EFFECTS OF ISCHEMIC PRECONDITIONING ON Na⁺–Ca²⁺ EXCHANGER ACTIVITY AND ION REGULATION IN ISOLATED PERFUSED RAT HEARTS

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Summary. We investigated the effects of ischemic preconditioning (IP) on the incidence of reperfusion-induced ventricular fibrillation (VF), intracellular ion regulation, and Na⁺–Ca²⁺ exchanger activity using isolated perfused rat hearts. The hearts perfused in a working-heart mode were exposed to sustained global ischemia for 15 minutes and were reperfused for 20 minutes. For preconditioning, the hearts were exposed to two short periods (3 or 5 minutes) of ischemia and reperfusion prior to induction of sustained ischemia. The incidence of VF decreased from 90% in the control hearts to 20% in the preconditioned hearts (p < 0.05). Treatment with an Na⁺–Ca²⁺ exchanger blocker, Ni²⁺ (0.5 μM), reduced the antiarrhythmic effect of IP. Thus, 70% of the preconditioned hearts treated with Ni²⁺ developed VF on reperfusion. To investigate the effect of IP on changes in intracellular ion levels, rat hearts perfused in Langendorff's mode were exposed to low-flow ischemia for 15 minutes and were reperfused for 15 minutes. Intracellular pH (pH_i) and Ca²⁺ concentrations ([Ca²⁺]) were measured ratiometrically using the fluorescent ion indicators 2',7'-bis(2-carboxylethyl)-5(6)-carboxyfluorescein (BCECF) or fura-2 with the simultaneous measurement of left ventricular pressure. IP limited the development of intracellular acidosis and prevented the rise in diastolic [Ca²⁺] during sustained ischemia. Ni²⁺ treatment reversed this effect of IP on diastolic [Ca²⁺]. During exposure to an Na⁺ free extracellular medium, which reversed the Na⁺–Ca²⁺ exchanger mode, IP significantly suppressed the peak amplitude (65.2% ± 7.8% of control, p < 0.005) and prolonged the time to peak (16.7 ± 0.9 seconds vs. 12.8 ± 1.5 seconds, p < 0.05) of the diastolic [Ca²⁺] increase. Results indicated that the Na⁺–Ca²⁺ exchanger may be important in ion regulation during IP.
INTRODUCTION

It is well established that ischemic preconditioning provides cardioprotective effects during the subsequent ischemia and reperfusion, reducing infarct size [1-3], improving contractile function on reperfusion [4,5], and suppressing reperfusion arrhythmias [6]. Several mechanisms have been proposed to explain these effects of preconditioning, including the preservation of myocardial high-energy stores [7], stimulation of the adenosine A1 receptor [8], activation of ATP-sensitive K+ channels [9], and the translocation and activation of protein kinase C [10].

In addition, alterations in some ionic states, such as reductions in intracellular acidosis or in Ca2+ overload, have been reported as consequences of preconditioning. Intracellular Ca2+ overload is one of the most important factors contributing to cell injury [11]. Using the fluorescent indicator fura-2 in rat hearts perfused in Langendorff’s mode, we previously demonstrated an increase in diastolic [Ca2+]i during the early phase of ischemia [12]. Moreover, Steenbergen et al., who used nuclear magnetic resonance, reported that ischemic preconditioning suppressed the accumulation of intracellular H+, Na+, and Ca2+ during sustained ischemia [5]. These authors hypothesized that these changes in ion levels were mediated by the Na+-H+ exchanger and the Na+-Ca2+ exchanger. Although a stimulation of Ca2+ influx via the Na+-Ca2+ exchanger has been emphasized in ischemia and reperfusion, the involvement of this exchanger in ischemic preconditioning remains unclear.

We analyzed the effect of preconditioning on the incidence of reperfusion-induced ventricular arrhythmias using the working rat heart model in the presence or absence of the Na+-Ca2+ exchanger blocker nickel chloride (Ni2+). We also investigated the effect of preconditioning on changes in intracellular Ca2+ or pH during ischemia and reperfusion using an isolated Langendorff’s perfused rat heart and the fluorescent ion indicators fura-2 or 2',7'-bis(2-carboxylethyl)-5(6)-carboxyfluorescein (BCECF). Finally, we determined the changes in sarcolemmal Na+-Ca2+ exchanger activity before and after preconditioning and evaluated the possible role of this exchanger in ion regulation during preconditioning.

METHODS

Isolated rat heart preparation

Adult male Sprague–Dawley rats (300–350 g) were anesthetized with pentobarbital sodium (50 mg/kg ip), and the hearts were removed promptly. The hearts then were perfused in a working-heart mode using Krebs–Henseleit bicarbonate buffer [13]. The perfusate contained 118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO3, 2.5 mM CaCl2, 1.2 mM MgSO4, 0.5 mM EDTA, 1.2 mM KH2PO4, and 11 mM glucose (pH 7.4, 37°C) and was gassed with 95% O2, 5% CO2.

Whole-heart ischemia was induced by clamping the bypass to activate the one-way ball valve and cut the backflow of the perfusate from the aorta to the coronary artery during diastole [13]. During ischemia the hearts were paced electrically at 300 beats/min. This procedure reduced the coronary flow to less than 10% of the