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Involvement of the mitochondrial calcium uniporter in cardioprotection by ischemic preconditioning

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Abstract

The objective of the present study was to determine whether the mitochondrial calcium uniporter plays a role in the cardioprotection induced by ischemic preconditioning (IPC). Isolated rat hearts were subjected to 30 min of regional ischemia by ligation of the left anterior descending artery followed by 120 min of reperfusion. IPC was achieved by two 5-min periods of global ischemia separated by 5 min of reperfusion. IPC reduced the infarct size and lactate dehydrogenase release in coronary effluent, which was associated with improved recovery of left ventricular contractility. Treatment with ruthenium red (RR, 5 μM), an inhibitor of the uniporter, or with Ru360 (10 μM), a highly specific uniporter inhibitor, provided cardioprotective effects like those of IPC. The cardioprotection induced by IPC was abolished by spermine (20 μM), an activator of the uniporter. Cyclosporin A (CsA, 0.2 μM), an inhibitor of the mitochondrial permeability transition pore, reversed the effects caused by spermine. In mitochondria isolated from untreated hearts, both Ru360 (10 μM) and RR (1 μM) decreased pore opening, while spermine (20 μM) increased pore opening which was blocked by CsA (0.2 μM). In mitochondria from preconditioned hearts, the opening of the pore was inhibited, but this inhibition did not occur in the mitochondria from hearts treated with IPC plus spermine. These results indicate that the mitochondrial calcium uniporter is involved in the cardioprotection conferred by ischemic preconditioning.

Keywords: Heart; Mitochondrial calcium uniporter; Ischemic preconditioning

Introduction

Ischemic preconditioning (IPC), which was originally identified by Murry and co-workers (Murry et al., 1986), provides powerful protection to the ischemic myocardium. Although a variety of intracellular signaling pathways have been implicated in this protection (O'Rourke, 2000), the precise mechanisms remain elusive.

Intensive studies showed that prolonged ischemia results in increased intracellular calcium concentration (Allard et al., 1994), and this calcium overload was thought to play a pivotal role in ischemia–reperfusion injury. It has been demonstrated that IPC can lower both intracellular and mitochondrial calcium during reperfusion (Wang et al., 2001). During myocardial performance, mitochondria accumulate significant amounts of calcium from the cytosol through the mitochondrial calcium uniporter (for simplicity, referred to as "uniporter" below), a highly selective calcium ion channel (Kirichok et al., 2004), implying that the uniporter may participate in the cardioprotection conferred by IPC.

Opening of the mitochondrial permeability transition pore (referred to below as "pore"), which is located in the inner mitochondrial membrane, can lead to mitochondrial swelling and cytochrome C release resulting in cell death (Kroemer et al., 1998). The cardioprotection by IPC may be achieved via inhibiting pore opening during reperfusion (Hausenloy et al., 2002).

Therefore, our working hypotheses are that the uniporter may participate in the cardioprotection induced by IPC and that the uniporter may interact with the pore in this process. To test the first hypothesis, we examined the effects of blockade or activation of the uniporter on the cardioprotective effect of IPC as measured by ventricular performance, infarct size and lactate dehydrogenase release in rat hearts

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perfused on a Langendorff apparatus. As a preliminary approach to the second hypothesis, the effects of uniporter activity and IPC on pore opening were also assessed.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 210–240 g were housed in a temperature-controlled (22–24 °C) room under a 12 h light/12 h dark cycle with water and food freely available. All procedures used in this study were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University.

Langendorff heart preparation

Rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and sacrificed by decapitation. The hearts were excised and immediately placed in ice-cold Krebs–Henseleit (K–H) bicarbonate buffer containing (mM): NaCl 118.0, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 2.5, $MgSO_4$ 1.2, $CaCl_2$ 1.4, glucose 10.0. The hearts were then mounted to the Langendorff apparatus and perfused retrogradely with K–H buffer gassed with 95% O_2/5% CO_2 equilibrated at pH 7.3–7.4 and maintained at 37 °C. A water-filled latex balloon was inserted into the left ventricle through the left atrium and the pressure was continuously monitored by a transducer connected to the balloon. The initial value of end-diastolic pressure was set to 5–8 mm Hg by adjusting the volume of the balloon. The parameters measured were left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and maximal rise/fall velocity ($\pm dP/dt_{max}$). All hearts were allowed to equilibrate for 20 min before any additional treatment.

Perfusion protocol of isolated hearts

The experimental protocols for Langendorff perfused hearts are shown in Fig. 1. All isolated hearts received 30 min of

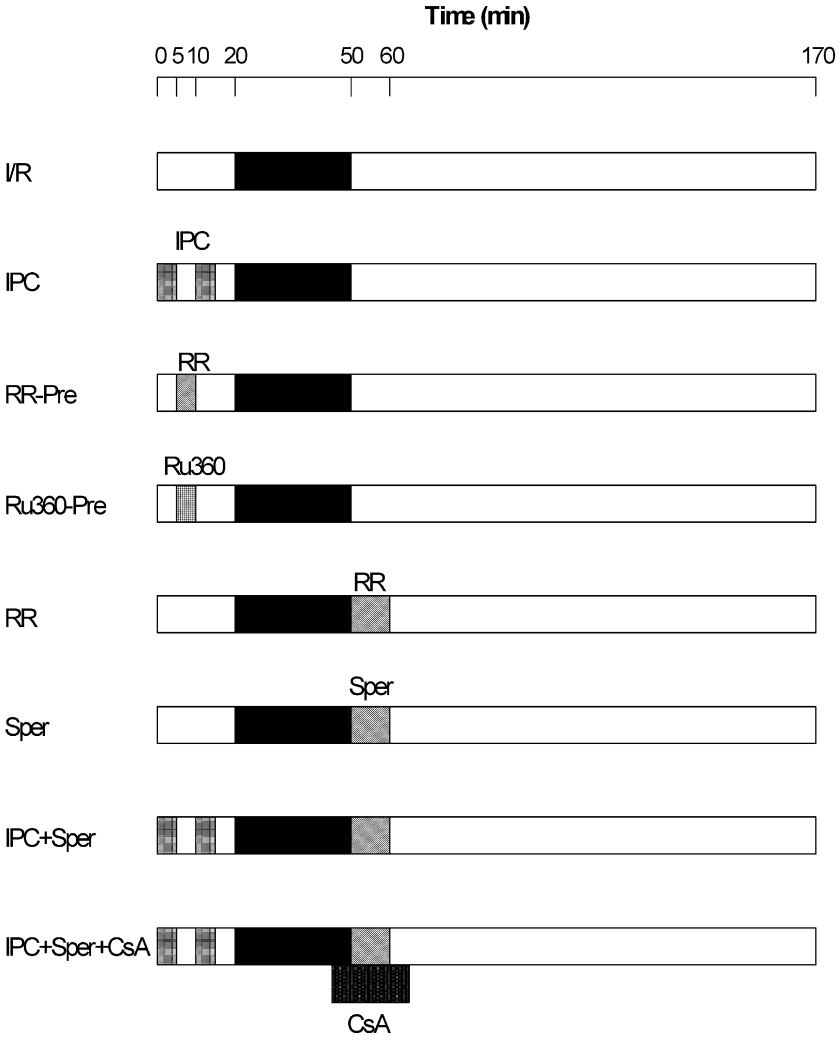

Fig. 1. Perfusion protocols. Perfusion protocols used for hemodynamic measurements in isolated rat hearts. RR: ruthenium red; RR-Pre: RR pretreatment; Ru360-Pre: Ru360 pretreatment; Sper: spermine; CsA: cyclosporin A.