On-pump inhibition of es-ENT1 nucleoside transporter and adenosine deaminase during aortic crossclamping entraps intracellular adenosine and protects against reperfusion injury: Role of adenosine A1 receptor

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Objective: The inhibition of adenosine deaminase with erythro-9 (2-hydroxy-3-nonyl)-adenine (EHNA) and the es-ENT1 transporter with p-nitro-benzylthioinosine (NBMPR), entraps myocardial intracellular adenosine during on-pump warm aortic crossclamping, leading to a complete recovery of cardiac function and adenosine triphosphate (ATP) during reperfusion. The differential role of entrapped intracellular and circulating adenosine in EHNA/NBMPR-mediated protection is unknown. Selective (8-cyclopentyl-1,3-dipropyl-xanthine) or nonselective [8-(p-sulfophenyl)theophyline] A1 receptor antagonists were used to block adenosine A1-receptor contribution in EHNA/NBMPR-mediated cardiac recovery.

Methods: Anesthetized dogs (n = 45), instrumented to measure heart performance using sonomicrometry, were subjected to 30 minutes of warm aortic crossclamping and 60 minutes of reperfusion. Three boluses of the vehicle (series A) or 100 μM EHNA and 25 μM NBMPR (series B) were infused into the pump at baseline, before ischemia and before reperfusion. 8-Cyclopentyl-1,3-dipropyl-xanthine (10 μM) or 8-(p-sulfophenyl)theophyline (100 μM) was intra-aortically infused immediately after aortic crossclamping distal to the clamp in series A and series B. The ATP pool and nicotinamide adenine dinucleotide was determined using high-performance liquid chromatography.

Results: Ischemia depleted ATP in all groups by 50%. The adenosine/inosine ratios were more than 10-fold greater in series B than in series A ($P < .001$). ATP and function recovered in the EHNA/NBMPR-treated group ($P < .05$ vs control group). 8-Cyclopentyl-1,3-dipropyl-xanthine and 8-(p-sulfophenyl)theophyline partially reduced cardiac function in series A and B to the same degree but did not abolish the EHNA/NBMPR-mediated protection in series B.

Conclusions: In addition to the cardioprotection mediated by activation of the adenosine receptors by extracellular adenosine, EHNA/NBMPR entrapment of intracellular adenosine provided a significant component of myocardial protection despite adenosine A1 receptor blockade. (J Thorac Cardiovasc Surg 2012;144:243-9)

Supplemental material is available online.

In addition to pre-existing ischemic injury, reperfusion injury has been implicated in poor cardiac recovery despite excellent cardiac repair.[1,2] Therefore, targeting postischemic reperfusion injury[3] is critical for improving intra- and postoperative outcomes. Adenosine has emerged as a promising adjuvant agent to augment cardioprotection in experimental models[4-6] and clinical trials[7-10] against ischemic and reperfusion injury. Blockade of the adenosine A1 receptor abolishes the cardioprotection mediated by ischemic preconditioning,[4] and treatment with exogenous adenosine potentiates ischemic preconditioning effects.[6] Unlike exogenous adenosine, endogenous adenosine generated during ischemia must be transported by way of the p-nitro-benzylthioinosine (NBMPR)-sensitive es-ENT1 transporter 1 (es-ENT1) to the extracellular domain (Figure E1, *A*) to activate the adenosine receptor subtype (A1, A2A, A2B, and A3)-mediated signaling mechanisms of cardioprotection. Because of the short half life of adenosine in humans and animals, inhibition of adenosine deaminase by erythro-9 (2-hydroxy-3-nonyl)-adenine (EHNA) prolongs its half life and potentiates its pharmacologic efficacy. In addition, selective blockade of es-ENT1 with NBMPR entraps myocardial adenosine and inosine

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Abbreviations and Acronyms

ATP = adenosine triphosphate
DPCPX = 8-cyclopentyl-1,3-dipropyl-xanthine
EHNA = erythro-9 (2-hydroxy-3-nonyl)-adenine
NBMPR = p-nitro-benzylthioinosine
SPT = 8-(p-sulfophenyl)theophyline

at the site of production during ischemia and prevents its loss during reperfusion. Preischemic treatment with EHNA/NBMPR entraps intracellular adenosine and results in complete recovery of adenosine triphosphate (ATP) and cardiac function,[11-13] suggesting that the entrapped adenosine is intracellularly compartmentalized (Figure E1, *B*). The specific binding sites of [^{3}H]-NBMPR were identified in human and animal myocardial preparations.[14] The relative contribution of entrapped intracellular adenosine and circulating extracellular adenosine in EHNA/NBMPR-mediated protection in relation to adenosine A1 receptor activation (Figure E1, *C*) is not known. The present study was designed to determine the role of the adenosine A1 receptor in EHNA/NBMPR-mediated postischemic recovery of function in a canine model of on-pump warm aortic crossclamping (ACC) and reperfusion. Selective blocking of the adenosine A1 receptor with 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX) or nonselective blocking of adenosine receptor subtypes with 8-(p-sulfophenyl)theophyline (SPT) partially reduces but does not abolish postischemic recovery mediated by EHNA/NBMPR, suggesting intracellular mechanisms of protection.

METHODS

Materials

The biochemical reagents, EHNA and NBMPR, were purchased from Sigma-Aldrich (St. Louis, Mo), and DPCPX and SPT were purchased from Research Biochemical (Natick, Mass).

Animal Model

The experiments conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication no. 5377-3; available at: http://www.nap.edu/catalog/5140.html).

A total of 45 microfilaria-free adult mongrel dogs, of either sex, weighing 17 to 25 kg were used in the present study. The dogs were initially anesthetized with 35 mg/kg intravenous sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, Ill). The surgical procedures and instrumentations were performed exactly as described previously.[11] Cardiopulmonary bypass was established by subclavian artery and atrial venous cannulation. A membrane oxygenator (Medtronic, Minneapolis, Minn) was used and primed with noncross-matched homologous blood. The mean arterial reperfusion pressure was maintained at 60 to 65 mm Hg during bypass. Arterial blood gases, pH, and hematocrit were routinely determined and maintained at the following levels: oxygen partial pressure, 100 to 140 mm Hg; carbon dioxide partial pressure, 30 to 40 mm Hg, pH, 7.32 to 7.48; and hematocrit, about 30%.

Assessment of Left Ventricular Performance

Left ventricular (LV) performance was assessed, off the bypass pump, from the relationship between stroke work and the end-diastolic dimension using a sensitive and load-independent index of contractility,[11] pulse transit sonomicrometry (Triton Technology, San Diego, Calif), and Millar pressure transducers. One pair of lead titanate zirconate piezoelectric hemispheric crystals was sutured to the anterior and posterior of the epicardial surface of the left ventricle wall in the minor axis (40–60 mm). Analog data were digitized at 200 Hz and stored on a personal computer hard disc and analyzed using interactive Crunch software developed in our laboratory. Data acquisition was performed at varying preloads by way of venous drainage, creating diminishing work loops. The slope was obtained from the relationship between the LV pressures (a surrogate of volume) against the end-diastolic lengths and is considered as a sensitive load-independent index of contractility.

Assessment of Adenine Nucleotide Pool Metabolism

Transmural serial Tru-Cut needle biopsy specimens (5–10 mg) were obtained and immediately frozen in liquid nitrogen (–196°C), extracted, analyzed, and quantified using high-performance liquid chromatography, and presented as nmoles/mg protein.[11]

Experimental Protocol

After a 30-minute stabilization period on pump, the dogs were assigned 1 of 2 series: A or B. Three boluses (500 mL each) of the vehicle solution (saline containing 0.05% dimethylsulfoxide) without (series A) or with (series B) 25 μM NBMPR and 100 μM EHNA, were infused into the pump oxygenator before ischemia, before ACC, and just before releasing ACC. The dogs in series A received a single intra-aortic infusion of 50 mL of saline (control group, n = 10), 100 μM SPT (n = 6) or 10 μM DPCPX (n = 7) distal to the clamp immediately after ACC. In series B, the dogs were treated with a single intra-aortic infusion of 50 mL of vehicle (EHNA/NBMPR group, n = 8), 100 μM SPT (n = 7) or 10 μM DPCPX (n = 7).

Statistical Analysis

The data are presented as the mean ± standard error of the mean. Sequential measurements were compared using repeated measures analysis of variance and Tukey's post hoc tests using SAS (SAS Institute, Cary, NC). Differences were considered significant if the probability value for the comparison of the least squares mean was less than .05; the F ratios are provided with the figures.

RESULTS

The results of the control group and EHNA/NBMPR-treated group have been previously published[9,10] and are included in the present report for comparison. This model represents a scenario of reversible global myocardial stunning without necrosis, and all dogs survived for the length of the experiments in all groups.

Myocardial ATP

On-pump normothermic ACC for 30 minutes reduced the myocardial ATP levels by about 50% in all groups (Figure 1). No recovery of ATP was observed in the series A groups during reperfusion. In series B, ATP had recovered at the end of reperfusion in the EHNA/NBMPR-treated