

mRNA and protein expression of sarcKATP channel subunit Kir6.2 after exercise-induced myocardial injury in rats

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Abstract. – OBJECTIVE: To investigate the expression of kir6.2 subunit of the sarcKATP channel in exercise-induced myocardial injury and to elucidate the underlying mechanism of myocardial protection by sarcK_{ATP} channels.

MATERIALS AND METHODS: Healthy male Sprague Dawley (SD) rats were divided into the Control (C) and the Exhaustive Exercise (EE) group. The one-time exhaustive exercise-induced myocardial injury model was established on a treadmill at a speed of 35 m/min. Alterations in myocardial ischemia and hypoxia were examined by hematoxylin-basic fuchsin-picric acid (HBFP) staining and the concentration of cardiac Troponin I (cTnI), a sensitive and specific marker for myocardial injury, was detected using immunochemiluminescence analysis. The mRNA expression level, localization, and protein expression of sarcKATP channel subunit kir6.2 were determined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), immunofluorescence, and Western blot analysis, respectively.

RESULTS: When compared to Group C, rats in Group EE demonstrated significantly increased areas of myocardial ischemia and hypoxia. Moreover, increased serum levels of cTnI were detected. Increased kir6.2 expression was found on the surface of cardiomyocytes and kir6.2 protein expression was also significantly increased.

CONCLUSIONS: Exercise-induced myocardial injury did not result in noticeable alterations in kir6.2 mRNA expression. However, kir6.2 protein expression was significantly increased and resulted in increased numbers of sarcKATP channel openings in the myocardium, thereby further inhibiting exercise-induced myocardial injury.

Key Words

Exercise-induced myocardial injury, SarcKATP channel, kir6.2 subunit, Exhaustive exercise, Myocardial protection, Rat.

Introduction

Among all myocardial ischemic injuries, ischemia/reperfusion (I/R)-induced myocardial infarction and arrhythmia are currently the most concerning I/R-related injuries¹. As seen in I/R-related injuries, vigorous or exhaustive exercise may result in myocardial injury, which is commonly observed in the fields of sports medicine and competitive sport. How to reduce myocardial injury has become a hot topic in sports medicine. It has been confirmed that myocardial I/R injury, ischemia, can lead to opening of the myocardial K_{ATP} channel². In addition, the K_{ATP} channels play important roles in cell energy metabolism, regulation of insulin secretion, as well as ischemia and hypoxia preconditioning of the myocardium and the brain. Opening of myocardial K_{ATP} channels may protect the myocardium under many stress-related conditions, including ischemia and hypoxia. Besides, myocardial K_{ATP} channel is an important component of the cardiomyocyte signal transduction pathway. However, there are no reports available on whether or not changes in myocardial K_{ATP} channel expression during exercise-induced myocardial injury resemble those in myocardial I/R injury. Moreover, these underlying mechanisms are still unknown. In this study, we have established a model of exercise-induced myocardial injury in which we examined the mRNA expression, protein distribution and expression of the sarcK_{ATP} channel subunit kir6.2 by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), immunofluorescence and Western blot, respectively. The aim was to explore the mechanism of the sarcKATP channel in protecting the myocardium and its role in alleviating exercise-induced myocardial injury.

Materials and Methods

Animal Model

Healthy male Sprague Dawley (SD) rats weighing 256 ± 13 g were purchased from Shanghai Sippr/BK Laboratory Animals Ltd., (Shanghai, China). Rats were conventionally housed at 5 rats/cage, were given standard rodent chow and had access to food and water *ad libitum*. Room temperature was maintained at 20-22°C with 45-50% relative humidity and a 12 h/d light cycle. Each day, rats were subjected to adaptive treadmill training at a speed of 15 m/min at a running slope of 0° for 10-20 min for 5 consecutive days. Rats were allowed to rest for 1 day after adaptive treadmill training and those that failed to adapt to treadmill training were eliminated from the study. The remaining rats were randomly divided into the control group (Group C) and exhaustive exercise group (Group EE). Animals in Group C were not involved in any treadmill exercise, whereas rats in Group EE were subjected to running at a speed of 35 m/min until exhaustion to establish the myocardial injury model. This study was approved by the Animal Ethics Committee of Hainan Medical University Animal Center.

Sample Collection

Thirty-minutes after the exhaustive exercise, rats were anesthetized with 10% chloral hydrate (400 mg/kg, i.p.), secured on a dissecting table in supine position and the chest cavity was opened to expose the heart. The hearts of some rats were directly removed, rinsed in precooled sterile saline, frozen in liquid nitrogen and stored at -80°C for kir6.2 qRT-PCR and Western blot analysis. Hearts from the remaining animals were perfused and fixed through the apex of the heart by a slow infusion of 250-300 mL 0.85% saline and 2 mL 1% heparin. The inferior vena cava was excised to allow blood outflow. When blood was no longer visible in the effluent, the heart was slowly perfused with 300 mL 4% paraformaldehyde over the course of approximately 30 min, and fixed with 4% paraformaldehyde for 24 hours. Tissues were washed in phosphate-buffered saline (PBS), trimmed, dehydrated by a conventional ethanol gradient, cleared in xylene, immersed in wax and embedded in paraffin for kir6.2 immunofluorescence.

Haematoxylin Basic Fuchsin Picric Acid (HBFP) Staining

Tissue sections were dewaxed and hydrated, rinsed for 2 min in running tap water and stained

with hematoxylin for 5 min. Tissue sections were gently washed with tap water for 5 min, destained in 1% hydrochloric acid ethanol for 4-5 s, rinsed with running tap water and distilled water for 5 min. For specific hypoxia-ischemia staining, sections were immersed in 0.1% basic Fuchsin (in distilled water) solution for 3 min. Sections were then rinsed for 10 s with distilled water to remove residual Fuchsin solution and immersed in absolute acetone for 5-10 min. Sections were incubated in 0.1% picric acid in absolute acetone solution for 15-22 s for cytoplasmic staining. Tissue sections were cleared in xylene I, xylene II and xylene III for 3-5 min, sealed with neutral balsam and left to dry. Five tissue sections were examined under an Olympus microscope (Tokyo, Japan) and altogether 25 images (five images per section) were taken.

Hematoxylin-Eosin Staining of Myocardial Tissue

Myocardial tissue sections were dewaxed, dehydrated and stained in hematoxylin solution 5 min. Sections were then gently washed with running tap water for 5 min, destained in 1% hydrochloric acid ethanol for 4-5 s, rinsed in running tap water and then distilled water for 5 min. The cytoplasm was stained by incubation in a 0.5% eosin solution for 2 min, after which the sections were gently washed in running tap water for 5 min. After staining, the slides were dehydrated using an ethanol gradient for 3 min, cleared in xylene I, xylene II and xylene III for 3-5 min, sealed with neutral balsam and left to dry. Five sections were examined using an Olympus microscope and 25 images (five images per section) were taken.

Detection of Rat Serum cTnI

When the endpoint of the study was reached, rats were anesthetized and secured on a dissecting table. 5 mL of blood were collected from the inferior vena cava and stored at room temperature for 30 min for the formation of serum. The serum was collected and centrifuged and used to determine the concentration of cTnI by immunochemiluminescence assay (Beckman Coulter Access 2 Immunoassay System, Fullerton, CA USA). The reagents were supplied by the instrument manufacturer and had a linear range of 0.01 to 99.99 ng/mL. The primary antibody used was an anti-human cTnI antibody, which showed roughly 92.8% homology between the amino acid sequences of rat and human cTnI.

Detection of Rat Serum NT-proBNP

Rat serum NT-proBNP levels were detected by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA). NT-proBNP standards were diluted using the Standard Diluent and the diluted standards were added to the ELISA plate. Blank control wells were included and contained diluent only, whereas the test wells contained the diluent and samples to be measured. Concentrated wash buffer was diluted 30-fold in medical double distilled water and mixed before use. The plate sealer was carefully lifted from the plate and the plate was washed 5 times with wash buffer, followed by the addition of 50 μ L enzyme-labeled reagent per well. After incubation, the plate sealer was carefully lifted and the plate was washed 5 times in wash buffer. Chromogenic substrates were added and the plate was gently shaken. Color development was allowed in the dark. Stop solution was added to terminate the reaction. The absorbance was measured at 450 nm and NT-proBNP levels concentrations were calculated using the standard curve generated in the assay.

Determination of Myocardial kir6.2 mRNA by qRT-PCR

A total of 50-100 mg of myocardial tissue each rat were harvested and placed in a glass homogenizer. The tissue was homogenized in TRIzol to extract total RNA (Invitrogen, Carlsbad, CA, USA). RNA quality was evaluated by denaturing agarose gel electrophoresis and RNA concentration was determined by a UV spectrophotometer (Unico UV-2000) at an absorbance of 260 nm. The annealing mixture consisted of 1.5 μ g RNA, 1 μ L of Oligo (dT) 18 (0.5 μ g/ μ L), 3.2 μ L of dNTPs Mix (2.5 mM) and RNase-free water in a total volume of 13 μ L. Complementary Deoxyribose Nucleic Acid (cDNA) was synthesized by reverse transcription and stored on ice for immediate use or at -20°C for long-term storage. The nucleotide sequences of the rat target gene and the internal reference gene were obtained from the NCBI nucleotide database. Specific primers for Kir6.2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were synthesized by KangChen Bio-tech Inc. (Shanghai, China) using the Primer 5.0 design software. The primers used for PCR were as follows: Kir6.2 primers (25 pmol/L): Upstream 5'-TATGTCCCTGCCCAACGAT-3'; Downstream 5'-AACAGCAATAGGCTCCCAACT-3'. The amplified Kir6.2 fragment was 158 bp in length. GAPDH primers (25 pmol/L): upstream

5'GGAAAGCTGTGGCGTGAT3'; downstream 5'AAGGTGGAAGAATGGGAGTT3'. The amplified GAPDH fragment was 308 bp in length. PCR amplification was performed in a total volume of 8 μ L. The reaction mixture contained 1.0 μ L 10 \times PCR buffer, 0.5 units Taq polymerase, 0.4 μ L PCR specific primer F, 0.4 μ L PCR specific primer R, 0.2 μ L 2 \times ROX Reference Dye and 2.0 μ L cDNA. PCR was carried out on an ABI 7900 Real-time PCR instrument (Waltham, MA, USA) under the following conditions: (95°C, 3 min) for 1 cycle and (95°C, 15 s; 59°C, 20 s; 72°C, 20 s; 82.5°C, 20 s) for 40 cycles. The results were expressed as absolute copies of gene expression and normalized against GAPDH.

Detection of kir6.2 Expression by Immunofluorescence

For detection of kir6.2 expression by immunofluorescence, paraffin-embedded sections were dewaxed and hydrated with xylene and a descending ethanol gradient, respectively. The sections were washed three times with distilled water and 0.01 M phosphate-buffered saline-tween (PBST) for 5 min and blocked with normal goat serum at room temperature for 20-30 min. A 1000-fold diluted primary anti-kir6.2 antibody (rabbit anti-goat, Alomone Labs, Israel) was added and incubated at 37°C for 1 hr. After incubation sections were rinsed three times with 0.01 M PBST 5 min and sections were incubated with a 600-fold diluted secondary anti-kir6.2 antibody (goat anti-mouse, KanChen Bio-tech Inc. Wuhan, China) in a 37°C incubator for 30 min. The sections were rinsed three times in 0.01M PBST 5 min and incubated with 100-fold diluted fluorescein solution in a humidified dark chamber at room temperature for 30 min. The slides were sealed in the presence of 20-50 μ L 4',6-diamidino-2-phenylindole (DAPI) Fluoromount, placed in the dark for about 5 min and examined under a Leica inverted fluorescence microscope with image acquisition. For negative controls, sections were incubated with PBS instead of the primary antibody.

Detection of Myocardial kir6.2 Protein by Western Blot Analysis

Myocardial tissue was weighed, cut into small pieces and placed in Eppendorf (EP) Micro Test tubes. Lysis buffer was added and tissue was homogenized at low-speed and centrifuged at 14000 rpm for 15 min (4°C) to collect the supernatant for further analysis. The samples were boiled in

2x sodium dodecyl sulfate (SDS) loading buffer, subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred overnight to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) at 30 mA constant current. The membrane was blocked with 5% bovine serum albumin (BSA) (Gibco, Rockville, MD, USA) for 1 h at room temperature and incubated with 5000-fold diluted rabbit anti-kir6.2 primary antibody (Alomone Labs, Jerusalem, Israel) and 10000-fold diluted anti-GAPDH primary antibody (goat anti-mouse, KanChen Bio-tech Inc. Wuhan, China) at 4°C overnight. Membranes were washed 3 times with TBST for 5 min and incubated with 5000-fold diluted goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at room temperature. Membranes were washed 3 times with Tris-buffered saline-tween (TBST) for 5 min at room temperature and visualized using a KC™ chemiluminescence kit. After the removal of excess chemiluminescence solution, the membrane was sandwiched between two plastic sheets and exposed to X-ray film. Images were taken and analyzed using ImageJ analysis software (Rawak Software, Inc., Hamburg, Germany).

Statistical Analysis

All data were analyzed using statistical product and service solutions (SPSS 12.0 Inc., Chicago, IL, USA) software and were expressed as mean \pm standard deviation. One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference) was used for the comparison between groups. $p < 0.05$ was considered statistically significant.

Results

Myocardial Ischemia and Hypoxia Staining

Representative images of hematoxylin-basic fuchsin-picric acid (HBFP) staining of rat myocardium are shown in Figure 1. We found that the myocardial fibers in Group C rats were evenly stained and were represented with a light yellow cytoplasm and distinct cell structures and borders. The nuclei appeared oval in shape, were located in the center of the cardiomyocytes and did not show any signs of reddish ischemia and hypoxia staining (Figure 1A). In contrast, the myocardial fibers of rats in Group EE displayed significant morphological and structural changes as demonstrated by the unevenly stained myocardial fibers, indistinct cell structures, blurred cell boundaries and fractured myocardial fibers (Figure 1B). A large number of bright-red dots/patches was present in the cytoplasm, some of which had fused into large and densely stained red areas (Figure 1B).

Changes in Rat Myocardium by HE Staining

The myocardial fibers in group C rats were evenly stained and distinct structures were present. The myofibrils appeared as short columns in a net of interconnected branches. The cytoplasm had a red appearance and the nuclei were bluish black and located in the center of the cells (Figure 2A). However, the myocardial fibers of group EE rats showed significant morphological changes, including unevenly stained myocardial fibers, indistinct structures, blurred cell boundaries and bent or fractured myocardial fibers (Figure 2B).

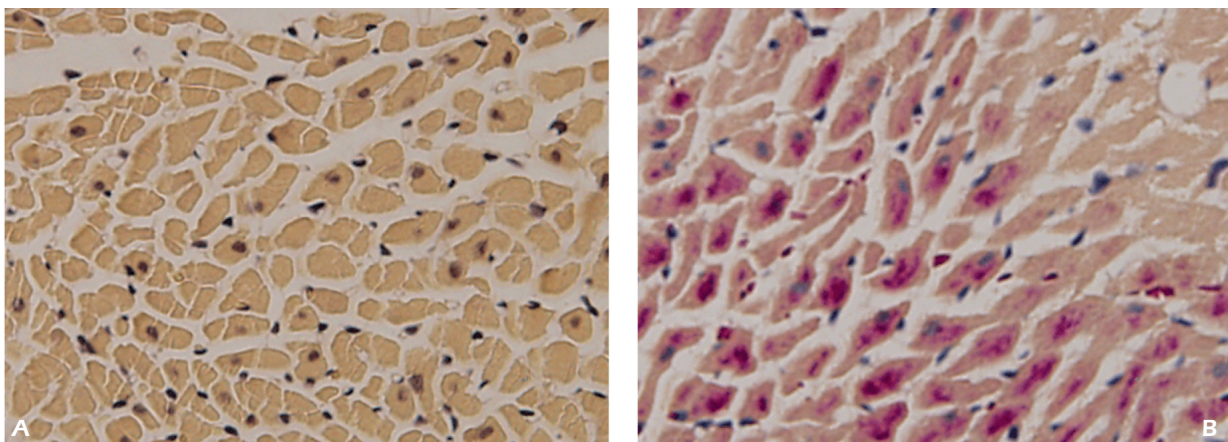


Figure 1. HBFP staining of rat myocardial tissue by HBFP staining. **A**, Myocardial fibers in Group C rats; **B**, Myocardial fibers of rats in Group EE rats (40 \times).

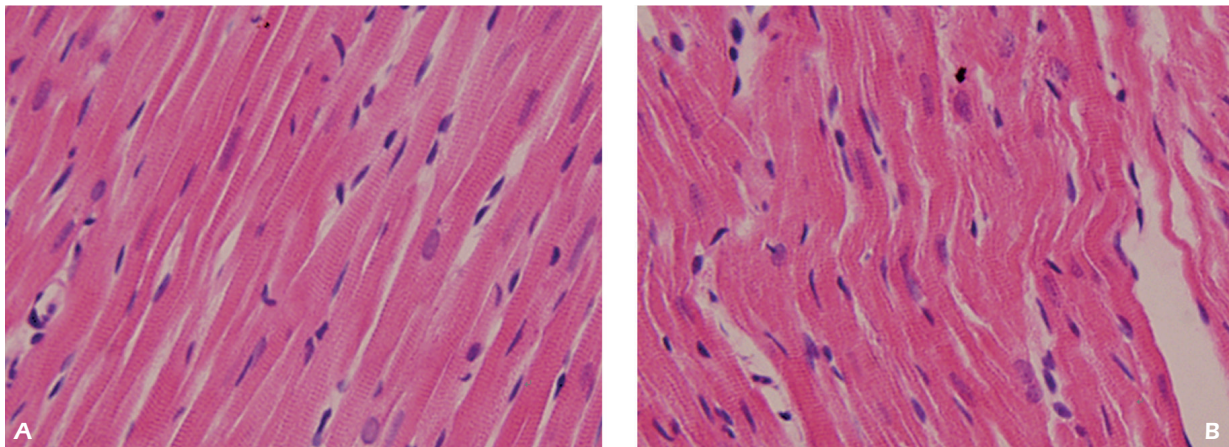


Figure 2. Changes in rat myocardium by HE staining. **A**, Myocardial fibers in Group C rats; **B**, Myocardial fibers of rats in Group EE rats (40×).

Changes in Rat Serum cTnI Concentration

Rat serum cTnI concentration are shown in Table I. Serum cTnI concentrations were significantly elevated in group EE rats compared to those in group C ($p < 0.05$).

Changes in Rat Serum NT-proBNP Concentration

Serum NT-proBNP concentrations are shown in Table II. The serum concentration of NT-proBNP in group EE rats were significantly higher than those in group C ($p < 0.05$).

Detection of Myocardial kir6.2 mRNA by RT-qPCR

Myocardial kir6.2 mRNA levels are shown in Table III. Kir6.2 mRNA level in group EE was lower than that in group C; however, the difference was not statistically significant.

Evaluation of Myocardial kir6.2 by Immunofluorescence

Positive staining of myocardial tissue of rats in group C could be identified as bright green immunofluorescence. In the longitudinal sections of the myocardium, kir6.2 appeared as regular

bright green bands that were mainly distributed on the surface of cardiomyocytes (Figure 3A). However, in the cross sections of the myocardium, kir6.2 immunofluorescence appeared as bright green dots that were distributed in the cytoplasm of cardiomyocytes (Figure 3B). Immunofluorescence staining of kir6.2 in group EE was stronger than that in group C (Figure 3C). In the negative control, myocardial tissue appeared dark green, which indicated background staining with no significant immunofluorescence (Figure 3D).

Detection of Myocardial Kir6.2 Protein by Western Blot Analysis

Kir6.2 protein expression results detected by Western blot analysis are shown in Table IV. Compared with group C, the expression of kir6.2 protein in group EE was significantly increased ($p < 0.05$).

Table I. Rat serum cTnI concentration.

Group	Serum cTnI concentration (µg/L)
Group C	0.02±0.01
Group EE	3.87±5.04*

* $p < 0.05$ when compared with Group C.

Table II. Rat serum NT-proBNP concentration.

Group	Serum NT-proBNP concentration (µg/L)
Group C	47.13±4.80
Group EE	50.72±3.44*

* $p < 0.05$ when compared with Group C.

Table III. Myocardial kir6.2 mRNA levels in rats.

Group	Serum NT-proBNP concentration (µg/L)
Group C	0.35±1.04
Group EE	3.00±0.47*

Myocardial kir6.2 levels were determined by qRT-PCR.

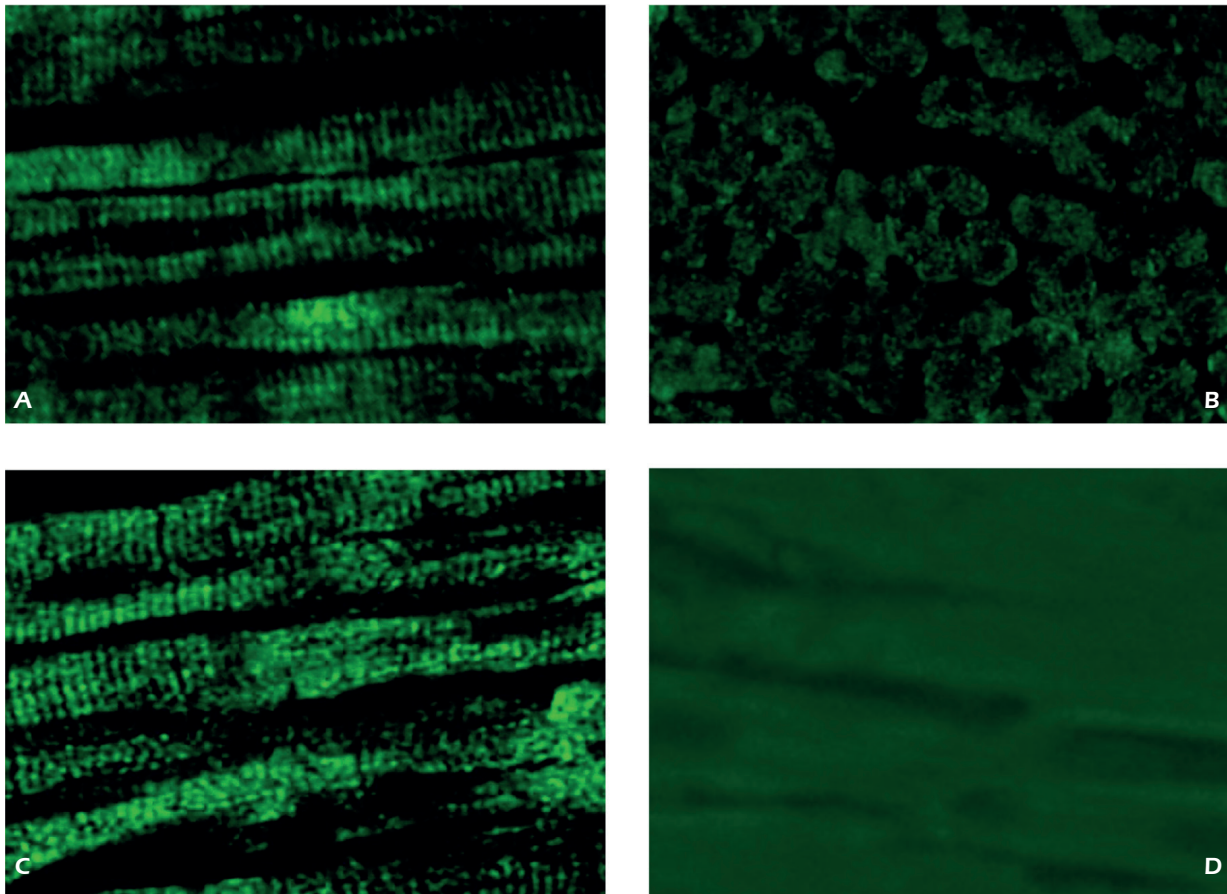


Figure 3. Immunofluorescence analysis of rat myocardial tissues. **A**, Longitudinal sections of the myocardium; **B**, Cross sections of the myocardium; **C**, Immunofluorescence staining of kir6.2 in group EE; **D**, Immunofluorescence staining of kir6.2 in the negative control group (40 \times).

Table IV. Rat myocardial kir6.2 protein expression by Western blot analysis.

Group	kir6.2 protein expression
Group C	0.28 \pm 0.07
Group EE	0.56 \pm 0.07*

* p <0.05 when compared with Group C.

Discussion

In this study, we identified that exhaustive exercise could result in exercise-induced myocardial injury in rats. In addition, we found that although no significant changes in the mRNA expression of myocardial sarcK_{ATP} channel subunit kir6.2 were observed, kir6.2 protein expression was significantly increased during exercise-induced myocardial injury. Similar to myocardial

ischemia/reperfusion (I/R) injury, vigorous or exhaustive exercises may lead to exercise-induced myocardial injury. In this study, the exercise-induced myocardial injury rat model was established by using high-intensity exhaustive treadmill exercise. The results showed that after exhaustive exercise, rat myocardial fibers had an unorganized appearance, along with indistinct structures, blurred cell boundaries, fractured myocardial fibers and numerous reddish-stained areas in the cytoplasm. Moreover, exhaustive exercise significantly increases rat serum cTnI levels. In this study, we demonstrated that exhaustive exercise in rats leads to exercise-induced myocardial injury. These findings were consistent with those presented in previous reports^{3,4}.

ATP-sensitive potassium channels (K_{ATP} channels) belong to a group of inwardly rectifying potassium channels, which are divided into two types, namely sarcolemmal K_{ATP} channel (or sarcK_{ATP} channel) and mitochondrial K_{ATP} channel (or mitoK_{ATP}

channel). SarcK_{ATP} channels are widely expressed *in vivo* and have a unique property of being inhibited by intracellular ATP (hence the name ATP-sensitive potassium channel). In contrast, adenosine diphosphate (ADP) activates sarcK_{ATP} channels and keeps them in an opened state. Therefore, the ATP/ADP ratio is one of the main factors for controlling sarcK_{ATP} channel activity^{5,6}. It had been demonstrated that mRNA expression of the myocardial sarcK_{ATP} channel Kir6.2 could undergo alterations during intense metabolism-induced myocardial ischemia or hypoxia. For example, Akaom et al⁷ proved that when myocardial kir6.1 mRNA expression was elevated, kir6.2 mRNA levels were not significantly changed during continuous myocardial ischemia with or without reperfusion. Du et al⁸ reported that myocardial ischemia in transgenic rats did not induce significant changes in kir6.2 mRNA expression. Therefore, no significant changes in myocardial kir6.2 mRNA expression were observed in the myocardial ischemia studies described above. However, in contrast to the above findings, Raeis-Dauvé et al⁹ investigated mRNA expression of the sarcK_{ATP} channel kir6.2 under hypoxic conditions and found that hypoxic conditions significantly reduced kir6.2 mRNA levels. These results indicated that different stress conditions may lead to alterations in sarcK_{ATP} channel kir6.2 mRNA expression. This may be due to the fact that ischemia and hypoxia are two different stress factors with different physiological mechanisms and, therefore, may have different effects on myocardial sarcK_{ATP} channel subunit expression. The specific mechanisms for differential Kir6.2 mRNA expressions under different stimulating conditions remain to be elucidated.

In the present research, the mRNA expression of the myocardial sarcK_{ATP} channel subunit Kir6.2 was investigated after exercise-induced myocardial injury in rats. The results showed that, compared with the control group, Kir6.2 mRNA levels were not significantly changed after exercise-induced myocardial injury, which is in accordance with the results from Zingman et al¹⁰. Our findings were also consistent with a study performed by Masaharu et al¹¹, where no significant changes in myocardial Kir6.2 mRNA expression were observed during myocardial ischemia in rats. The changes of kir6.2 mRNA expression in a myocardial I/R injury model were similar to those during exhaustive exercise-induced myocardial injury. These data suggested that the mechanism of exercise-induced myocardial injury may be similar to that of myocardial I/R injury. Morrissey et al¹² analyzed the localization of myocardial sarcK_{ATP} channel sub-

unit Kir6.2 protein and found that kir6.2 was distributed as regular striated bands on the surface of cardiomyocytes. In a subsequent study by Korchev et al¹³, it was observed that myocardial sarcK_{ATP} channels were localized as clusters around the transverse tubule openings of Z-lines of the sarcolemma. Consistent with Korchev's findings, Li et al¹⁴ reported that the myocardial sarcK_{ATP} channel kir6.2 subunit was mainly localized on cardiomyocyte transverse tubules and appeared as regular striated bands. The above studies demonstrated that the myocardial sarcK_{ATP} channel kir6.2 is primarily located on the surface of cardiomyocytes and on the transverse tubules formed by cardiomyocyte membrane invagination.

In this work, an exercise-induced myocardial injury model was successfully established to identify the localization of the sarcK_{ATP} channel kir6.2 subunit during exercise-induced myocardial injury. Consistent with the results observed by Morrissey et al¹², our findings showed that, in longitudinal sections, myocardial kir6.2 was present as bright green regular striated bands on the cell surface of cardiomyocytes. In the cross-section of cardiomyocytes, the kir6.2 was present as bright green dots in the cytoplasm of cardiomyocytes, which was different from the results previously reported. Therefore, based on the above results, we speculate that the sarcK_{ATP} channels may be located on the transverse tubules formed by cardiomyocyte membrane invagination and extend into the cytoplasm by wrapping around each myofibril along the transverse tubules. The main function of the ion channels on the transverse tubules at the horizontal Z-line is to control the inward transmission action potential into the cardiomyocytes by transmitting cell membrane potentials resulted from myocyte excitation into the cells. This finding suggests that the regulation of myocardial action potentials by myocardial sarcK_{ATP} channels mediates the protective effects of alleviated myocardial ischemia and hypoxia. Previous researches have shown that myocardial sarcK_{ATP} channel kir6.2 protein levels were significantly increased in exercise-induced myocardial protection. Zingman et al¹⁵ found that 5 consecutive days of exercise led to a significant increase in kir6.2 protein levels. Consistent with this finding, Brown et al¹⁶ reported that 5 days of continuous exercise significantly increased myocardial kir6.2 protein expression. Kraljevic et al¹⁷ examined changes in kir6.2 protein expression during the myocardial protective effect induced by long-term exercise and found that kir6.2 protein level was significantly elevated after endurance training. Similarly, Bayat

et al¹⁸ observed significantly elevated kir6.2 protein levels after exercise and training. The above investigations indicated that both long-term and short-term exercise could increase kir6.2 protein levels, suggesting that sarcK_{ATP} channels may increase the number of openings by increasing the protein level of kir6.2. Increased number of channel openings may alleviate the shortened myocardial action potential duration (APD) and reduced myocardial energy consumption induced by increased heart rate, thus conferring exercise-induced myocardial protection.

In the present study, it was found that kir6.2 protein levels in the exhaustive exercise group were significantly higher than that in control group. This finding was consistent with the changes in kir6.2 protein expression observed in cases of myocardial protection induced by long-term and short-term aerobic exercise. In addition, the increase of myocardial kir6.2 protein levels were consistent with the immunofluorescence results in that kir6.2 immunoreactivity in the exhaustive exercise group was significantly greater compared to the control group. This study demonstrated that exhaustive exercise as a high-intensity activity could lead to exercise-induced myocardial injury, resulting in myocardial ischemia, increased hypoxia and decreased ATP concentration in cardiomyocytes. Increased myocardial sarcK_{ATP} channel kir6.2 protein levels resulted in an increased number of channel openings, thereby reducing the intracellular ATP consumption and consequently alleviating exhaustive exercise-induced myocardial injury. The increase in myocardial sarcK_{ATP} channel kir6.2 protein during exercise-induced myocardial injury may promote sarcK_{ATP} channel opening, leading to cardiomyocyte membrane hyperpolarization and reduced closure of L-type Ca²⁺ channels. The opening of sarcK_{ATP} channels increased K⁺ efflux, shortened APD and reduced Ca²⁺ influx during the plateau phase. Furthermore, the increase of the K⁺ efflux accelerates the recovery of the resting membrane potential, so that intracellular Ca²⁺ can be easily removed by the Na⁺-Ca²⁺ exchange system at a relatively low resting membrane potential⁹. These mechanisms can reduce myocardial Ca²⁺ overload, resulting in decreased myocardial Ca²⁺ that weakens the contractility of the ischemic myocardium and to a certain extent, reduces ATP consumption. In exercise-induced ischemia, the sarcK_{ATP} channel can be regarded as a cellular energy sensor capable of regulating a myriad of cardioprotective effects to maintain cell integrity. The increase in sarcK_{ATP} channel protein expression after exercise may be as-

sociated with the maintenance of ATP levels during ischemia. It has been reported that the opening of sarcK_{ATP} channels could increase the concentration of intracellular ATP of cardiomyocytes, which in turn restored the energy balance in cardiomyocytes and reduced myocardial ischemic injury and thereby played an important role in maintaining normal cellular structure and function¹⁹⁻²².

In summary, during exercise-induced myocardial injury, myocardial sarcK_{ATP} channels increase the number of openings by increasing the protein levels of the kir6.2 subunit. This increase of channel openings can in turn suppress exhaustive exercise-induced myocardial injury by reducing Ca²⁺ overload, increasing the concentration of intracellular ATP and shortening of the myocardial action potential.

Conclusions

Although no significant alterations in kir6.2 mRNA expression were observed, kir6.2 protein levels were found to be elevated during exercise-induced myocardial injury. This suggested that myocardial sarcK_{ATP} channels may increase the number of channel openings by increasing protein levels of kir6.2 protein, thereby reducing intracellular ATP consumption and further inhibiting exhaustive exercise-induced myocardial injury.

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Conflict of interest

The authors declared no conflict of interest.

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