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Effects of intravenous cariporide on release of norepinephrine and myoglobin during myocardial ischemia/reperfusion in rabbits

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ABSTRACT

Aims: To examine the effects of cariporide, a Na^+/H^+ exchanger-1 inhibitor, on cardiac norepinephrine (NE) and myoglobin release during myocardial ischemia/reperfusion by applying a microdialysis technique to the rabbit heart.

Main methods: In anesthetized rabbits, two dialysis probes were implanted into the left ventricular myocardium and were perfused with Ringer's solution. Cariporide (0.3 mg/kg) was injected intravenously, followed by occlusion of the left circumflex coronary artery. During 30-min coronary occlusion followed by 30-min reperfusion, four consecutive 15-min dialysate samples (two during ischemia and two during reperfusion) were collected in vehicle and cariporide-treated groups. Dialysate myoglobin and NE concentrations were measured by immunochemistry and high-performance liquid chromatography, respectively.

Key findings: Dialysate myoglobin and NE concentrations increased significantly during myocardial ischemia/reperfusion in both vehicle and cariporide-treated groups ($P < 0.01$ vs. baseline). In cariporide-treated group, dialysate myoglobin concentrations were significantly lower than those in vehicle group throughout ischemia/reperfusion ($P < 0.01$ at 0–15 min of ischemia, $P < 0.05$ at 15–30 min of ischemia, $P < 0.01$ at 0–15 min of reperfusion, and $P < 0.01$ at 15–30 min of reperfusion). However, dialysate NE concentrations in cariporide-treated group were lower than those in vehicle group only during ischemia ($P < 0.01$ at 0–15 min of ischemia, and $P < 0.05$ at 15–30 min of ischemia).

Significance: When administered before ischemia, cariporide reduces myoglobin release during ischemia/reperfusion and decreases NE release during ischemia.

Introduction

The Na^+/H^+ exchanger isoform-1 (NHE-1) is a ubiquitously expressed integral membrane protein transporter that regulates intracellular pH by removing intracellular H^+ in exchange for extracellular Na^+ (Fliegel, 2005). NHE-1 has been reported to play an important role in the pathogenesis of myocardial ischemia/reperfusion injuries (Avkiran, 1999, 2003). During myocardial ischemia/reperfusion, the activity or quantity of NHE-1 increases, leading to an accumulation of intracellular Na^+, which in turn reduces and eventually reverses the driving force for the Na^+/Ca^{2+} exchanger, thereby decreasing Ca^{2+} efflux and eventually increasing Ca^{2+} influx. This process subsequently induces intracellular Ca^{2+} overload and promotes structural (apoptosis) and functional (arrhythmias, hypercontraction) damages (Leineweber et al., 2007). In sympathetic nerve endings, increased NHE-1 activity results in accumulation of axoplasmic Na^+ that diminishes the inward transport and eventually favors the outward transport of norepinephrine (NE) via the neuronal NE transporter (a bidirectional NE carrier, NET) (Leineweber et al., 2007). Thus, inhibition of NHE-1 may reduce NE release into the synaptic cleft. Because excessive NE release from sympathetic nerve endings is a prominent cause of arrhythmias and cardiac dysfunction (Leineweber et al., 2007), NHE-1 inhibitors may provide cardioprotection against functional damage during ischemia/reperfusion.

Cariporide, a NHE-1 inhibitor, has been reported to be a pharmacologically preconditioning agent. Several experimental studies have demonstrated that pretreatment with cariporide reduces infarct size (Kristo et al., 2004; Miura et al., 1997), suggesting that the inhibition of NHE-1 protects the heart from structural damage during ischemia/reperfusion. Furthermore, Létienne et al. (2006) reported that cariporide significantly reduced plasma myoglobin and troponin I

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levels that strongly correlated with myocardial necrosis. Therefore, cariporide treatment before ischemia may reduce both pathological NE release and structural damage of the heart during ischemia/reperfusion. However, because of the limited methodology for simultaneous monitoring NE release and structural heart damage in the past, the mechanism of cardioprotection by cariporide has not been fully elucidated. Our group has already demonstrated that cardiac microdialysis technique can simultaneously monitor interstitial NE and myoglobin levels in the ischemic region of the left ventricle (Kitagawa et al., 2005). Because there is less blood flow in ischemic lesion, diffusion of myoglobin should be limited. Therefore interstitial myoglobin level monitored by cardiac microdialysis technique may serve as a more accurate index of structural damage of the heart than plasma myoglobin level. Using this technique, we investigated the effects of cariporide on both NE and myoglobin releases in the left ventricle during ischemia/reperfusion.

Materials and methods

Animal preparation

Animal care was provided in accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences* approved by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Twelve adult male Japanese white rabbits weighing from 2.5 to 3.5 kg were anesthetized with an intravenous injection of pentobarbital sodium (40 mg/kg) via the marginal ear vein, followed by continuous intravenous infusion of pentobarbital sodium (2 mg/kg/h). Butorphanol (0.1 mg/kg) was injected intramuscularly every 2–3 h for analgesia. Adequate anesthesia level was confirmed by loss of the ear pinch response. The rabbits were intubated and ventilated mechanically with room air mixed with oxygen. Systemic arterial pressure was monitored by a catheter inserted into the femoral artery. Heparin sodium (10 IU/kg/h) was infused to prevent blood coagulation in the femoral artery catheter. Heart rate was monitored on body surface electrocardiogram. Arterial pressure and heart rate were recorded by a PowerLab Data Acquisition System (ADInstruments, Dunedin, New Zealand). Esophageal temperature was maintained between 38 and 39 °C using a heating pad.

With the animal in the lateral position, the fifth or sixth rib on the left side was partially removed and a small incision was made in the pericardium to expose the heart. A snare was placed around the main branch of the left circumflex coronary artery (LCX) to act as an occluder for later coronary occlusion. Two dialysis probes were implanted in the left ventricular wall corresponding to the region perfused by the LCX. To confirm that the dialysis probes were properly located inside the ischemic region, we examined the color and motion of the ventricular wall during a brief occlusion. To avoid a preconditioning effect, the duration of brief occlusion was limited to a few seconds.

At the end of each experiment, the LCX was again occluded. Evans blue (1%) was intravenously injected to confirm that the dialysis probes were properly implanted within the ischemic area. The rabbits were euthanized by injecting an overdose of pentobarbital sodium. At postmortem, the heart was excised from the euthanized rabbit and was transversely sliced into 3 or 4 pieces. The left ventricular cavity was macroscopically examined to confirm that the dialysis membranes were not exposed to the left ventricular cavity.

Dialysis technique

Materials for cardiac microdialysis probe have been described in detail previously (Akiyama et al., 1991; Kitagawa et al., 2005). The long transverse dialysis probes were custom made. For monitoring myocardial interstitial NE levels, a dialysis fiber (length 8 mm, o.d. 0.31 mm, i.d. 0.20 mm; PAN-1200 50,000 molecular weight cutoff; Asahi Chemical Japan) was glued at both ends to polyethylene tubes. This dialysis probe was perfused with Ringer's solution at a rate of 2 μl/min using a microinjection pump (Carnegie Medicine CMA/102, Sweden). Each dialysate sample was collected over 15 min (1 sampling volume = 30 μl) into a microtube containing 3 μl of 0.1 N HCl to prevent amine oxidation. Dialysate NE level was measured by high-performance liquid chromatography with electrochemical detection (ECD-300, Eicom, Japan) as described in the Analytical procedures section.

For monitoring myocardial interstitial myoglobin levels, another dialysis probe (length 8 mm, o.d. 0.215 mm, i.d. 0.175 mm, 300 Å pore size; Evaflux type 5A; Kuraray Medical, Japan) was used as described previously (Kitagawa et al., 2005). This dialysis probe was perfused with Ringer's solution at a rate of 5 μl/min. Dialysate sampling period was 15 min (1 sampling volume = 75 μl). Dialysate myoglobin concentration was measured by immunochemistry using a Cardiac Reader (Roche Diagnostics, Basel, Switzerland) as described in the Analytical procedures section.

Experimental protocols were started 2 h after implantation of the dialysis probes. During dialysate sampling, we took into account the dead space between the dialysis membrane and the sample tube.

Analytical procedures

Dialysate NE concentrations were measured by high-performance liquid chromatography with electrochemical detection. An alumina procedure was employed to remove the interfering compounds from the dialysate samples. The liquid chromatography system consisted of a pump (EP-300, Eicom) with a degasser (DG-300, Eicom), a separation column (Eicompak CA-5ODS, Eicom), and an electrochemical detector (ECD-300, Eicom). The temperature of the separation was maintained at 25 °C by a column oven (ATC-300, Eicom). The electrochemical detector was operated with a graphite electrode (WE-3G, Eicom) at +0.45 V vs. an Ag/AgCl reference electrode. Mobile phase consisted of 12% (v/v) methanol, 1-octanesulfonic acid sodium (600 mg/l) and 88% (v/v) 100-mM phosphate buffer adjusted to pH 5.68. The pump flow rate was 0.23 ml/min. Chromatograms were recorded and analyzed by a laboratory computer connected with an A–D converter (Power Chrom EPC-500, Eicom). NE concentrations were determined by measuring the peak areas. The absolute detection limit of NE was 0.1 pg per injection (signal-to-noise ratio = 3).

Dialysate myoglobin concentrations were measured by the Cardiac Reader system (Roche Diagnostics). Single-use immunochemical test strips were used in the Cardiac Reader system. When a sample was added to the test well, the sample migrated along the strip due to capillary action, and myoglobin combined with two specific monoclonal antibodies. The resulting sandwich complex was immobilized by streptavidin in a stripe along the read window, producing a reddish line with an intensity related to myoglobin concentration. The CCD (Charge Coupled Device) camera quantified the intensity of the signal line and control line on the immunochemical test strips via reflectance measurements. The reflectance measurements were then converted into myoglobin concentration using electronically stored lot-specific calibration curves. The measuring range was between 30 and 700 ng/ml (Ambrose et al. 2002). When dialysate myoglobin concentrations were expected to be higher than 700 ng/ml, dialysate samples were diluted 10 or 100 times with saline.

Experimental protocols

Time courses of dialysate NE and myoglobin concentrations during acute myocardial ischemia/reperfusion (n = 6, vehicle group)

We examined the time courses of dialysate NE and myoglobin concentrations during 30 min of ischemia followed by 30 min of reperfusion. After 15-min baseline sampling, the main branch of the LCX was occluded for 30 min and then was released. Four consecutive 15-min