Ischemic Preconditioning and Nicorandil Pretreatment Improve Donor Heart Preservation

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The present study investigated the effects of ischemic preconditioning (IPC) and nicorandil pretreatment on myocardial storage in a donor heart preservation model. Isolated rat hearts were separated into groups: group 1, non-preconditioned control group; group 2, 2.5 min of normothermic ischemia followed by 15 min of normothermic Langendorff perfusion (one IPC cycle); and group 3, 2 cycles of IPC. All hearts were subsequently stored in University of Wisconsin solution at 4°C for 2, 4 and 6 h, and the concentrations of high-energy phosphate metabolites were measured for each time point. Heart function parameters (aortic flow, coronary flow and cardiac output) were measured when the heart was reperfused following the 2, 4 or 6 h of preservation. The effects of nicorandil, an ATP-sensitive potassium channel opener, on heart function following preservation were also evaluated. Nicorandil was injected intravenously before heart harvesting. The results showed that the energy status was well preserved in the IPC groups. The 2-cycle IPC group showed better recovery of heart function following preservation. Pretreatment with nicorandil also improved functional recovery of the heart following preservation. The present study showed that IPC of the rat heart resulted in improved myocardial energy metabolism and functional recovery after hypothermic preservation, and that nicorandil has potential for pharmacological preconditioning in heart preservation for transplantation. (Jpn Circ J 2001; 65: 678–682)

Key Words: Heart preservation; Ischemic preconditioning; Nicorandil; Rat

Repeated brief episodes of ischemia and reperfusion render the myocardium more resistant to subsequent sustained ischemia and reperfusion, a tolerance known as ischemic preconditioning (IPC) and which was first described by Murry et al who found in a canine model that 4 consecutive periods of 5 min of coronary occlusion were able to reduce the infarct size caused by a subsequent 40-min occlusion by as much as 75%. Beneficial effects of IPC have been observed in other animal species and recently evidence was presented for IPC in human myocardium, emphasizing the potential clinical significance of its cardioprotective effect. Although a number of studies have investigated this phenomenon, its mechanism is still not fully understood. Recent studies reported that the ATP-sensitive potassium channel opener plays an important role in the process so the present study investigated whether IPC and nicorandil, an ATP-sensitive potassium channel opener, have protective effects for the myocardium during prolonged heart preservation in an isolated rat heart model.

Methods

Isolated Rat Heart Perfusion and Preservation

All experimental animals received humane care in compliance with the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy Sciences and published by the National Institute of Health (NIH Publication No. 85-23, revised 1985). Male Wistar rats (250-300 g) were anesthetized with sodium pentobarbital (65 mg/kg ip) and systemically heparinized (500 U ip). Each heart was rapidly excised and immediately immersed in ice-cold Krebs-Henseleit bicarbonate (KHB) buffer. The heart was then mounted on a Langendorff apparatus (IPH-W, Labo Support, Osaka, Japan) via the aorta and Langendorff perfusion was initiated at a pressure of 60 mmHg using modified KHB buffer (mmol/L: NaCl, 118; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 2.5; and glucose, 11.0). The perfusate (pH 7.4) was equilibrated with 95% oxygen and 5% carbon dioxide and maintained at 37°C. Each heart was housed in a thermostatically controlled heart chamber and maintained at 37°C during the perfusion periods. The pulmonary outflow tract was incised to allow better drainage of the coronary effluent. During the Langendorff perfusion the left atrium was cannulated via the pulmonary vein for conversion to working heart mode, which was achieved by stopping aortic perfusion and starting left atrial perfusion at a filling pressure of 10 mmHg. Under these conditions, the perfusate was ejected through the aortic cannula against an afterload of 60 mmHg. During preparation, the excised hearts were rapidly cannulated to minimize the ischemic time and to protect the coronary orifices from damage. Baseline function was determined after 5 min of working mode perfusion. Aortic flow (AF, ml/min), coronary flow (CF, ml/min) and cardiac output (CO, ml/min) were measured, AF and CF by timed collection of the effluent. All perfusion fluids were passed through a 5-μm porosity filter to remove particulate matter. The perfusate was not recycled.
Experimental Protocol

After cannulating the aorta, the heart was perfused in the Langendorff mode for 10 min to wash out blood and stabilize the myocardium, and then converted to working mode for 5 min to measure AF, CF and CO. After these measurements, all the hearts were switched back to the Langendorff mode and divided into 3 groups: group 1 (n=8), non-preconditioned control hearts preserved with University of Wisconsin solution (UW solution); group 2 (n=8), 2.5 min of normothermic ischemia followed by 15 min of normothermic Langendorff perfusion (one IPC cycle); and group 3 (n=9), 2 cycles of IPC. At the final perfusion, the hearts were arrested by administering UW solution (60 ml/kg at 4°C) via the aortic cannula at a pressure of 60 mmHg and stored in UW solution (30 ml) at 4°C for 2, 4 and 6 h.

Measurement of Adenosine Nucleotides

Following preservation, muscle from the left ventricle was rapidly cut and weighed, homogenized in 0.6 mol/L ice-cold perchloric acid, stabilized at 4°C for 10 min, then centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was neutralized with KOH at a ratio of 10:1 for 1 h and the sample was again centrifuged for 10 min at 3,000 rpm at 4°C, before the supernatant was frozen in liquid nitrogen and stored at –40°C until assayed. Adenosine triphosphate (ATP), diphosphate (ADP) and monophosphate (AMP) were measured by high-performance liquid chromatography at a wavelength of 340 nm. Data are expressed as μmol/g wet weight. The energy charge was calculated from the formula (ATP + 1/2ADP)/(ATP + ADP + AMP).

Measurement of Post-Preservation Heart Function

After metabolite measurement, contractile function cannot be measured in the same heart because of the myocardial damage, so another 3 groups of hearts were processed in the same manner. Following hypothermic preservation, these hearts were mounted on the Langendorff apparatus for reperfusion. The aorta was cannulated and the heart was immediately perfused in the Langendorff mode, after which the pulmonary vein was cannulated for conversion to working mode. Air was prevented from entering the coronary system. The heart was initially perfused in Langendorff mode for 20 min then switched to working mode for 20 min, after which CF, AF and CO were measured and expressed as a percentage of the baseline value.

Evaluation of the Effects of Nicorandil on Heart Preservation

We also examined the effects of nicorandil, an ATP-sensitive potassium channel opener, in 2 randomized groups of rats (Fig 2). The nicorandil group (group 4, n=7) were intubated after anesthesia and placed on a volume-controlled ventilator. Nicorandil [N-(2-hydroxyethyl)-nicotinamide nitrate, Chugai, Tokyo, Japan] was injected intravenously via the femoral vein (1 mg/kg) over a period of 10 min to avoid a sharp decrease in blood pressure. Thirty minutes later, the heart was harvested and placed on the Langendorff apparatus and the pulmonary vein was cannulated for heart function measurement as described before. After a 10-min perfusion to wash out blood and for stabilization, the heart was switched to working mode for baseline measurement. Finally, the heart was stopped and preserved with 4°C UW solution as described before. In the paired control group (group 5, n=7), the same volume of saline was injected intravenously and all other procedures were the same. After 6 h of hypothermic preservation with UW solution, the heart was put back on the Langendorff apparatus to reassess heart function.

Statistical Analysis

All results are expressed as mean values ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance. When a significant F-value was identified, intergroup comparison was performed with Scheffe’s test using the SPSS program package (SPSS Inc, Chicago, IL, USA). The comparison between the 2 groups in the nicorandil experiment was conducted with Student’s t test. A p value less than 0.05 was considered statistically significant.

Results

Myocardial Energy Metabolites Change

The absolute values of ATP and the energy charge are
shown in Table 1. Baseline ATP and energy index values did not significantly differ between the 3 groups and over the course of the experiment, the ATP content gradually decreased in all groups. Following 2h and 4h of preservation, the ATP levels in the IPC groups were higher than in the control group (p<0.01 and p<0.05, respectively, after 2h; p=0.057 and p=0.055, respectively; after 4h). After 6h of preservation, energy charge was nearly identical in the 3 groups.

Baseline (Pre-Ischemic) Cardiac Function (Table 2)

The baseline cardiac function measurements in the 5 groups did not significantly differ, although CF in the rats given nicorandil (group 4) appeared to be higher (p>0.05).

Recovery of Cardiac Function (Table 3)

In group 2, the single cycle of IPC failed to protect contractile function, but in group 3 the 2 cycles of IPC significantly increased AF, CF and CO after 4 and 6h of hypothermic preservation (Table 2). Rat hearts pretreated with nicorandil (group 4) recovered better in terms of AF (p<0.01), CF (p<0.05) and CO (p<0.01) than the control group 5 (Table 4).

Discussion

Since Murry et al originally reported that brief periods of regional myocardial ischemia and reperfusion prior to a prolonged occlusion reduced infarct size! other studies have verified the protective effect of IPC in different animal species2–4 and this has led to speculation on the potential clinical applications of this phenomenon. The first to demonstrate that IPC could be performed in humans were Deutsch et al who reported that patients undergoing elective percutaneous transluminal coronary angioplasty of
conflicting results appear to be because of differences in surgery, and heart transplantation is now being considered for the possible therapeutic application of IPC. Although the mechanism of this phenomenon has not been fully elucidated, the ATP-sensitive potassium channel seems to play an important role and so we designed an experimental protocol clinically relevant to donor heart preservation, with which to evaluate the effects of IPC and nicorandil, a potential mediator of IPC, on rat heart preservation with UW solution at 4°C.

The present study demonstrates that IPC improved both myocardial energy metabolism and cardiac function following preservation. The myocardial ATP content remained higher in the preconditioned groups following 2 h of preservation compared with the control group, and following 4 h of preservation, the ATP values in groups 2 and 3 still tended to be higher than in the control, and the energy charge index in the preconditioned groups was significantly higher than that in the control group. Murry et al. showed that although ATP was decreased during the preconditioning itself, the myocardial ATP levels were higher after 10 min of subsequent sustained ischemia in preconditioned dogs than in the non-preconditioned ones, and this higher level of high-energy phosphates in preconditioned hearts is considered to be a result of slower ATP consumption during ischemia.

Systolic function was assessed in our experimental model by measuring CO and the results showed that it was well preserved in the 2-cycle preconditioned group following 4 and 6 h of preservation. Other studies have examined the effects of IPC on myocardial recovery in isolated hearts. Valen et al., using a Langendorff model, preconditioned rat hearts with two 3-min episodes of ischemia following by 5 min of reperfusion. Diastolic function was better preserved in the preconditioned hearts subjected to warm ischemia, but not in hearts stored for 3–3.5 h at temperature between 6 and 8°C. In contrast, Ogino et al. showed that 5 min of global ischemia and 10 min of reperfusion, preceding 6 h of cold storage, preserved diastolic function. Karck et al. subjected isolated working rat hearts to 5 min of reperfusion and then stored the hearts at 4°C for 10 h in 5 preservation solutions. There was very little recovery in the control groups, but systolic function was preserved in the preconditioned hearts.

The optimal duration of preconditioning ischemia is species- and strain-dependent. In the present rat experimental model, we found that 2 cycles only of IPC induced better cardiac functional recovery than a single cycle and this is supported by Saitoh et al who found that 2 cycles of 2.5-min ischemia followed by 10-min reperfusion were necessary to protect contractile functional recovery in preserved rat hearts. On the other hand, Landymore et al reported that 1 cycle of 5-min ischemia followed by 10 min of reperfusion protected myocardial systolic function in a heart transplantation model and Liu and Downey reported that three 5-min cycles of ischemia were required to delay infarction in a rat ischemia–reperfusion model. These conflicting results appear to be because of differences in the preconditioning procedures. We found that after 2 h of preservation, the preconditioned groups did not recover any better than the control group, which may be because UW solution can satisfactorily protect the rat heart in a cold environment within a short time period.

Myocardial ATP and cardiac functional recovery did not correlate in this experimental model. Following 2 h of preservation, myocardial ATP in both the preconditioned groups was better preserved, and after 6 h the ATP content and energy charge were identical in the 3 groups. In the 2-cycle IPC group, recovery of both CO and CF was better following 4 and 6 h of preservation, a result that is supported by Cave and Hearse who also did not find any correlation between preconditioning-induced protection of function and metabolic recovery in isolated rat hearts subjected to sustained ischemia at 20°C. Murry et al. showed that IPC in dog hearts reduced ATP depletion during sustained ischemia; however, differences in the myocardial ATP content of control and preconditioned hearts disappeared as early as 40 min after the induction of normothermic ischemia. The good correlation between IPC and high-energy phosphate metabolism that was observed during sustained normothermic ischemia may become more complex when prolonged hypothermic ischemia is involved. IPC may enhance recovery of post-ischemic contractile function, irrespective of the ATP level, after sustained ischemia.

Ischemic preconditioning is effective during cardiopulmonary bypass in humans but its clinical application in donor heart harvesting requires a preoperative cardiopulmonary bypass or postoperative IPC, both of which are time-consuming, so we therefore evaluated whether nicorandil could be used for pharmacological preconditioning before heart harvesting. Nicorandil is a nitrate and an ATP-sensitive potassium channel opener that improves regional myocardial function in acute myocardial infarction. The mechanism that nicorandil exerts for its cardioprotective effects during ischemia remains unclear, but may be related to lowering of the threshold of potassium channel opening so that the channels are more promptly activated during the subsequent prolonged ischemia. Shigematsu et al. have reported that when cardiac ATP-sensitive potassium channels are activated by prolonged ischemia, it is the resulting shortened action potential with the decreasing intracellular ATP that could prevent the heart from developing myocardial stunning. Our preliminary experiment showed that the slow administration of nicorandil over a period of 10 min avoided a sharp decrease in blood pressure, which may not cause the massive release of catecholamine that is the receptor mediator reported to play a role in initiating the protective effects during IPC. We found that pretreatment with nicorandil gave better recovery of heart function following 6 h of cold preservation compared with the control group and thus it may have potential for improving the preservation of donor hearts before transplantation.

There are many differences between the isolated perfused rat heart and the human heart in situ. We used an aqueous perfusion solution free of blood cells, fatty acids and protein, so the present findings cannot be directly extrapolated to the blood-perfused transplanted heart. However, the present study has demonstrated that IPC or pretreatment with nicorandil improved preservation of the rat heart in hypothermic UW solution.
References


