Functional Roles of Cardiac and Vascular ATP-Sensitive Potassium Channels Clarified by Kir6.2-Knockout Mice

Masashi Suzuki, Ronald A. Li, Takashi Miki, Hiroko Uemura, Naoya Sakamoto, Yuki Ohmoto-Sekine, Masaji Tamagawa, Takehiko Ogura, Susumu Seino, Eduardo Marbán, Haruaki Nakaya

Abstract—ATP-sensitive potassium (K<sub>ATP</sub>) channels were discovered in ventricular cells, but their roles in the heart remain mysterious. K<sub>ATP</sub> channels have also been found in numerous other tissues, including vascular smooth muscle. Two pore-forming subunits, Kir6.1 and Kir6.2, contribute to the diversity of K<sub>ATP</sub> channels. To determine which subunits are operative in the cardiovascular system and their functional roles, we characterized the effects of pharmacological K<sup>+</sup> channel openers (KCOs, ie, pinacidil, P-1075, and diazoxide) in Kir6.2-deficient mice. Sarcolemmal K<sub>ATP</sub> channels could be recorded electrophysiologically in ventricular cells from Kir6.2<sup>−/−</sup> (wild-type [WT]) but not from Kir6.2<sup>−/−</sup> (knockout [KO]) mice. In WT ventricular cells, pinacidil induced an outward current and action potential shortening, effects that were blocked by glibenclamide, a K<sub>ATP</sub> channel blocker. KO ventricular cells exhibited no response to KCOs, but gene transfer of Kir6.2 into neonatal ventricular cells rescued the electrophysiological response to P-1075. In terms of contractile function, pinacidil decreased force generation in WT but not KO hearts. Pinacidil and diazoxide produced concentration-dependent relaxation in both WT and KO aortas precontracted with norepinephrine. In addition, pinacidil induced a glibenclamide-sensitive current of similar magnitude in WT and KO aortic smooth muscle cells and comparable levels of hypotension in anesthetized WT and KO mice. In both WT and KO aortas, only Kir6.1 mRNA was expressed. These findings indicate that the Kir6.2 subunit mediates the depression of cardiac excitability and contractility induced by KCOs; in contrast, Kir6.2 plays no discernible role in the arterial tree. (Circ Res. 2001;88:570-577.)

Key Words: gene targeting ▪ heart ▪ vascular smooth muscle ▪ ATP-sensitive K<sup>+</sup> current ▪ action potential

ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels, originally discovered in cardiac myocytes, have been described in many other tissues including pancreatic β cells, skeletal and smooth muscle cells, and neurons. They play important roles in the physiology and pathophysiology of various tissues by coupling the metabolic state of the cell to its electrical activity. Recent progress in molecular biology and electrophysiology resulted in cloning of K<sub>ATP</sub> channel genes and elucidation of their subunit composition. K<sub>ATP</sub> channels are assembled with a hetero-octameric stoichiometry from the following 2 structurally distinct subunits: an inwardly rectifying potassium channel openers forming the pore (Kir6.X) and a regulatory subunit, a sulfonylurea receptor (SUR) belonging to the ATP binding cassette superfamily. The pancreatic β-cell K<sub>ATP</sub> channels have been shown to consist of SUR1 and Kir6.2. In fact, our previous report indicated that insulin secretion in response to glucose or sulfonylurea was absent in pancreatic β cells from K<sub>ATP</sub> channel-deficient mice generated by genetic disruption of Kir6.2. Cardiac K<sub>ATP</sub> channels are thought to be formed by SUR2A and Kir6.2, because coexpression of these clones in a cell line reconstitutes the basic electrophysiological and pharmacological properties of the native cardiac channel. In terms of vascular K<sub>ATP</sub> channels, Isomoto et al showed that coexpression of Kir6.2 and SUR2B, a splice variant of SUR2A, reconstituted the pharmacological and electrophysiological properties of K<sub>ATP</sub> channels described in some smooth muscle cells. Indeed, it has been suggested that K<sub>ATP</sub> channels of smooth muscle cells of murine colon and guinea pig bladder comprise SUR2B and Kir6.2. However, Yamada et al have suggested that K<sup>+</sup> channel composed of SUR2B and Kir6.1 closely resembles the NDP-dependent K<sup>+</sup> (<i>K<sub>N</sub>DP</sub>) channel observed in vascular smooth muscle cells. To clarify the functional role(s) of a defined K<sub>ATP</sub>-channel subunit in cardiac and vascular tissues,
we studied \( K_{\text{ATP}} \) channel-deficient mice (Kir6.2\(^{−/−}\)) produced by knockout (KO) of the Kir6.2 gene. We find that Kir6.2 is essential for the depression of cardiac excitation and contraction, but not for vasodilation, in response to \( K^+ \) channel openers (KCOs), highlighting major functional and genetic distinctions between cardiac and vascular \( K_{\text{ATP}} \) channels.

Materials and Methods

Kir6.2\(^{−/−}\) Mice

All procedures complied with the standards for the care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1986). A mouse line deficient in \( K_{\text{ATP}} \) channel was generated by targeted disruption of the gene coding for Kir6.2, as described previously.\(^9\) C57BL/6 mice were used as control because they had been backcrossed to a C57BL/6 strain for 5 generations.

Electrophysiology

Adult Ventricular Cells

Single ventricular cells of the adult mouse heart were isolated by conventional enzymatic digestion. Single-channel and whole-cell membrane currents were recorded by the patch-clamp method as previously described.\(^{17,18}\) For single-channel recordings from the open cell-attached patches, symmetrical high-K\(^+\) pipette solution (solution A) and internal solution (solution B) were used. After the gigahm seal formation, part of the surface membrane of a rod-shaped cell was disrupted by penetrating the membrane with a glass pipette (1 to 2 \( \mu \)m in tip diameter) and the ruptured cell was exposed to the solution containing only a trace amount of ATP (solution B). The single-channel currents were recorded at room temperature and analyzed with pClamp software (version 5.5, Axon Instruments). Single-cell current recordings were performed at 36.0°C using glass patch pipettes filled with solution C (pCa 8.0). The external solution used was HEPES-Tyrode solution (solution D). A liquid junction potential between the internal solution and the bath solution of \(-8 \text{ mV}\) was corrected. A ramp pulse protocol was used to record the quasi–steady-state membrane current as previously described.\(^{18}\) Current-clamp experiments were also performed in the whole-cell recording mode at 36.0°C.

Isolation of Neonatal Ventricular Myocytes and Viral Gene Transfer

Neonatal (1- to 2-day-old) ventricular myocytes were prepared by trypsin digestion as previously described.\(^{18}\) Briefly, ventricles of a single-litter of mouse pups (\( \sim 10\), 1 to 2 days old) were aseptically removed immediately after decapitation. Isolated hearts were pooled, minced, and digested at room temperature with trypsin. After digestion, cells were centrifuged, plated at low density (2×10\(^5\) cells mL\(^{-1}\)), and incubated at 37°C in a humidified atmosphere of 95% \( \text{O}_2 /5\% \text{CO}_2 \).

The edcsyne-inducible recombinant adenovirus (AdKir6.2) carrying the wild-type (WT) Kir6.2 channel and the reporter (enhanced green fluorescent protein) genes were generated by Cre-lox recombination.\(^{20,22}\) Neonatal myocytes were coinfected with the channel virus AdKir6.2 and the hormone receptor virus AdVgKRR (ratio=1:10) at a final concentration of 10,000 particles mL\(^{-1}\). Protein expression was induced by adding 1 mmol/L ponasterone A (Invitrogen) to the culture medium.

Electrical recordings were performed at room temperature after 72 hours in culture using the whole-cell patch clamp technique with a pipette solution (solution E) and a bath solution (solution F). Only successfully infected cells, as identified by their green fluorescence using epifluorescent microscopy, were selected for experiments.

Vascular Smooth Muscle Cells

Single smooth muscle cells were enzymatically isolated from the adult mouse aorta, and the membrane currents were recorded at room temperature using the whole-cell patch clamp technique with high-K\(^+\) HEPES-buffered bath solution (solution G) and pipette solution (solution H).

Mechanical Function Study

Langendorff-Perfused Heart Preparation

The heart was quickly removed from the mouse anesthetized with urethane (1.5 mg/g, IP). The heart was retrogradely perfused at a constant flow (~3 mL/min) with the Krebs-Henseleit solution (solution I, 37°C) gassed with 95% \( \text{O}_2 /5\% \text{CO}_2 \). 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 baseline end-diastolic pressure to 5 to 10 mm Hg. Left ventricular pressure and its dp/dt were measured continuously.

Isolated Aortic Preparation

The aorta was carefully removed and cut into rings (4 mm in length). The endothelium was removed carefully by rubbing with a small steel pin, and the rings were mounted in a thermostatic organ bath for isometric tension recording under a resting tension of 0.5 grams. The bath was continuously perfused with the Krebs-Henseleit solution (solution H) gassed with 95% \( \text{O}_2 /5\% \text{CO}_2 \) at 37°C.

Hemodynamic Measurement

Blood pressure and heart rate (HR) were measured in mice anesthetized with urethane. Subcutaneous needle electrodes were inserted in the limbs for ECG recording. Femoral artery and vein were cannulated with small polyethylene catheters for the measurement of blood pressure and the injection of saline or drugs, respectively. Body temperature was monitored with a rectal probe and maintained at 37°C using a heating lamp.

Northern Blot Analysis

Total cellular RNA was isolated using the RNeasy Mini kit (Qiagen). Total RNA (10 \( \mu \)g) from the hearts and aortas of the mice were denatured with formaldehyde, subjected to electrophoresis on a 1% agarose gel, and transferred to nylon membranes. Hybridization was carried out under high-stringency conditions with a \( ^3\)P-labeled full-length cDNA of Kir6.1 or Kir6.2. For autoradiography, the nylon membranes were exposed to x-ray film with an intensifying screen at \(-80°C\) for 1 day. Results from these analyses were quantified through densitometry.

Drugs

The following drugs were used: pinacidil (Research Biochemicals International), diazoxide and glibenclamide (Sigma), norepinephrine (Wako), P-1075 (Leo Pharmaceutical Products), and HMR1098 (Aventis Pharmaceuticals). Pinacidil was dissolved in 0.1N HCl (Aventis Pharmaceuticals). Diazoxide and glibenclamide (Sigma), norepinephrine were dissolved in saline. Diazoxide and P-1075 were dissolved in DMSO (final concentration of 0.1%). L-Ascorbic acid (0.1 mmol/L) was added to the Krebs-Henseleit solutions to prevent the oxidation of norepinephrine.

Statistics

All data are presented as mean±SE. Statistical analyses of the data were performed using the Student \( t \) test or ANOVA. Probability values <0.05 were considered significant. EC\(_{50}\) values were obtained by use of Delta Graph Professional (Delta Point). A table listing the composition of the various solutions can be found in an online data supplement available at http://www.circresaha.org.

Results

Electrophysiology in Cardiomyocytes

The open cell-attached patches of ventricular cells were held at \(-40 \text{ mV}\) and exposed to a solution containing only a trace amount of ATP (1 \( \mu \)mol/L). Single \( K_{\text{ATP}} \) channel activity could be recorded from 32 of 32 patches of 6 WT mice (Figure 1a). The channel openings were inhibited by addition
of 1 mmol/L ATP or 10 μmol/L glibenclamide to the solution. The linear slope conductance obtained from the current-voltage relationship from -80 to 0 mV for the single-channel current of WT ventricular cells was 79 ± 1 pS (6 cells from 3 animals) (Figures 1c and 1d). In contrast, openings of K<sub>ATP</sub> channels could not be recorded from any open-cell-attached patches of KO mouse (22 cells from 4 animals) (Figure 1b). The channel activity could not be activated even by addition of Mg-UDP, Mg-ATP, and/or pinacidil.

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 (Figures 2a and 2b). There was no significant difference in the density of the outward current at 0 mV between ventricular cells isolated from WT (2.67 ± 0.83 pA/pF) and KO (3.47 ± 0.91 pA/pF) mice under control conditions. Pinacidil produced concentration-dependent increases of an outward current, which, by virtue of its blockade by 1 μmol/L glibenclamide, was confirmed as the ATP-sensitive K<sup>+</sup> current (I<sub>KATP</sub>); such a current was observed in WT ventricular cells but not in KO cells (Figure 2c). There were no significant differences in the basal parameter values of the action potentials recorded from the ventricular cells stimulated at 0.2 Hz. Exposure to pinacidil revealed dramatic differences. The action potential duration (APD) was shortened by pinacidil in WT but not in KO ventricular cells (Figures 2d and 2e). APD at 90% repolarization level (APD<sub>90</sub>) of WT cells was significantly decreased from 34.0 ± 1.5 to 10.8 ± 3.1 ms by 100 μmol/L pinacidil and reversed to 37.9 ± 2.5 ms after the addition of 1 μmol/L glibenclamide (Figure 2f). However, the APD<sub>90</sub> of the KO ventricular cells was not significantly changed from 35.6 ± 2.4 ms after exposure to pinacidil (Figure 2f).

**Figure 1.** Single-channel current recordings in the open-cell attached mode from ventricular cells of Kir6.2<sup>+/+</sup> and Kir6.2<sup>−/−</sup> mice. K<sub>ATP</sub> channel activity that was sensitive to ATP and glibenclamide was observed in membrane patches of Kir6.2<sup>+/+</sup> (a) but not Kir6.2<sup>−/−</sup> (b) ventricular cells. c, Unitary K<sup>+</sup> currents in a membrane patch held at various voltage levels. d, Current-voltage relationship for the single-channel current; slope conductance at 0 to -80 mV was 80 pS.

**Figure 2.** Effects of the KCO pinacidil (PIN) and coapplication of glibenclamide (GLB) on whole-cell membrane currents (a and b) and action potentials (d and e) recorded from ventricular cells of WT (a and d) and KO (b and e) mice. Current densities at 0 mV in Kir6.2<sup>+/+</sup> (12 cells from 11 mice) and Kir6.2<sup>−/−</sup> (7 cells from 5 mice) ventricular cells are summarized in panel c. f, Summary of changes in APD<sub>90</sub> after (in μmol/L) PIN 100 and PIN 100 plus GLB 1 in Kir6.2<sup>+/+</sup> (8 cells from 8 mice) and Kir6.2<sup>−/−</sup> ventricular cells (8 cells from 5 mice). *P<0.05, **P<0.01 vs control (CON); #P<0.01 vs 100 μmol/L pinacidil.
Restoration of $I_{\text{K,ATP}}$ by Adenoviral Gene Transfer of Kir6.2

As anticipated from our data in adult mouse cells, application of 100 $\mu$mol/L P-1075, a pinacidil derivative and a potent specific opener of surface $I_{\text{K,ATP}}$, induced sarcolemmal $I_{\text{K,ATP}}$ only in neonatal myocytes isolated from WT but not from KO mice (Figure 3a). If this absence of the surface current in KO is solely due to the absence of functional Kir6.2 channels, we should be able to restore the missing current by expressing Kir6.2. Therefore, we attempted to rescue the KO phenotype by delivering Kir6.2 to cells via adenoviral gene transfer. Indeed, $I_{\text{K,ATP}}$ was observed in KO cells infected with Ad-Kir6.2 on addition of 100 $\mu$mol/L P-1075 (Figures 3a and 3b). The identity of this virally induced and P-1075–induced current as $I_{\text{K,ATP}}$ was confirmed by its complete suppression by coapplication with 100 $\mu$mol/L P-1075 of the specific surface channel blocker HMR1098.23

Electrophysiology in Aortic Smooth Muscle Cells

Effects of pinacidil on the whole-cell membrane current were examined in aortic smooth muscle cells held at $-40$ mV in high-K$^+$ (140 mmol/L) solution. Pinacidil induced an inward current that was sensitive to glibenclamide in a concentration-dependent manner in both Kir6.2$^{-/-}$ and Kir6.2$^{-/-}$ aortic smooth muscle cells held at $-40$ mV in high-K$^+$ solution. The concentration-response curves for the stimulatory effects of pinacidil in WT and KO smooth muscle cells were almost superimposable (Figure 4c). The calculated EC$_{50}$ values of pinacidil for activating the $I_{\text{K,ATP}}$...
were 0.21 and 0.16 μmol/L in aortic smooth muscle cells of WT and KO mice, respectively.

**Functional Experiments in Isolated Cardiac and Vascular Tissues**

Cardiac function of the hearts isolated from WT and KO mice was evaluated by measuring the left ventricular developed pressure (LVDP) and its derivative. In WT hearts, pinacidil at a concentration of 100 μmol/L significantly decreased LVDP and maximal dp/dt to 70.7 ± 2.0% (n = 10, P < 0.05) and 71.5 ± 2.1% (n = 10, P < 0.05) of the control, respectively (Figure 5a). In WT hearts, the addition of 1 μmol/L glibenclamide reversed the pinacidil-induced decreases in LVDP and maximal dp/dt to 93.4 ± 2.4% and 92.4 ± 2.6% of the control, respectively. In contrast, the LVDP and maximal dp/dt of KO hearts were 99.1 ± 3.2% (n = 10, NS) and 98.4 ± 3.1% (n = 10, NS) of the control after 100 μmol/L pinacidil in KO hearts (Figure 5b). To assess vascular reactivity, effects of KCOs on aortic preparations precontracted with 0.1 μmol/L norepinephrine were examined. In both the aortic rings isolated from WT and KO mice, pinacidil and diazoxide produced a concentration-dependent vasorelaxing effect (Figure 6). The EC<sub>50</sub> values of pinacidil for the vasorelaxing effect were 1.29 and 1.66 μmol/L in aortic preparations isolated from WT and KO mice, respectively. The EC<sub>50</sub> values of diazoxide for vasorelaxing effects were 3.42 and 4.61 μmol/L in WT and KO aortas, respectively (Figure 6d). There were no significant differences in these EC<sub>50</sub> values of pinacidil or diazoxide between aortic preparations of WT and KO mice. Thus, KCOs produced vasodilation in vascular tissues isolated from WT and KO mice with similar potency.

**Hemodynamic Measurements**

There were no significant differences in the basal hemodynamic values between WT and KO mice anesthetized with urethane. The basal values of HR in WT and KO mice were 642 ± 24 and 638 ± 12 bpm, respectively. The basal values of mean arterial pressure (MAP) in WT and KO mice were 65.1 ± 2.9 mm Hg and 73.5 ± 4.0 mm Hg, respectively. Intravenous injection of pinacidil (0.3 mg/kg) decreased MAP and increased HR in WT and KO to a similar extent (Figure 7). There were no significant differences in the magnitude of MAP decrease and HR increase in response to pinacidil between WT and KO mice.

**Northern Blot Analysis**

Kir6.2 mRNA was expressed in hearts of WT mice (n = 4) but not in those of KO mice (n = 4), whereas Kir6.1 mRNA was expressed in hearts of both WT and KO mice (Figure 8a). Kir6.1 mRNA was expressed in both aortic preparations of WT (n = 4) and KO mice (n = 4), whereas Kir6.2 mRNA was not detectable in either preparation. Expressed levels of Kir6.1 mRNA in KO hearts (Figure 8b) and aortas (Figure 8c) were similar to those observed in respective preparations of WT mice.
Discussion

Coexpression of SUR2A and Kir6.2 produces a \( \approx 80 \) pS \( K_{\text{ATP}} \) channel resembling that found in native cardiac cells.\(^{11}\) The channel activity is inhibited by ATP with an \( IC_{50} \) value of \( \approx 100 \ \mu\text{mol/L} \) and by glibenclamide with less sensitivity compared with pancreatic-type \( K_{\text{ATP}} \) channel (ie, SUR1/Kir6.2). SUR2A, which showed 68\% identity of amino acids with SUR1, was expressed at high levels in heart and skeletal muscle. Such pharmacological data, conductances, and tissue distributions suggested that the cardiac \( K_{\text{ATP}} \) channel may be composed of SUR2A and Kir6.2. The present study has confirmed directly that Kir6.2 is essential for the function of sarcolemmal \( K_{\text{ATP}} \) channels in cardiac cells. In open-cell attached patches of ventricular cells of Kir6.2\(^{-/-} \) mice, we could record the \( K_{\text{ATP}} \) channel activity, which showed a slope conductance of 79 pS and was inhibited by application of ATP or glibenclamide. However, we did not observe any \( K_{\text{ATP}} \) channel activity in membrane patches of Kir6.2\(^{-/-} \) ventricular cells even in the presence of Mg-UDP and/or pinacidil. In addition, the KCO pinacidil activated the glibenclamide-sensitive outward current in ventricular cells of WT but not of KO mice. These findings indicate that Kir6.2 forms the pore of cardiac \( K_{\text{ATP}} \) channels. This concept was further strengthened by Kir6.2 rescue experiments. In neonatal myocytes of Kir6.2\(^{-/-} \) mice, adenoviral gene transfer of Kir6.2 restored the surface \( K_{\text{ATP}} \) current that was activated by P-1075 and blocked by HMR1098. P-1075 and HMR1098 have been shown to be a specific activator and a blocker of sarcolemmal \( K_{\text{ATP}} \) channel, respectively.\(^{23}\) Therefore, Kir6.2 is essential for the function of sarcolemmal \( K_{\text{ATP}} \) channel in cardiac cells.

The present study has also demonstrated that sarcolemmal \( K_{\text{ATP}} \) channels mediate the depression of cardiac function by KCO. Pinacidil markedly shortened APD and decreased cardiac contractile function assessed by LVDP and dp/dt in WT but not in KO hearts. In Kir6.2\(^{-/-} \) hearts, the action potential shortening induced by pinacidil might lead to a decrease in \( \text{Ca}^{2+} \) influx via \( \text{Ca}^{2+} \) channels and/or \( \text{Na}^{+}-\text{Ca}^{2+} \) exchange system, resulting in a negative inotropic response. Similar pinacidil-induced negative inotropy associated with action potential shortening was observed with guinea pig papillary muscles.\(^{17}\) However, such electromechanical response to pinacidil was absent in Kir6.2\(^{-/-} \) hearts. We also found that the action potential shortening under metabolic inhibition was absent in Kir6.2\(^{-/-} \) ventricular cells (M.S., N.S., H.N., unpublished observations, 1999). The action potential shortening in hypoxic and ischemic conditions is expected to decrease \( \text{Ca}^{2+} \) influx, resulting in reduction of mechanical contraction, amelioration of intracellular \( \text{Ca}^{2+} \) overload, and energy sparing. Administration of KCO would accelerate the functional alterations and thereby protect ischemic myocardium. However, there is recent evidence that mitochondria may harbor another type of \( K_{\text{ATP}} \) channel, an additional site of action of KCOs, and that the mitochondrial...
K_ATP channel rather than the sarcolemmal K_ATP channel may be important for cardioprotection by KCOs and ischemic preconditioning.24,25 Because action potential shortening would not be observed during myocardial ischemia or administration of KCO, the Kir6.2-deficient mouse may be a useful model to define the underlying mechanism(s) of cardioprotection afforded by KCOs or ischemic preconditioning.

Currents attributed to K_ATP channels have been observed in a number of vascular and visceral smooth muscle preparations, such as rabbit mesenteric artery,26 portal vein,27 guinea pig trachealis,28 and urinary bladder.29 In terms of SUR of smooth muscle cells, Isomoto et al13 have proposed that SUR2B, a splice variant of SUR2A having different carboxyl-terminal amino acids, is the subunit constituting the smooth muscle–type K_ATP channel as inferred from pharmacological properties and tissue distribution. In smooth muscle cells of murine colon14 and guinea pig bladder,15 the pore region of the K_ATP channels was reported to comprise SUR2B and Kir6.2.

There is still controversy as to physiological properties of K_ATP channels in vascular smooth muscle cells. K_ATP channels of large conductance (120 to 258 pS) were reported in vascular smooth muscle cells from rabbit mesenteric artery30,31 and rat tail artery.32 Edwards and Weston33 postulated that the large-conductance K^+ channel might be a Ca^{2+}-activated K^+ (K_{Ca}) channel. K_ATP channels having small or intermediate conductance (15 to 50 pS) were observed in various vascular smooth muscle cells.33–35 They were inhibited by glibenclamide and activated by KCOs and NDPs. Because these characteristics were closely similar to the “KNDP channel” composed of SUR2B and Kir6.1, Yamada et al16 suggested that vascular K_ATP channel may be composed of Kir6.1 rather than Kir6.2. The present study has demonstrated that KCOs relaxed the WT and KO aortic preparations precontracted by norepinephrine with similar potency and pinacidil induced the glibenclamide-sensitive current to a similar extent in vascular smooth muscle cells isolated from WT and KO aortas. These findings indicate that Kir6.2 is not necessary for the vasodilation by KCOs in mouse aorta. Strong support for this concept is the finding that Kir6.1 mRNA, but not Kir6.2 mRNA, was detected in both WT and KO aortas. In this study, not only pinacidil but also diazoxide relaxed the precontracted aortic preparations. Because the benzothiadiazine KCO, diazoxide, activated the KATP channels composed of Kir6.2/SUR2B13 but not Kir6.2/SUR2A,11 the SUR subunit in mouse aorta might be SUR2B.

It may be of importance to examine the effect of KCO on the resistance vessels rather than conductance vessels such as thoracic aortas. Therefore, we examined the effect of pinacidil on systemic blood pressure in anesthetized WT and KO mice. There were no significant differences in baseline values and decrease of MAP in response to WT

**Figure 7.** Effects of pinacidil on MAP and HR in anesthetized mice. Changes of MAP and HR after intravenous injection of 0.3 mg/kg pinacidil are indicated in panels a and b, respectively. ■ and ○ indicate data obtained from Kir6.2^{+/+} and Kir6.2^{−/−} mice, respectively. Ordinates indicate changes in MAP and HR from baseline values at the time point of 0 minutes. Points are mean±SE of 6 animals for both groups.

**Figure 8.** Northern blot analyses of Kir6.1 and Kir6.2 mRNA. a. Comparison of expressed levels in WT (lanes 1 through 4) and KO mice (lanes 5 through 8). Left and right panels show data obtained from 8 individual animals. b and c, Quantitative analyses of Kir6.1 mRNA expression. Expressed levels of Kir6.1 mRNA in hearts (b) and aortas (c) were normalized by those of GAPDH mRNA, respectively.
and KO mice. The target of pinacidil in resistance vessels might not be the K$_{ATP}$ channel having Kir6.2 as a pore subunit. It has been suggested that K$_{ATP}$ channels in vascular smooth muscle cells may be activated by metabolic inhibition and endogenous substances such as adenosine, calcitonin gene-related peptide, and prostacyclin. Further studies may be needed to identify the pore subunit of K$_{ATP}$ channels involved in the regulation of vascular tones under metabolic changes and released endogenous substances using the genetically targeted animal.

In conclusion, we provide direct evidence that Kir6.2 forms the pore region of cardiac K$_{ATP}$ channel but not of vascular K$_{ATP}$ channel and that Kir6.2 is essential for the action potential shortening and depressed cardiac contractility in response to KC$_{o}$s.

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References

14. Suzuki et al Role of Kir6.2 in Cardiovascular Function 577

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