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The mitochondrial permeability transition pore and the Ca^{2+}-activated K^+ channel contribute to the cardioprotection conferred by tumor necrosis factor-α

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Abstract

Pretreatment with tumor necrosis factor-α (TNF-α) is known to trigger cardioprotection and it can activate multiple downstream signaling cascades. However, it is not known whether the mitochondrial permeability transition pore and the Ca^{2+}-activated K^+ channel (K_{Ca} channel) are involved in the TNF-α-induced cardioprotection. In the present study, we examined whether TNF-α inhibits pore opening and activates the K_{Ca} channel in the cardioprotection. In isolated rat hearts subjected to 30 min of regional ischemia and 120 min of reperfusion, pretreatment with 10 U/ml TNF-α for 7 min followed by 10 min washout improved the recovery of rate-pressure product (RPP = left ventricular developed pressure × heart rate) and coronary flow (CF) during reperfusion, and reduced the infarct size and release of lactate dehydrogenase (LDH). Administration of 20 μmol/L atractyloside, a pore opener, for the last 5 min of ischemia and first 15 min of reperfusion, and pretreatment with 1 μmol/L paxilline, an inhibitor of the K_{Ca} channel, for 5 min before ischemia, attenuated the recovery of RPP and CF, and the reductions of infarct size and release of LDH induced by TNF-α. On the other hand, administration of 10 μmol/L NS 1619, an opener of the K_{Ca} channel, for 10 min before ischemia, decreased the infarct size and LDH release, and improved contractile functions and CF; these effects were attenuated by atractyloside. Pretreatment with 0.2 μmol/L cyclosporin A for the last 5 min of ischemia and first 15 min of reperfusion showed similar effects to those of TNF-α, and they were not attenuated by paxilline. In mitochondria isolated from hearts pretreated with 10 U/ml TNF-α for 7 min, a significant inhibition of Ca^{2+}-induced swelling was observed. Furthermore, paxilline attenuated the inhibition of Ca^{2+}-induced mitochondrial swelling by TNF-α. These findings indicate that TNF-α protects the myocardium against ischemia and reperfusion injury by inhibiting mitochondrial permeability transition pore opening as well as activating K_{Ca} channels, probably the mitochondrial K_{Ca} channel, which is upstream from the pore.

Keywords: Calcium-activated potassium channel; Heart; Ischemia and reperfusion; Mitochondrial permeability transition pore; Tumor necrosis factor-α

1. Introduction

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine with pleiotropic biological effects, including the ability to evoke protective preconditioning against ischemia and reperfusion injury in isolated perfused rat and rabbit hearts [1,2]. Hearts from TNF-α-pretreated animals contain lower levels of lactate dehydrogenase than hearts from untreated rats, and TNF-α induces the messenger RNA for manganous superoxide dismutase [3,4]. TNF-α receptor knockout mice show accelerated myocardial apoptosis and increased infarct size after ischemia [5] and ischemic preconditioning fails to decrease infarct size in these mice [6]. This evidence indicates that endogenous TNF-α is required for cardioprotection. Although TNF-α activates multiple downstream signaling cascades related to mitochondria, the relationship between the cardioprotection evoked by TNF-α and mitochondria has not been elucidated.

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The mitochondrial permeability transition pore (abbreviated to "pore" below) is a non-specific pore across the inner and outer mitochondrial membranes. Pore opening causes a Ca^{2+}-dependent increase of mitochondrial permeability to ions and solutes with molecular masses of up to 1500 Da, and then induces matrix swelling and mitochondrial de-energization [7]. Several studies performed on isolated cardiomyocytes and perfused hearts support the idea that pore opening might be pivotal in determining the transition of ischemic damage to the irreversible phase [8]. It was reported that inhibition of pore opening in the presence of 0.2 μmol/L cyclosporin A (CsA) or 1.0 μmol/L sanglifehrin A plays a cardioprotective role in isolated rat heart subjected to ischemia and reperfusion [9,10]. Therefore, we hypothesized that inhibiting the pore may participate in the protective effect of TNF-α in hearts subjected to ischemia and reperfusion.

The α-subunits of the Ca^{2+}-activated K^+ channel (K_{Ca} channel) are expressed in samples from the inner mitochondrial membranes of guinea pig ventricular myocytes and activation of this channel is cardioprotective against ischemic myocardial injury in the isolated perfused guinea pig heart [11]. Previous studies from our lab and others found that the mitochondrial ATP-sensitive potassium channel is involved in the protective effect of TNF-α [6,12], suggesting that influx of potassium from cytosol to mitochondria is beneficial for the myocardium during ischemia and reperfusion. However, whether the K_{Ca} channel is involved in the cardioprotection conferred by TNF-α is not clear.

Therefore, the present study was designed to test whether stimulation or inhibition of pore opening affects the cardioprotective effect of TNF-α and whether the K_{Ca} channel participates in this effect. The relationship between the K_{Ca} channel and the pore in the cardiac effect of TNF-α was also explored.

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats (200–250 g) were obtained from the Animal Center of Zhejiang Academy of Medical Sciences and treated in accordance with the Guide for the Care and Use of Laboratory Animals of Zhejiang University.

Tumor necrosis factor-α (TNF-α) was purchased from Beijing Biosea Biotechnology Co., Ltd. Cyclosporin A (CsA), atractyloside (Atr), paxilline (Pax), 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS 1619), Evans blue and 2,3,5-triphenyl-tetrazolium chloride (TTC) were purchased from Sigma Chemical Co.

2.2. Isolated perfused heart preparation

Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg). Hearts were 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 7.4) 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 (MedLab, Nanjing, China) to assess contractile function. The left ventricular end-diastolic pressure (LVEDP) was adjusted to between 4 and 8 mmHg. The cardiac parameters, namely, heart rate (HR), left ventricular developed pressure (LVDP: difference between left ventricular end-systolic pressure and end-diastolic pressure), 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.

2.3. Infarct size measurement

At the end of the 120 min reperfusion period, the coronary artery was re-occluded and the heart was slowly perfused with a solution of 0.5% Evans blue to delineate the non-ischemic zone of the myocardium as a dark blue area. Hearts were then frozen and sliced into 2 mm thick transverse sections and incubated in a sodium phosphate buffer containing 1% w/v TTC for 10–15 min, then fixed in 10% formalin for 15 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.

2.4. Lactate dehydrogenase (LDH) measurement

To assess the extent of myocardial injury, the effluent from the isolated perfused heart was collected at 5 min of reperfusion and LDH was spectrophotometrically assayed. LDH activity was expressed as units per liter.

2.5. Isolated cardiac mitochondria preparation

Mitochondria were isolated from rat hearts by differential centrifugation [13]. 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 pellets were 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. The pellets were used for determination of mitochondrial swelling.