Sodium/hydrogen-exchanger inhibition during cardioplegic arrest and cardiopulmonary bypass: An experimental study

Charles S. Cox, Jr, MD
Henning Sauer, MD
Steven J. Allen, MD
L. Maximilian Buja, MD
Glen A. Laine, PhD

Objective: We sought to determine whether pretreatment with a sodium/hydrogen-exchange inhibitor (EMD 96 785) improves myocardial performance and reduces myocardial edema after cardioplegic arrest and cardiopulmonary bypass.

Methods: Anesthetized dogs (n = 13) were instrumented with vascular catheters, myocardial ultrasonic crystals, and left ventricular micromanometers to measure preload recruitable stroke work, maximum rate of pressure rise (positive and negative), and left ventricular end-diastolic volume and pressure. Cardiac output was measured by means of thermodilution. Myocardial tissue water content was determined from sequential biopsy. After baseline measurements, hypothermic (28°C) cardiopulmonary bypass was initiated. Cardioplegic arrest (4°C Bretschneider crystalloid cardioplegic solution) was maintained for 2 hours, followed by reperfusion-rewarming and separation from cardiopulmonary bypass. Preload recruitable stroke work and myocardial tissue water content were measured at 30, 60, and 120 minutes after bypass. EMD 96 785 (3 mg/kg) was given 15 minutes before bypass, and 2 μmol was given in the cardioplegic solution. Control animals received the same volume of saline vehicle. Arterial–coronary sinus lactate difference was similar in both animals receiving EMD 96 785 and control animals, suggesting equivalent myocardial ischemia in each group.

Results: Myocardial tissue water content increased from baseline in both animals receiving EMD 96 785 and control animals with cardiopulmonary bypass and cardioplegic arrest but was statistically lower in animals receiving EMD 96 785 compared with control animals (range, 1.0%-1.5% lower in animals receiving EMD 96 785). Preload recruitable stroke work decreased from baseline (97 ± 2 mm Hg) at 30 (59 ± 6 mm Hg) and 60 (72 ± 9 mm Hg) minutes after cardiopulmonary bypass and cardioplegic arrest in control animals; preload recruitable stroke work did not decrease from baseline (98 ± 2 mm Hg) in animals receiving EMD 96 785 and was statistically greater at 30 (88 ± 5 mm Hg) and 60 (99 ± 4 mm Hg) minutes after bypass and arrest compared with control animals.

Conclusions: Sodium/hydrogen-exchanger inhibition decreases myocardial edema immediately after cardiopulmonary bypass and cardioplegic arrest and improves preload recruitable stroke work. Sodium/hydrogen-exchange inhibition during cardiac procedures with cardiopulmonary bypass and cardioplegic arrest may be a useful adjunct to improve myocardial performance in the immediate postbypass or arrest period.

From the Departments of Surgery and Anesthesiology-Center for Microvascular and Lymphatic Studies, and Pathology and Laboratory Medicine at the University of Texas-Houston, Medical School, and the Michael E. DeBakey Institute, Texas A&M University, Houston, Tex.

Supported in part by National Heart, Lung, and Blood Institute grant No. HL-36115 and National Institute of General Medical Sciences grant No. GM-00675. Dr Sauer is the recipient of a scholarship from Dr F. Kohler Chemie GmbH, Germany.

Received for publication July 11, 2001; revisions requested Aug 13, 2001; revisions received Aug 29, 2001; accepted for publication Oct 2, 2001.

Address for reprints: Charles S. Cox, Jr, MD, 6431 Fannin, Suite 5.246. Houston, TX 77030 (E-mail: Charles.S.Cox@uth.tmc.edu).

J Thorac Cardiovasc Surg 2002;123:959-66

0022-5223/2002 $35.00+0 **12/1/120715**

doi:10.1067/mtc.2002.120715

The introduction of cardioplegia has dramatically improved myocardial protection during cardiac surgery.[1] However, cardiopulmonary bypass (CPB) and cardioplegic arrest (CPA) have been shown to be a form of global myocardial ischemia-reperfusion (I-R) that can induce post-CPB myocardial dysfunction. Although most patients undergoing CPB and CPA do well, some patients with limited myocardial reserve may be difficult to separate from CPB. Although post-CPB myocardial contractile dysfunction is multifactorial, we have focused on the relationship between myocardial edema and post-CPB myocardial dysfunction.[2-6] Because post-CPB/CPA myocardial dysfunction is a potentially devastating complication, we have investigated cardioprotective strategies to reduce post-CPB/CPA myocardial edema, thus improving myocardial function.

Recent studies have shown that myocardial sodium/hydrogen (Na^+/H^+)-exchanger inhibition preserves myocardial performance after global I-R.[7-10] Myocardial ischemia results in intracellular lactate accumulation and a concomitant decrease in intracellular pH. Na^+/H^+-exchange activity serves to normalize intracellular pH by exchanging intracellular H^+ for Na^+ during intracellular lactate accumulation.[11,12] However, intracellular Na^+ accumulation may lead to a concomitant increase in intracellular water. EMD 96 785 is a Na^+/H^+-exchanger inhibitor that is selective for the myocardial isoform NHE-1. We hypothesized that Na^+/H^+-exchanger inhibition during I-R associated with CPB/CPA would decrease myocardial edema and improve post-CPB/CPA myocardial performance.[13] In formulating our hypothesis, we noted ex vivo studies of hypoxia-reoxygenation and simulated normothermic CPA by using Na^+/H^+-exchanger inhibition that reduced total myocardial water content (MWC) and improved myocardial performance.[14,15] To test this hypothesis, we studied the effects of Na^+/H^+-exchanger inhibition using EMD 96 785 before myocardial I-R on myocardial function and myocardial fluid balance after CPB/CPA.

Na^+/H^+-exchanger inhibition has also been shown to attenuate neutrophil activation and subsequent neutrophil-mediated myocardial reperfusion injury.[16] A secondary hypothesis was that Na^+/H^+-exchanger inhibition decreased myocardial leukosequestration. We measured myocardial myeloperoxidase (MPO) after CPB/CPA to determine whether Na^+/H^+-exchanger inhibition decreased myocardial leukosequestration and whether this was associated with improved post-CPB/CPA myocardial function.

Methods

Animal Preparation

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. Conditioned dogs of either sex were anesthetized by means of intravenous administration of 25 mg/kg thiopental sodium (Pentothal, Abbott Laboratories, North Chicago, Ill) and underwent tracheal intubation and mechanical ventilation with 100% oxygen by using a volume-cycled respirator (Siemens-Elema AB, Solna, Sweden). Anesthesia was maintained with intravenous infusion of 1% thiopental sodium in Ringer solution.

Fluid-filled catheters were placed into the left femoral artery and vein and connected to pressure transducers for arterial pressure monitoring, arterial blood sampling, and fluid administration, respectively. A 7F Swan-Ganz thermodilution catheter (Baxter Healthcare Corp, Edwards Divison, Santa Ana, Calif) was inserted into the pulmonary artery through the right jugular vein for pressure and cardiac output measurements. We then exposed the right femoral artery for subsequent CPB cannulation. After a median sternotomy and pericardiotomy, a 5F catheter was advanced into the coronary sinus through a snare-secured purse-string stitch for coronary sinus blood sampling. An umbilical tape was placed around the inferior vena cava for cardiac preload manipulation. A micromanometer-tipped pressure transducer (Millar Instruments, Inc, Houston, Tex) was introduced into the left ventricular cavity through the apex. Sonomicrometry crystals (10 MHz; Sonometrics Corporation, London, Ontario, Canada) were placed in the left ventricular subendocardium across the septum/free-wall axis of the left ventricle. The crystals were then connected to a sonomicrometer (Triton Technology Inc, Watsonville, Calif) for processing of signals.

Hemodynamics and Preload Recruitable Stroke Work

Hemodynamic data were simultaneously logged into a Macintosh Quadra 700 computer through an analog-to-digital data-acquisition device (MacLab, World Precision Instruments, Inc, Sarasota, Fla). Cardiac output was determined in duplicate by injecting 10 mL of ice-cold Ringer solution. Left ventricular pressure was measured with a micromanometer, and the left ventricular septum/free-wall diameter was obtained with a sonomicrometer. Preload recruitable stroke work (PRSW) was calculated as the slope of the relation between left ventricular end-diastolic volume and left ventricular stroke work, as previously described.[2-5]

MWC Determination

MWC for edema quantification was measured by using a microgravimetric technique, as previously described.[2,3] With a biopsy needle (Tru-Cut; Baxter Healthcare Corp), transmyocardial biopsy specimens were taken from the left ventricular anterior or anterolateral wall. Measurements were performed in triplicate.

Microscopic Analysis

From each dog, myocardial biopsy specimens were collected for both light and electron microscopy. Samples were placed in 3% buffered glutaraldehyde fixative (4°C) for later processing. In brief, after fixation in 2% osmium tetroxide, dehydration in an ethanol series, and substitution with propylene oxide, specimens were embedded in epoxy resin and polymerized at 60°C overnight. Semithin (0.5-1 μm) sections were prepared and stained with toluidine blue for light optic selection of areas of interest. Samples were reviewed by a blinded investigator. A modification of a point-counting technique was used to obtain morphometric quantification during light microscopic observation of the toluidine blue–stained sections. Percentage volumes were calculated from (1) interstitial space, which is reflective of interstitial fluid content; (2) cytologically normal cardiomyocytes; and (3) cardiomyocytes with significant injury with cytoplasmic clearing (edema) and contraction band formation.[17]

Determination of Myocardial MPO

MPO tissue levels were analyzed as an index of neutrophil infiltration in the tissue. The presence of MPO, an enzyme specific for neutrophils, was determined in myocardial tissue by using the method described by Bradley and colleagues[18] and modified by Mullane and colleagues.[19]

Na^+/H^+-Exchanger Inhibition

EMD 96 785 is a specific Na^+/H^+-exchanger inhibitor. In vitro assays studying the inhibitory effects at 10 μmol/L showed only a 13% reduction in Na^+/Ca^{2+}-exchanger activity (84% reduction in Na^+/H^+-exchanger activity at the same dose). This level of Na^+/Ca^{2+}-exchanger inhibition is considered insignificant. Plasma concentrations of EMD 96 785 were measured in some dogs and ranged from 1900 ng/mL after bolus injection to 500 ng/mL at the end of the experiment.

CPB Techniques

After instrumentation, heparin (250 IU/kg) was given intravenously for systemic anticoagulation, followed by additional doses of 100 IU/kg given every 60 minutes throughout the experiment. Extracorporeal circulation was performed by using a 14F arterial perfusion cannula placed into the right femoral artery and a 2-stage venous cannula (36F and 46F) in the right atrium–inferior vena cava. The left ventricle was vented with a 12F catheter inserted through the left atrium. The extracorporeal circuit (model 7000; Sarns, Inc, Ann Arbor, Mich) and the membrane oxygenator (HVRF 3700, COBE Cardiovascular, Inc, Arvada, Colo) were primed with 800 mL of Ringer solution and 1000 IU of heparin. Approximately 5 minutes after initiating CPB, CPA was initiated with 500 mL of 4°C crystalloid cardioplegic solution (Bretschneider) and lasted for 2 hours.[20] We gave an additional dose of 100 mL of cardioplegic solution after 60 minutes of cardiac arrest. External myocardial cooling was accomplished by means of intermittent instillation of iced (4°C) saline solution into the pericardium. Hearts were then reperfused on normothermic CPB for 40 minutes. Thereafter, we weaned the dogs from CPB and removed all cannulas. During ischemia, animals were systemically cooled to 28°C with a heat exchanger. We kept CPB flow between 40 and 60 $mL \cdot kg^{-1} \cdot min^{-1}$ at 28°C to maintain a mean systemic perfusion pressure of 40 to 70 mm Hg.

Experimental Design

Systemic pretreatment was used to ensure drug availability during the ischemic period before CPA. Direct infusion by means of cardioplegia ensures drug delivery at the time of ischemia (CPA), and it decreases the variability of drug delivery that may occur during the hemodilution that occurs with initiation of CPB. EMD 96 785 (3 mg/kg) was given intravenously 15 minutes before CPB and was present at a final concentration of 2 μmol in the cardioplegic solution. Treated dogs ($n = 6$) were compared with control animals ($n = 7$) that were subjected to the same protocol, except that an equal volume of saline vehicle was used instead of EMD 96 785. These doses were chosen after in vitro and in vivo experiments evaluating binding specificity and efficacy, as well as experiments with CPB circuits, to ensure that no significant binding occurred to the oxygenator.

Experimental Protocol

After at least 30 minutes for stabilization after the completion of instrumentation, preischemic hemodynamic values were recorded. Myocardial samples for MWC determination were collected, as described above. Arterial and coronary sinus blood samples were frozen at −20°C for later determination of arterio–coronary sinus lactate difference and creatine kinase, lactate dehydrogenase, and troponin I levels (Sigma Diagnostics, Poole, Dorset, United Kingdom).

Coronary sinus and arterial blood samples were collected simultaneously at 1 and 15 minutes during reperfusion. Additionally, MWC was determined at 15 minutes of reperfusion. Postischemic hemodynamic recovery data were recorded at 30, 60, and 120 minutes after separation from CPB.

Statistical Analysis

All data presented are means ± SEM. Data analysis was carried out with Statistica software (StatSoft Inc, Tulsa, Okla). Time courses of each measured parameter were examined within groups by using analysis of variance for repeated measures and the Tukey HSD (honestly significant difference) test. Time-point comparisons between groups were made with the Student *t* test.

Results

Figure 1 demonstrates an increase in MWC with CPB/CPA in both groups. MWC in animals receiving EMD 96 785 was statistically lower compared with that in control animals at each time point. These control data are similar to those previously reported from our laboratory.[2,3] Figure 2 demonstrates a statistically significant decrease of PRSW in control animals at 30 minutes after CPB and 60 minutes after CPB compared with baseline values and values in EMD 96 785–treated animals. Table 1 demonstrates the hemodynamic data. At the same left ventricular end-diastolic volume and left ventricular end-diastolic pressure (LVEDP), EMD 96 785 had a significantly greater $+dp/dt_{max}$ compared with that seen in control animals at the 30- and 60-minute post-CPB/CPA time periods. This parallels the PRSW data. Figure 3 shows no difference between groups in arterio–coronary sinus lactate difference, which suggests an equivalent global ischemic insult. There was no difference in levels of the cardiac enzymes creatine kinase, lactate dehydrogenase, or troponin I between groups. Myocardial tissue MPO was almost identical between groups (control: 0.29 ± 0.04 U/100 mg of tissue vs EMD 96 785: 0.31 ± 0.07 U/100 mg of tissue).

Morphometric evaluation demonstrated myocardium with

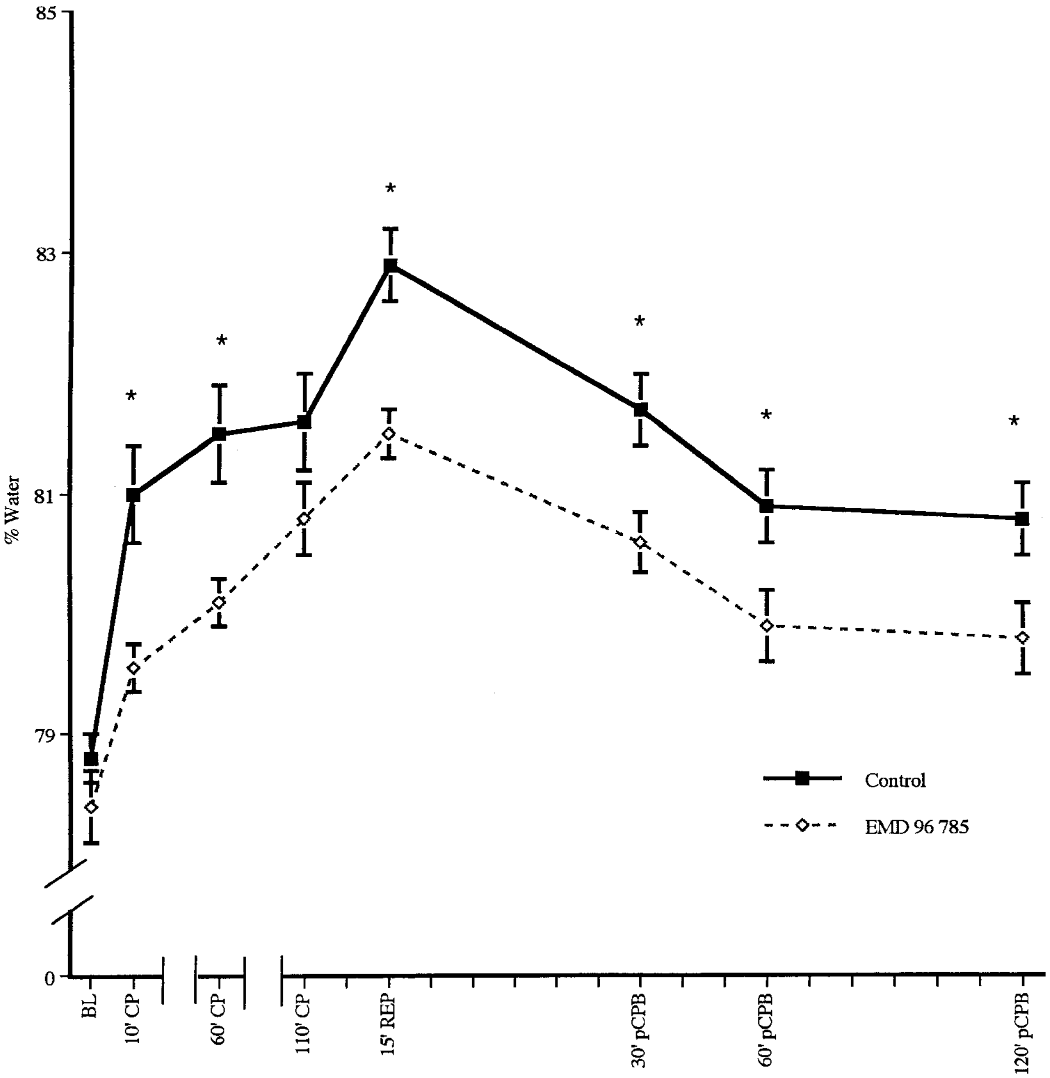

Figure 1. MWC increases in both groups after CPB/CPA. EMD 96 785 pretreatment results in significantly less myocardial edema compared with that seen in control animals. *$P < .05$ compared with control animals. *BL*, Baseline; *CP*, cardioplegia; *REP*, reperfusion.

25.6% ± 1.8% volume interstitial space in control animals compared with 24.7% ± 2.0% in animals receiving EMD 96 785 ($P = .37$). Normal cardiomyocytes were 60.0% ± 3.9% in control animals and 53.0% ± 4.0% in animals receiving EMD 96 785 ($P = .1$). Cardiomyocytes with contraction band formation were 14.3% ± 4.0% in control animals and 22.3% ± 4.5% in animals receiving EMD 96 785 ($P = .11$).

Discussion

Our study shows that Na^+/H^+-exchanger inhibition before and during myocardial ischemia reduces myocardial edema formation during CPB/CPA. In addition, the rapid increase of reperfusion-induced myocardial water accumulation in control animals was attenuated by Na^+/H^+-exchanger inhibition. Apparently, post-CPB functional recovery was enhanced by edema reduction. Our central hypothesis was that Na^+/H^+-exchanger inhibition reduces myocardial edema formation associated with CPB/CPA. However, the differentiation between interstitial myocardial edema versus intracellular myocardial edema was not determined. Interstitial myocardial edema is primarily related to altered Starling forces and a loss of the myocardial edema safety factors during CPA. These include the cessation of myocardial lymph flow without organized contraction, reduction of interstitial pressure with diastolic arrest, and reduction of plasma oncotic pressure with crystalloid cardioplegia.[2] This is apparent in Figure 1, which shows that MWC increases abruptly. Thus, we would not expect Na^+/H^+-exchanger inhibition to affect interstitial edema. EMD 96 785 affects intracellular edema by reducing Na^+ and water influx into

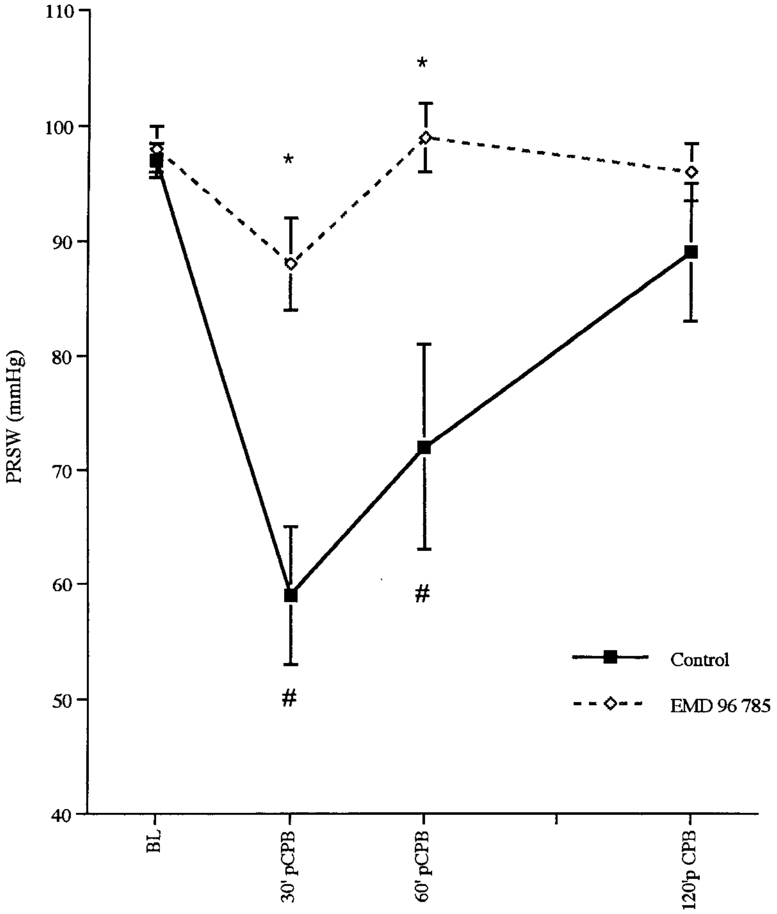

Figure 2. PRSW decreases in both groups after CPB/CPA. EMD 96 785 pretreatment results in improved myocardial contractility after CPB/CPA. *$P < .05$ compared with control animals; #$P < .05$ compared with baseline. *BL,* Baseline.

the cell.[11-13] Although morphometric analysis can yield variable information caused by sampling errors or fixation artifact, our data show no significant difference in the interstitial space volume between control animals and animals receiving EMD 96 785. This suggests that the increase in MWC occurred in the intracellular, and not in the interstitial, space. Because these samples were taken at the end of the experiment, when the differences in MWC were not as pronounced, the microscopic techniques may have not differentiated between the differences in intracellular and interstitial water.

The sequence of events that determines the degree of cellular edema and Na^+ gain after I-R has not been irrefutably determined. However, a number of studies describe mechanisms explaining how Na^+/H^+-exchanger inhibition can reduce myocardial edema after I-R.[11,21,22] Na^+/H^+ exchange is an osmolar equivalent exchange. During hypoxia-ischemia, Na^+/H^+ exchange alone has no effect on osmolality, and Cl^-/HCO_3^- exchange that operates in the absence of intracellular acidosis is inhibited. However, with reperfusion, cytosolic Na^+ is not rapidly normalized but remains increased for a prolonged period of time. During this time, intracellular pH rapidly increases, allowing Cl^-/HCO_3^- exchange to operate and driving external Cl^- inward. Thus, even though the antiporter catalyzes the strictly coupled exchange of one osmolyte for another, exchange of Na^+ for H^+ results in net osmotic gain for 2 reasons. As Na^+ moves inward and H^+ outward, the intracellular concentration and activity of H^+ is simultaneously compensated by dissociation of intracellular buffers. The associated increase in intracellular pH prompts exchange of intracellular HCO_3^- with inward flow of Cl^-. Together, the parallel stimulation of the cation and anion antiporters promotes the uptake of NaCl coupled to osmotic water uptake, thereby favoring intracellular myocardial edema.

The contribution of myocardial edema to the multifactorial post-CPB/CPA myocardial dysfunction is controversial. It is reasonably well established that myocardial edema impairs diastolic function, increasing myocardial stiffness. Ludwig and colleagues[23] showed that modest myocardial edema without preceding I-R depressed systolic function as

Table 1. Hemodynamic parameters

	Baseline	30 min after CPB	60 min after CPB	120 min after CPB
MAP (mm Hg)				
Control	125 ± 2	75 ± 5*†	90 ± 6†	106 ± 7†
EMD 96 785	120 ± 6	98 ± 3^{+}	109 ± 3	107 ± 7
$+dp/dt_{max}$				
Control	2159 ± 153	1514 ± 92*†	1824 ± 81*	2147 ± 73
EMD 96 785	2340 ± 267	2261 ± 102	2601 ± 213	2088 ± 143
$-dp/dt_{max}$				
Control	3082 ± 260	1827 ± 208*†	2267 ± 186*†	2477 ± 131
EMD 96 785	3643 ± 170	2949 ± 271†	2956 ± 200	2342 ± 219†
LVEDP (mm Hg)				
Control	2 ± 0	2 ± 0	2 ± 0	2 ± 0
EMD 96 785	1 ± 0	2 ± 0	2 ± 0	3 ± 0
LVEDV (mL)				
Control	33 ± 6	28 ± 6	28 ± 5	30 ± 6
EMD 96 785	27 ± 4	26 ± 3	27 ± 4	27 ± 5
PAOP (mm Hg)				
Control	10 ± 1	10 ± 0	10 ± 0	10 ± 1
EMD 96 785	9 ± 0	9 ± 0	9 ± 0	9 ± 0
CVP (mm Hg)				
Control	7 ± 0	7 ± 0	7 ± 0	8 ± 0
EMD 96 785	6 ± 0	6 ± 0	6 ± 0	7 ± 0
CI				
Control (L · min^{-1} · m^{-2})	2.9 ± 0.2	1.7 ± 0.3†	1.9 ± 0.2†	1.9 ± 0.3†
EMD 96 785	2.5 ± 0.2	1.9 ± 0.1	1.9 ± 0.2	2.0 ± 0.3

At the same left ventricular end-diastolic volume in each group, $+dp/dt_{max}$ is significantly greater at 30 and 60 minutes after CPB in animals receiving EMD 96 785 compared with that in control animals. This parallels the PRSW data. Mean arterial pressure is decreased to a greater degree in control animals compared with animals receiving EMD 96 785 at 30 and 60 minutes after CPB. $-dp/dt_{max}$ is greater in animals receiving EMD 96 785 compared with control animals at 30 and 60 minutes after CPB. This suggests an improvement in diastolic function in the EMD 96 785 group compared with that in the control group. *MAP*, Mean arterial pressure; *dp/dt*, derivative change in left ventricular pressure over time; *LVEDP*, left ventricular end-diastolic pressure; *PAOP*, pulmonary artery occlusion pressure; *CVP*, central venous pressure; *CI*, cardiac index.

*$P < .05$ compared with control value at same time point.

†$P < .05$ compared with baseline value within group.

well. In contrast, Miyamoto and coworkers[24] later demonstrated no depression of systolic function with induction of myocardial edema without preceding I-R. However, we have shown that myocardial edema without preceding ischemia impairs cardiac performance.[25] Data from the current study are similar to those of previous studies demonstrating that increases of 1% to 2% in MWC after CPB/CPA significantly depress PRSW.[2-5] The degree and time course of myocardial edema development-resolution after CPB/CPA are clinically relevant because this is the critical period immediately after separation from CPB. Myocardial performance during this time determines the need to reinstitute CPB or circulatory support devices, as well as inotropes.

Our data show an improvement in post-CPB/CPA myocardial contractility after pretreatment with EMD 96 785, primarily in the first hour after separation from CPB, with a return to control values at 2 hours after separation from CPB. The observed preservation of myocardial contractility in animals receiving EMD 96 785 is associated with a reduction in MWC compared with that seen in control animals. However, Na^+/H^+-exchanger inhibition during myocardial I-R could affect contractility directly, irrespective of changes in myocardial water. The Na^+/H^+ exchanger normalizes intracellular pH during intracellular acid (lactate) accumulation that can occur with myocardial ischemia.[11,12,26] During reperfusion, lactate is removed, and there is an alkaline overshoot.[9] Intracellular alkalinization has a potent sensitizing effect of myocardial contractile proteins to Ca^{2+}. Thus, impaired diastolic relaxation may occur not only because of increased intracellular Ca^{2+} but also because of increased Ca^{2+}-troponin interactions. This is one of the reported mechanisms of postreperfusion-induced myocardial contracture and loss of myocardial compliance.[9]

Increased Na^+/H^+-exchanger activity during ischemia results in an increased Na^+ influx into the cell. A secondary effect of this phenomenon is a reversal of the Na^+/Ca^{2+} exchanger, resulting in an increase in intracellular Ca^{2+}.[11,12,26] Increased intracellular Ca^{2+} within the myocyte may alter the Ca^{2+} kinetics of excitation-contraction coupling or Ca^{2+} cycling between the contractile apparatus

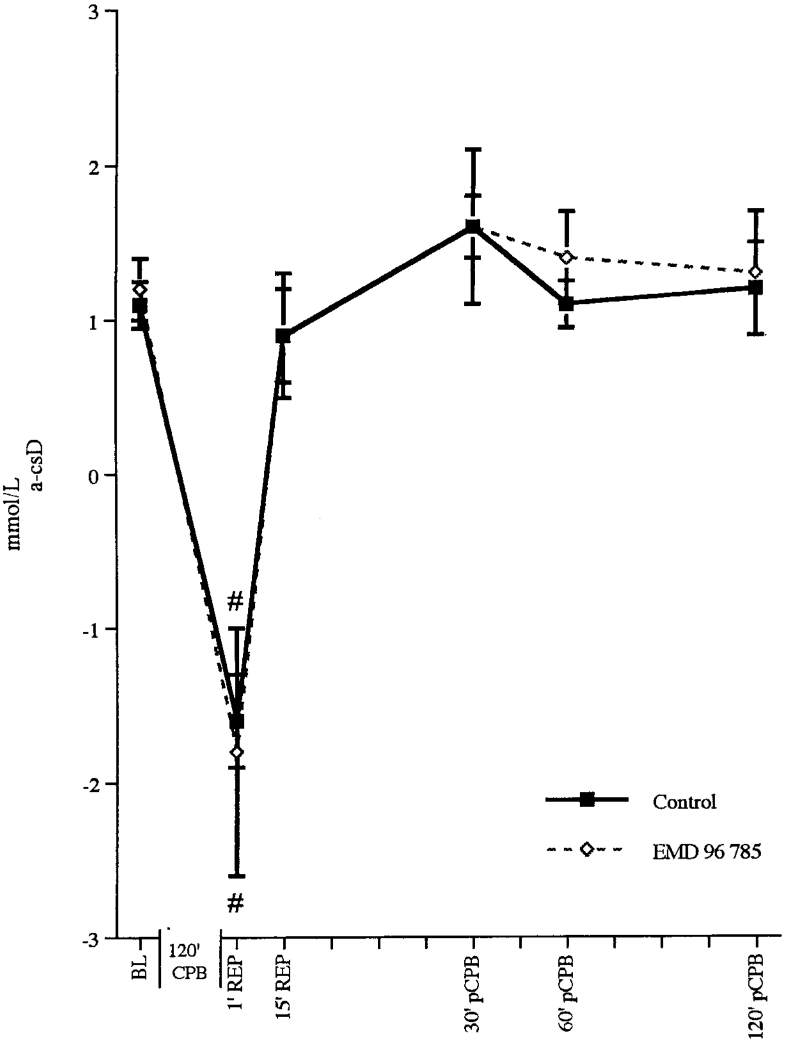

Figure 3. Arterio–coronary sinus lactate difference is used as a marker of degree of myocardial ischemia during CPB/CPA. There is no difference between groups, indicating equivalent I-R injury. #*P* < .05 compared with baseline. *BL*, Baseline; *REP*, reperfusion.

and the sarcoplasmic reticulum, leading to myocardial contracture with a resultant rise in LVEDP, stone heart, or both. Numerous studies have demonstrated attenuation in the expected rise in LVEDP after myocardial I-R by using Na^+/H^+-exchanger inhibition.[7-9] Increased intracellular Ca^{2+} may initiate reperfusion-induced arrhythmias through a mechanism involving oscillatory release of Ca^{2+} from the sarcoplasmic reticulum, thus inducing delayed afterpolarizations.[10,27] Therefore, Na^+/H^+-exchanger inhibition may improve post-CPB/CPA myocardial contractility and function in 4 ways: (1) limiting intracellular Na^+ gain (and water) during myocardial ischemia; (2) suppressing postreperfusion contractile and diastolic dysfunction caused by myofilament sensitization by the alkaline overshoot; (3) limiting intracellular Ca^{2+} overload with secondary hypercontracture, progression to infarction,[28] or both; and (4) preventing Ca^{2+} overload–mediated reperfusion arrythmias.[10,27] We were unable to determine the relative contribution of a reduction of MWC toward improved post-CPB/CPA myocardial contractility.

Our secondary hypothesis was that Na^+/H^+-exchanger inhibition attenuated polymorphonuclear neutrophil (PMN)-mediated myocardial reperfusion injury. Faes and colleagues[16] demonstrated that Na^+/H^+-exchanger inhibition improved in vitro contractile function and decreased myocardial injury when hearts were perfused with stimulated PMNs. Our study did not directly evaluate PMN-mediated myocardial injury. However, we infer that Na^+/H^+-exchanger inhibition probably does not alter PMN-mediated myocardial I-R injury because there is no difference in myocardial leukosequestration between groups. If Na^+/H^+-exchanger inhibition altered local or systemic PMN priming-activation, myocardial leukosequestration should decrease and be reflected in decreased myocardial MPO.

As with any technique that models the clinical situation, there are inherent limitations to this study. First and foremost is that healthy animals, and not human subjects, with ischemic hearts were used. Moreover, dogs may be more prone to development of myocardial edema than human subjects. Finally, many myocardial protection techniques in use today may alter these results, including temperature, methods of reperfusion, and the use of blood and various other drugs as perfusate additives.

In summary, Na^+/H^+-exchanger inhibition delivered before global myocardial I-R in a clinically relevant model of CPB/CPA improved post-CPB/CPA myocardial contractility and edema formation. This effect does not appear to be related to PMN-mediated myocardial injury.

We thank COBE Cardiovascular, Inc, Arvada, Colorado, for their donation of perfusion equipment and oxygenators. EMD 96 785 was provided by Drs Norbert Beier, Wolfgang Scholz, and Claudia Wilm of Merck KGaA, Darmstadt, Germany. We also thank John Frederick and Matthew Murray for technical assistance.

References

1. Hearse DJ, Stewart DA, Braimbridge MV. Cellular protection during myocardial ischemia: the development and characterization of a procedure for the induction of reversible ischemic arrest. *Circulation.* 1976;54:193-202.
2. Mehlhorn U, Davis KL, Burke EJ, Adams D, Laine GA, Allen SJ. Impact of cardiopulmonary bypass and cardioplegic arrest on myocardial lymphatic function. *Am J Physiol.* 1995;268:H178-83.
3. Mehlhorn U, Allen SJ, Davis KL, Geissler HJ, Warters RD, de Vivie ER. Increasing the colloid osmotic pressure of cardiopulmonary bypass prime and normothermic blood cardioplegia minimizes myocardial oedema and prevents cardiac dysfunction. *Cardiovasc Surg.* 1998; 6:274-81.
4. Mehlhorn U, Allen SJ, Adams DL, Davis KL, Gogola GR, Warters RD. Cardiac surgical conditions induced by β-blockade: effect on myocardial fluid balance. *Ann Thorac Surg.* 1996;62:143-50.
5. Allen SJ, Geissler HJ, Davis KL, Gogola GR, Warters RD, de Vivie ER. Augmenting cardiac contractility hastens myocardial edema resolution after cardiopulmonary bypass and cardioplegic arrest. *Anesth Analg.* 1997;85:987-92.
6. Laine GA, Allen SJ. Left ventricular myocardial edema- Lymph flow, interstitial fibrosis and cardiac function. *Circ Res.* 1991;68:1713-21.
7. Choy IO, Schepkin VD, Budinger TF, Obayashi DY, Young JN, DeCampli WM. Effects of specific sodium/hydrogen exchange inhibitor during cardioplegic arrest. *Ann Thorac Surg.* 1997;64:94-9.
8. Klein HH, Pich SP, Bohle RM, Wollenweber J, Nebendahal K. Myocardial protection by Na^+/H^+ exchange inhibition in ischemic reperfused porcine hearts. *Circulation.* 1995;92:912-7.
9. Hendrixx M, Mubagwa K, Verdonck F, Overloop K, Van Hecke P, Vanstapel F, et al. New Na^+/H^+ exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca^{2+} overload in isolated perfused rabbit heart. *Circulation.* 1994;89:87-98.
10. Yasutake M, Ibuki C, Hearse DJ, Avkiran M. Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am J Physiol.* 1994;267:H2430-40.
11. Piper HM, Balser C, Ladilov YV, Schafer M, Siegmund B, Ruiz-Meana M, et al. The role of Na^+/H^+ exchange in ischemia-reperfusion. *Basic Res Cardiol.* 1996;91:191-202.
12. Pierce GN, Czubryt MP. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J Mol Cell Cardiol.* 1995;27:53-63.
13. TenHove M, Van Emous JG, vanEchteld CJA. Na^+ overload during ischemia and reperfusion in rat hearts: Comparison of the Na^+/H^+-exchange blockers EIPA, HOE642, and EMD96785. In: Proceedings of the 5th International Symposium of Magnetic Resonance in Cardiovascular Research. In press.
14. Inserte J, Garcia-Dorado D, Ruiz-Meana M, Solares J, Soler J. The role of Na^+-H^+ exchange occurring during hypoxia in the genesis of reoxygenation-induced myocardial oedema. *J Mol Cell Cardiol.* 1997; 29:1167-75.
15. Tritto FP, Inserte J, Garcia-Dorado D, Ruiz-Meana M, Soler-Soler J. Sodium/hydrogen exchanger inhibition reduces myocardial reperfusion edema after normothermic cardioplegia. *J Thorac Cardiovasc Surg.* 1998;115:709-15.
16. Faes FC, Sawa Y, Ichikawa H, Shimazaki Y, Ohashi T, Fukuda H, et al. Inhibition of Na^+/H^+ exchanger attenuates neutrophil-mediated reperfusion injury. *Ann Thorac Surg.* 1995;60:377-81.
17. Muntz KH, Hagler HK, Boulas HJ, Willerson JT, Buja LM. Redistribution of catecholamines in the ischemic zone of the dog heart. *Am J Pathol.* 1984;114:64-78.
18. Bradley PP, Priebat DA, Christensen RD, Rothstein GR. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol.* 1982;78:206-10.
19. Mullane KM, Kraemer R, Smith G. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J Pharmacol Methods.* 1985;14:157-60.
20. Bretschneider HJ, Hubner G, Knoll D. Myocardial resistance and tolerance to ischemia: physiological and biochemical basis. *J Cardiovasc Surg.* 1975;16:241-60.
21. Askenasy N, Vivi A, Tassini M, Navon G. The relation between cellular sodium, pH, and volumes and the activity of Na^+/H^+ antiport during hypothermic ischemia: multinuclear NMR studies of rat hearts. *J Mol Cell Cardiol.* 1996;28:589-601.
22. Demaurex N, Grinstein S. Na^+/H^+ antiport: modulation by ATP and role in cell volume regulation. *J Exp Biol.* 1994;196:389-404.
23. Ludwig LL, Schertel ER, Pratt JW, McClure DE, Ying AJ, Heck CF, et al. Impairement of left ventricular function by acute cardiac lymphatic obstruction. *Cardiovasc Res.* 1997;33:164-71.
24. Miyamoto M, McClure DE, Schertel ER, Andrews PJ, Jones GA, Pratt JW, et al. Effects of hypoproteinemia-induced myocardial edema on left ventricular function. *Am J Physiol.* 1998;274:H937-44.
25. Laine GA, Allen SJ. Left ventricular myocardial edema. *Circ Res.* 1991;68:1713-21.
26. Fliegel L, Wang H. Regulation of the Na^+/H^+ exchanger in the mammalian myocardium. *J Mol Cell Cardiol.* 1997;29:1991-7.
27. Opie LH, Coetzee WA. Role of calcium ions in reperfusion arrythmias: relevance to pharmacologic intervention. *Cardiovasc Drugs Ther.* 1988;2:623-36.
28. Gumina RJ, Mizumura T, Beier N. A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before and after coronary artery occlusion. *J Pharmacol Exp Ther.* 1998;286:175-83.