Abstract

The protective effects of ischemic preconditioning (IP) and Na\textsuperscript{+}/H\textsuperscript{+} exchanger blockade (NHE\textsubscript{b}) by two blockers [ethylisopropylamiloride (EIPA) and HOE 642] were compared in the isovolumic perfused rat heart. The impairment in systolic and diastolic function detected in control ischemic hearts (C) exposed to 20 min of ischemia and 30 min of reperfusion was diminished in similar extent by IP and by NHE\textsubscript{b} with EIPA and HOE 642. At the end of the reperfusion period $+dP/dt_{max}$ values were 57±9% in C hearts and 94±6%, 82±6% and 104±6% after IP and NHE\textsubscript{b} with EIPA and HOE 642, respectively. A depletion of ATP levels detected in C hearts after reperfusion (from 20.2±0.8 \textmu mol/g dry weight before ischemia to 6.9±0.7 \textmu mol/g dry weight) was partially prevented by both IP and NHE\textsubscript{b} with EIPA (9.2±0.7 \textmu mol/g dry weight and 11.1±0.5 \textmu mol/g dry weight, respectively). The ischemic contracture (IC), assessed by the left ventricular end diastolic pressure (LVEDP), observed in C hearts (35±4 mmHg) was not decreased by IP (40±4 mmHg) but it was prevented by NHE\textsubscript{b} (18±4 mmHg and 10±3 mmHg with EIPA and HOE 642, respectively). The ATP levels at the end of the ischemic period were similar in C and IP hearts (4.1±0.2 \textmu mol/g dry wt vs. 3.3±0.4 \textmu mol/g dry wt) but they were significantly higher after NHE\textsubscript{b} with HOE 642 (7.0±1.0 \textmu mol/g dry wt). PKC inhibition by chelerythrine abolished the protection induced by IP after reperfusion although not the improvement induced by NHE\textsubscript{b} with EIPA.

According to the present results, we can conclude that despite the fact that IP and NHE\textsubscript{b} are protecting the postischemic function in a similar magnitude, both interventions are different in terms of modifying IC that develops during the ischemic period. IC was prevented by NHE\textsubscript{b} whereas it was not by IP. Furthermore, IP protection and not that obtained by NHE\textsubscript{b} is abolished by PKC.

Key words Ischemia · Reperfusion · Contracture · Contractility · Ischemic preconditioning · Na\textsuperscript{+}/H\textsuperscript{+} exchanger · HOE 642 · Protein kinase C · Chelerythrine

Introduction

When blood flow is restarted after a short ischemic episode, isovolumic rat hearts show a depression of contractility and a decreased diastolic compliance (Braunwald and Kloner 1982; Bolli 1990). This altered ventricular function is the result of changes occurring during both ischemic and reperfusion periods. In some species, like in rat, the contracture (decreased diastolic compliance) develops during the ischemic period and is maintained during reperfusion (Steenbergen et al. 1990; Armstrong and Ganote 1991). Although many reports have correlated the degree of ischemic contracture (IC) with the impairment of postischemic function (Hearse et al. 1977; Ganote 1983; García-Dorado et al. 1992), this relationship remains controversial. An example of the dissociation is observed in the protection induced by one or more brief cycles of ischemia and reperfusion previously applied to a more prolonged ischemia, called ischemic preconditioning (IP). This phenomenon protects the diastolic and systolic function after reperfusion, whereas the contracture during the ischemic period is not modified or even increased (Cave 1995; Kolocassides et al. 1995, 1996).

One way of protection of the myocardium from ischemia/reperfusion seems to be the Na\textsuperscript{+}/H\textsuperscript{+} exchanger blockade (NHE\textsubscript{b}; Meng and Pierce 1990; Scholz et al. 1992, 1993, 1995; Bugge et al. 1996). The possibility that NHE can be involved in the mechanism of protection of the IP is controversial (Bugge and Ytrehus 1995; Ramasamy et al. 1995; Shipolini et al. 1997). The NHE can be activated by a protein kinase C (PKC; Fliegel and Fröhlich 1993) and PKC activation also seems to be the necessary
trigger for the protection brought about by IP (Liu et al. 1994; Speechly-Dick et al. 1994; Hu and Nattel 1995).

The objective of the present study was to compare the protection induced by IP with that obtained by blocking NHE.

**Materials and methods**

**Isolated heart preparation.** Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body wt). The heart was rapidly excised and perfused by the non-recirculating Langendorff technique with Ringer’s solution containing (in mM): 118 NaCl, 5.9 KCl, 1.2 MgSO₄, 1.35 CaCl₂, 20 NaHCO₃ and 11.1 dextrose. The buffer was saturated with a mixture of 95% O₂/5% CO₂, had a pH of 7.4, and was maintained at 37°C. The conductive tissue in the atrial septum was damaged with a fine needle to achieve atrioventricular block, and the right ventricle was paced at 280±10 beats/min. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve; the opposite end of the tube was then connected to a Statham P23XL pressure transducer. The balloon was filled with water to give an end-diastolic pressure (LVEDP) of 8–12 mmHg, and this volume was unchanged for the remainder of the experiment. Coronary perfusion pressure was monitored at the point of cannulation of the aorta and adjusted to approximately 60–70 mmHg. Coronary flow, controlled with a peristaltic pump, was 11±2 ml/min. Left ventricular pressure (P) and its first derivative (dP/dt) were recorded with a direct writing recorder.

**Experimental protocols.** After 10 min of stabilization, the following experimental protocols were performed (Fig. 1). Control ischemic hearts (C): Hearts were submitted to 20 min of normothermic global ischemia followed by 30 min of reperfusion. Global ischemia was induced by stopping the perfusate inflow line and the heart was placed in a saline bath held at 37°C. Preconditioned hearts (IP): IP was induced by only one cycle of 5 min of ischemia and 10 min of reperfusion followed by the same protocol as in the C group.

For examining the NHE₆₅ effects, alternatively EIPA or HOE 642 was used. Twelve C hearts received 1 μmol/l HOE 642 (gift from Hoechst, Frankfurt/Main, Germany; n=6) 10 min before the 20-min ischemic period or 1 μmol/l ethylisopropyllumiloride (EIPA; bought from Research Biochemicals International; n=6). In other C (n=6) and IP (n=7) hearts 25 μg/min chelerythrine (Ch), a PKC inhibitor, was added to perfusion solution through an infusion pump during 10 min.

In five hearts we examined the effects of the combined administration of 1 μmol/l EIPA and 25 μg/min Ch before the long ischemic period.

Four hearts from each of the C, IP and NHE₆₅ (with HOE 642) groups were freeze-clamped with liquid nitrogen-cooled aluminium clamps at the end of the ischemic period and six hearts from each of the same groups (EIPA was used as NHE blocker) were frozen at the end of the reperfusion period while they were being perfused. Another six hearts were frozen after 10 min of stabilization (Pre-I). All the hearts were stored in an ultra-low-temperature freezer (−70°C) until ATP extraction. The hearts were crushed with nitrogen-cooled mortar and pestle, and neutralized perchloric acid extracts were assayed for adenosine triphosphate (ATP) levels by standard enzymatic procedure (Lamprecht et al. 1974).

**Systolic function.** Myocardial contractility was assessed by the maximal velocity of rise of left ventricular pressure (+dP/dt max) values. Data were expressed as percentage of their respective preischemic values.

**Ischemic contracture.** The contracture during ischemia (IC) was assessed by LVEDP. The time to onset of ischemic contracture (tₒ) was defined as the time required to reach an LVEDP value 5 mmHg greater than its preischemic value.

**Statistical analysis.** Data are given as means ± SEM. The analysis of +dP/dt max, LVEDP and ATP levels was performed using repeated measures of one-way analysis of variance (ANOVA) with the Newman-Keuls’s test for multiple comparisons among groups. Student’s t-test was used to analyze the difference of tₒ between C and IP hearts. Values of P<0.05 were considered to be significant.

**Results**

**Effects of 20 min of ischemia and IP**

The recovery of systolic function after reperfusion, assessed by +dP/dt max, was significantly improved by IP. After 30 min of reperfusion, +dP/dt max values were 57±9%