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K_{ATP} Channels Are Involved in Regulatory Volume Decrease in Rat Cardiac Myocytes

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Summary

Regulatory volume decrease (RVD) is essential for the survival of animal cells. The aim of this study was to observe the RVD process in rat ventricular myocytes, and to determine if the K_{ATP} channels are involved in the RVD process in these cells. By using reverse transcriptase polymerase chain reaction and Western blot analysis, we demonstrated that there are two types of K_{ATP} channels expressed in rat ventricular myocytes: Kir6.1 and Kir6.2. When rat cardiac myocytes were exposed to hypotonic solution, cell volume increased significantly within 15 min and then gradually recovered. This typical RVD process could be inhibited by a Cl^- channel blocker (0.5 mM 9-anthracene-carboxylic acid, 9-AC), a K^+ channel blocker (5.0 mM CsCl) and a K_{ATP} channel blocker glibenclamide (10 μM). Electrophysiological results showed that hypotonic solution activated a whole-cell current, which had similar biophysical characteristics with K_{ATP} opener (pinacidil)-induced currents. This current could be blocked by glibenclamide. Our data suggested that the RVD process in rat ventricular myocytes is dependent on the activation of K^+ channels, and that K_{ATP} channels are involved in this process.

Key words

Whole-cell patch clamp • ATP-sensitive K^+ channel • Regulatory volume decrease

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Introduction

After hypoosmotic cell swelling, regulatory volume decrease (RVD) occurs due to efflux of K^+, Cl^- and organic osmolytes, accompanied by osmotically obliged water loss (Hoffmann and Dunham 1995). RVD is an essential function for animal cells because osmotic perturbation is coupled to various physiological and pathological processes (e.g. proliferation, differentiation and programmed cell death) (Lang *et al.* 1998, Okada *et al.* 2001). In cardiac myocytes, cell swelling occurs during acute myocardial ischemia (Tranum-Jensen *et al.* 1981) and is exacerbated after reperfusion (Jennings *et al.* 1985). During the ischemic period, there is an intracellular accumulation of metabolites (e.g. lactate), which leads to an increase in cellular osmolality (Tranum-Jensen *et al.* 1981). Hence, water enters the cells, increases the cell volume, and alters the function of ion channel. Excessive changes in cell volume in the heart may cause profound alteration of structural integrity and constancy of the intracellular *milieu*, which affects many cellular functions and causes cell death. Reduction of cardiomyocyte swelling during myocardial ischemia by enhancing the regulation of cell volume may be a potential mechanism of cardioprotection.

Osmotic cell swelling has been reported to be associated with the activation of different ion channels, including Cl^- and K^+ channels. The biophysical characteristics of the volume-sensitive Cl^- channel that is relatively constant in different cell types, including

cardiac myocytes, (Wang *et al.* 2005, Wang *et al.* 2006) has been described by ourselves and other research groups (Okada *et al.* 2001). The K^+ channel that is responsible for the volume regulation of cardiac myocytes has not been investigated intensively.

The volume-sensitive K^+ channel may have different molecular identification in different cell types. We have demonstrated that Ca^{2+}-activated intermediate conductance K^+ channels are involved in the RVD process in human intestine 407 cells (Wang *et al.* 2003). In cardiac myocytes, the modulation effect of mechanical stretch on K_{ATP} channel had been reported in atrial myocytes (van Wagoner 1993). It is well known that myocardial K_{ATP} channels play the key role in myocardial protective effect *via* ischemic preconditioning (IPC). Whereas, in rabbit ventricular myocytes, it was suggested that enhanced cell volume regulation was a key protective mechanism of IPC (Diaz *et al.* 2003). Thus, it is reasonable the postulate that K_{ATP} channels may be a molecular entity that link IPC protection process and cell volume regulation. But till now, the information regarding the function of K_{ATP} channels in the RVD process in rat cardiac myocytes is basically unavailable. In the present study, we sought to determine the role of K_{ATP} channels in RVD process in rat ventricular cardiac myocytes.

Materials and Methods

Isolation of single cardiac myocytes

Adult female Sprague-Dawley rats (250-300 g) from the Experimental Animal Center of Capital Medical University (Beijing, China) were treated in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Committee of Science and Technology of China (1988) and approved by the State Council of China (1988). Isolation of single cardiac myocytes involved a previously described, well-established method (Wang *et al.* 2005, Wang *et al.* 2006). Briefly, rats were anesthetized by injection of sodium phenobarbitone (50 mg/kg, i.p.). Hearts were excised, arrested in ice-cold normal Tyrode solution and perfused using a Langendorff apparatus with normal Tyrode solution for 1 min, and Ca^{2+}-free Tyrode solution for another 7 min. Then the perfusate was changed to Ca^{2+}-free Tyrode solution containing collagenase type II (1 mg/ml, Worthington, USA) and BSA (1 mg/ml) for 20-25 min. All solutions were maintained at 37 °C and equilibrated with 95 % O_2 - 5 % CO_2. The ventricles were cut off and minced to disperse the cells, stored at 4 °C in KB solution, used for further experiments (patch clamp and volume measurement) within 6 h of harvesting, and only quiescent cells with regular striations and no evidence of membrane bleb were selected for study.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from cardiac mycyotes by using TRIzol reagent (Sigma, USA) according to the manufacturer's instructions. The quality of the RNA was determined from the ratio of absorbance at 260 nm to that at 280 nm. RNA was reverse-transcribed to synthesize first-strand cDNA and PCR was done by using an RNA PCR Kit (TaKaRa, Dalian, China). The specific primers were designed from coding regions of human Kir6.1 (forward primer: 5'-CCGTCTGTGTGACCAATGTC-3', reverse primer: 5'-CTGGTGAATAGGCACCACTC-3') and Kir6.2 (forward primer: 5'-GAAAGGGGGACAA GAAGGAG-3', reverse primer: 5'-ATGGTCCCCCAGA CAAAGTG-3'). As control for RNA integrity, the primer of glyceraldehyde phosphate dehydrogenase (GAPDH) was used (forward primer: 5'-ACCACAGTCCATGCCA TCAC-3' and reverse primer: 5'-TCCACCACCCTGTTG CTGTA-3'). PCR was done with an Icycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: the PCR reaction mixture was subjected to 30 cycles of denaturation at 94 °C for 0.5 min, annealing at 55 °C for 0.5 min and extension at 72 °C for 0.5 min. This was followed by a final extension at 72 °C for 10 min to ensure complete product extension. Amplified products were separated by 1.5 % agarose gel electrophoresis and stained with ethidium bromide. PCR product bands were visualized by UV light and sequenced with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit and an ABI PRISM 310 genetic analyzer (Applied Biosystems, CA, USA).

Western blot analysis

The cardiac myocytes were harvested and lysed in Eukaryotic Cell Lysis Buffer (BioDev-Tech. Beijing, China) following the manufacturer's instructions. Protein (100 μg) was mixed and boiled in SDS-PAGE sample buffer for 5 min, separated by SDS-PAGE (12 % polyacrylamide gel) and then electro-transferred onto a nitrocellulose membranes by electroblotting in a Mini Trans-Blot. The transferred membrane was washed for 10 min with TTBS buffer containing (in mM): 10 Tris-

HCl, pH 7.4, 150 NaCl, and 0.05 % (w/v) Tween-20, followed by the blocking solution with 5 % nonfat milk in TTBS for 1 h. The blocked membrane was incubated with primary purified goat polyclonal antibody against Kir6.1 and Kir6.2 (Santa Cruz Biotechnology, CA, USA) at a 1:2000 dilution for 3 h at room temperature. For the second antibody, Fluorescently labeled secondary antibodies (IRDye 680 donkey anti-rabbit IgG) were diluted in TTBS buffer and incubated for 1 h at room temperature. Membranes were scanned with Odyssey infrared imaging system at 680 nm and 780 nm wavelength (Li-COR, Biosciences, Lincoln, NE).

Measurement of cell volume

Measurement of cell volume was done as previously described (Diaz *et al.* 2003, Drewnowska and Baumgarten 1991, Wang *et al.* 2005). Briefly, a digital video-camera (Spot RF/SE; Diagnostic) mounted on an inverted microscope (Eclipse TE2000-U; Nikon) was used to acquire myocyte images (400× magnification) at intervals of 1-5 min during the entire experimental protocol. Each image was used for computer tracing all myocyte edges to calculate myocyte area (NIH Image, version 1.62). Cell dimensions (diameter or width and length) were measured using two calibrated graticules (one for width and the other for length) in the microscope. Cell volume was estimated with assumed right cylindrical geometry according to the following equation: $V = \pi L(D/2)^2$ (where V, L and D are cell volume, length and diameter, respectively) (Wang, *et al.* 2005). Using each cell as its own control, relative cell volume was calculated as follows:

$$Vol_t/Vol_c = \pi(L_t \times (D_t/2)^2)/\pi(L_c \times (D_c/2)^2)$$

where t and c refer to test (e.g. hypotonic solution) and control (isotonic solution) solutions respectively (Clemo *et al.* 1999). From these cell volume measurements, the RVD for each myocyte was calculated according to the following formula:

$$RVD = \frac{\%\text{ cell volume at peak time - }\%\text{ cell volume at 40 min}}{\%\text{ cell volume at peak time}}$$

During volume measurement, cells were first stabilized in isotonic solution for at least 10 min, and then exposed to hypotonic solution for 30 min, followed by 10 min of re-exposure to isotonic solution. Blockers were added 2-3 min before and during application of hypotonic solution.

Cell volume data are reported as a decimal fraction of baseline cell volume (e.g. an increase in cell volume of 13 % is 1.13). RVD calculations are also reported as decimal fractions (e.g. 0.50) (Diaz *et al.* 2003). Experiments were carried out at room temperature.

Electrophysiological measurements

The tight-seal, whole-cell voltage-clamp technique was used as previously described (Wang *et al.* 2005, Wang *et al.* 2006, Wang *et al.* 2003). Pipettes were pulled from borosilicate glass capillaries with a micropipette puller (P-2000; Sutter Instruments, Novato, CA). The electrode had a resistance of 2.0-4.0 MΩ when filled with pipette solution. Data were acquired using an EPC-10 amplifier and Pulse software (HEKA Electronics, Lambrecht, Germany). Current signals were low-pass filtered at 2.9 and 1.0 kHz using a four-pole Bessel filter and digitized at 10 kHz. Sampled data were analyzed by an original software application called PatchMaster and Origin 7.0 (Origin Lab, Northampton, MA). In most experiments, a grounded Ag-AgCl pellet electrode was placed in the perfusion solution. When Cl^--free bath solution was used, a 3.0 M KCl-agar bridge was employed. Series resistance was compensated 60-70 % to minimize voltage errors. The time course of whole-cell current activation and recovery was monitored repetitively by applying (every 15 s) ramp pulses (of duration 1 s) from –100 mV to +100 mV from a holding potential of –40 mV. Cell membrane capacitance was estimated from the integral of the transient current response to a 5 mV hyperpolarizing clamp step, and all whole-cell currents were normalized to this value. To obtain whole cell I-V relations, step pulses were applied from a holding potential –40 mV to test potentials (1 s duration) of –100 to +100 mV (in 20 mV increments). To minimize Ca^{2+} and/or swelling-activated Cl^- currents, whole-cell recordings were carried out using low-Cl^- intracellular solution and Cl^--free external solution containing a Cl^- channel blocker, 9-anthracenecarboxylic acid (9-AC).

Solutions and chemicals

Normal Tyrode solution for isolation of single cardiac myocytes contained the following (mM): 137.0 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 0.33 Na_2HPO_4, 5.0 glucose, and 10.0 Hepes (pH 7.3 with NaOH). $CaCl_2$ was omitted from normal Tyrode solution to produce Ca^{2+}-free Tyrode solution. KB solution comprised of (mM): 70.0 KOH, 40.0 KCl, 50.0 L-glutamic acid,

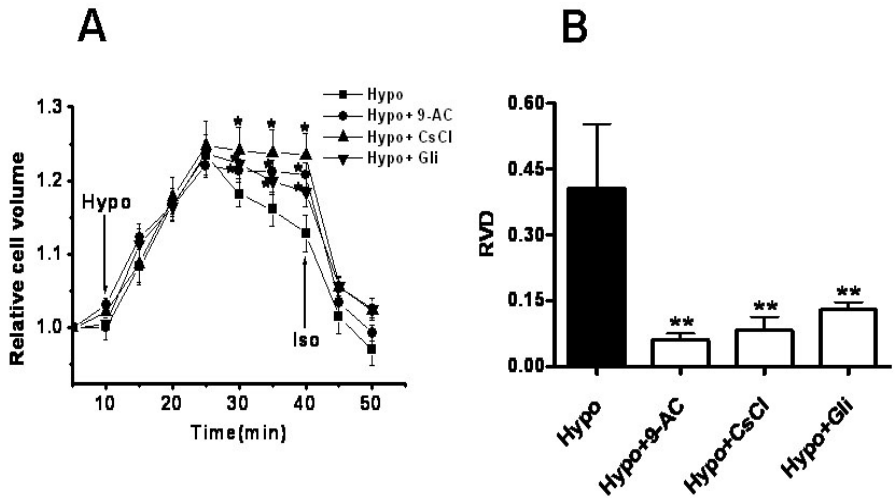

Fig. 1. Effect of Cl^- channel and K^+ channel blockers on cell volume regulation in rat ventricular myocytes. **A.** After 10 min in isotonic solution, cells were exposed to hypotonic solution for 30 min, followed by 10 min of re-exposure to isotonic solution. Blockers were added 2-3 min before and during application of hypotonic solution. Each symbol represents the mean ± SE (vertical bar) of 10 observations. **P*<0.01 vs. hypotonic. **B.** Blockade effect of different blockers on the RVD capability. After 30 min exposure to the hypo-osmotic solution, RVD in hypotonic solution was 0.41±0.15. Addition of 9-AC, CsCl and glibenclamide produced a significant reduction in RVD (hypo+9-AC: 0.06±0.01; hypo+ CsCl: 0.08±0.03; hypo+ glibenclamide: 0.13±0.02) respectively. Data are expressed as mean ± SEM. ***P*<0.01 vs. hypotonic condition. (n=10).

20.0 taurine, 0.5 $MgCl_2$, 1.0 K_2HPO_4, 0.5 EGTA, 10.0 Hepes, 5.0 creatine, and 5.0 pyruvic acid (pH 7.38 with KOH). The isotonic (~312 mosmol/kg H_2O) or hypotonic (~212 mosmol/kg H_2O) solution used for cell volume measurement contained (mM): 5.4 KCl, 100.0 NaCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 100.0 or 0 mannitol, and 10.0 Hepes (pH 7.4 with NaOH). The pipette solution contained (mM): 140.0 K-aspartate, 10.0 Na-aspartate, 1.0 $MgCl_2$, 1.0 Mg-ATP, 2.0 EGTA, and 5.0 Hepes (pH 7.3 with KOH). The Cl^--free isotonic (~310 mosmol/kg H_2O) or hypotonic (~210 mosmol/kg H_2O) bath solution contained (mM): 5.4 K-aspartate, 100.0 Na-aspartate, 1.0 $MgSO_4$, 1.8 $CaSO_4$, 100.0 or 0 mannitol, and 10.0 Hepes (pH 7.4 with NaOH), as well as 0.5 9-AC. To test the K^+ selectivity of current, K^+-aspartate in the bath solution was replaced by equimolar of Na^+-aspartate.

Reagents were obtained from Sigma-Aldrich Chemical Company (Shanghai, China). Stock solutions of 9-AC, pinacidil and glibenclamide were prepared in dimethyl sulfoxide (DMSO). The concentration of DMSO in the experimental solutions was <0.1 %.

Statistical analysis

Data are presented as means ± SE of n observations. Statistical differences in data were evaluated by one-dimensional ANOVA and Scheffe's post-hoc multiple comparison tests. P<0.05 was considered significant.

Results

KATP channels participated in the RVD process of rat cardiac myocytes

Figure 1A shows the time course of a typical volume response of single cardiac myocyte during hypoosmotic perfusion. Under isotonic condition (~312 mosmol/kg H_2O), the volume of cardiac myocytes remained stable throughout the entire perfusion period（data not shown）. Upon exposure to hypotonic solution (~212 mosmol/kg H_2O), the mean volume of cardiac myocytes increased significantly (123.45±2.72 %, *P*<0.05 versus isotonic condition) within 15 min, and then gradually recovered as described previously in single chick cardiac myocytes (Hall *et al.* 1997). After restoration to isotonic solution, cell volume significantly decreased to the initial baseline within 10 min. This result demonstrated that rat ventricular myocytes exhibited a typical RVD process, which was inhibited by application of 0.5 mM 9-AC (a Cl^- channel blocker), 5.0 mM CsCl (a K^+ channel blocker), or 10 μM glibenclamide (which is known to be a specific blocker of K_{ATP} channels at this concentration) 2-3 min before and during application of hypotonic solution. After a 30-min exposure to the hypo-osmotic solution, RVD in control myocytes was 0.41±0.15 (Fig. 1B). Whereas addition of 9-AC, CsCl and glibenclamide in hypotonic solution produced a significant reduction in RVD (hypo+9-AC: 0.06±0.01; hypo+CsCl: 0.08±0.03; hypo+glibenclamide: 0.13±0.02),

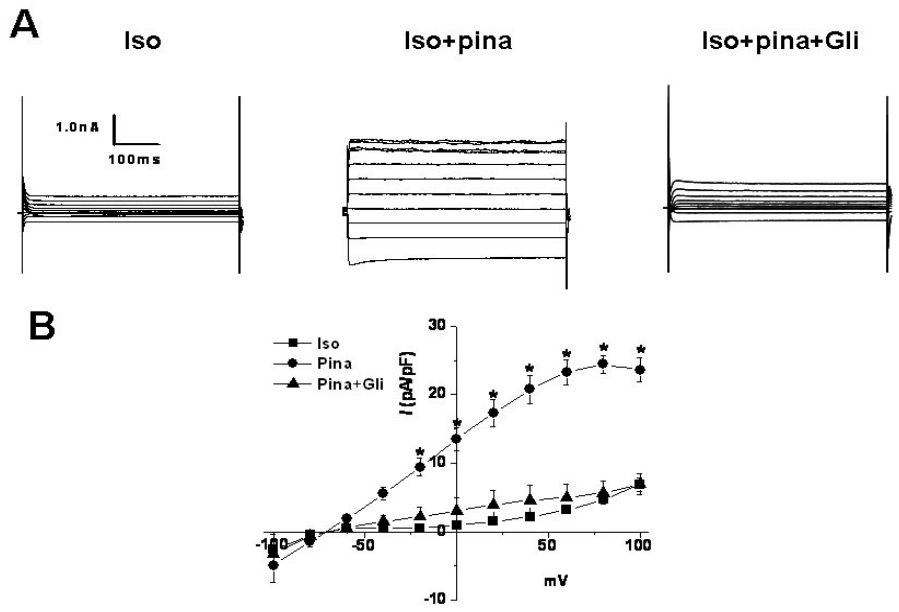

Fig. 2. Whole-cell K_{ATP} currents activated by pinacidil in rat ventricular myocytes. **A.** The whole-cell currents elicited by a series of voltage steps from a holding potential of –40 mV to potentials between –100 mV and +100 mV in 20 mV increments. **B.** I-V curve of the pinacidil activated currents with or without glibenclamide. Similar results were obtained from 5 cells.

no significant difference in cell swelling was found between the hypotonic group with or without blockers. The increase in cell volume mainly resulted from a distinct increase in the radial dimensions of the cell in hypotonic solution with little or no change in longitudinal dimension, as previously reported (Vandenberg *et al.* 1996). In the light of these data, we conclude that Cl^- channels and K^+ channels are responsible for the RVD process of rat cardiac myocytes, and that K_{ATP} channels are at least partially involved in this process.

The characteristics of K_{ATP} currents in rat cardiac myocytes

The characteristics of K_{ATP} currents in rat ventricular myocytes was observed by using a K_{ATP} opener, pinacidil to induce the whole cell current. When cardiac myocytes were dialyzed with low-Cl^- pipette solution and exposed to isotonic Cl^--free bath solution containing 9-AC (0.5 mM), voltage step from holding potential of –40 mV to potentials between –100 mV and +100 mV activated only a small current under control conditions. Whereas, application of pinacidil (50 μM) in the bath solution resulted in a dramatic increase in the amplitude of the current. Subsequent application of glibenclamide (10 μM) in addition to pinacidil reduced the current to nearly control levels (Fig. 2A). The reversal potential for the pinacidil-induced current (–71.5 mV) is near the calculated equilibrium potential for K^+ (–84.1 mV) (Fig. 2B).

Cardiac myocyte swelling activated K_{ATP} current

We used whole cell patch clamp technique to record the hypotonic-activated K^+ current to demonstrate further that K_{ATP} channel participated in the RVD process. A hypotonic challenge (~210 mosmol/kg H_2O) reversibly induced an increase in cell membrane currents in single ventricular myocytes with low-Cl^- pipette solution and Cl^--free bath solution containing 0.5 mM 9-AC (Fig. 3). The profile of the swelling-induced current (Fig. 3A) and the I–V relation (Fig. 3B) were similar to that of the pinacidil-induced current (Fig. 2). This current was also sensitive to K_{ATP} channel blocker, glibenclamide (Fig. 3C). The reversal potential (*E*rev) for the hypotonic induced glibenclamide-sensitive current was –60.2 mV, which is near to the *E*rev of K^+. When extracellular K^+ concentration ($[K^+]_o$) was increased, the *E*rev shifted in the positive direction. The *E*rev shift per ten fold increase in $[K^+]_o/[K^+]_i$ was 43 mV, the linear equation ($E = -42.89842\text{Log}[K^+]_o/[K^+]_i + 0.36831$) comes from Figure 3D, indicating the high selectivity of K^+. These results suggested that K_{ATP} channels were activated by cell swelling.

Molecular expression of K_{ATP} channels in rat ventricular myocytes

RT-PCR and western blot were carried out to examine the expression of the splice variants for Kir6.1 and Kir6.2 in rat ventricular myocytes. Specific primers were chosen on each side of the splicing site, thus amplifying two fragments of different sizes when both isoforms were expressed. As shown in Figure 4A (lanes 3 and 4), DNA fragments of expected size at 383 bp and 282 bp were amplified by Kir6.1 and Kir6.2 specific primers from reverse-transcribed cDNA. The nucleotide

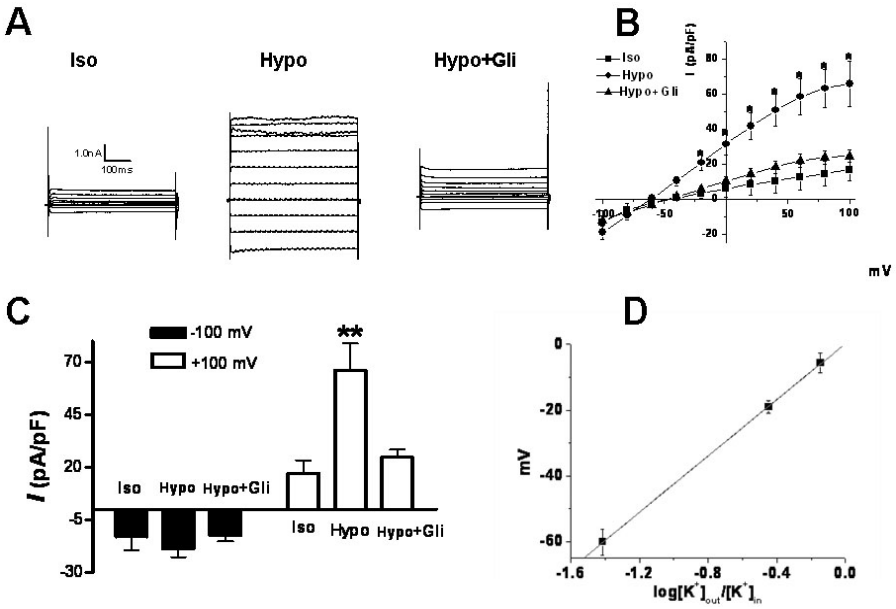

Fig. 3. Whole-cell currents activated by hypotonic solution in rat ventricular myocytes. **A.** The original traces obtained from whole-cell clamp in isotonic solution, in hypotonic solution and in hypotonic solution with glibenclamide (Hypo+Gli). **B.** I-V curves of hypotonic activated current with or without glibenclamide. **C.** Average data of the amplitude of the K_{ATP} currents evoked by hypotonic solution with or without glibenclamide, measured at +100 mV and −100 mV, respectively (n=7). ***P<0.01 vs* isotonic and Gli group at +100 mV. **D.** The fitting line of the reversal potential shift with different extracellular potassium concentrations (n=8).

sequence of these PCR products was identical to the corresponding sequence in the rat Kir channel. No PCR product was amplified when reverse transcriptase was omitted from the reaction (data not shown). Fig. 4B showed the protein expression of Kir6.1 and Kir6.2 in rat cardiac myocytes analyzed using western blot. The specific Kir6.1 and Kir6.2 bands were detected clearly at 51 kDa and 40 kDa respectively. These results consistent with the result of Wu *et al.* (2007).

Discussion

Volume regulatory mechanisms are essential to maintain the structural integrity and efficient functioning of cells. Various factors modify intra- or extracellular osmolarity and thus challenge the osmotic equilibrium across the cell membrane, and therefore cell volume. Defects in volume regulation of cardiac myocytes can occur as a result of metabolic imbalance during myocardial ischemia and reperfusion. Uncontrolled cardiac swelling leads to rupture of the sarcolemma and cell death.

A complex series of cellular control mechanisms have been shown to have roles in the control and regulation of cardiac cell volume under normal and adverse conditions. The most rapid and efficient means to accomplish cell volume regulation is ion transport across the cell membrane. The modulation of several K^+-selective channels by hypotonic condition in cardiac myocytes (including fast-inactivating voltage-dependent transient outward K^+ channel, rapid and slow components of delayed rectifier potassium current) has been reported from various preparations (Wang *et al.* 2005, Wehner *et al.* 2003, Rees *et al.* 1995, Sasaki *et al.* 1994).

The K_{ATP} channel couples the metabolic state of the cardiac myocyte to its electrical activity. It was known to play an important part in IPC, which has been shown to ameliorate ischemia-induced cell swelling at the cellular and mitochondrial level. Cell swelling is an important cause of cell death induced by ischemia and reperfusion. Cell volume regulation has been suggested to be a key mechanism accounting for most of the IPC protection in cardiomyocytes in ventricular myocytes (Diaz *et al.* 2003). Enhanced cell volume regulation may be a key protective mechanism of ischemic preconditioning in rabbit ventricular myocytes (Diaz *et al.* 2003). IPC protection has a close relationship with cell volume regulation, but the molecular identity for the link is not clear.

Mechanical gating properties of the K_{ATP} channel (van Wagoner 1993) have been reported. Hypotonic stress could enhance the slope conductivity of K_{ATP} channels activated by K_{ATP} openers (Kocic *et al.* 2007). In guinea-pig ventricular myocytes, prolonged myocardial ischemia has been shown to cause the duration of the ventricular action potential (AP) to decrease, (Priebe and Beuckelmann 1998), which protects the cell by decreasing calcium influx, thereby reducing inotropy and energy consumption. The possible mechanism underlying this shortening of AP duration is the activation of K_{ATP} channels. The activation of K_{ATP} in this situation can not be explained by the reduction in

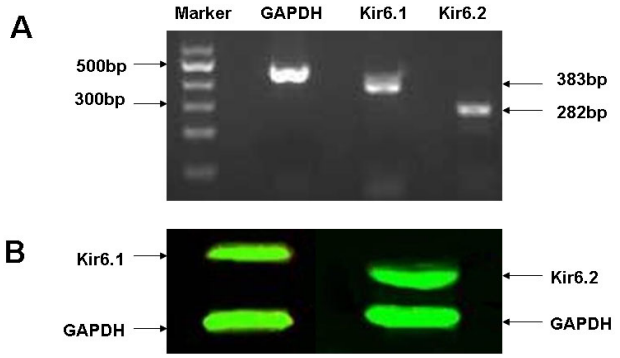

Fig. 4. Molecular expression of K_{ATP} channel in rat cardiac myocytes. **A.** RT-PCR for Kir6.x mRNA in rat ventricular myocytes. Expected fragment sizes in the graph are as follows: Kir6.1, 383 bp (lane 3); Kir6.2, 282 bp (lane 4). **B.** Western blot results for Kir6.1 and Kir6.2. The specific Kir6.1 and Kir6.2 bands were detected clearly at 51 kDa and 40 kDa respectively.

ATP concentration in cardiac myocytes because AP duration decreases significantly when the ATP concentration is >3 mM. Cell swelling could also shorten AP duration in guinea-pig ventricular myocytes by activating K_{ATP} (Priebe and Beuckelmann 1998). This phenomenon could partially explain why K_{ATP} channels appear to open during early ischemia, before ATP levels have decreased to the levels below which the channels can be activated in isolated patch experiments. But till now, there is no direct evidence to demonstrate that K_{ATP} channels are involved in the RVD process in rat cardiac myocytes.

In the present experiments, the RVD process could be maximally blocked by Cl^- channel blocker (9-AC) and K^+ channel blocker (CsCl), demonstrating that the parallel activation of Cl^- and K^+ channel was responsible for the RVD process. However, glibenclamide (10 μM), a K_{ATP} channel blocker, could also inhibit the RVD process. These results suggested the K_{ATP} channels were involved in RVD in rat cardiac myocytes. Although glibenclamide has been reported to block the volume-sensitive Cl^- channel, but at the concentration of 10 μM, it could only blocks the K_{ATP} current without affecting the volume-sensitive Cl^- channel.

Our results provide the following evidences for K_{ATP} channels in the rat heart: (i) there are two types of K_{ATP} channels expressed in rat ventricular myocytes: Kir6.1 and Kir 6.2 and (ii) the pharmacological and electrophysiological evidence indicates that K_{ATP} channels are partially responsible for the RVD process in rat cardiac myocytes (i.e. K_{ATP} channels are regulated by cell volume changes). These findings are potentially important because the Kir6.1/Kir6.2-encoded K_{ATP} plays a crucial part in protecting against ischemia and reperfusion in the heart. It may provide an alternative mechanism to the molecular linkage between ischemia cell death and volume regulation.

The mechanism underlying the activation of K_{ATP} channel by cell swelling remains unknown. Future studies will focus on signal pathways by which swelling activates the K_{ATP} channels, and the possible contribution such changes in membrane conductance to ischemia- and reperfusion-induced injury.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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