Myocardial Ischemia Induces Differential Regulation of K_{ATP} Channel Gene Expression in Rat Hearts

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Abstract

The cardiac ATP-sensitive potassium (K_{ATP}) channel is thought to be a complex composed of an inward rectifier potassium channel (Kir6.1 and/or Kir6.2) subunit and the sulfonylurea receptor (SUR2). This channel is activated during myocardial ischemia and protects the heart from ischemic injury. We examined the transcriptional expression of these genes in rats with myocardial ischemia. 60 min of myocardial regional ischemia followed by 24–72 h, but not 3–6 h, of reperfusion specifically upregulated Kir6.1 mRNA not only in the ischemic (~ 2.7–3.1-fold) but also in the nonischemic (~ 2.0–2.6-fold) region of the left ventricle. 24 h of continuous ischemia without reperfusion also induced an increase in Kir6.1 mRNA in both regions, whereas 15–30 min of ischemia followed by 24 h of reperfusion did not induce such expression. In contrast, mRNAs for Kir6.2 and SUR2 remained unchanged under these ischemic procedures. Western blotting demonstrated similar increases in the Kir6.1 protein level both in the ischemic (2.4-fold) and the nonischemic (2.2-fold) region of rat hearts subjected to 60 min of ischemia followed by 24 h of reperfusion. Thus, prolonged myocardial ischemia rather than reperfusion induces delayed and differential regulation of cardiac K_{ATP} channel gene expression. (*J. Clin. Invest.* 1997. 100:3053–3059.) Key words: K_{ATP} channel • myocardial ischemia • gene expression

Introduction

The ATP-sensitive potassium channel (K_{ATP} channel) was originally discovered in cardiac muscle (1). The K_{ATP} channels play a crucial role in coupling metabolic energy to the membrane potential of cells, thereby functioning as cellular "metabolic sensors." They are important regulators of muscle contraction and secretory processes. In myocardial tissues, K_{ATP} channels are thought to be one of the central regulators of cardioprotection (2, 3). Therefore, these channels are therapeutic targets, and it is no surprise that there has been tremendous interest in cloning this class of channels.

Cloning of a putative K_{ATP} channel, Kir6.1 (4) of relative molecular mass of 49,000 D, revealed it to be a member of the inward rectifier potassium channels, with two membrane-spanning regions. This clone is ubiquitously expressed in a range of rat tissues including brain, heart, skeletal, and smooth muscle. It has been reported (5) that the pancreatic β cell K_{ATP} channel comprises a complex of at least two subunits: the inward rectifier subunit BIR (or Kir6.2: a homologue of Kir6.1) as K_{ATP}-α, and the sulfonylurea receptor (SUR)[1] (6) which is a member of the ATP-binding cassette (ABC) superfamily (7) as K_{ATP}-β. In this model, K_{ATP}-α constitutes the pore-forming channel "core," and K_{ATP}-β is associated with the channel core with a molar ratio of 1:1 (8) and determines the pharmacological and electrophysiological properties of the K_{ATP} channels (9).

The molecular constitution of K_{ATP} channels in myocardial cells has not yet been determined. In rat hearts, however, both Kir6.1 and Kir6.2 mRNAs are abundantly expressed (4, 10, 11). On the other hand, whereas the mRNA for SUR1, which was first discovered by Aguilar-Bryan, is not expressed in rat hearts (6), the mRNA for the cardiac type of sulfonylurea receptor, SUR2, is expressed at high levels (12). Moreover, Inagaki et al. (12) have shown that the coexpression of SUR2 and Kir6.2 mRNA in COS1 cells reconstitutes K_{ATP} channels with properties similar to those in native cardiac muscle. These findings indicate that cardiac K_{ATP}-α is inward rectifier subunit Kir6.1 and/or Kir6.2, and that K_{ATP}-β is SUR2.

In experimental and clinical settings, it has been revealed that activation of cardiac K_{ATP} channels plays a crucial role in cardioprotection against myocardial ischemia. The role of these channels in the phenomenon of ischemic preconditioning in several mammalian species, including rats, has drawn considerable attention (13–17). Opening of the K_{ATP} channel increases the outward K^+ current, resulting in the shortening of the action potential duration (18), which inhibits the entry of Ca^{2+} into the myocyte through the voltage-gated calcium channel. Decreased intracellular Ca^{2+} then results in a reduction of ischemic injury in myocytes. However, the cardioprotective mechanism by this channel activation could not be uniquely attributed to the shortening of the action potential duration via sarcolemmal K_{ATP} channels. There is also new interest in the potential role of a recently identified mitochondrial K_{ATP} channel which may be important in the cardioprotective actions of several K_{ATP} channel openers (19).

Despite the key physiological role of this channel, the

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1. *Abbreviations used in this paper:* IR, ischemic region of the left ventricle; nIR, nonischemic region of the left ventricle; SUR, sulfonylurea receptor.

regulation of cardiac K_{ATP} channel gene expression under pathological conditions has up to now not been reported. The purpose of this study was to examine the changes in the transcriptional expression of the potential cardiac K_{ATP} channel genes (Kir6.1, Kir6.2, and SUR2) induced by acute myocardial ischemia and reperfusion.

Methods

Animals with myocardial ischemia. Male Wistar rats weighing 250–300 g (Shimizu Experimental Supplies, Kyoto, Japan) were used for this study. Rats were anesthetized with intraperitoneal injection of sodium pentobarbital. Under mechanical ventilation with a volume-cycled respirator (SN-480-7; Shinano Manufacturing Co., Ltd., Tokyo, Japan), the chest was opened via a left thoracotomy, the pericardium was opened, and the heart was rapidly exposed. Myocardial ischemia was elicited by the method described previously (20). In brief, a 7-0 silk suture on a small curved needle was passed around the root of the left coronary artery beneath left atrial appendage, and the ends of the suture were passed through a small vinyl tube to make a snare. Coronary occlusion was achieved by pulling the snare and clamping the tube firmly against the heart with a mosquito hemostat, and myocardial ischemia was confirmed by regional cyanosis of the myocardial surface. Reperfusion was achieved by releasing the clamp and was confirmed by myocardial reactive hyperemia over the risk area. The heart was returned to its original position, and the incision was closed. Sham-operated rats were prepared with the coronary artery loosely ligated so as not to obstruct coronary flow. After the operation, rats were allowed free access to standard rat chow, and water was provided ad libitum.

Study groups and experimental protocols. Messenger RNA expression of cardiac K_{ATP} subunits was evaluated in nine groups of ischemic animals, varying the duration of ischemia and reperfusion. These groups included four groups of animals subjected to 60 min of coronary artery occlusion followed by 3, 6, 24, or 72 h of reperfusion ($n = 6$ for each). Two other groups of animals received 15 or 30 min of ischemia followed by 24 h of reperfusion ($n = 6$ for each). The remaining three groups of animals were subjected to 3 ($n = 4$), 6 ($n = 4$), or 24 h ($n = 6$) of continuous coronary artery ligation without reperfusion. Sham-operated animals were killed at four time points: 3, 6, 24, or 72 h after the sham operation ($n = 6$ for each).

In addition, the hearts of six other rats subjected to 60 min of ischemia followed by 24 h of reperfusion and those of six corresponding sham-operated rats were prepared for Western blot analysis.

Hemodynamic measurements. Hemodynamic measurements were performed in sham rats and rats receiving prolonged ischemia with or without reperfusion ($n = 4$–6 for each). The hemodynamic parameters were obtained immediately before the animals were killed at the end of the protocols. Left ventricular pressure measurements were made by inserting a fluid-filled 22-gauge needle into the left ventricle through the apex. The needle was connected directly to a transducer (Statham p-23Db; Gould, Cleveland, OH). Left ventricular pressure development (dP/dt) was obtained from a differentiating circuit in the recorder with the high-frequency filter cutoff set at 70 Hz. Heart rate was determined from the pressure tracing. Rats were allowed at least 10 min after surgical preparation to reach steady state. The left ventricular peak systolic and end-diastolic pressures, and maximum dP/dt were recorded at a paper speed of 100 mm/s (model R-60; TEAC Co., Musashino, Tokyo, Japan). All values obtained were calculated as a mean of at least five cardiac cycles in sinus rhythm.

All experiments reported here conformed to the American Physiological Society's guidelines regarding the use and care of laboratory animals and were approved by the institutional animal care committee.

Tissue preparation for Northern and Western blot analyses. In a preliminary study, we assessed the extent of myocardial infarction in cross sections of the left ventricle in rats receiving 60 min of ischemia followed by 24 h of reperfusion ($n = 3$), using triphenyl tetrazolium chloride staining technique. Since we confirmed that the infarcted area covered most of the left ventricular free wall and did not extend to the interventricular septum, we defined the free wall as the ischemic region (IR) and the septum as the nonischemic region (nIR).

After the end of each experimental protocol, thoracotomy was performed under pentobarbital anesthesia and the heart was removed immediately. The heart was cut transversely 2 mm beneath the atrioventricular groove to avoid contamination of atrial tissues. Then the right ventricle was excised from the remaining heart. The left ventricle was further divided into the interventricular septum and the left ventricular free wall. These pieces of cardiac tissues were snap-frozen in liquid nitrogen and stored at −80°C until use. The cardiac tissues obtained were subjected to RNA or peptide extractions.

Total RNA extraction and Northern blot analysis. Total RNA was extracted from frozen cardiac tissues by the single-step guanidinium thiocyanate method described by Chomczynski and Sacchi (21). 20 μg of total RNA from sham-operated or experimentally ischemic heart was electrophoresed in 1.2% agarose gels containing 6% formaldehyde, and transferred overnight onto nylon membranes (GeneScreenPlus; DuPont, Wilmington, DE). RNA was fixed by ultraviolet cross-linking and by baking at 80°C for 2 h, followed by prehybridization for 2 h at 42°C in 50% formamide, 6× SSC (1× is 150 mM sodium chloride, 15 mM sodium citrate), 5× Denhardt's solution (1× is 0.02% Ficoll, 0.02% povidone, 0.02% BSA), 0.5% SDS, and 10 μg/ml salmon sperm DNA. Hybridization was then performed with the addition of random-primed [^{32}P]DNA probes prepared from corresponding cDNAs using a commercial kit (TaKaRa, Tokyo, Japan) at the same temperature for 16–24 h. The hybridized membranes were washed twice in 2× SSC + 1% SDS and once in 0.1× SSC + 0.1% SDS at 50°C. Autoradiograph was developed on an intensifying screen at −70°C for 24–72 h and radioactivity was measured densitometrically. The membranes were then washed by boiling and rehybridized with another probe. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as an internal control to correct for differences in RNA loading. The resultant densitometrical data of each mRNA were compared with their GAPDH mRNA and expressed as a ratio.

Preparation for cDNA probes. The Kir6.1 cDNA is a generous gift from Dr. Inagaki (Chiba University School of Medicine, Chiba, Japan). The Kir6.2 cDNA was cloned from rat brain in our laboratory (22). DNA probes were 1.2-kb BamHI-HindIII full-length restriction fragment of rat Kir6.1 (4), and 3-kb NotI-EcoRI full-length restriction fragment of rat Kir6.2.

The SUR2 cDNA was obtained by using RT-PCR. The gene specific primers (descending 5′-CGCGGATCCATGAGCCTTTCCTTCTGTGGTAA-3′, ascending 5′-ATATATAAGCTTAAGCGCAGGTCTGACATTCCC-3′) corresponding to the 5′ terminal of rat SUR2 cDNA (12) were synthesized on an automated DNA synthesizer. The expected size of the amplified fragment was 500 bp. 4 μg of the total RNA from rat heart was mixed, in a final volume of 25 μl, with specific primer (ascending primer), dNTPs (1 mM), RNase inhibitor (40 U), sodium pyrophosphate (4 mM), and AMV reverse transcriptase (15 U; Promega, Madison, WI) in 1× reverse transcription buffer (mM: 50 KCl, 5 $MgCl_2$, 5 DTT, 50 Tris-HCl, pH 8.3, at 42°C). The mixtures were incubated at 42°C for 60 min. PCR was performed at a final concentration of 1× PCR buffer (TaKaRa), one-fifth of RT products, 100 μM dNTPs, 50 pmol of both ascending and descending primers, and 2 U of LA-*Taq* polymerase (TaKaRa), in a total volume of 50 μl, by repeated 30 cycles using a thermal cycler (Perkin Elmer, Norwalk, CT). The amplification profiles involved denaturation at 95°C for 1 min, primer annealing at 60°C for 2 min, and extension at 72°C for 2 min. The PCR product was sequenced and was identical to the published sequence of SUR2.

Antibody production. The COOH-terminal region on the rat Kir6.1 was chosen for antibody production. The sequence in this region is confined to the Kir6.1 isoform and was predicted to produce isoform-specific antibodies. Antiserum against the COOH-terminal epitope was produced in rabbits using a synthetic peptide, NSLRKRNSMRRNNS.

Western blot analysis. Cardiac tissues from sham rats and ischemic rats were homogenized with a polytron PT10 in 50 mM Tris-Cl, pH 8.0, 80 mM NaCl, and 100 μg/ml PMSF. The debris was removed by centrifugation at 1,000 *g* at 4°C for 15 min. The cardiac membranes were sedimented from the supernatant at 30,000 *g* at 4°C for 1 h. The final pellet was solubilized in 1 ml of Triton X-100 buffer (250 mM Tris-Cl, pH 6.8, 1% Triton X-100, 100 μg/ml PMSF). The protein was fractionated by SDS-PAGE, electrotransferred to nitrocellulose filter (Hybond C; Amersham Corp., Arlington Heights, IL), and incubated with antibody according to standard protocols. The primary Kir6.1 antiserum was diluted 1:500, whereas the secondary antibody, horseradish peroxidase–conjugated goat anti–rabbit IgG (Amersham Corp.), was diluted 1:2,000. Detection was done using an ECL kit (Amersham Corp.) according to the manufacturer's directions. Exposure was for 2–5 min. Quantitative immunoreactivity was determined by densitometry of the developed film that was in the linear ranges with respect to film exposure. Linearity between amounts of protein and immunoreactive signals was proved by plotting different amounts of protein at varying exposure times against corresponding densitometric units.

Reagents and statistical analysis. All reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless otherwise indicated below. All quantitative data are described as mean±SEM. Statistical analyses of hemodynamics among the two ischemic groups and the sham group, and that of changes in mRNA levels for Kir6.1, Kir6.2, and SUR2 over the time course were performed using one-way ANOVA with Fisher's least significant difference as the post-hoc test. Kir6.1 protein levels between ischemic and sham groups in both the IR and the nIR were compared by an unpaired *t* test. A level of $P < 0.05$ was accepted as statistically significant.

Results

Cardiac K_{ATP} channel gene expression under myocardial ischemia. Fig. 1 presents Northern blot analysis of rat cardiac Kir6.1, Kir6.2, and SUR2 as putative components of the K_{ATP} channel, and GAPDH as an internal control to correct for differences in RNA loading, in rats with 60 min of coronary occlusion followed by varying times of reperfusion. 60 min of coronary artery occlusion followed by 24–72 h, not 3–6 h, of reperfusion significantly increased Kir6.1 mRNA levels both in the IR (Fig. 1 *A*) and in the nIR (Fig. 1 *B*), while increment of Kir6.2 mRNA was only slight. In contrast, the same ischemia and reperfusion procedures did not alter the SUR2 mRNA expression. Interestingly, there were no significant differences between the increased Kir6.1 mRNA levels in the IR and those in the nIR.

The resultant densitometrical semiquantifications are demonstrated in Fig. 2. Kir6.1 mRNA showed a time-dependent increase not only in the IR (Fig. 2 *A*; 3.09±0.31-fold increase at 24 h and 2.72±0.47-fold increase at 72 h), but also in the nIR (Fig. 2 *B*; 2.57±0.30-fold increase at 24 h and 1.99±0.25-fold increase at 72 h) ($P < 0.05$ vs. sham animals [$n = 6$] for all). The Kir6.2 mRNA expression showed a slight and not statistically significant increase at 24 h of reperfusion (1.69±0.29-fold increase in the IR, and 1.47±0.12-fold increase in the nIR).

Fig. 3 *A* shows Kir6.1 mRNA levels in rats receiving 15–60 min of ischemia followed by 24 h of reperfusion. Despite a prolonged 24 h of reperfusion, a short duration of 15–30 min of is-

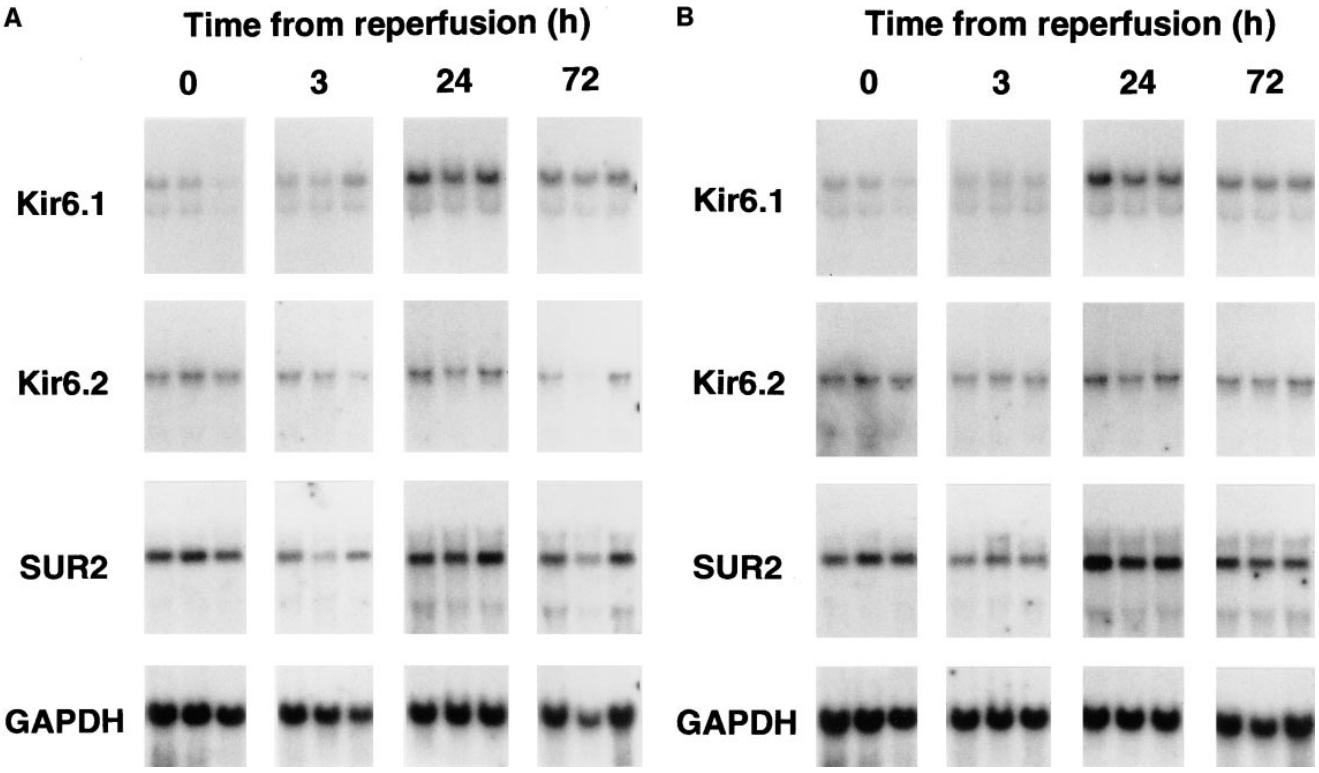

Figure 1. Northern blot analysis showing time-dependent changes in the amount of mRNAs for Kir6.1, Kir6.2, SUR2, and GAPDH in the IR (*A*) and the nIR (*B*) of rat hearts subjected to 60 min of coronary artery occlusion followed by indicated duration of reperfusion. Since we confirmed that the sham operation did not affect these mRNA levels, samples from 24 h after the sham operation were used as controls (0 h in the figure).

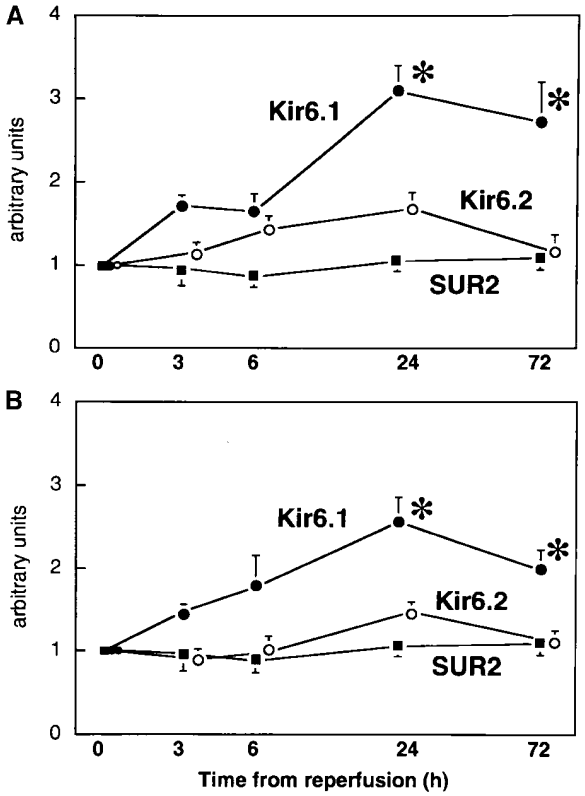

Figure 2. Densitometrical analysis of mRNAs for Kir6.1 (*filled circles*), Kir6.2 (*open circles*), and SUR2 (*filled squares*) in the IR (*A*) and the nIR (*B*). The resultant data of each mRNA were compared with their GAPDH mRNA and expressed as a ratio. The normalized value in sham-operated controls is arbitrarily expressed in 1 U. $*P <$ 0.05 vs. control.

chemia did not induce a significant increase in Kir6.1 mRNA. Fig. 3 *B* shows Kir6.1 mRNA levels in rats receiving 3, 6, or 24 h of continuous coronary artery occlusion without reperfusion. 24 h of continuous ischemia without reperfusion also induced the upregulation of Kir6.1 mRNA, while 3 or 6 h of such ischemia did not upregulate the expression of this message.

Protein levels of Kir6.1 under myocardial ischemia. In six other rats receiving 60 min of myocardial ischemia followed by 24 h of reperfusion, Western blotting was performed using antiserum raised against Kir6.1 peptide, as shown in Fig. 4. Each band was semiquantified using a computing densitometer. In parallel with the change of mRNA for Kir6.1 at 24 h of reperfusion, the protein level showed a 2.4±0.15-fold increase in the IR and a 2.2±0.20-fold increase in the nIR.

Hemodynamics. Table I represents the hemodynamic parameters in sham-operated rats, rats receiving 60 min of ischemia followed by 0, 3, 6, or 24 h of reperfusion, and those receiving 3, 6, or 24 h of continuous ischemia without reperfusion. Heart rate in the ischemic rats receiving 60 min of ischemia followed by 0, 3, or 6 h of reperfusion and in those receiving 3 or 6 h of continuous ischemia was lower than that in the sham rats. In all of the ischemic animals compared with the sham animals, left ventricular systolic pressure and peak dP/dt were significantly reduced, and left ventricular end-diastolic pressure was significantly elevated.

Discussion

This study demonstrates that (*a*) potential cardiac K_{ATP} channel genes (Kir6.1, Kir6.2, and SUR2) are differentially regulated under prolonged myocardial ischemia: among them, mRNA for the pore-forming subunit, Kir6.1, alone is significantly upregulated; (*b*) this upregulation of Kir6.1 mRNA is observed not only in the IR but also in the nIR; and (*c*) these increases in the transcription of Kir6.1 are associated with similar increases in its protein levels. To the best of our knowledge, this is the first report to describe the dynamic regulation of cardiac K_{ATP} channel gene expression under pathological conditions.

Differential regulation of cardiac K_{ATP} channel gene expression. In this study, 60 min of myocardial ischemia followed by 3–6 h of reperfusion changed none of Kir6.1, Kir6.2, or SUR2 mRNA, while the same duration of ischemia followed by 24–72 h of reperfusion specifically upregulated Kir6.1 mRNA. On the other hand, we found that 24 h of continuous ischemia

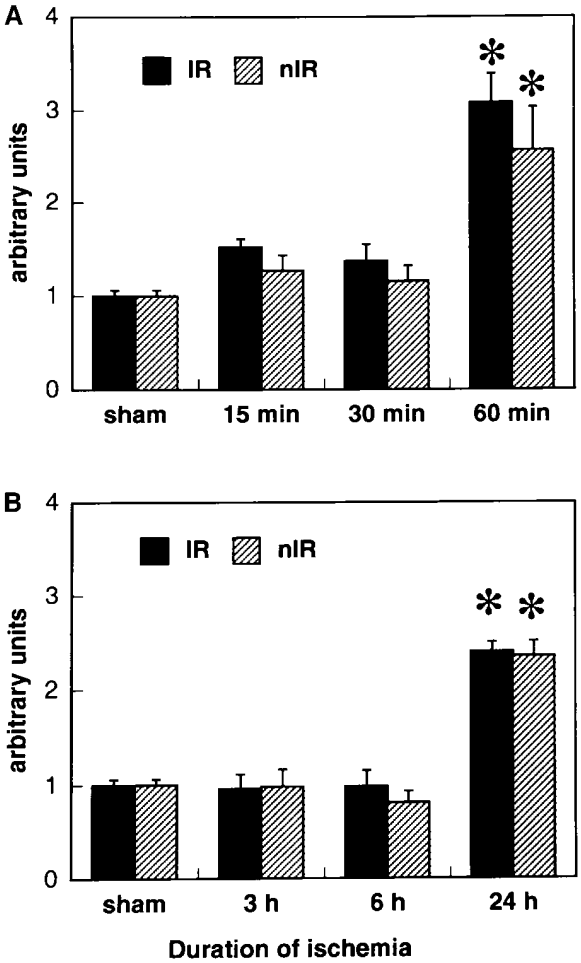

Figure 3. Kir6.1 mRNA levels of rats receiving various durations of coronary artery occlusion (15, 30, or 60 min) followed by 24 h of reperfusion (*A*) and those of rats receiving various durations of continuous ischemia (3, 6, or 24 h) without reperfusion (*B*). *sham*, Sham rats killed 24 h after the sham operation; *15, 30, 60 min*, rats subjected to each time of myocardial ischemia followed by 24 h of reperfusion; *3, 6, 24 h*, rats subjected to each time of continuous ischemia without reperfusion. $*P <$ 0.05 vs. sham group.

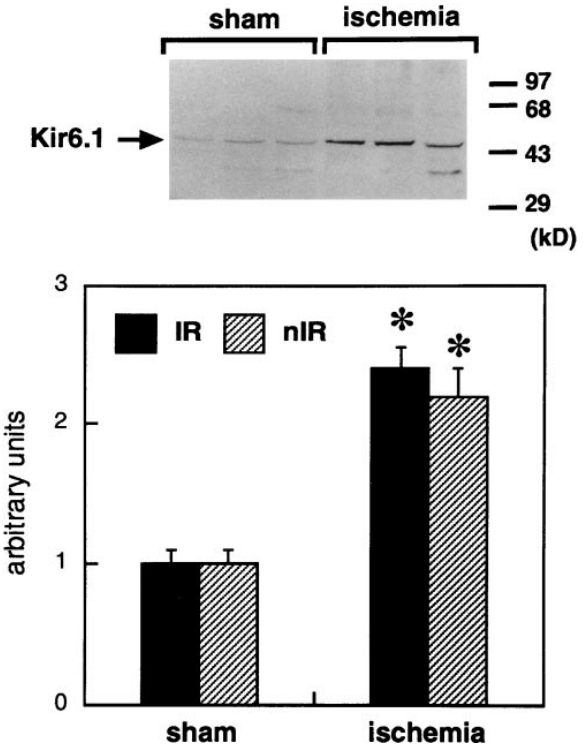

Figure 4. Western blot analysis showing the expression of Kir6.1 immunoreactive protein level in rats ($n = 6$) receiving 60 min of ischemia followed by 24 h of reperfusion and in sham rats ($n = 6$) killed at 24 h after the sham operation. The Kir6.1 protein detected as the 49-kD band in the IR (*top*) and resultant densitometrical data in the IR and nIR (*bottom*). $*P < 0.05$ vs. sham group.

without reperfusion also induced an increase in Kir6.1 mRNA expression, whereas neither 15–30 min of ischemia followed by 24 h of reperfusion nor 3–6 h of continuous ischemia induced such expression. These findings indicate that this specific expression of Kir6.1 mRNA is a delayed response to prolonged ischemia rather than reperfusion.

There are some differences between the time course of cardiac K_{ATP} channel activation in ischemic preconditioning and that of the K_{ATP} channel gene expression shown in the present study (15). The duration of ischemic insult necessary to turn on the K_{ATP} channel in ischemic preconditioning is much shorter than that to upregulate Kir6.1 mRNA. In addition, ischemic preconditioning has been shown to require a short period of reperfusion to activate these channels before sustained ischemia, while such a reperfusion process is not essential for the change in the regulation of Kir6.1 mRNA. Therefore, delayed augmentation of Kir6.1 mRNA that we found in this study may not relate to the fundamental mechanism of ischemic preconditioning.

Two inward rectifier K channels, Kir6.1 and Kir6.2, share high homology in primary structures (they have 69% identity in nucleotide sequence, and 72% in amino acid sequences) and are strongly expressed in rat hearts (4, 10). However, their electrophysiological properties differ from each other in several points. Heterologous expression of Kir6.1 alone results in functional channel activity, while that of Kir6.2 does not. Whole-cell current recordings from Kir6.1-SUR1 or Kir6.2-SUR1 cotransfected cells revealed that the currents formed by Kir6.1-SUR1 are less sensitive to ATP than those formed by Kir6.2-SUR1 (23). K_{ATP} channels are considered to be composed of several subunits. However, since the conductances of Kir6.1 and Kir6.2 are close, it is difficult to analyze K_{ATP} channel stoichiometry in native myocardial cells. Our result, that Kir6.1 was more steeply upregulated than Kir6.2 in ischemic rat hearts, may suggest that the K_{ATP} channel consists of different molar ratios of Kir6.1 and Kir6.2 under different pathological conditions. This could enable K_{ATP} channels to be more readily activated once the heart is subjected to pathological conditions where intracellular ATP is comparatively high.

SUR1 and Kir6.2 form the functional K_{ATP} channel of the pancreatic β cell and these two K_{ATP} subunits map to the same locale on human chromosome 11 (5). Human Kir6.1, recently described by Inagaki et al. (24), is located on chromosome 12, position p11.23. Human SUR2 is also located on chromosome 12 and maps to the same site as Kir6.1, suggesting a common ancestor for K_{ATP} subunits (25). Nevertheless, the present study demonstrates that in contrast with the dynamic regulation of the potassium pore protein, Kir6.1, the regulatory β subunit SUR2 remained unchanged at the transcriptional level. It is possible that although SUR2 couples to Kir6.1, the quantitative change of the dynamically regulated Kir6.1 may determine the electrophysiological properties in our ischemic model of rat hearts.

Kir6.1 mRNA expression in the nIR. Another important finding in this study is that upregulation of Kir6.1 mRNA was observed in nIR as well as in IR. In addition, these changes in

Table I. Hemodynamic Parameters

		Rats with ischemia/reperfusion				Rats with continuous ischemia		
	Sham	0 h	3 h	6 h	24 h	3 h	6 h	24 h
	n = 6	*n = 5*	*n = 5*	*n = 5*	*n = 6*	*n = 4*	*n = 4*	*n = 6*
HR (bpm)	403±20	293±19*	295±5*	274±10*	403±24	351±14*	300±7*	419±21
LVSP (mmHg)	117±7	86±3*	89±3*	78±2*	71±5*	84±5*	78±3*	77±5*
LVEDP (mmHg)	1.3±0.5	5.6±0.8*	7.8±1.5*	6.2±1*	7.5±1*	5.4±0.7*	5.8±0.6*	5.2±0.4*
LV (+) dP/dt (mmHg/s)	5067±530	3740±169*	3200±140*	2620±204*	2116±218*	3000±415*	2720±259*	3633±214*

HR, Heart rate; *LVSP*, left ventricular systolic pressure; *LVEDP*, left ventricular end-diastolic pressure; *Sham*, sham-operated rats; *Rats with ischemia/reperfusion*, rats subjected to 60 min of myocardial ischemia followed by indicated time of reperfusion; *Rats with continuous ischemia*, rats subjected to indicated duration of continuous ischemia without reperfusion. All hemodynamic parameters were obtained at the end of the protocol. Values are mean±SEM. $*P < 0.05$ vs. sham.

transcriptional levels are indeed accompanied by changes in protein levels. In rats subjected to 60 min of ischemia followed by 24 h of reperfusion, immunoreactive Kir6.1 protein levels increased not only in the IR but also in the nIR. This finding suggests that these upregulations of the gene transcription and protein expression of cardiac Kir6.1 are not the direct effect of myocardial ischemia. In another experiment, we compared the mRNA levels of Kir6.1 in the right ventricle and in the skeletal muscle (quadriceps femoris muscle) between ischemic rats receiving 60-min ischemia followed by 24 h of reperfusion (n = 6) and sham rats killed 24 h after the sham operation (n = 6). Unlike the upregulation in the left ventricle, Kir6.1 mRNA levels were unaltered in these tissues (data not shown). Therefore, it is unlikely that regulatory processes are controlled by systemically acting circulating neurohumoral factors derived from the ischemic left ventricle. Alternatively, previous studies have also demonstrated increased gene transcription in the nIR in animal models of regional myocardial ischemia. These genes include ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide) (26), fetal phenotypic genes (27) (β-myosin heavy chain and α-skeletal actin), collagen types I and III (28), TGF-β1 (29), genes related to renin-angiotensin system (30–32), and fetal isoforms of the voltage-dependent calcium channel (33). Importantly, in these studies, the upregulation of these genes is associated with hemodynamic deterioration and geometric remodeling in the noninfarcted as well as infarcted myocardium. Sadoshima and Izumo have shown that stretch-induced cardiac hypertrophy is mediated by tissue angiotensin II, released from cardiac myocytes (34). In this study, we observed prolonged elevation of left ventricular end-diastolic pressure in rats with an upregulated Kir6.1 mRNA. It is possible that this elevation increases wall stress in the ischemic and nonischemic myocardium and thus represents a trigger for induction of Kir6.1 mRNA expression.

In summary, prolonged myocardial ischemia induces delayed upregulation of Kir6.1 mRNA in rat hearts, while it does not change SUR2 mRNA expression. This suggests that different regulatory mechanisms are responsible for the gene transcription of the potential α and β subunits of cardiac K_{ATP} channels. Moreover, this upregulation of Kir6.1 mRNA is observed not only in the IR but also in the nIR, which may be caused by mechanical overload due to myocardial infarction. This delayed and differential upregulation of Kir6.1 can vary the composition of the cardiac K_{ATP} channel, thus altering the channel properties. However, further studies are required to elucidate more precise mechanism(s) for this differential regulation and its pathophysiological significance.

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