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Blockers of sulfonylureas receptor 1 subunits may lead to cardiac protection against isoprenaline-induced injury in obese rats

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ABSTRACT

Recent studies have found that blockers of sulfonylureas receptor 1(SUR1) might have cardiac ischemic protective effects. We evaluated the effects of a selective SUR1 blocker gliclazide on cardiac function and arrhythmia after isoprenaline-induced myocardial injury in obese rats. Diet-induced obese rats received isoprenaline or saline shots subcutaneously. Gliclazide or saline was given q12h for 48 h to rats received isoprenaline. We measured ECG and hemodynamic parameters and collected blood samples for CK-MB, glucose and lipid profile determination, and then harvested hearts for water content, histological and immunohistochemical analysis and infarct size measurements. The obese rats' hearts receiving isoprenaline-induced myocardial injury showed up-regulated SUR-1 expression in the peri-microvascular area. Obese rats receiving gliclazide lavage had less severe arrhythmia (ASI: 4.00 ± 0.61 vs. 2.14 ± 0.39, $P < 0.05$) and myocardial edema (water percentage: $85.16 \pm 0.46\%$ vs. $81.56 \pm 0.57\%$, $P < 0.05$). Less infarct size ($47.6 \pm 12.8\%$ vs. $32.7 \pm 9.1\%$, $P < 0.05$) and improved diastolic function (LVEDP: $6.86 \pm 0.85\%$ vs. $2.51 \pm 1.09\%$, $P < 0.05$; $-(dp/dt)_{max}$: -1663.6 ± 387.91 mmHg/s vs. -2834.8 ± 290.76 mmHg/s, $P < 0.05$) were also observed in rats receiving gliclazide lavage. Blocking of the SUR1 thus exerts a protective effect on the isoprenaline-induced myocardial injury in obese rats. That SUR1 blocker leads to ischemic protection suggesting a critical biological role of SUR1 in regulating the function of the cardiovascular system than previously recognized under pathophysiological conditions.

1. Introduction

In recent decades, the prevalence of obesity in the world has risen to epidemic/pandemic proportions. Risks of cardiovascular diseases are substantially higher under obesity, but the weight loss is associated with benefits in all of the obesity-related comorbidities. However, most weight loss interventions are associated with weight re-gain and are therefore not successful in the longer term. It is for these reasons that exploring mechanisms and new treatments of obesity related cardiovascular diseases remains a crucial role in improving the prognosis of obesity.

Sulfonylureas receptor 1(SUR1) is one of the receptors of sulfonylureas (SUs), a widely used hypoglycemic agent, and new understanding of molecular cloning studies responsible for ion channels has provided novel insights into biophysical and pharmacological properties of SUR1 subunits. SUR1 has further been recognized as the regulatory subunit of a newly discovered calcium-activated nonselective cation channel (NSC_{Ca}), identified in cultured human and mouse endothelial cells in central nervous systems subjected to hypoxia (Chen et al., 2003). Another recent discovery focusing on the heart have shown that SUR1 is one of the regulatory subunit of K_{ATP} channels (abundantly expressed in the heart but are generally thought to consist of Kir6.2/SUR2A subunits) and serves as an ATP-binding cassette transporter. Other recent studies have also found the presence of SUR1 subunits in mouse cardiac tissue and a surprising protection from myocardial ischemia-reperfusion in SUR1-null mice (Lefer et al., 2009).

We hypothesized that SUR1 regulated cardiac NSC_{Ca} channel and/or K_{ATP} channel is involved in heart ischemic injury and inhibiting these channels with a highly selective SUR1 blocker may have beneficial cardio-protective effects. We created an isoprenaline-induced myocardial injury model on diet-induced obese Wistar rats to observe the possible up-regulation of SUR1 subunits in the myocardium. The effects of low-dose gliclazide on infarct size, cardiac function and arrhythmia was examined by blocking SUR1 to shut down SUR1-regulated cardiac K_{ATP} channel and/or NSC_{Ca} channel.

2. Materials and methods

2.1. Experimental animals

Four-week-old male Wistar rats (200–220 g body weight) obtained from the Experimental Animal Center of the Chengdu

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Dashuo Biotechnology Co, Ltd. (Chengdu, China) were used in our experiments. The rats were handled following the Guidelines of the Chinese Council on Animal Care and the protocols were approved by the institutional ethics committee. Animals were randomly divided into 2 groups: Obese group (fed with carbohydrate–lipid-rich diet; 50.1% fat; 33.6% carbohydrate; 16.3% protein; 493 kcal 100 g^{-1}) for 12 weeks, and Normal-Control (NC) group (given normal diet). Body weight was monitored and recorded weekly for the entire 12 weeks.

2.2. Study protocols

2.2.1. Determination of SUR1 expression in isoprenaline-induced ischemic hearts

Twelve (12) obese rats (OB-MI group) received subcutaneous isoprenaline (100 mg/kg, purchased from Sigma Chemical Co., St. Louis, MO, USA) shots at an interval of 24 h for 2 day to induce myocardial injury, 6 normal control rats (NC group) and 18 obese rats (OB-C group) received subcutaneous normal saline shots. At the end of 48 h after the first shot, rats were euthanized and the blood collected for analysis and measurement of serum CK-MB, glucose and lipid profile, followed by collection and weighing of abdominal visceral fat. Hearts were harvested and prepared for water content determination, macroscopic enzyme mapping and histological mean cell diameter (MCD) measurements. These protocols are illustrated in Fig. 1A.

2.2.2. The effects of selective block of SUR1subunits on isoprenaline-induced ischemic hearts

We randomly selected 44 obese rats and divided them into 2 groups. SU group ($n=22$) were given gliclazide (1 mg/kg) lavage Q12h for 48 h and control group ($n=22$) given normal saline lavage. They were then subjected to myocardial injury by isoprenaline (100 mg/kg) injection subcutaneously before first lavage, at an interval of 24 h for 2 day. We recorded the electrocardiogram (ECG) at the baseline, extended for 3 h after the isoprenaline injection. After 48 h of the first isoprenaline shots, hemodynamic parameters were measured with a probe inserted into the left ventricle via a percutaneous cervical surgery. The rats were then euthanized, and the blood collected for serum CK-MB level examinations. Sixteen (16) hearts from each group were harvested and cut into 1 mm slides, and the infarct size evaluated with macroscopic enzyme mapping (calculated with Image-Pro Plus 6.0), followed by wet and dry weights determination. This protocol is illustrated in Fig. 1B.

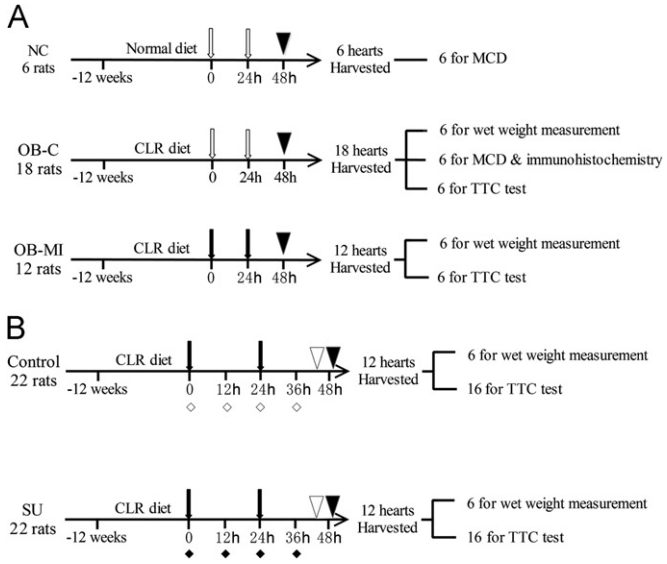

Fig. 1. General study protocols. (A) Determination of SUR-1 expression in ISO-induced ischemic hearts. (B) The effects of selective block of SUR1-subunits on ISO-induced ischemic hearts. White arrow: subcutaneous saline shots (same volume as ISO); Black arrow: subcutaneous ISO shots (100 mg/kg); Black arrowhead: blood collected and rats euthanized; White diamond: saline lavage; Black diamond: gliclazide (1 mg/kg) lavage; White arrowhead: hemodynamic parameters determination.

2.3. Experimental methods

2.3.1. Determination of serum biochemical parameters

We obtained blood samples by intracardiac puncture. The serum CK-MB activity was assayed by immune inhibition with a commercial kit from Shengneng Biotech Ltd., Shanghai, and the plasma glucose measured by glucose oxidase method. Serum triglyceride (TG), cholesterol (TC), free fatty acid (FFA) concentrations were determined by using colorimetric assays.

2.3.2. Histology and immunohistochemistry

2.3.2.1. Determination of cardiac MCD. The determination of MCD were performed following Ito et al. protocols (Ito et al., 1994). The obese rats' left ventricles were fixed and stained with hematoxylin and eosin (H & E stain), and MCDs were measured at a magnification of $\times 400$. A total of 6 to 8 fields from the left ventricle wall were chosen for analysis. Diameters were measured across the nuclei and were restricted to myocytes containing nuclei in the center of the fiber in both transversely and longitudinally cut cells, and 5 to 10 myocytes per field (a total of 50 myocytes) per animal analyzed.

2.3.2.2. Immunohistochemistry. To observe the distribution and dynamic change of the SUR1 expression on myocardium with isoprenaline-induced injury, frozen slides (1 mm) of the hearts from both OB-MI and OB-C groups were fixed by 4% paraformaldehyde (PFA). The primary antibodies used were goat anti-SUR1 C-16 antibody (sc-5789; Santa Cruz Biotechnology, CA, USA), while secondary antibodies were Cy3-conjugated donkey anti-goat IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA). The tissue sections were also counterstained with 0.5 mg/mL 40, 6-diamidino-2-phenylindole (DAPI), and specimens examined using a Nikon Eclipse E1000 microscope. Images were captured using a SenSys digital camera (Roper Scientific) and processed using a personal computer running IPLab software (version 3.0, Scanalytics). Post-processing of images was performed using Adobe Photoshop (version 5.0, Adobe Systems).

2.3.3. Evaluation of isoprenaline-induced arrhythmia

The ECG was recorded continuously by lead II limb electrodes. Arrhythmias were defined following the Lambeth Conventions guideline (Walker et al., 1988), involving measurements of the starting time and length of arrhythmic episodes. The severity of arrhythmias was evaluated with the aid of the arrhythmia severity index (ASI) (Bernauer, 1986). The occurrence of upto 10 premature ectopic beats during the respective observation time was weighted with the value 1, and the occurrence of more than 10 ectopic beats weighted 2. Ventricular tachycardia/ventricular flutter and ventricular fibrillation were weighted with the value 3 and 4, respectively. For appearance of more than one kind of arrhythmia, the respective values are added up to yield the ASI value—representing a numerical term for the severity of the dysrhythmia.