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The mitochondrial Ca^{2+}-activated K^+ channel contributes to cardioprotection by limb remote ischemic preconditioning in rat

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ABSTRACT

Aims: To investigate the role of the mitochondrial Ca^{2+}-activated K^+ channel in cardioprotection induced by limb remote ischemic preconditioning.
Main methods: Male Sprague–Dawley rats (250–300 g) were randomized into control, ischemia/reperfusion (I/R), remote ischemic preconditioning (RPC), NS1619 (a specific mitochondrial Ca^{2+}-activated K^+ channel opener), and RPC + paxilline (a specific mitochondrial Ca^{2+}-activated K^+ channel inhibitor) groups. RPC was induced by 4 cycles of 5 min of ligation followed by 5 min of reperfusion of the left femoral artery. Myocardial I/R was achieved by ligation of the left anterior descending coronary artery for 30 min, followed by 120 min of reperfusion. Infarct size was determined by 2,3,5-triphenyltetrazolium chloride staining, the hemodynamics were monitored, and lactate dehydrogenase (LDH) levels in the coronary effluent, manganese superoxide dismutase (Mn-SOD) content in mitochondria and mitochondrial membrane potential were measured spectrophotometrically. The ultrastructure of cardiomyocyte mitochondria was assessed by electron microscopy.
Key findings: NS1619 (10 μM) improved heart function, decreased infarct size, reduced LDH release, maintained mitochondrial structural integrity and mitochondrial membrane potential, and increased the mitochondrial content of Mn-SOD to the same degree as RPC treatment. However, paxilline (1 μM) eliminated the cardioprotective effect conferred by RPC.
Significance: The mitochondrial Ca^{2+}-activated K^+ channel participates in the myocardial protection by limb remote ischemic preconditioning.

Introduction

Remote ischemic preconditioning (RPC) protects the heart from injury evoked by subsequent exposure to severe ischemia followed by reperfusion (Przyklenk et al., 1993), but its mechanisms remain unclear. While there have been reports that adenosine (Pell et al., 1998), bradykinin (Schoemaker and van Heijningen, 2000), calcitonin gene-related peptide (Wolfrum et al., 2005), TNF-α (Ates et al., 2002), IL-1β (Harkin et al., 2002), opioids (Patel et al., 2002), and PKC (Tokuno et al., 2002) participate in this cardioprotection, the mitochondrion is thought to be one of the most important end-effectors (Marin-Garcia and Goldenthal, 2004). The mitochondrial Ca^{2+}-activated K^+ channel (KCa) is an important potassium channel located in the inner membrane, and plays an important role in cardioprotection (Xu et al., 2002). However, no study has yet been undertaken to determine whether the KCa channel confers the cardioprotection of RPC. Therefore, the aim of the present study was to determine whether the KCa channel plays a role in RPC.

Methods

Animals

Male Sprague–Dawley rats (250–300 g) were obtained from the Animal Center of Tongji Academy of Medical Sciences and treated in accordance with the Guide for the Care and Use of Laboratory Animals of China Three Gorges University.

Chemicals

1,3-dihydro-1-[2-hydroxy5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619), paxilline(Pax), Evans blue, 2,3,5-triphenyl-tetrazolium chloride (TTC) and rhodamine-123 were from Sigma Chemical Co. and the lactate dehydrogenase (LDH) and manganese superoxide dismutase (Mn-SOD) kits were from Nanjing Jiancheng Reagent Co.

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Langendorff heart preparation

Rats were anesthetized by intraperitoneal injection of chloral hydrate (3.3 g/kg). The heart was excised rapidly and placed in ice-cold Krebs–Henseleit (K–H) buffer, then mounted on a constant pressure (100 cm H_2O) Langendorff apparatus and perfused at 37 °C with K–H buffer. The buffer was equilibrated with 95% O_2/5% CO_2 (pH 7.3–7.4) and had the following composition (mmol/L): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0. For hearts subjected to regional ischemia, a 5/0 silk suture was passed under the left coronary artery to form a snare. The artery was occluded by pulling the snare to produce ischemia, while reperfusion was achieved by releasing it. A latex, fluid-filled balloon was introduced into the left ventricle through the left atrial appendage and the balloon catheter was linked to a pressure transducer connected to a data acquisition system (Biopac, USA) to assess contractile function. The left ventricular end-diastolic pressure (LVEDP) was adjusted to between 4 and 8 mm Hg. Left ventricular developed pressure (LVDP), heart rate (HR) and rate-pressure product (RPP = LVDP × HR) were monitored continuously. Coronary flow (CF) was measured by timed collection of effluent at regular intervals using a calibrated tube, and expressed in mL/min.

Perfusion protocols of isolated heart

Each heart was allowed to equilibrate for at least 20 min and received 30 min of regional ischemia followed by 120 min of reperfusion. The hearts were randomly assigned to one of the following groups: (1) hearts subjected to regional ischemia and reperfusion only (I/R); (2) hearts from animals in which RPC, done five minutes before sacrifice, was induced by four cycles of 5 min of limb ischemia, followed by 5 min of reperfusion; (3) hearts treated with NS1619 (10 μmol/L), an activator of the KCa channel (Cao et al., 2005), which was perfused 15 min before ischemia for 10 min; (4) hearts treated with Pax (1 μmol/L), an inhibitor of the KCa channel (Huhn et al., 2010), which was administered 30 min before ischemia.

Infarct size

At the end of 120 min reperfusion, the coronary artery was re-occluded and the heart was perfused with a solution of 1% Evans blue to delineate the non-ischemic zone as a dark blue area. The heart was then frozen at −20 °C and sliced into 2 mm thick transverse sections and incubated in a sodium phosphate buffer containing 1% w/v TTC for 15 min, then fixed in 10% formalin for 10 min. In the risk zone, the viable tissue was stained red and the infarcted tissue appeared pale. Infarct and risk zone areas were determined by planimetry with Image/J software from NIH. Infarct size was expressed as a percentage of the risk zone.

LDH in coronary effluent

The coronary effluent from the isolated perfused heart was collected at 5 min of reperfusion and the LDH activity was spectrophotometrically assayed and expressed as units per liter.

Myocardial ultrastructure

At the end of reperfusion, a small piece of myocardium was immediately dissected from each cardiac apex (left ventricle) for ultrastructural examination using an H-600 transmission electron microscope.

Mitochondria preparation

Mitochondria were isolated from the Langendorff-perfused rat hearts with the same protocol as used for perfusion of the isolated heart (Tanonaka et al., 2003). In brief, heart tissues were homogenized in ice-cold buffer containing 160 mmol/L KCl, 10 mmol/L EGTA (pH 7.4), and 0.5% fatty acid-free BSA. The homogenate was centrifuged at 1000 *g* for 10 min at 2 °C, and the supernatant was centrifuged at 8000 *g* for 10 min at 2 °C. The pellet was re-suspended in buffer containing 320 mmol/L sucrose and 10 mmol/L Tris–HCl (pH 7.4), and centrifuged at 8000 *g* for 10 min at 2 °C. Then we acquired the purified mitochondria and the mitochondrial protein was quantitated with Coomassie brilliant blue.

Mitochondrial membrane potential

Mitochondrial potential changes were evaluated by measuring rhodamine-123 fluorescence quenching under the following conditions: 460 μL of distilled water was added to 2.5 mL buffer (in mM: sucrose 15, Hepes 20, sodium succinate 5, $MgCl_2$ 5, KH_2PO_4 5, pH 7.4) containing 40 μL rhodamine-123 (26 μM). Then the experiment was performed by exciting the rhodamine-123 at 503 nm and detecting the fluorescence emission at 527 nm. This gave the fundamental fluorescence value F1. After addition of 0.01 mg mitochondria to the buffer and incubation for 5 min at room temperature, the fluorescence value F2 was recorded. The change of mitochondrial membrane potential was represented by $\Delta F = (F1 - F2)/(0.01\ mL \times 1\ mg/mL)$.

Mn-SOD in mitochondria

Mitochondrial membranes were broken by an ultrasonic disintegrator under the following conditions: 400 A repeated for four cycles of 5 s at 10 s intervals. The Mn-SOD content was assayed spectrophotometrically.

Statistical analysis

All values are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance and the Newman–Keuls test. Differences of $p < 0.05$ were regarded as significant.

Results

Hemodynamics

In the I/R group, LVDP and RPP decreased at 30 min of reperfusion, while LVEDP was elevated. However, in the RPC and NS1619 groups, LVDP and RPP were higher and LVEDP was lower than in the I/R group (Table 1).

Myocardial infarct size and LDH

The infarct size was 39.7 ± 4.7 (Fig. 1A) and the LDH content was 74.88 ± 7.68 U/L after 5 min of reperfusion when the LDH in effluent reached a peak in the I/R group (Fig. 1B). The results were similar in the RPC + Pax group. The infarct size and LDH content were reduced both in RPC- and NS1619-treated groups. These differences were significant relative to the I/R group and there were no significant differences between the RPC- and NS1619-treated groups.

Morphology of myocardium

After the hearts were subjected to 30 min global ischemia and 120 min reperfusion, cardiac muscle fibers were arranged irregularly, the structure of the myocomma was unclear, and the myocardial cell membrane was disrupted. The number of mitochondria was reduced