Exercise Preconditioning to Improve Myocardial Ischemia Tolerance Via the NO-PKC-K_{ATP} Signaling Pathway

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Abstract

Background: Exercise preconditioning (EP) has a similar effect on ischemic preconditioning. Yet the specific endogenous trigger substance, intermediary substance and effect substance involved in EP have yet to be fully defined.

Methods: 32 SD rats were divided into four groups: group A (control sham operation group); group B (control ischemia/reperfusion (I/R) model group); group C (train and I/R model group); and group D (train, inhibitor, and I/R model group). Rat model of acute endurance exercise was established. Then, in vivo I/R experiment and index test of the serum myocardial enzymes, the heart functions, Kir6.1 and Kir6.2 gene expressions and NOS and PKC protein expressions were carried out.

Results: Compared with the A group, the heart function of the B group decreased significantly, and the serum myocardial enzymes increased significantly. Compared with the B group, the C group had less heart function decline and less myocardial injury. Injection L-NAME attenuated the cardioprotection induced by exercise preconditioning. B group's PKC protein expression was higher than the A group's; the C group's PKC protein expression was significantly higher than the B group's, and the D group's PKC protein expression was significantly lower than the C group's. In addition, the B group's Kir6.1 and Kir6.2 gene expressions were higher than the A group's; the C group's Kir6.1 and Kir6.2 gene expressions were significantly higher than the B group's, and the D group's Kir6.1 and Kir6.2 gene expressions were significantly lower than the C group's.

Conclusions: Moderate intensity endurance exercise can induce a cardioprotective effect on I/R myocardium. Exercise preconditioning induces myocardial protective function via the NO-PKC-KATP signaling pathway.

Background

Ischemic preconditioning (IP) has a protective effect on the myocardium. It has been proven through studies that exercise, as a stimulating factor, to have a similar effect on IP. The phenomenon that exercise can induce the body to produce endogenous self-protection and improve the myocardial tolerance to ischemia and hypoxia is known as exercise preconditioning (EP) [1-3].

The mechanism of the protective effect of exercise on the heart still requires much experimental research to be fully explained. The protective effect of EP on the I/R myocardium via the signaling pathway of trigger substance - intermediary substance - effect substance has been confirmed by many studies performed throughout the world, yet the specific endogenous trigger substance, intermediary substance, and effect substance involved in EP have yet to be fully defined. In this study, moderate-intensity endurance training was used as an intervention means to explore the preconditioning protection of moderate-intensity endurance exercise on the heart, and to explore the role of preconditioning signaling pathway in EP by means of inhibitor technology, so as to provide an experimental basis for the theory of preconditioning, as well as for the prevention and rehabilitation of cardiovascular diseases.

Methods

Experimental Animals and Grouping

A total of 32 healthy and clean male SD rats, which were six weeks old and had a weight of 120 ± 10 g, were provided by the experimental animal center of Guangxi Medical University. The animals were randomly divided into four groups: group A (control sham operation group); group B (control I/R model group); group C (train and I/R model group); and group D (train, inhibitor, and I/R model group). The animals were each kept in separate cages. The indoor temperature was controlled at $22 \pm 6^{\circ}\text{C}$, and the humidity at 60.10%. The circadian rhythm was controlled by a fluorescent lamp, and the daily illumination time was 12 hours (8:00 am - 8:00 pm). The standard toothed animals were given free access to food and water. The padding was replaced once a day.

Animal training programme

The training method used was incremental load treadmill training. The exercise load of the training model was designed according to the treadmill exercise of rats as reported by Bedford [4] and slightly adjusted according to the actual situation. The adaptive training period was one week, and the formal training period was eight weeks; there was one day off after continuous training for three days, and the training time was 18:00-22:00. The gradient of the adaptive training treadmill was 0° , the speed was controlled at 10-15 m/min, and the exercise duration was 20 min. The 8-week endurance treadmill training programme of the train and I/R model group and train, inhibitor, and I/R model group was as follows: the exercise speed was gradually increased from 10 m/min in the first week to 20 m/min in the fourth week, while the speed of 20 m/min was maintained until the eighth week. The exercise time was gradually increased from 30 min in the first week to 60 min in the third week, and the time of 60 min was maintained until the eighth week. The gradient increased from 0° in the first week to 5° in the second week, and the gradient of 5° was maintained until the eighth week. The control sham operation group and control I/R model group was given regular cage feeding, free drinking and eating, and no endurance treadmill exercise.

Preparation of the I/R model for in-situ heart

The in-situ I/R rats had fasted for 12 hours before the operation, and urethane (1.2g/kg) was injected into the abdominal cavity for anesthesia. The rats were fixed on an anatomic table and intubated after trachea separation, an animal ventilator was connected to support respiration, an electrode was inserted into the subcutaneous skin of the limbs, an electrocardiograph was connected, a biological signal analysis system was connected, and a standard lead electrocardiograph was recorded. Then we opened the chest, inserted the needle 2 mm below the root of the left atrial appendage, and fed the 5/0 ligation line through the myocardial surface, and withdrew the needle at the side of the pulmonary artery cone. Then we placed a silk ring at both ends of the ligation line as the reperfusion cable and tightened the ligation line to incite myocardial ischemia. We then pulled the reperfusion cable to relax the ligation line, then conducted the reperfusion. The model was successful if the S-T segment of the ECG was raised after tightening the ligation line, and the S-T segment was lowered by more than 1/2 after loosening the

ligation line, after which the chest would be closed. Ischemia was performed for 40 min, and reperfusion for 50 min. The rats in the sham operation group underwent thoracotomy and threading without ligation of the coronary artery. Other operations were the same as those in the I/R group. After in-situ I/R experiment, the animals were killed by blood sampling from aorta before waking up.

Preparation and use of inhibitors

The rats in the inhibitor group were injected with NOS inhibitor L-nitro-arginine methyl ester (L-NAME) 30 minutes prior to ischemia-reperfusion. The L-NAME was prepared into a 50 mg/ml aqueous solution, and the tail vein was injected at a dose of 30 mg/kg.

Measurement of cardiac function indexes

After the successful establishment of the ischemia-reperfusion model, the biological signal analysis system was connected to record the arterial blood pressure, the peak value of left ventricular systolic pressure, left ventricular diastolic pressure, and maximum development rate of left ventricular pressure.

Measurement of serum myocardial enzymes

Blood was collected from the abdominal aorta after ischemia-reperfusion modeling. After blood coagulation, it was centrifuged at 2,500 r/min for 15 min, and the upper serum was drawn. The serum CK, LDH, and AST/GOT indexes were measured by colorimetry, and the experiment was carried out according to the instructions.

Detection of NOS and PKC protein expression by Western Blot

We placed the apical tissue in a centrifuge tube placed on ice and cut the tissue into small tissue fragments with scissors. Then we dissolved the RIPA cracking solution at room temperature to homogenize it. According to the ratio of 99:1 of cracking liquid to PMSF, we added PMSF a few minutes prior to use. We added lysates in the proportion of 300-500 microliters per 40 mg of tissue. We used a glass homogenizer to homogenize the ice until it became fully cracked. We implemented centrifugation of 10,000-150,000 g for 3-5 min, and took the supernatant to determine the protein concentration via the BCA method. Then we implemented the 10% and 12% SDS-PAGE separation gel electrophoresis (adjusted to 80 V electrophoresis for about 80 min after 60 V for 40 min) until the bromphenol blue ran out of the rubber plate. We transferred the PVDF membrane by the wet transfer method and sealed it for overnight storage. We placed the PVDF in monoclonal antibody Mouse Anti-NOS1 (1:1000) solution, where it was gently shaken for 2 hours. Then we performed PBST membrane washing for 3 cycles, for 10 minutes each time. We placed the PVDF was in an IgG-HRP (1:2000) solution and slowly shook it at room temperature for 2 hours. This was followed by PBST membrane washing for 3 cycles, at 10 min each time. We then mixed 150-200 microliters of solution A and solution B (1:1), added them to the membrane, and incubated them for 1 min. Next, we performed dark room pressing for 1-10 min, development for 1 min, and fixation for 1 min. Finally, we used a scanning film and gel image processing system to analyze the molecular weight and net optical density of the target strip.

Detection of Kir6.1 and Kir6.2 gene expression by RT-PCR

We used a TRIzol reagent to extract the total RNA from the apical tissue, then synthesized the cDNA and amplified it by PCR. We used Primer Premier 5.0 to design the primers as follows: Kir6.1 (133 bp): upstream primer 5'-TGA CTGAGGAGGAGGGAGTG-3', downstream primer 5'-CTCTTTTGGAGGGTCTGAATC-3'; Kir6.2 (117 bp): upstream primer 5'-CAAGCCCAAGTTTAGCATCTCT-3', downstream primer 5'-GCACCCAGCACTCTACATAC-3'; β -actin (165 bp): upstream primer 5'-TGTCACCAACTGGGACGATA-3', downstream primer 5'-GGGGTGTTGAAGGTCTCAA -3'. PCR amplification conditions: 96°C pre denaturation for 3 min, 96°C denaturation for 30 s, 57°C annealing for 30 s, 75°C extension for 30 s, 28 cycles, and the thickest 75°C extension for 5 min.

Statistical analysis

We used SPSS12.0 statistical software to perform the data analysis, and the measurement data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). We used one-way ANOVA for analysis and the LDS method for post comparisons. The significance level was $P < 0.05$, and the very significant level was $P < 0.01$.

Results

The detection results of the left ventricular systolic pressure peak (LVSP) and left ventricular end diastolic pressure (LVEDP) at different times of the ischemia-reperfusion experiment in each group are shown in Table 1. The maximum rate of left ventricular pressure rise ($+dp/dt_{\max}$) in each group is shown in Table 2, and the maximum rate of left ventricular pressure drop ($-dp/dt_{\max}$) in each group is shown in Table 3.

After 40 min of ischemia and 50 min of reperfusion, the activities of CK, LDH, and AST in the serum of rats of the control I/R model group were shown to be significantly lower than those of the control sham operation group ($P < 0.01$), thus indicating that the I/R had caused damage to the body. Compared with the control I/R model group, the activities of CK, LDH, and AST in the train and I/R model group were significantly higher than those of the control I/R model group ($P < 0.01$), indicating that the endurance exercise training had a protective effect on the body damage caused by I/R. Compared with the train and I/R model group. The activity of CK, LDH, and AST in the train, inhibitor, and I/R model group was significantly lower than that in the train and I/R model group ($P < 0.01$), and the activity of AST was lower than that in the train and I/R model group ($P < 0.05$), thus indicating that the L-NAME inhibited the protective effect of endurance exercise on the body.

The detection results of eNOS and iNOS protein expression in the myocardial tissue of rats by immunoblotting showed that the eNOS expression in the myocardial tissue of rats in the control I/R model group was higher than that in control sham operation group ($P < 0.05$), while the iNOS expression was significantly higher than that of the control sham operation group ($P < 0.01$). In addition, compared with the control I/R model group, the expression of eNOS and iNOS in the train and I/R model group was

significantly higher ($P < 0.01$); compared with the control group, the expression of eNOS and iNOS in the train and I/R model group was significantly higher than that of the control I/R model group ($P < 0.01$); and compared with the train and I/R model group, the expression of eNOS and iNOS in the train, inhibitor, and I/R model group was significantly lower than that in the train and I/R model group ($P < 0.01$), as shown in Fig. 1.

The detection results of PKC protein expression in myocardial tissue of rats performed by immunoblotting showed that the expression of PKC in the myocardium of the control I/R model group was higher than that of the control sham operation group ($P < 0.01$); compared with the control I/R model group, the expression of PKC in the train and I/R model group was significantly higher than that in the control I/R model group ($P < 0.01$); and compared with the train and I/R model group, the expression of PKC in the train, inhibitor, and I/R model group was significantly lower than that in the exercise training model group ($P < 0.01$), as shown in Fig. 2.

The results of Kri6.1 and Kri6.2 gene expression in the myocardial tissue of the rats, performed by RT-PCR, showed that the expressions of the Kir6.1 and Kir6.2 genes in the control I/R model group were higher than those in control sham operation group ($P < 0.01$), thus indicating that the expressions of the Kir6.1 and Kir6.2 genes had increased at the same time of myocardial injury. In addition, the expressions of Kir6.1 and Kir6.2 in the train and I/R model group were significantly higher than those in the control I/R model group ($P < 0.01$), and it was shown that exercise had a protective effect on myocardial I/R injury while increasing the expressions of Kir6.1 and Kir6.2. Finally, the expressions of Kir6.1 and Kir6.2 in the train, inhibitor, and I/R model group were significantly lower than those in the train and I/R model group ($P < 0.01$), and the gene expressions of Kir6.1 and Kir6.2 showed the same trend with iNOS, eNOS, and PKC protein expression, as shown in Figs. 3 and 4.

Discussion

In this study, VSPP and $+dp/dt_{\max}$ indexes were used to evaluate the systolic function of the heart. A high LVSP value indicates strong myocardial contractility. In addition, an increase in the $+dp/dt_{\max}$ value indicates an increase in myocardial contractility, while a decrease in the $+dp/dt_{\max}$ reflects an impairment of myocardial contractility [5]. The diastolic function was evaluated by LVEDP and $-dp/dt_{\max}$. LVEDP refers to the preload of the left ventricle, reflecting the diastolic function and compliance of the left ventricle. $-dp/dt_{\max}$ is the most sensitive index by which to reflect diastolic function [6, 7].

The experimental results showed that the left ventricular systolic and diastolic function decreased after I/R and that endurance exercise had a significant protective effect on myocardial systolic and diastolic function. This showed that the LVSSP and $\pm dp/dt_{\max}$ of the rat heart after exercise training were significantly higher than those of the control sham operation group after 40 min of ischemia and 50 min of reperfusion, and the LVEDP was significantly lower than that of the control sham operation group after 40 min of ischemia and 50 min of reperfusion. The results also showed that exercise training could increase the anti-ischemic ability of the heart and protect the heart function during ischemia-reperfusion

injury. The LVSSP and $\pm dp/dt_{\max}$ in the train, inhibitor, and I/R model group were significantly lower than those in the train and I/R model group after 40 min of ischemia and 50 min of reperfusion, and the LVEDP after 40 min of ischemia and 50 min of reperfusion were significantly higher than those in the train and I/R model group. The results revealed that the inhibitor inhibited the action of exercise-induced protection of myocardial systolic and diastolic function during reperfusion.

Some possible reasons for the decrease in function during myocardial ischemia are that myocardial ischemia leads to the destruction of contractile components, insufficient uptake of Ca^{2+} by the sarcoplasmic reticulum, insufficiency of myocardial energy supply, acidosis, etc. Some possible reasons for the decline of myocardial function during reperfusion are Ca^{2+} overload, energy metabolism disorder, white blood cell mediated injury, etc. Endurance exercise training can alter the shape of the heart and cause the thickening of the exercise-induced myocardium, which is characterized by the increase of MHC activity of cardiac myosin, the coincidence of capillary proliferation and ratio of muscle fiber, the matching of myocardial cell growth, and non-myocardial cell growth, and the adaptable increase of the heart volume. One possible mechanism of the improvement of the anti-injury ability of the heart in I/R after the intervention of moderate-intensity endurance exercise is that the capillary hyperplasia and the increase of oxygen supply ability after exercise, thereby improving the energy supply of the heart, along with the increase of the synthesis of some other endogenous protective factors, such as NO, which in turn activates the related mediators and effectors, etc. The LVSSP and $\pm dp/dt_{\max}$ of the heart in the train, inhibitor, and I/R model group were shown to be significantly lower than those in the train and I/R model group, and the LVEDP was significantly higher than that in the train and I/R model group. It is suggested that the inhibitor inhibits the synthesis of NO in vivo, blocks its reaction with mediators and effectors, and cancels the protective effect of endurance exercise on I/R-injured hearts, thus indicating that the protective effect of exercise adaptation to myocardial injury is at least partially mediated by the NO pathway.

The change of serum myocardial enzymes is one of the main indicators of the degree of myocardial injury. Under normal conditions, the activities of CK, LDH, and AST in serum are lower than those in the tissues. When the cell membrane is damaged and the permeability is increased, it can seep from the cell to the extracellular level [8]. The experimental results in this study showed that the activities of CK, LDH, and AST in the serum of the control I/R model group were significantly lower than those of the control sham operation group ($P < 0.01$). Compared with the control group, the activities of CK, LDH, and AST in the train and I/R model group were significantly higher than those of the control I/R group ($P < 0.01$). Compared with the exercise training model group, the activities of CK, LDH, and AST in the train, inhibitor, and I/R model group were significantly lower than those in the exercise training model group ($P < 0.01$ Or $P < 0.05$), thus indicating that I/R causes myocardial damage. It was observed that endurance exercise has a protective effect on myocardial damage caused by I/R. Finally, L-NAME inhibits the protective effect of endurance exercise on the myocardium, further indicating that NO plays an important role in the protection of exercise pre-adaptation myocardium.

The experimental results showed that, compared with the control sham operation group, the expression of iNOS protein in control I/R model group was higher than that in the control sham operation group ($P < 0.05$), and the expression of eNOS protein in control I/R model group was significantly higher than that in the control sham operation group ($P < 0.01$). Compared with the control I/R model group, the protein expressions of iNOS and eNOS in the rat myocardium of the train and I/R model group were significantly higher than those of the control I/R model group ($P < 0.01$), thus indicating that endurance exercise can improve the protein expression of iNOS and eNOS in the myocardium of I/R. One possible mechanism of resistance to I/R injury of moderate-intensity endurance training is to activate the expression of iNOS and eNOS, and increase the synthesis of NO through exercise. The increase of NO biosynthesis is necessary to trigger the protection of the EP delayed phase. At the same time, NO can also participate in the signal transduction pathway as a signal molecule to regulate some physiological processes and achieve the purpose of protecting the heart [9]. For example, NO can activate soluble guanylate cyclase (SGC), increase the concentration of cGMP in cells, reduce Ca^{2+} influx through cGMP acting on the L-calcium channel, or stimulate cGMP to reduce cAMP content and reduce myocardial contractility, thus ultimately reducing oxygen consumption and energy demand. NO can also directly act on PKC, MARK, mitochondria, etc., or on KATP, heat shock protein, sodium hydrogen exchanger, etc., through PKC to play a role. Compared with the train and I/R model group, the expression of iNOS in the train, inhibitor, and I/R model group significantly decreased ($P < 0.01$), and the expression of eNOS in the train, inhibitor, and I/R model group significantly decreased ($P < 0.05$). Previous studies have shown that L-NAME can slightly reduce the synthesis of endogenous NO in resting rats, including the content of NO in the liver, spleen, bone marrow, and other tissues and cells [10]. The experimental results showed that L-NAME inhibited the protein expressions of iNOS and eNOS blocked the biosynthesis of NO and caused the protective effect of endurance exercise to disappear. Therefore, it is observed that NO plays an important role in the myocardial protection induced by exercise preconditioning.

PKC is a serine threonine kinase with a single peptide chain structure, which widely exists in many tissues, organs, and cells. PKC is a key part of the NO intracellular signal transduction pathway [11, 12]. The experimental results showed that the expression of PKC in the myocardial tissue of the control I/R model group was higher than that of the control sham operation group ($P < 0.01$). Compared with the control I/R model group, the expression of PKC in the train and I/R model group was significantly higher than that in the control I/R model group ($P < 0.01$). The results showed that the expression of PKC increased at the same time of myocardial injury. Exercise had a protective effect on myocardial I/R injury and increased the PKC expression at the same time. Compared with the train and I/R model group, the expression of PKC in the train, inhibitor, and I/R model group was significantly lower than that in the train and I/R model group ($P < 0.01$). The results also showed that the NO inhibitor decreased the expressions of iNOS and eNOS protein, while at the same time, the protein expression of PKC also showed the same trend of decrease. One possible mechanism causing the increased trend of PKC expression at the same as that of NO expression is that exercise causes relative hypoxia of myocardial tissue and stimulates the myocardium to release endogenous trigger substances such as NO, adrenaline, bradykinin, opioid titanium, etc. These substances are coupled with Guanosine regulatory protein (G-protein) after binding

with the receptor and activate the inhibitory G protein (Gi), which then decreases the activity of acylate cyclase, reduces the number of Ca^{2+} channels on the cell membrane, decreases the Ca^{2+} influx, and reduces the myocardial contractility. At the same time, the binding of the endogenous active substance with the receptor can activate another G protein (Gq). GQ is the membrane effector enzyme that activates PLC. PLC initiates the release of the intracellular calcium pool and activates PKC, and then initiates a series of downstream cascade reactions [13]. The protein expression trend of PKC was the same as that of the NO inhibitor group. This revealed that moderate-intensity endurance training stimulated cardiomyocytes to release endogenous active substances NO and NO combined with the corresponding receptors to activate Gq protein and then activate PLC. PLC decomposed PIP_2 into IP_3 and DAG, then activated PKC, which had a protective effect on the heart. This further illustrates that PKC is downstream of the trigger substance of NO, and plays an intermediary role in the signaling pathway of exercise preconditioning to improve myocardial ischemic tolerance.

The expression of K_{ATP} is different in various tissues. K_{ATP} in the myocardium is composed of Kir6.2 and SUR2A, K_{ATP} in smooth muscle is composed of Kir6.2 and SUR2B, K_{ATP} in pancreas β cell, brain nerve cells are composed of Kir6.2 and SUR1, and K_{ATP} in the kidney is composed of Kir1.1 and CFTR [14, 15]. In this study, the Kir6.1 and Kir6.2 gene expressions in rat myocardial tissue were consistent with those observed in previous studies.

The role of K_{ATP} in the pancreas and nervous system has been widely studied [16, 17]. K_{ATP} in the heart is normally closed. When ischemia, hypoxia, and energy shortage occur, K_{ATP} channels open to play a role in protecting the heart muscle. Previous research shows that the activity of the K_{ATP} channel depends on the number and the opening degree of the K_{ATP} channel in cells. Exercise increases the expression of Kir6.1, Kir6.2, and SUR2 in cardiomyocytes, thus indicating that increasing the expression of various subunits of the K_{ATP} channel is of great significance in improving myocardial anti-I/R injury [18]. As an effective substance, K_{ATP} is an important part of the signal transduction pathway of EP-induced myocardial protection.

The study results showed that the expressions of Kir6.1 and Kir6.2 in control I/R model group were significantly higher than those in the control sham operation group ($P < 0.01$), thus indicating that K_{ATP} was open to protect myocardium when the myocardial injury occurred. Compared with the control I/R model group, the expressions of Kir6.1 and Kir6.2 in the train and I/R model group were significantly higher than those in the control I/R model group ($P < 0.01$), thus indicating that endurance exercise increased the activity of the K_{ATP} channel and enhanced the protective effect on injured myocardium. One possible mechanism of the increase of K_{ATP} gene expression is that I/R injury causes the decrease of blood glucose level and muscle glycogen content, causes the K_{ATP} to open, leads to the outflow of K^+ , increases the potassium conductance of cardiomyocytes and vascular smooth muscle cells, accelerates repolarization, shortens the action potential time course, closes the Ca^{2+} channel, reduces Ca^{2+} overload in cardiomyocytes, and reduces the exchange of Na^{2+} - Ca^{2+} , in turn reducing myocardial injury. Previous

studies have shown that I/R causes the sympathetic adrenomedullin system to secrete a large number of catecholamines, while the oxidation of catecholamines may produce oxygen free radicals [19, 20]. Oxygen free radicals may cause the loss of selective permeability of finger membrane and hinder the transport of Ca^{2+} ions in cells. Endurance exercise can accelerate the opening of K_{ATP} , increase the intake of catecholamine, inhibit the production of interleukin-oxygen free radicals in cells when the I/R is cleared, reduce the formation of free fatty acids and other harmful metabolites, increase the energy supply of intracellular ATP decomposition and coupling with calcium pump, and stabilize ATP production. Compared with the train and I/R model group, the expressions of Kir6.1 and Kri6.2 in the train, inhibitor, and I/R model group decreased significantly ($P < 0.01$). The results showed that L-NAME inhibited the expression of iNOS and eNOS protein, as well as the gene expression of mediated PKC and effector K_{ATP} .

Conclusions

Moderate intensity endurance exercise is capable of effectively improving the myocardial ischemic tolerance of rats and protecting the ischemia-reperfusion heart. Activation of the NO-PKC- K_{ATP} signaling pathway is one of the ways that moderate-intensity endurance exercise produces myocardial protection.

Abbreviations

AST: Alanine aminotransferase; cAMP: cyclic adenosine monophosphate; cGMP cyclic guanosine monophosphate; CK: creatine kinase; DAG: diacylglycerol; EP: exercise preconditioning; GOT: Glutamic-oxalacetic Transaminase; IP: ischemic preconditioning; I/R: ischemia-reperfusion; K_{ATP} : ATP-sensitive potassium channel; LDH: lactate dehydrogenase; L-NAME: L-nitroso-L-arginine methyl ester; LVEDP: left ventricular end diastolic pressure; LVSP: left ventricular systolic pressure peak; MAPK: mitogen-activated protein kinase; NO: nitric oxide; NOS: Nitric oxide synthase; PKC: protein kinase C; PLC: phospholipase C; SGC: soluble guanylate cyclase; $+\text{dp}/\text{dt max}$: maximum rate of left ventricular pressure rise; $-\text{dp}/\text{dt max}$: maximum rate of left ventricular pressure drop.

Declarations

Ethics approval and consent to participate

This study was approved by the Experimental Animal Ethics Committee of Guangxi Normal University (No. 202105-001).

Consent for publication

Not applicable.

Availability of data and material

The raw data are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

Prof. FP participated in the design, conducted data analysis and drafted the manuscript, provided technical support and commented on the manuscript drafts. Mr. CG collected and analyzed part of the data. Mr. YL aided interpretation of data and commented on this study design. All authors have read and approved the manuscript.

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Tables

Due to technical limitations, table 1-4 is only available as a download in the Supplemental Files section.

Figures

Fig. 1 Expression of iNOS and eNOS protein in myocardium of rats in each group (GAPDH as internal reference). A, control sham operation group; B, control I/R model group; C, train and I/R model group; D, train, inhibitor, and I/R model group. $\Delta P < 0.05$, $\Delta\Delta P < 0.01$, compared with the sham operation group; $\blacktriangle P < 0.05$, $\blacktriangle\blacktriangle P < 0.01$, compared with the control I/R model group; $\star P < 0.05$, $\star\star P < 0.01$, compared with the train, inhibitor, and I/R model group.

Figure 1

See image above for figure legend.

Fig. 2 PKC protein expression in myocardium of rats in each group (GAPDH as internal reference). A, control sham operation group; B, control I/R model group; C, train and I/R model group; D, train, inhibitor, and I/R model group. $\Delta P < 0.05$, $\Delta\Delta P < 0.01$, compared with the sham operation group; $\blacktriangle P < 0.05$, $\blacktriangle\blacktriangle P < 0.01$, compared with the control I/R model group; $\star P < 0.05$, $\star\star P < 0.01$, compared with the train, inhibitor, and I/R model group.

Figure 2

See image above for figure legend.

Fig. 3 Expression of Kir6.1 mRNA in rat myocardium (β - actin as internal reference). A, control sham operation group; B, control I/R model group; C, train and I/R model group; D, train, inhibitor, and I/R model group. $\Delta P < 0.05$, $\Delta\Delta P < 0.01$, compared with the sham operation group; $\blacktriangle P < 0.05$, $\blacktriangle\blacktriangle P < 0.01$, compared with the control I/R model group; $*P < 0.05$, $**P < 0.01$, compared with the train, inhibitor, and I/R model group.

Figure 3

See image above for figure legend.

Fig. 4 Expression of Kir6.2 mRNA in rat myocardium. A, control sham operation group; B, control I/R model group; C, train and I/R model group; D, train, inhibitor, and I/R model group. $\Delta P < 0.05$, $\Delta\Delta P < 0.01$, compared with the sham operation group; $\blacktriangle P < 0.05$, $\blacktriangle\blacktriangle P < 0.01$, compared with the control I/R model group; $*P < 0.05$, $**P < 0.01$, compared with the train, inhibitor, and I/R model group.

Figure 4

See image above for figure legend.

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