Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice

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Received for publication September 21, 2001, and accepted in revised form January 7, 2002.

Recently it has been postulated that mitochondrial ATP-sensitive K^+ (mitoK_{ATP}) channels rather than sarcolemmal K_{ATP} (sarcK_{ATP}) channels are important as end effectors and/or triggers of ischemic preconditioning (IPC). To define the pathophysiological significance of sarcK_{ATP} channels, we conducted functional experiments using Kir6.2-deficient (KO) mice. Metabolic inhibition with glucose-free, dinitrophenol-containing solution activated sarcK_{ATP} current and shortened the action potential duration in ventricular cells isolated from wild-type (WT) but not KO mice. MitoK_{ATP} channel function was preserved in KO ventricular cells. In anesthetized mice, IPC reduced the infarct size in WT but not KO mice. Following global ischemia/reperfusion, the increase of left ventricular end-diastolic pressure during ischemia was more marked, and the recovery of contractile function was worse, in KO hearts than in WT hearts. Treatment with HMR1098, a sarcK_{ATP} channel blocker, but not 5-hydroxydecanoate, a mitoK_{ATP} channel blocker, produced a deterioration of contractile function in WT hearts comparable to that of KO hearts. These findings suggest that sarcKATP channels figures prominently in modulating ischemia/reperfusion injury in the mouse. The rapid heart rate of the mouse (>600 beats per minute) may magnify the relative importance of sarcK_{ATP} channels during ischemia, prompting caution in the extrapolation of the conclusions to larger mammals.

J. Clin. Invest. **109**:509–516 (2002). DOI:10.1172/JCI200214270.

Introduction

Brief periods of ischemia that precede sustained ischemia lead to a reduction of infarct size (1–4). This phenomenon is known as ischemic preconditioning (IPC) and the mechanisms underlying it remain unclear, despite extensive study (5–7). Sarcolemmal ATP-sensitive potassium (sarcK_{ATP}) channels were proposed to play an important role in the cardioprotective effect, because potassium channel openers (KCOs) mimicked the cardioprotection and the K_{ATP} channel blocker glibenclamide abolished the IPC (8–13). Since the discovery of sarcK_{ATP} channels (14), it has been recognized that activation of these channels may serve an endogenous cardioprotective mechanism: action potential shortening due to K_{ATP} channel activation is expected to reduce Ca^{2+} influx in ischemic heart cells, producing a cardioplegic effect. However, recent studies indicate that mitochondria harbor another type of K_{ATP} channel (15, 16), and activation of the mitochondrial K_{ATP} (mitoK_{ATP}) channel rather than the sarcK_{ATP} channel has been proposed to underlie the cardioprotective effect of IPC or KCOs (17–20). Because the mitoK_{ATP} channel has yet to be identified at a molecular level, the existing evidence is almost entirely based on pharmacological studies.

The molecular structure of sarcK_{ATP} channels has been clarified by cloning members of the inwardly rectifying K^+ channel subfamily Kir6.0 (Kir6.1 and Kir6.2) and the receptors for sulfonylureas (SUR1, SUR2A, and SUR2B) (21, 22). Accumulating evidence indicates that native sarcK_{ATP} channels are composed of these two structurally distinct subunits. Various combinations of Kir6.0 and SUR convey the heterogeneity in channel properties observed in native cells of various tissues, such as heart, pancreatic β cells, skeletal and smooth muscles, and neurons (22). Cardiac sarcK_{ATP} channels have been suggested in a reconstitution study to comprise SUR2A and Kir6.2 (23). Our recent functional study using Kir6.2-deficient mice has provided direct evidence that Kir6.2 forms the pore region of cardiac sarcK_{ATP} channels but not of vascular sarcK_{ATP} channels (24). Although the molecular identity of mitoK_{ATP} channel remains unclear, experiments using dominant negative gene transfer have indicated that neither Kir6.1 nor Kir6.2 is a functionally important part of the mitoK_{ATP} channel in native heart cells (25). Despite the paucity of molecular tools available to probe the role of mitoK_{ATP} channels, Kir6.2-deficient mice are potentially useful to define the pathophysiological significance

of sarcK_{ATP} channels. Such mice have no functional sarcK_{ATP} channels; the absence of such channels may inferentially shed light on the relative roles of sarcK_{ATP} channels and mitoK_{ATP} channels. One potential caveat is the fact that sarcK_{ATP} channels have been proposed to be protective by virtue of a cardioplegic effect: if they open, excitability is suppressed, and ischemic injury may be secondarily mitigated. Such an effect may be magnified in the mouse, a species which has a resting heart rate more than tenfold greater than that of humans and thus stands to derive greater potential benefit from electrical silencing. Nevertheless, mice are the only mammalian species in which germline manipulation is routine, and the availability of Kir6.2-deficient mice presents a unique opportunity to investigate the mechanisms of cardioprotection.

In this study, we tested whether IPC is preserved in Kir6.2-deficient mice. IPC is abolished in the Kir6.2-deficient mice, and the degree of ischemic injury in these mice appears to be greater even under basal conditions. Accordingly, we also evaluated sarcK_{ATP} and mitoK_{ATP} channel function in cardiac cells isolated from the knockout mice and the contribution of these K_{ATP} channels to mechanical dysfunction after ischemia/reperfusion in isolated hearts.

Methods

Kir6.2$^{-/-}$ mice. All procedures complied with NIH standards for the care and use of animal subjects. A mouse line deficient in K_{ATP} channels was generated by targeted disruption of the gene coding for Kir6.2, as described previously (26). It has been demonstrated that sarcK_{ATP} channel activity is absent in pancreatic β cells (26), neurons (27), and heart cells (24), but not in vascular smooth muscle cells (24). C57BL/6 mice were used as controls because the knockout animals had been backcrossed to a C57BL/6 strain for five generations.

In vivo study. Mice were anesthetized by intraperitoneal injection of urethane (1.5 g/kg). Additional doses were given during the procedure as needed to maintain anesthesia. The skin on the neck was opened and the trachea was cut down to guide the placement of an endotracheal tube. This consisted of a blunt-ended 22-gauge needle. The animals were ventilated with room air supplemented with oxygen using a rodent ventilator (model SN-480-7; Shinano Manufacturing Co., Tokyo, Japan). During the operation, body temperature was monitored with a rectal probe and maintained at 37°C using a heat lamp. The chest was opened and the heart was exposed. Ischemia was achieved by ligating the left anterior descending artery (LAD) using an 8-0 silk suture 1–3 mm from the tip of the normally positioned left atrial auricle. Regional ischemia was confirmed by visual inspection of pale color in the occluded distal myocardium. After the coronary occlusion of 45 minutes, the myocardium was reperfused by releasing the ligature. Successful reperfusion was confirmed by visualization of the return of a bright red color in the previously pale region. Mice of IPC groups underwent three cycles of 3 minutes of coronary occlusion followed by 5 minutes of reperfusion before being subjected to the same protocol used in the control group. The chest was loosely closed and the chest wall was covered by wet paper to avoid desiccation of the heart and lung. After reperfusion for 120 minutes, the LAD was occluded again and blue dye (Blue Black Dye; Nihon Kohden Supply Co., Tokyo, Japan) was injected into the left ventricular chamber via the apex by needle puncture to stain the nonischemic area, using methods analogous to those employed by Yang et al. (28) and Izumi et al. (29). This procedure delineated the nonischemic myocardium as dark blue. The heart was cut into six transverse slices, which were incubated for 5 minutes at 37°C in a 1% solution of triphenyltetrazolium chloride (TTC). All atrial and right ventricular tissues were excised. To measure myocardial infarction, the slices were photographed (Camedia C-1400L; Olympus Optical Co., Tokyo, Japan) and weighed. The infarcted (pale), viable ischemic/reperfused (red), and nonischemic (blue) areas were measured by computed planimetry (Scion Image 1.62; Scion Corp., Frederick, Maryland, USA). Noninfarcted viable myocardium containing dehydrogenase stained brick red by reacting with TTC, whereas the infarcted tissue remained unstained because of lack of the enzyme. The area at risk (AAR), the portion of the left ventricle supplied by the previously occluded coronary artery, was identified by the absence of blue dye. Infarct weights were calculated as $(A_1 \times W_1) + (A_2 \times W_2) + (A_3 \times W_3) + (A_4 \times W_4) + (A_5 \times W_5) + (A_6 \times W_6)$, where A is the area of infarct for the slice and W is the weight of the respective section. The weight of the AAR was calculated in similar fashion. Infarct size was expressed as a percentage of AAR. The individuals conducting the measurements were blinded to the experimental groups. The interobserver error of the infarct size expressed as a percentage of AAR was less than 2% when repeated determinations were made on all the samples by blinded observers.

Electrophysiology. Single ventricular cells were isolated from adult mouse hearts by conventional enzymatic digestion (24). Whole-cell membrane currents were recorded by nystatin-perforated patch configuration of the patch-clamp method as previously described (30). Single ventricular cells were superfused with the HEPES-Tyrode solution (in mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5, and HEPES-NaOH buffer 10 (pH 7.4). The pipette solution contained (in mM): potassium aspartate 110, KCl 20, $MgCl_2$ 1.0, $CaCl_2$ 1.0, EGTA 0.1, HEPES-KOH buffer 5.0 (pH 7.4), and added nystatin. Nystatin was dissolved in methanol at a concentration of 10 mg/ml and added to the pipette solution at a concentration of 100–200 μg/ml just before experiments. After the gigaohm seal between tip and cell membrane was formed, negative pressure was released to await gradual opening of nystatin-induced pores. A ramp-pulse protocol was used to record the quasi–steady-state

membrane current as previously described (24). Current-clamp experiments were also recorded in perforated-patch recording mode. The isolated ventricular cells were exposed to a glucose-free HEPES-Tyrode solution containing 50 μM dinitrophenol (DNP), and changes of the membrane current and action potential were observed. The experiments were performed at 36°C and analyzed with pClamp software (version 5.5; Axon Instruments Inc., Foster City, California, USA).

Flavoprotein fluorescence measurement. MitoK_{ATP} channel activities were assessed at Johns Hopkins University by measuring changes in flavoprotein fluorescence before and after application of diazoxide (18). Experiments were performed on adult mouse cardiomyocytes isolated from wild-type (WT) and knockout (KO) animals. Cells were superfused with an external bath solution containing (in mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, 3-nitropropionic acid 1, HEPES 10 (pH 7.4). Endogenous flavoprotein fluorescence was excited at 480 nm for 100 milliseconds and emission was collected at 530 nm by a photomultiplier and digitized (Digidata 1200; Axon Instruments Inc.). Relative fluorescence during excitation was averaged and calibrated with signals recorded after application of cyanide (CN) and DNP, which led to complete reduction and oxidation, respectively.

In vitro functional study. Mice were anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and heparinized (0.1 units/g body weight) by intravenous injection. Hearts were rapidly excised and connected to the perfusion canula via the aorta, as previously described (24). Retrograde perfusion was maintained at a constant flow (3 ml/min) with modified Krebs-Henseleit solution containing (in mM): NaCl 119, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.0, glucose 10, $NaHCO_3$ 24.9. The perfusate was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4, 37°C). A polyethylene film balloon was inserted into the cavity of the left ventricle through the left atrium. The balloon was filled with saline to adjust the base-line end-diastolic pressure (EDP) to 5–10 mmHg. After a stabilization period of more than 20 minutes, hearts were subjected to no-flow, global ischemia by clamping the perfusion line. During all procedures, the hearts were immersed in the perfusate and the temperature was maintained at 37°C. Drug treatment was initiated 15 minutes before the ischemic period and was continued throughout the experiments. After 20 minutes of ischemia, the clamp was released and the hearts were reperfused for 60 minutes. Left ventricular pressure (LVP) was measured continuously. Left ventricular developed pressure (LVDP) was designated as differences between systolic and diastolic pressures of the left ventricle.

Drugs. The following drugs were used: nystatin (Wako Pure Chemicals Industries, Osaka, Japan); 5-hydroxydecanoate (5-HD), glibenclamide, and diazoxide (Sigma-Aldrich Japan, Tokyo, Japan); and HMR1098 (1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl-3-methylthiourea, sodium salt; Aventis Pharma, Tokyo, Japan). Diazoxide was dissolved in DMSO, and the final concentration of solvent was 0.01%. 5-HD and HMR1098 were dissolved in the perfusate.

Statistics. All data are presented as mean ± SE. Statistical analyses of the data were performed using Student's *t* test or ANOVA. Probability values less than 0.05 were considered significant.

Results

Myocardial infarction and IPC. There were no significant differences in the basal hemodynamic parameters, including heart rate, between WT and KO mice, as previously described (24). There were also no significant differences in the size of the AAR among the groups of control WT (WT-CON; $n = 6$), WT with IPC (WT-IPC; $n = 6$), control KO (KO-CON; $n = 6$) and KO with IPC (KO-IPC; $n = 6$); the AARs of these four groups were 33% ± 3%, 41% ± 3%, 33% ± 2%, and 37% ± 2% of left ventricular weight, respectively. The infarct size of the WT-IPC group (24% ± 5% of AAR; Figure 1, b and e) was significantly smaller than that of the WT-CON group (45% ± 4% of AAR; Figure 1, a and e). Although the infarct size was reduced to about half of control by IPC in WT mice, there was no significant difference

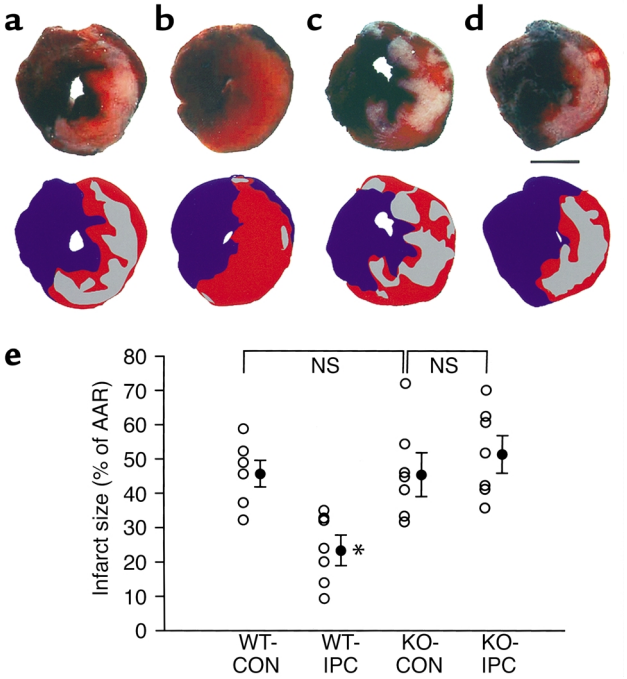

Figure 1
In vivo myocardial infarction studies. (**a**–**d**) Representative photographs of myocardial slices of WT-CON mice (**a**), WT-IPC mice (**b**), KO-CON mice (**c**), and KO-IPC mice (**d**) are shown. A schema of each photograph is indicated below; infarct area is expressed as gray, viable myocardium in AAR as red, and nonischemic area as blue. Scale bar = 2 mm. (**e**) Myocardial infarct size expressed as percentage of AAR for WT-CON ($n = 6$), WT-IPC ($n = 6$), KO-CON ($n = 6$), and KO-IPC ($n = 6$). There were no significant differences in the infarct size between WT-CON and KO-CON or between KO-CON and KO-IPC. Values are expressed as mean ± SE. NS, not significant. *$P < 0.01$ versus WT-CON.

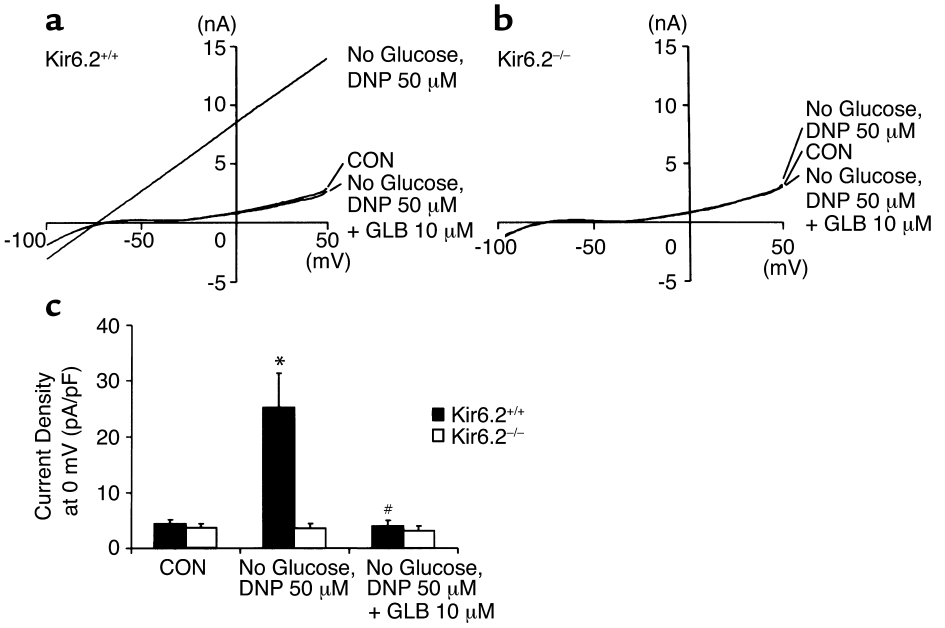

Figure 2
Effects of metabolic inhibition with a glucose-free, DNP-containing (50 μM) solution and coapplication of glibenclamide (GLB; 10 μM) on the whole-cell membrane currents recorded from ventricular cells of WT (**a**) and KO mice (**b**). (**c**) Current densities at 0 mV in WT (n = 10) and KO ventricular cells (n = 10) are summarized. Values are expressed as mean ± SE. *P < 0.01 versus control (CON); #P < 0.01 versus DNP, no glucose.

between the infarct size of the KO-CON (45% ± 6% of AAR; Figure 1, c and e) and that of the KO-IPC (51% ± 6% of AAR; Figure 1, d and e). Thus, in KO mice, IPC produced no significant cardioprotective effect against myocardial infarction.

SarcK$_{ATP}$ channel function. Whole-cell membrane currents were recorded using a ramp-pulse protocol. The reversal potential was close to the potassium equilibrium potential in ventricular cells of both WT and KO mice (Figure 2, a and b). There was no significant difference in the density of the outward current at 0 mV between ventricular cells isolated from WT (4.3 ± 0.8 pA/pF, ten cells from three animals) and from KO mice (3.8 ± 0.7 pA/pF, ten cells from three animals) in the control condition (Figure 2c). In WT mice, metabolic inhibition with a glucose-free, DNP-containing (50 μM) solution induced an outward current (25.2 ± 6.1 pA/pF at 0 mV), which, by virtue of its blockade by 10 μM glibenclamide (3.9 ± 1.0 pA/pF), was confirmed to be the ATP-sensitive K^+ current ($I_{K,ATP}$). However, such a membrane current change was not observed in KO cells (3.6 ± 0.8 pA/pF after metabolic inhibition; Figure 2, a–c).

The action potential duration (APD) was measured from the ventricular cells stimulated at 0.2 Hz by current-clamp mode. There were no significant differences in the basal action potential parameters, such as the resting membrane potential, action potential amplitude, and APD, between WT (eight cells from five animals) and KO myocytes (eight cells from four animals). The APD_{90} (APD at 90% repolarization level) was markedly shortened from 40.4 ± 3.3 milliseconds to 19.1 ± 2.3 milliseconds by metabolic inhibition in WT ventricular cells (n = 8, P < 0.01; Figure 3a). The shortened APD in WT ventricular cells reverted to the control level after the addition of 10 μM glibenclamide (40.1 ± 3.1 milliseconds; Figure 3, a and c) or 30 μM HMR1098 (n = 4; data not shown). However, in KO ventricular cells, APD_{90} values before and after metabolic inhibition were 45.7 ± 5.3 milliseconds and 48.3 ± 5.2 milliseconds (n = 8), respectively, and there was no significant difference between these APD_{90} values (Figure 3, b and c).

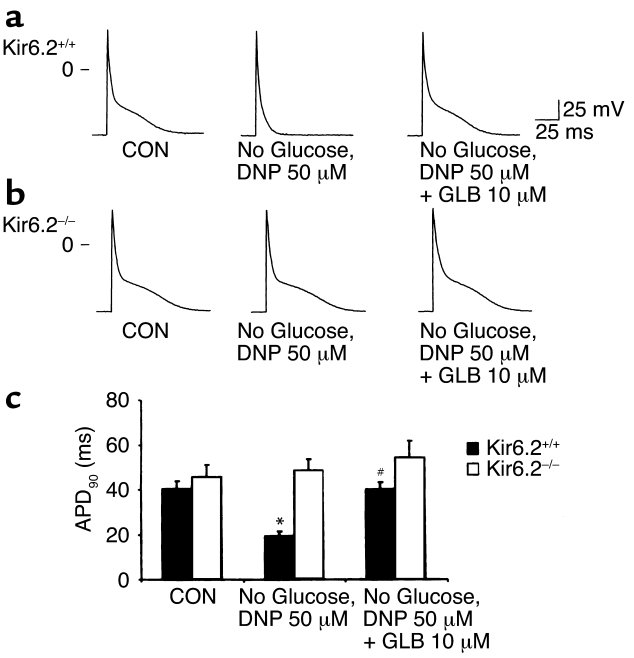

Figure 3
Effects of metabolic inhibition with a glucose-free, DNP-containing (50 μM) solution and coapplication of glibenclamide (GLB; 10 μM) on the action potentials recorded from ventricular cells of WT (**a**) and KO mice (**b**). (**c**) Summarized changes of APD_{90} in WT (n = 8) and KO cells (n = 8). Values are expressed as mean ± SE. *P < 0.01 versus control (CON); #P < 0.01 versus DNP, no glucose.

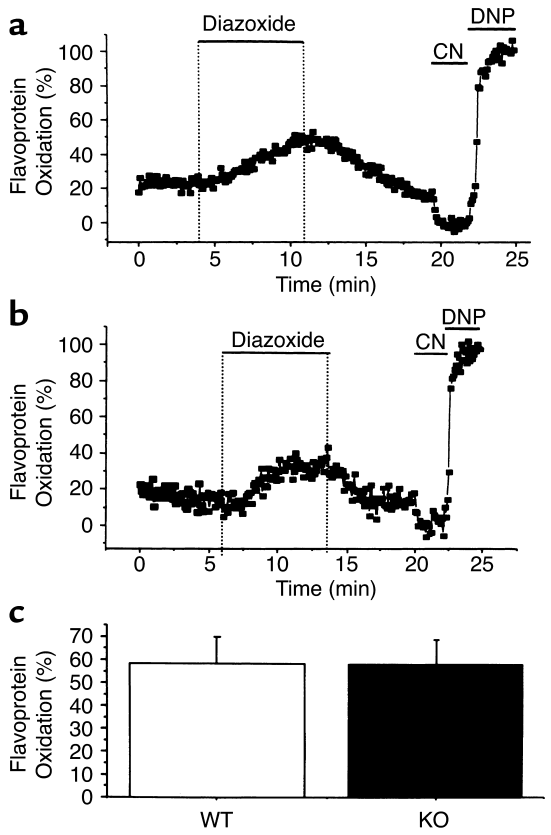

Figure 4
Mitochondrial K_{ATP} channel activities assessed by changes in flavoprotein fluorescence. The effects of 100 μM diazoxide on flavoprotein fluorescence of representative cells isolated from (**a**) WT and (**b**) KO mice. Bars indicate periods of diazoxide application. (**c**) Summarized data for percentage of flavoprotein oxidation. Despite the absence of surface $I_{K,ATP}$ in KO cells, they displayed response to diazoxide similar to that of WT.

MitoK$_{ATP}$ channel function. Opening of mitoK$_{ATP}$ channels partially dissipates the inner mitochondrial membrane potential established by the proton pump and thereby accelerates electron transfer by the respiratory chain. If uncompensated by increased production of electron donors, this leads to net oxidation of the mitochondria. Therefore, mitochondrial redox state, which can be monitored by examining changes in fluorescence of flavin adenine nucleotide–linked enzymes in the mitochondrial matrix, can be used as an indirect index of mitoK$_{ATP}$ channel activity (18, 31, 32). We studied mitoK$_{ATP}$ activity by examining the effects of the K$_{ATP}$ opener diazoxide on flavoprotein fluorescence of WT and KO ventricular cells. Figure 4a shows the time course of changes in flavoprotein fluorescence of a representative WT cell after exposure to 100 μM diazoxide. Diazoxide induced reversible flavoprotein oxidation in WT cells to 58.2% ± 11.5% (n = 6), relative to the maximal oxidation (100%) and complete reduction (0%) that followed applications of DNP and CN, respectively. This induction by diazoxide could be blocked by the mitoK$_{ATP}$ blocker 5-HD (data not shown). Figure 4b shows a typical example of the effects of diazoxide on flavoprotein fluorescence of KO cells. Similarly, diazoxide also reversibly induced flavoprotein oxidation in KO cells (58.1% ± 10.9%, n = 3) to a level not different from that of WT cells (P > 0.05) (Figure 4c), implying that mitoK$_{ATP}$ channel function is preserved in KO ventricular cells.

Mechanical function of isolated hearts during ischemia/reperfusion. There were no significant differences in heart rate or LVDP between WT and KO hearts: heart rates in WT and KO hearts were 409 ± 18 beats per minute (bpm) and 384 ± 27 bpm, respectively, and LVDPs in WT and KO hearts were 58.6 ± 2.7 mmHg and 48.8 ± 2.8 mmHg, respectively. Treatment with 5-HD or HMR1098 did not significantly affect these parameters. During global ischemia, LVDP gradually declined and the left ventricular EDP (LVEDP) increased (Figure 5a). It took

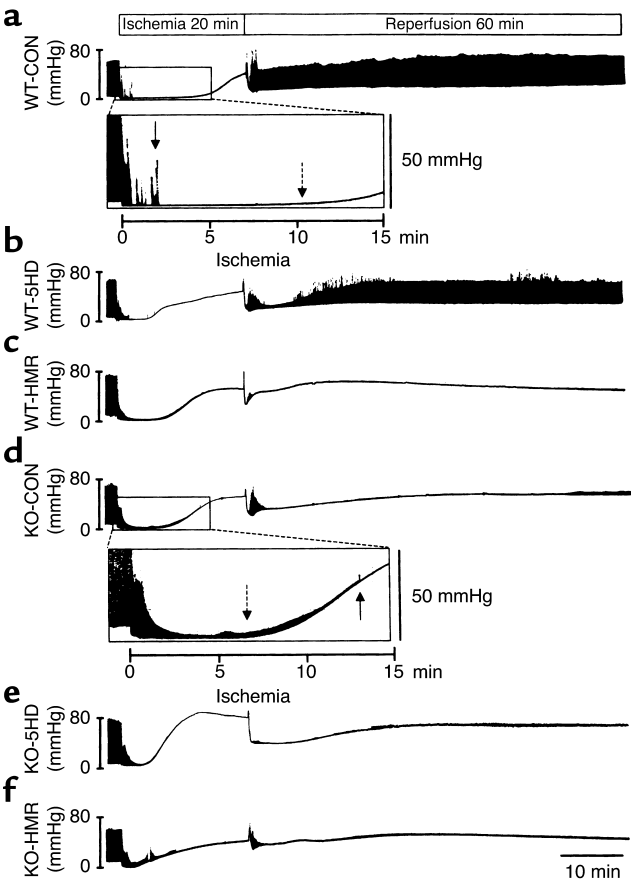

Figure 5
Changes in LVP during ischemia and reperfusion. Representative LVP changes in untreated WT (WT-CON, **a**), 5-HD–treated WT (WT-5HD, **b**), HMR1098-treated WT (WT-HMR, **c**), untreated KO (KO-CON, **d**), 5-HD–treated KO (KO-5HD, **e**), and HMR1098-treated KO hearts (KO-HMR, **f**) are shown. The traces from WT-CON and KO-CON hearts are partly expanded in the insets. Arrows with full line and those with dashed line show the time points of cessation of contraction and onsets of contracture, respectively. Note that KO hearts continued to contract for a longer time than the WT hearts did during ischemia.

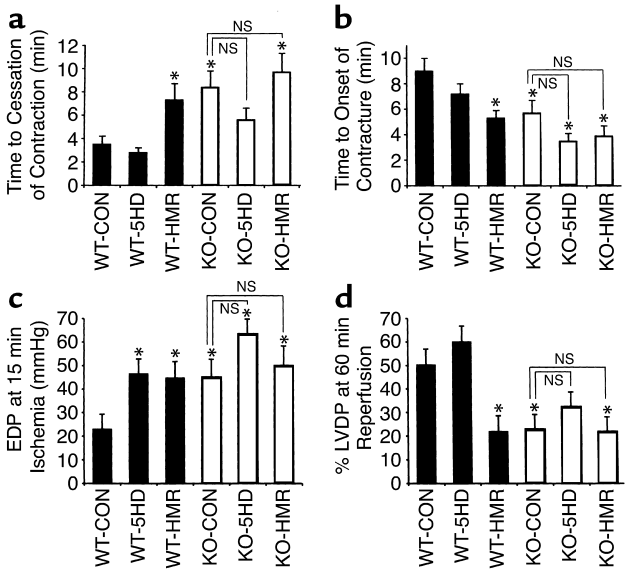

Figure 6
Summarized data of mechanical function of isolated hearts during ischemia/reperfusion. Time to cessation of contraction (**a**), time to onset of contracture (**b**), EDP at 15 minutes of ischemia (**c**), and LVDP at 60 minutes of reperfusion (**d**) in untreated WT (WT-CON, $n = 7$), 5-HD–treated WT (WT-5HD, $n = 7$), HMR1098-treated WT (WT-HMR, $n = 7$), control KO (KO-CON, $n = 7$), 5-HD–treated KO (KO-5HD, $n = 7$), and HMR1098-treated KO hearts (KO-HMR, $n = 7$) are shown. Values are expressed as mean ± SE. *$P < 0.05$ versus WT-CON.

significantly more time for KO hearts to cease contracting during ischemia (Figure 5d). The time to cessation of left ventricular contraction in WT and KO hearts was 3.5 ± 0.7 minutes and 8.4 ± 1.4 minutes, respectively (Figure 6a). Significantly more time was required for the cessation of ventricular contraction in HMR1098-treated WT hearts (7.3 ± 1.4 minutes) compared with untreated WT hearts. The KO and HMR1098-treated WT hearts developed ischemic contracture more rapidly than did untreated WT hearts ($P < 0.05$) (Figure 6b); accordingly, EDP at 15 minutes after global ischemia was significantly greater in untreated KO, HMR1098-treated WT, and 5-HD–treated WT hearts than in untreated WT hearts (Figure 6c). After reperfusion, left ventricular function recovered gradually (Figure 6). Recovery of the LVDP in HMR1098-treated WT and KO hearts was worse than in WT-CON hearts (Figure 6d). Treatment with 500 μM 5-HD did not significantly worsen the recovery of left ventricular function, although it increased EDP more markedly during myocardial ischemia (Figure 6c). Treatment with HMR1098 in KO hearts failed to increase EDP or deteriorate the recovery of left ventricular function further (Figure 6, c and d). These results are noteworthy because they reveal a greater susceptibility of KO hearts to ischemia, under basal conditions. Such a shift in susceptibility may influence the threshold for preconditioning, complicating the interpretation of the data in Figure 1; however, this possibility remains to be evaluated.

Discussion

Recently we have demonstrated that Kir6.2 forms the pore of sarcK_{ATP} channels in cardiac cells but not in vascular smooth muscle cells based upon functional experiments using Kir6.2-deficient mice (24). The KCO pinacidil markedly shortened APD in WT but not in KO ventricular cells. In the present study (see also ref. 33), metabolic inhibition shortened APD in WT ventricular cells but hardly affected APD in KO ventricular cells, suggesting that activation of sarcK_{ATP} channel composed of the Kir6.2 pore subunit is exclusively responsible for the action potential shortening during metabolic inhibition.

Although it is acknowledged that sarcK_{ATP} channel in heart cells is formed by a muscle-type sulfonylurea receptor (SUR2A) and Kir6.2 (22, 23), the molecular identity of mitoK_{ATP} channels remains elusive. Recently it was demonstrated that dominant negative Kir6.2 gene transfer suppressed the function of sarcK_{ATP} channel function but not mitoK_{ATP} channel function in rabbit ventricular myocytes, suggesting no discernible role for Kir6.2 in the mitoK_{ATP} channel (25). In the present study, mitoK_{ATP} channel function, assessed by flavoprotein oxidation, was preserved in the ventricular myocytes isolated from Kir6.2-deficient mice, providing additional evidence that Kir6.2 is not a critical subunit of the mitoK_{ATP} channel.

IPC is a phenomenon in which brief intermittent periods of ischemia paradoxically protect the myocardium against a more prolonged ischemic insult, the result of which is a marked reduction of infarct size (1). Originally it was proposed that activation of sarcK_{ATP} channels plays an important role in IPC (8, 9). Indeed, several studies have demonstrated that IPC was mimicked by KCOs (10–12) and abolished by the sulfonylurea K_{ATP} channel blocker glibenclamide (8). However, Grover et al. (34) found that there was no correlation between APD shortening and cardioprotection produced by cromakalim in dogs. In addition, the class III antiarrhythmic drugs dofetilide and terikalant (which do not target K_{ATP} channels) each failed to antagonize the cardioprotective effect of IPC (35, 36). A more recent, alternative suggestion postulates that mitochondria harbor another type of K_{ATP} channel, and activation of mitoK_{ATP} channels rather than sarcK_{ATP} channels may be important for the cardioprotection including IPC (15–20). In addition, it has been shown that diazoxide and 5-HD are relatively selective activators and blockers of mitoK_{ATP} channel in cardiomyocytes, respectively, at least under nonischemic conditions (20).

Taking this background into consideration, it might have been expected that IPC would be preserved in Kir6.2-deficient mice (37). Surprisingly, however, IPC was abolished in KO mice. Why could IPC not be observed in Kir6.2-deficient mice in spite of intact mitoK_{ATP} channel function in KO ventricular cells? In order to gain greater insights into the cause, we conducted in vitro experiments using Langendorff-perfused hearts. Such experiments revealed that the

increase in LVEDP during global ischemia was more marked, and the recovery of contractile function was worse, in KO hearts than in WT hearts. Since it took more time for the KO hearts to stop beating during ischemic period than for WT hearts, massive Ca^{2+} influx might occur in ischemic heart cells of Kir6.2-deficient mice. This concept is supported by the experiments using HMR1098, a selective sarcK_{ATP} channel blocker (20). Treatment with HMR1098 significantly increased LVEDP during ischemia in WT hearts but not in KO hearts. Treatment with the mitoK_{ATP} channel blocker 5-HD did not worsen the recovery of contractile function in WT or KO hearts, although the increase in LVEDP was greater during global ischemia in these hearts. These findings illustrate that sarcK_{ATP} channels influence the degree of ischemic injury independent of any preconditioning protocol: the extent of basal ischemic damage is greater in the KO hearts than in WT hearts, as judged by the greater magnitude of ischemic contracture and the poorer recovery of ventricular function after reperfusion. The fact that sarcK_{ATP} channels affect the severity of ischemia at base line may affect the outcome of IPC protocols, for example by shifting the "threshold" for IPC. Such a possibility was not examined in the present study.

It was originally hypothesized by Noma (14) that activation of sarcK_{ATP} channels may serve as an endogenous cardioprotective mechanism. Action potential shortening due to activation of sarcK_{ATP} channels is expected to reduce the time for Ca^{2+} influx via L-type Ca^{2+} channels and to increase the time for Ca^{2+} extrusion through the Na^+-Ca^{2+} exchange system. The resultant decrease in Ca^{2+} influx would lead to a reduction of mechanical contraction, blunting of intracellular Ca^{2+} overload, and energy sparing. Genetic disruption of sarcK_{ATP} channel might deteriorate the recovery of mechanical function after ischemia/reperfusion and abolish the IPC due to an increase in intracellular Ca^{2+} overload during ischemia. Indeed, in untreated KO hearts and HMR1098-treated WT hearts, it took a longer time for the cessation of contraction during ischemia than in untreated WT hearts, which might be mainly ascribed to the absence of depressed cardiac contractility due to action potential shortening. In this context, it is noteworthy that gene delivery of Kir6.2 and SUR2A to COS-7 cells can afford the cytoprotective properties against Ca^{2+} overload induced by chemical hypoxia/reperfusion (38). Since action potentials were not generated in the cultured cells, hyperpolarization of the cell membrane resulting from the activation of sarcK_{ATP} channel might play an important role in the cytoprotection. It has been suggested that activation of mitoK_{ATP} channels can depolarize the mitochondrial membrane, thereby preventing mitochondrial Ca^{2+} overload (39). If attenuation of mitochondrial Ca^{2+} overload is one of primary mechanisms of mitoK_{ATP} channel–mediated cardioprotection, intracellular Ca^{2+} overload during ischemia in KO heart cells might be too much for the mitochondria to handle. Activation of sarcK_{ATP} channel has also been shown to be important for cell protection in other tissues including the brain (27) and skeletal muscle (40).

Numerous studies have indicated that activation of mitoK_{ATP} channel is important for early and delayed preconditioning in animal (17–20, 34–37, 41, 42) as well as human myocardium (43). When nonbeating myocardial preparations (43) or quiescent ventricular myocytes (20) are used for preconditioning experiments, mitoK_{ATP} channels might play a more predominant role in the cardioprotection. In addition, inhibition of action potential shortening by sulfonylureas or other K^+ channel blockers such as dofetilide and terikalant might not be complete under severe ischemic conditions, in striking contrast with KO hearts, which show a complete lack of APD shortening. Probably both sarcK_{ATP} and mitoK_{ATP} channels are important for the cardioprotection, although there are some differences in their importance among animal species and experimental conditions.

It has been controversial whether sarcK_{ATP} or mitoK_{ATP} channel is more important in IPC (44). The present study has indicated that intact sarcK_{ATP} channel function is necessary for IPC, at least in the mouse, which is a high-heart-rate species. It has been demonstrated that administration of digoxin, which might inhibit sarcK_{ATP} channel and produce intracellular Ca^{2+} overload by Na^+-K^+ ATPase inhibition, abolished IPC in vivo in rabbit hearts (45). More recently it has been reported that, in the canine heart, both mitoK_{ATP} and sarcK_{ATP} channels play an important role in the limitation of infarct size by IPC (46) and a volatile anesthetic (47). Two features of the current experiment limit its potential interpretability at face value. First, the results were derived from the mouse, a species in which the physiologic heart rate is tenfold higher than in humans. The higher the heart rate, the greater one would predict the cardioplegic effect of sarcK_{ATP} channel activation to be. Thus, the functional effects of sarcK_{ATP} channels may be exaggerated in mice relative to larger mammals. Second, the fact that ischemic injury is greater at base line in KO hearts than in WT hearts may undermine the rationale of comparing identical IPC protocols in the two strains. Nevertheless, the present findings obtained from Kir6.2-deficient mice prompt reconsideration of the importance of sarcK_{ATP} channel in cardioprotection.

Acknowledgments

We thank T. Sato, H. Uemura, and T. Ogura for helpful discussion. We are grateful to Y. Reien and I. Sakashita for excellent technical and secretarial assistance. HMR1098 was kindly provided by Aventis Pharma. This work was supported by a Scientific Research Grant and a Grant-in-Aid for Creative Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Mitsui Life Insurance Research Foundation; and K. Watanabe

Research Fund. The work on mitochondrial fluorescence was performed at Johns Hopkins University and funded by the NIH (R37 HL-36957 to E. Marbán). E. Marbán holds the Michel Mirowski, M.D., Professorship in Cardiology at Johns Hopkins University.

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