells with low forward scatter (FSC) on side scatter (SSC)/FSC dot plots, PI dim staining on FSC/PI dot plots, and annexin V-positive and PI-negative staining on annexin V/PI dot plots (5, 14, 19). The mean fluorescence intensity of Rh-123 on the histograms measured by flow cytometry was used to determine the loss of mitochondrial membrane potential (ΔΨ). Fluorescence probes were excited with an air-cooled 488-nm argon laser. The emission fluorescence was monitored at 525 nm for Rh-123 or annexin V-FITC and 620 nm for PI. Data were analyzed with the Coulter software package (Phoenix Flow).

**Analysis of apoptotic nuclei by fluorescent microscopy.** To detect the characteristic features of apoptotic nuclei, unfixed cardiomyocytes were stained with 0.12 μM Hoechst 33342 (Wako), a fluorescent DNA-binding dye, for 10 min. Fluorescence of Hoechst 33342 (excited at 365 nm and emitted at 400 nm) was captured with a charge-coupled device camera under a fluorescent microscope. Apoptotic cells were identified by their typical morphological appearance, with chromatin condensation and nuclear fragmentation. An average of >500 nuclei from random fields was analyzed for each data point.

**Determination of lactate dehydrogenase in culture medium.** Lactate dehydrogenase (LDH) released to the culture medium was determined with an LDH assay kit (Eiken Chemical, Tokyo, Japan). Approximately 5 × 10⁶ cardiomyocytes were placed into 35-mm culture dishes containing 1 ml of culture medium (DMEM supplemented with 5% fetal bovine serum). After 2-h exposure to H₂O₂, 500 μl of supernatant was carefully collected for LDH determination.

**Experimental protocols.** The experimental protocols are depicted in Fig. 1. Apoptosis was induced by exposing cardiomyocytes to 500 μM H₂O₂. The dose of H₂O₂ was chosen on the basis of previous reports that the induction of apoptosis in neonatal rat cardiomyocytes occurred via activation of the mitochondrial apoptotic pathway (4, 32). To investigate the role of the mitoKATP channel as a trigger of cardioprotection against H₂O₂-induced apoptosis, cardiomyocytes were pretreated with diazoxide for 30 min and then washed twice with PBS before incubation with H₂O₂ for 2 h. In the control group, cardiomyocytes obtained from sister cultures received vehicle only without exposure to H₂O₂. In the H₂O₂ group, cardiomyocytes were incubated with H₂O₂ alone. In the H₂O₂+DZ(P) group, cardiomyocytes were pretreated with 100 μM diazoxide (Sigma, St. Louis, MO) for 30 min before H₂O₂ incubation. In the H₂O₂+DZ(P)+5HD(P) group, cardiomyocytes were pretreated with diazoxide and 500 μM 5-hydroxydecanoate (5-HD; Sigma) before H₂O₂ incubation. In the H₂O₂+DZ(P)+5HD(C) group, cardiomyocytes were pretreated with diazoxide before H₂O₂ incubation and 5-HD was coadministered with H₂O₂. In the H₂O₂+DZ(P)+HMR(P) group, cardiomyocytes were pretreated with diazoxide and 30 μM HMR-1098 (kindly provided by Aventis Pharma) before H₂O₂ incubation.

**Statistical analysis.** Data are expressed as means ± SE. Between two groups were examined for statistical significance by ANOVA with Fisher’s post hoc test. A P value < 0.05 denoted the presence of a statistically significant difference.

**RESULTS**

H₂O₂ induces apoptosis of cardiomyocytes. Figure 2A shows the representative flow cytometric analysis of cardiomyocytes stained with PI and annexin V-FITC. Side and forward scatter (SSC/FSC) dot plots identified two distinct cell subpopulations, and cells with high FSC signals (R1) were predominant in controls. Exposure to H₂O₂ for 2 h shifted the cells into the lower portion of the diagram (R2). When cell subpopulations were detected on FSC/PI dot plots, apoptotic cells in the dim PI fluorescence region (R4) increased after exposure to H₂O₂. Moreover, annexin V-positive and PI-negative apoptotic cells (R9) were prominent after exposure to H₂O₂. Figure 2A further shows the nuclear morphology of cardiomyocytes stained with Hoechst 33342. The apoptotic features of chromatin condensation and nuclear fragmentation were observed after 2-h exposure to H₂O₂. As summarized in Fig. 2B, the percentage of apoptotic cells in the low-FSC subpopulation (R2) was significantly increased from 18 ± 1% in the control sister culture to 45 ± 2% after exposure to H₂O₂ (n = 5; P < 0.01). Similarly, H₂O₂ significantly increased the degree of apoptosis assessed by PI staining (36 ± 2% vs. 16 ± 2%, n = 5; P < 0.01), annexin V-PI double staining (40 ± 2% vs. 19 ± 1%, n = 5; P < 0.01), and Hoechst 33342 staining (35 ± 4% vs. 7 ± 1%, n = 11; P < 0.01). These results indicate that H₂O₂ induced apoptosis of rat neonatal cardiomyocytes and that the degree of apoptosis determined by four different methods was comparable.

**Time course of H₂O₂-induced apoptosis and ΔΨ loss.** Figure 3 shows the time courses of apoptotic cell death and ΔΨ loss during exposure to H₂O₂. Here, apoptotic cells were identified as the low-FSC subpopulation and ΔΨ loss was assessed by relative change in the mean...
fluorescence intensity of Rh-123. The percentage of apoptotic cell death increased progressively and peaked at 2 h (285 ± 78% of control sister culture, n = 8; \(P < 0.01\)). The time course of decrease in Rh-123 fluorescence paralleled that of apoptotic cell death, and after 2-h exposure to \(H_2O_2\) decreased to 53 ± 10% (n = 8; \(P < 0.01\)) of the control sister culture. These results suggest that \(H_2O_2\)-induced apoptosis was associated with loss of \(\Delta \Psi\).

Diazoxide prevents \(H_2O_2\)-induced apoptosis and \(\Delta \Psi\) loss. We then examined whether pretreatment with diazoxide attenuated \(H_2O_2\)-induced apoptosis and \(\Delta \Psi\) loss. As shown in Fig. 4, \(H_2O_2\)-induced apoptotic cells in the low-FSC subpopulation accompanied the dissipation of \(\Delta \Psi\), which was evident by the leftward shift in Rh-123 fluorescence. The mean intensity of Rh-123 fluorescence measured on the histogram was 40.0 in the sister control culture and 12.4 after \(H_2O_2\) incubation for 2 h. Pretreatment with diazoxide for 30 min attenuated both apoptotic cell death and \(\Delta \Psi\) loss induced by exposure to \(H_2O_2\), and the mean intensity of Rh-123 fluorescence was restored to 24.7. Summarized data shown in Fig. 5 confirm that the results in Fig. 4 are indeed representative. \(H_2O_2\) significantly increased apoptotic cell death to 338 ± 41% of the control sister culture (n = 15; \(P < 0.01\)) and decreased the \(\Delta \Psi\) assessed by mean intensity of Rh-123 fluorescence to 56 ± 8% of control (n = 15; \(P < 0.01\)). Diazoxide pretreatment \([H_2O_2+DZ(P)\) group\] significantly attenuated apoptotic cell death to 228 ± 19% of control (n = 15; \(P < 0.01\) vs. \(H_2O_2\) group) and restored the loss of \(\Delta \Psi\) to 78 ± 8% of control (n = 15; \(P < 0.01\) vs. \(H_2O_2\) group). The selective mitoKATP channel blocker 5-HD (30) applied together with diazoxide \([H_2O_2+DZ(P)\) and 5-HD(P) group\] prevented the effects of diazoxide, and the extent of apoptotic cell death and loss of \(\Delta \Psi\) was similar to that induced by \(H_2O_2\) alone. In contrast, when 5-HD was coadministered with \(H_2O_2\) after the...
application of diazoxide \([H_2O_2+DZ(P)+5-HD(C)\) group], the drug failed to inhibit the effect of diazoxide. These effects of 5-HD suggest that opening of the mitoKATP channel by diazoxide indeed acts as a trigger of cardioprotection against \(H_2O_2\)-induced apoptosis. Moreover, HMR-1098, a selective sarcolemmal KATP channel blocker \((31)\), applied together with diazoxide before \(H_2O_2\) \([H_2O_2+DZ(P)+HMR(P)\) group\], did not affect the protective effects of diazoxide, suggesting that the sarcolemmal KATP channel is not involved in the antiapoptotic effect of diazoxide.

**Diazoxide prevents \(H_2O_2\)-induced necrosis.** \(H_2O_2\) induced not only apoptosis but also necrosis. The level of LDH in the culture medium was significantly increased from \(0.059 \pm 0.013\) IU/ml \((n = 6)\) in the control sister culture to \(0.278 \pm 0.015\) IU/ml after 2-h exposure to \(H_2O_2\) \((n = 6; P < 0.001)\). Pretreatment with diazoxide for 30 min significantly reduced the LDH level to \(0.222 \pm 0.014\) IU/ml \((n = 6; P < 0.05\) vs. \(H_2O_2\) group). When necrotic cell death was assessed as a percentage of the bright PI fluorescence region (R3), \(H_2O_2\) increased necrosis from \(4.7 \pm 0.5\%\) \((n = 6)\) in control to \(9.3 \pm 1.3\%\) \((n = 6; P < 0.01)\) after \(H_2O_2\) exposure. Pretreatment with diazoxide again attenuated necrotic cell death to \(5.6 \pm 0.3\%\) \((n = 6; P < 0.01\) vs. \(H_2O_2\) group).

**Transient depolarization of \(\Delta\Psi\) triggers cardioprotection.** We investigated the effect of diazoxide on \(\Delta\Psi\) during the triggering period (Fig. 6). Diazoxide significantly depolarized the \(\Delta\Psi\) and decreased the intensity of Rh-123 fluorescence to \(74 \pm 5\%\) of the control sister culture \((n = 5; P < 0.01)\) after a 15-min application. This depolarization of \(\Delta\Psi\) restored to \(87 \pm 4\%\) of control \((n = 5; P = \text{not significant})\) after 30 min. Transient depolarization of \(\Delta\Psi\) observed after a 15-min application of diazoxide was abolished by 5-HD \((88 \pm 6\%, n = 5)\). Thus the \(\Delta\Psi\) measured just before application of \(H_2O_2\) was comparable among the groups. Nevertheless, diazoxide prevented the loss of \(\Delta\Psi\) induced by subsequent exposure to \(H_2O_2\) for 2 h. These results suggest that the transient depolarization of \(\Delta\Psi\) during the triggering period might contribute to the mechanism of cardioprotection.

**DISCUSSION**

The major findings of the present study were that 1) pretreatment of rat cardiomyocytes with diazoxide, a putative mitoKATP channel opener, elicited the transient depolarization and attenuated the subsequent apoptotic cell death and \(\Delta\Psi\) loss induced by exposure to \(H_2O_2\); and 2) these effects of diazoxide were antagonized by the mitoKATP channel blocker 5-HD but not by...
the sarcolemmal KATP channel blocker HMR-1098. Our data therefore suggest that mitoKATP channels serve as a trigger of cardioprotection against apoptosis induced by oxidative stress.

H2O2 has been reported to induce apoptosis of cardiomyocytes through activation of the mitochondrial apoptotic pathway (4, 32), where loss of $\Delta\Psi$ is a key step associated with cytochrome $c$ release from the mitochondria (10). Our results confirmed that this was indeed the case, and H2O2 induced the apoptosis of cultured neonatal rat cardiomyocytes, determined by flow cytometric analysis (light scatter, PI, and annexin V-FITC staining) and by nuclear morphology (Hoechst 33342 staining). Furthermore, consistent with a previous report (4), H2O2-induced apoptosis was associated with the loss of $\Delta\Psi$ as shown by Rh-123 fluorescence. Besides apoptosis, we also found that H2O2 increased necrotic cells, which is in agreement with a previous study in neonatal mouse cardiomyocytes (33). LDH release increased by approximately fivefold, whereas PI-positive cells increased by approximately twofold after exposure to H2O2. A probable reason for this is that necrotic cells become nonadherent and hence may be removed when the cell layer is washed and resuspended in PBS for flow cytometric analysis. The degree of necrotic cells assessed as a percentage of the bright PI fluorescence region (~9%) was substantially smaller than that of apoptotic cells (~40%). Accordingly, cultured neonatal rat cardiomyocytes predominantly showed apoptotic cell death after exposure to H2O2 under our experimental conditions.

Although the mitoKATP channel was initially proposed to be the end-effector of IPC (8, 18, 29), the triggering action of mitoKATP channels has also been proposed using infarct size as the end point. Pain et al. (27) showed that 5-HD administered early to bracket preconditioning ischemia could abolish the infarct size-limiting effect of IPC. Baines et al. (2) demonstrated that diazoxide administered before ischemia but not after the onset of index ischemia reduced infarct size. The results presented here confirm that pretreatment with diazoxide acts as a trigger, thereby attenuating the necrotic cell death induced by H2O2. Recently, in addition to reduction of infarct size, IPC has been reported to reduce apoptosis (6, 9, 20, 28, 36). Akao et al. (1) reported that diazoxide attenuated both apoptotic cell death and $\Delta\Psi$ loss induced by exposure to H2O2 for 16 h, and these effects of diazoxide were antagonized by 5-HD. Because diazoxide and/or 5-HD were applied together with H2O2 in their study, the results support the idea that mitoKATP channel acts as a mediator/effect of cardioprotection against apoptosis. A novel and interesting finding in the present study is that apoptotic cell death and $\Delta\Psi$ loss after 2-h exposure to H2O2 were significantly attenuated when diazoxide was applied for 30 min before exposure to H2O2. We confirmed that this indeed resulted from opening of mitoKATP channels: 5-HD, but not HMR-1098, completely abolished the antiapoptotic effects of diazoxide (Fig. 5). Moreover, once cardiomyocytes were pretreated with diazoxide, subsequent application of 5-HD together with H2O2 could not block the protection afforded by diazoxide. Together, these results indicate that the mitoKATP channel acts as a trigger of cardioprotection against apoptosis. We further found that the

![Fig. 5. Summarized effects of diazoxide, 5-HD, and HMR on apoptotic cell death and loss of $\Delta\Psi$ induced by exposure to H2O2 for 2 h in protocol. Data represent means ± SE of 11–15 experiments and are expressed as % of control sister culture. *P < 0.01 vs. sister control; #P < 0.01 vs. H2O2 group.](http://ajpheart.physiology.org/)

![Fig. 6. Time course of change in $\Delta\Psi$ during application of diazoxide and/or 5-HD and 2 h after exposure to H2O2. Diazoxide and/or 5-HD was applied for 30 min before H2O2 exposure. Data represent means ± SE of 5 experiments and are expressed as % of control sister culture. *P < 0.05 vs. sister control.](http://ajpheart.physiology.org/)
triggering action of diazoxide to reduce apoptotic cell death could not be observed after 16-h exposure to \( \text{H}_2\text{O}_2 \) (data not shown). Thus the mitoK\(_{\text{ATP}}\) channel apparently triggers an early phase of protection that lasts for \( \sim 2 \) h.

The precise mechanism by which diazoxide prevents apoptosis remains unclear. It has been reported that diazoxide-induced opening of mitoK\(_{\text{ATP}}\) channels causes mild depolarization of \( \Delta \psi \), thereby attenuating mitochondrial Ca\(^{2+} \) overload induced by ouabain and metabolic inhibition (13, 22). Mitochondrial Ca\(^{2+} \) overload results in the opening of permeability transition pore (PTP), which in turn causes the loss of \( \Delta \psi \) and release of cytochrome \( c \) (10). Therefore, prevention of PTP by attenuating mitochondrial Ca\(^{2+} \) overload may be a critical mechanism of cardioprotection. Indeed, Akao et al. (1) demonstrated that diazoxide inhibited the cytochrome \( c \) release and loss of \( \Delta \psi \) induced by \( \text{H}_2\text{O}_2 \). Recently, Minners et al. (21) reported that pharmacological preconditioning by diazoxide uncoupled mitochondria and decreased \( \Delta \psi \) in Girardi cells and \( \text{C}_2\text{C}_{12} \) myotubes. In the present study, we also found that diazoxide depolarized the \( \Delta \psi \) during triggering period (Fig. 6). However, it should be noted that depolarization of \( \Delta \psi \) induced by diazoxide was transient and that there was no significant depolarization of \( \Delta \psi \) just before application of \( \text{H}_2\text{O}_2 \). This finding implies that prevention of mitochondrial Ca\(^{2+} \) overload in association with \( \Delta \psi \) cannot account for the triggering mechanism of cardioprotection. Alternatively, ROS generation is thought to be a trigger of signaling pathways mediating IPC. Pain et al. (27) demonstrated that the triggering effect of diazoxide was lost when a scavenger of ROS was coadministered with diazoxide. Forbes et al. (7) provided direct evidence that diazoxide increases ROS production. Therefore, it has been hypothesized that opening of mitoK\(_{\text{ATP}}\) channels by diazoxide may lead to ROS generation. ROS may then activate downstream PKC. Okamura et al. (25) reported that the protective effect of IPC against apoptosis was blocked by a PKC inhibitor. Liu et al. (16) further demonstrated that PKC-\( \varepsilon \) is involved in inhibition of apoptosis by IPC. Moreover, Wang and Ashraf (34) demonstrated diazoxide-induced PKC translocation in Langendorff-perfused rat hearts and showed that these effects could be blocked by PKC inhibitors. Thus such a PKC-dependent mechanism mediated by ROS generation might contribute to the antiapoptotic effect of diazoxide. In a preliminary experiment, however, we observed that a ROS scavenger only partially inhibited the transient depolarization of \( \Delta \psi \) during application of diazoxide and could not completely abolish the antiapoptotic effect of diazoxide. Regarding the infarct size-limiting effects, ROS seems to be involved in the triggering action of mitoK\(_{\text{ATP}}\) channels. However, it remains unclear whether the ROS-dependent mechanism may be involved in the antiapoptotic effect of mitoK\(_{\text{ATP}}\) channels.

In conclusion, the results of our study provide novel evidence that opening of mitoK\(_{\text{ATP}}\) channels by diazoxide acts as a trigger and reduces apoptotic cell death. Although we used \( \text{H}_2\text{O}_2 \) to induce apoptosis, it will be interesting to see whether mitoK\(_{\text{ATP}}\) channels trigger antiapoptotic effects in an in vivo setting. More recently, it has been proposed that diazoxide inhibits respiratory chain, which may serve as a predictive mechanism for ROS generation (12). However, this idea is contrary to a study demonstrating that diazoxide can suppress ROS generation and reduce cytochrome \( c \) release at reoxygenation in a potassium-independent manner (26). Furthermore, although diazoxide has been shown to depolarize \( \Delta \psi \), a modest depolarization of \( \Delta \psi \) would be expected to decrease ROS generation rather than increase it (15). Obviously, how diazoxide might be linked to ROS generation remains unclear. Further studies are required to define the mechanism by which diazoxide sets the heart into a preconditioned state against apoptosis.

This study was supported by a grant from the Ministry of Education, Science, Sports and Culture of Japan (H. Yonemochi and T. Sato) and the Mitsui Life Social Welfare Foundation (T. Sato).

REFERENCES


15. Korshunov SS, Skulachev VP, and Starkov AA. High pro-
tonic potential actuates a mechanism of production of reactive
17. Liu Y and O’Rourke B. Opening of mitochondrial K\textsubscript{ATP} chan-
nels triggers cardioprotection: are reactive oxygen species in-
18. Liu Y, Sato T, O’Rourke B, and Marban E. Mitochondrial
ATP-dependent potassium channels: novel effectors of cardio-
19. Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA,
Van Schie RC, LaFace DM, and Green DR. Early redistribu-
tion of plasma membrane phosphatidylserine is a general
future of apoptosis regardless of the initiating stimulus: inhibi-
III, Rousou JA, and Das DK. Ischemic preconditioning atten-
uates apoptotic cell death associated with ischemia/reperfusion.
DM, and Sack MN. Ischemic and pharmacological precondi-
tioning in Girardi cells and C\textsubscript{2}C\textsubscript{12} myotubes induce mitochon-
22. Murata M, Akao M, O’Rourke B, and Marban E. Mitochon-
drial ATP-sensitive potassium channels attenuate matrix Ca\textsuperscript{2+}
overload during simulated ischemia and reperfusion: possible
23. Murry CE, Jennings RB, and Reimer KA. Preconditioning
with ischemia: a delay of lethal cell injury in ischemic myocard-
Mitochondrial K\textsubscript{ATP} channels: role in cardioprotection. Cardio-
25. Okamura T, Miura T, Iwamoto H, Shirakawa K,
Kawamura S, Ikeda Y, Iwatake M, and Matsuzaki M. Ische-
mic preconditioning attenuates apoptosis through protein kinase
26. Ozcan C, Bienengaerber M, Dzeja PP, and Terzie A. Potas-
ium channel openers protect cardiac mitochondria by attenuat-
ing oxidant stress at reoxygenation. Am J Physiol Heart Circ
27. Pain T, Yang XM, Critz SD, Yue Y, Nakano A, Liu GS,
Heusch G, Cohen MV, and Downey JM. Opening of mito-
chondrial K\textsubscript{ATP} channels triggers the preconditioned state by
CL. Ischemic preconditioning decreases apoptosis in rat heart in
29. Sato T and Marban E. The role of mitochondrial K\textsubscript{ATP} channels
30. Sato T, O’Rourke B, and Marban E. Modulation of mito-
31. Sato T, Sasaki N, Seharaseyon J, O’Rourke B, and Marban
E. Selective pharmacological agents implicate mitochondrial but
not sarcolemmal K\textsubscript{ATP} channels in ischemic cardioprotection. Cir-
32. Von Harsdorf R, Li PF, and Diet R. Signaling pathways in
reactive oxygen species-induced cardiomyocytes apoptosis. Cir-
33. Wang GW, Schuschke DA, and Kang YJ. Metallothionein-
overexpressing neonatal mouse cardiomyocytes are resistant to
H\textsubscript{2}O\textsubscript{2} toxicity. Am J Physiol Heart Circ Physiol 276: H167–H175,
1999.
34. Wang Y and Ashraf M. Role of protein kinase C in mito-
chondrial K\textsubscript{ATP} channel-mediated protect ion against Ca\textsuperscript{2+}
35. Yonemochi H, Yasunaga S, Teshima Y, Takahashi N, Na-
kagawa M, Ito M, and Saikawa T. Rapid electrical stimula-
tion of contraction reduces the density of β-adrenergic recep-
tors and responsiveness of cultured neonatal rat cardiomyocytes:
possible involvement of microtubule disassembly secondary to
36. Zhao ZQ and Vinten-Johansen J. Myocardial apoptosis and