Improved Myocardial Function Using a Na⁺/H⁺ Exchanger Inhibitor During Cardioplegic Arrest and Cardiopulmonary Bypass*

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Introduction: We have demonstrated that a component of post-cardiopulmonary bypass (CPB)/cardioplegic arrest (CPA) myocardial dysfunction is related to myocardial edema. Myocardial ischemia/reperfusion that occurs with CPB/CPA activates the Na⁺/H⁺ exchanger to normalize intracellular pH, with intracellular Na⁺ (and water) accumulation. We hypothesized that Na⁺/H⁺ exchanger inhibition with a selective inhibitor (EMD 87580) would decrease myocardial edema and improve myocardial performance after CPB/CPA.

Methods: Anesthetized dogs (n = 14) were instrumented with myocardial ultrasonic crystals, and left ventricular (LV) micromanometer, to study myocardial function. Myocardial tissue water (MWC) was determined using microgravimetry. Treated animals (n = 5) received EMD 87580 (5 mg/kg IV pretreatment and 10 mol/L cardioplegia); control animals (n = 9) received a saline vehicle. After baseline, hypothermic CPB/CPA was initiated for 2 h, followed by reperfusion/rewarming for 45 min and separation from CPB. Myocardial function parameters and MWC were measured at 30 min, 60 min, and 120 min after CPB.

Results: Preload recruitable stroke work did not decrease from baseline in EMD 87580-treated animals, and was significantly greater in EMD 87580-treated animals than control animals at 120 min after CPB. At a similar LV end-diastolic volume, the maximal rate of rise of LV pressure (dp/dtMAX) was significantly decreased from baseline at all time points in control animals, and unchanged in EMD 87580-treated animals. MWC increased with CPB/CPA in both groups, with no difference between groups. There was no difference in dp/dtMAX or slope of the end-diastolic pressure-volume relationship.

Conclusion: Na⁺/H⁺ exchanger inhibition improves systolic but not diastolic function after CPB/CPA. This is not due to a reduction in MWC. (CHEST 2003; 123:187–194)

Key words: edema; myocardial fluid balance; myocardial protection

Abbreviations: CPA = cardioplegic arrest; CPB = cardiopulmonary bypass; dp/dtMAX = maximal rate of rise of left ventricular pressure; EDPVR = end-diastolic pressure-volume relationship; I/R = ischemia/reperfusion; LV = left ventricular/left ventricle; LVEDV = left ventricular end-diastolic volume; MWC = myocardial tissue water; PRSW = preload recruitable stroke work; RLV = radius of the left ventricle; SGDRY = specific gravity of the dry myocardium; SGMYO = specific gravity of the myocardial sample; SW = stroke work; VLV = left ventricular volume.

The introduction of cardioplegia has dramatically improved myocardial protection during cardiac surgery. However, cardiopulmonary bypass (CPB) and cardioplegic arrest (CPA) is a form of global myocardial ischemia/reperfusion (I/R) that can induce post-CPB myocardial dysfunction. Although most patients undergoing CPB/CPA do well, some patients with limited myocardial reserve may be difficult to separate from CPB. While the causes of post-CPB myocardial contractile dysfunction are multifactorial, our laboratory has focused on the relationship between myocardial edema and post-CPB myocardial dysfunction. 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.
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. However, intracellular Na⁺ accumulation may lead to a concomitant increase in intracellular water. Studies have shown that myocardial Na⁺/H⁺ exchange inhibition preserves myocardial performance after global I/R. A multicenter clinical trial studied the effects of a selective Na⁺/H⁺ exchange inhibitor, cariporide, on myocardial revascularization using CPB/CPA. EMD 87580 is a Na⁺/H⁺ exchange inhibitor that is selective for the myocardial isoform (Na⁺/H⁺ exchange [NHE]-1). We hypothesized that Na⁺/H⁺ exchange inhibition during I/R associated with CPB/CPA would decrease myocardial edema by decreasing intracellular Na⁺ accumulation and intracellular edema, thus improving post-CPB/CPA myocardial performance.

**Materials and Methods**

**Animal Preparation**

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the 1985 guidelines for care and use of laboratory animals of the National Institutes of Health. Conditioned mongrel dogs (n = 14) of either sex (36% female/equally distributed) were anesthetized by IV administration of 25 mg/kg thiopental sodium, and underwent tracheal intubation and mechanical ventilation with 100% oxygen using a volume-cycled respirator (Siemens-Elema AB; Syndbyerg, Sweden). Anesthesia was maintained with IV infusion of 1% thiopental sodium in Ringer’s 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 thermolabelled catheter was inserted into the pulmonary artery via 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 via a snare-secured purse stitch for coronary sinus blood sampling. An unibladder tape was placed around the inferior vena cava for cardiac preload manipulation. A micromanometer-tipped pressure transducer (Millar Instruments; Houston, TX) was introduced into the left ventricular (LV) cavity through the apex. Sonomicrometry crystals (10 MHz, Sonometrics; London, ON, Canada) were placed in the LV subendocardium across the septum/free-wall axis of the LV. The crystals were then connected to a sonomicrometer (Triton Technology; Watsonville, CA) for processing of signals.

**Hemodynamics and Preload Recrutable Stroke Work**

Systolic function was determined using preload recruitable stroke work (PRSW) and the maximal rate of rise of LV pressure (dp/dtmax)/LV end-diastolic volume (LVEDV) relationship. Diastolic function was determined using the slope of the end-diastolic pressure-volume relationship (EDPVR) during preload manipulation (see below). The diastolic isovolumic relaxation constant (τ) was used as a measure of diastolic function. Hemodynamic data were simultaneously logged into a Macintosh Quadra 700 computer via an analog-to-digital data acquisition device (MacLab; World Precision Instruments; New Haven, CT). Cardiac output was determined in duplicate by injecting 10 mL of ice-cold Ringer’s solution. Mean arterial pressure and pulmonary artery pressure/pulmonary artery occlusion pressure were measured with arterial catheters interfaced to pressure transducers. LV pressure was measured with a micromanometer, and the LV septum/free-wall diameter was obtained with a sonomicrometer. These data were recorded at a frequency of 200 Hz during 10 s of inferior vena cava occlusion (Sonolabs/Sonoview; Sonometrics; London, ON, Canada). If we assume a spherical shape, the LV volume (V_LV) in milliliters can be calculated from the following equation:

\[ V_{LV} = \left(\frac{4}{3}\right) \times \pi \times (r_{LV})^3 \]

where \( r_{LV} \) is the radius of the LV. Replacing \( r_{LV} \) with \( (10^{-4} \times d_{LV})^2 \) yields:

\[ V_{LV} = \pi \times (d_{LV})^3 \times 6,000 \]

where \( d_{LV} \) is diameter of the LV.

PRSW was calculated as the slope of the relation between LVEDV and LV stroke work (SW) from pressure-volume loops obtained using inflow occlusion of the inferior vena cava. SW (in milliliters times millimeters of mercury) was calculated as follows:

\[ SW = SV \times (M EP - E DP) \]

where \( SV \) is the LV stroke volume; MEP is the LV mean ejection pressure, and is measured from ejection onset to end-ejection; ejection onset was defined at 10 ms after the time of + dp/dtmax; end-ejection was defined at the time of − dp/dtmax; and EDP is the LV end-diastolic pressure. LVEDV was plotted against + dp/dtmax at each time point. Comparisons were made from baseline within the group. The slope of the EDPVR was determined on each set of pressure-volume loops, and the linear portion of the slope was determined using computerized line-fitting software (Sonolabs/Sonoview), and expressed in millimeters of mercury per milliliter. The time constant for isovolumic relaxation (τ) was measured in milliseconds. All measurements were taken in duplicate.

**Myocardial Water Content Determination**

Myocardial water content for edema quantification was measured using a microgravimetric technique as previously described. With a biopsy needle (Tru-Cut; Baxter Healthcare; Deerfield, IL), transmyocardial biopsy samples were taken from the LV anterior or anterolateral wall. The specific density of these samples was measured in a linear density gradient consisting of bromobenzene and kerosene. Knowing myocardial specific density, the percentage of gram water per gram tissue or myocardial water content (MWC) can be calculated using the following equation:

\[ MWC = \left[1 - \left(SG_{MYO} - 1\right) / \left(1 - SG_{DRY} \times SG_{MYO}\right)\right] \times 100 \]

where \( SG_{MYO} \) and \( SG_{DRY} \) are the specific gravities of the myocardial sample and of dry myocardium, respectively. At the end of the experiment, the dogs were killed and the hearts rapidly frozen.
excised, after which both ventricles were weighed and dried to a constant weight at 60°C. We calculated SGDRY in grams using the equation:

\[
\text{SGDRY} = \frac{1}{1 - [(\text{SGMYO} - 1) \times \frac{W}{D} \times \text{SGMYO}]} \]

where W and D are wet and dry weights of both ventricles. We assumed that SGDRY did not change over time. Measurements were performed in triplicate.

**CPB Techniques**

After instrumentation, heparin, 250 IU/kg, was administered IV for systemic anticoagulation followed by additional doses of 100 IU/kg administered every 60 min throughout the experiment. Extracorporeal circulation was performed using a 14F arterial perfusion cannula placed into the right femoral artery and a two-stage venous cannula (36F and 46F) in the right atrium/inferior vena cava. The LV was vented with a 12F catheter inserted via the left atrium. The extracorporeal circuit (Model 7000; Sarns; Ann Arbor, MI) and the membrane oxygenator (HVRF 3700; Cobe Cardiovascular; Arvada, CO) were primed with 800 mL of Ringer’s solution and 1,000 IU of heparin. Cardioplegic arrest lasted for 2 h. We administered an additional dose of 100 mL of cardioplegia after 60 min of cardiac arrest. Two hours of CPA were chosen to mimic a longer-than-standard cardiac procedure in which adjuncts to myocardial protection would be most useful. We have not noticed resumption of electrical or mechanical activity using the single additional dose as described. External myocardial cooling was accomplished by iced (4°C) saline solution instillation into the pericardium. Hearts were then reperfused on normothermic CPB for 40 min. Thereafter, we weaned the dogs from CPB and removed all cannulae. No inotropes or antiarrhythmic agents were used. During ischemia, animals were systematically cooled to 28°C using a heat exchanger.\(^6\) We kept CPB flow between 40 mL/kg/min and 60 mL/kg/min at 28°C to maintain a mean systemic perfusion pressure of 40 to 70 mm Hg.

**Experimental Design**

Two groups were studied. The treatment group (n = 5) received EMD 87580 at a dose of 5 mg/kg IV bolus 5 min before aortic cross-clamping, and 10 mol/L EMD 87580 in the cardioplegic solution. The control group (n = 9) received the same volume of saline vehicle. Systemic pretreatment was used to ensure drug availability during the ischemic period prior to CPA. Direct infusion via cardioplegia ensures drug delivery at the time of ischemia (CPA), and it decreases the variability of drug delivery that may occur during the hemodilution with CPB initiation. Incorporation into the cardioplegia also minimizes the potential variable of drug/extracorporeal circuit interactions. Using this dosing regimen, the plasma concentration of EMD 87580 was in the 2,500 to 3,000 ng/mL range at 10 min after CPB. This concentration has been shown to be effective at Na⁺/H⁺ exchanger inhibition in vitro.

**Experimental Protocol**

After 30 min for stabilization following the completion of instrumentation, preischemic hemodynamic values were recorded. Myocardial samples for MWC determination were collected as described above. Additionally, MWC was determined at 30 min, 60 min, and 120 min after separation from CPB.

**Statistical Analysis**

All data presented are mean ± SEM. Data analysis was carried out using Statistica software (StatSoft; Tulsa, OK). Time courses of each measured parameter were examined using analysis of variance for repeated measures andTukey’s test. Between-group time-point comparisons were made using Student t test for unpaired data. A value of p < 0.05 was considered significantly different.

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**Table 1—Hemodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 min After Bypass</th>
<th>60 min After Bypass</th>
<th>120 min After Bypass</th>
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<tr>
<td><strong>Cardiac index, L/min/m²</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>2.8 ± 0.3</td>
<td>2.0 ± 0.01†</td>
<td>1.7 ± 0.1†</td>
<td>1.4 ± 0.1†</td>
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<td>2.8 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2†</td>
<td>1.7 ± 0.1†</td>
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<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>127 ± 6</td>
<td>96 ± 5†</td>
<td>94 ± 6†</td>
<td>97 ± 7†</td>
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<tr>
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<td>130 ± 7</td>
<td>112 ± 10</td>
<td>128 ± 6†</td>
<td>120 ± 11</td>
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<td></td>
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<tr>
<td>Control</td>
<td>18 ± 1</td>
<td>21 ± 1†</td>
<td>21 ± 1†</td>
<td>21 ± 1†</td>
</tr>
<tr>
<td>EMD 87580</td>
<td>20 ± 1</td>
<td>20 ± 1</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
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<tr>
<td><strong>Pulmonary artery occlusion pressure, mm Hg</strong></td>
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<tr>
<td>Control</td>
<td>14 ± 1</td>
<td>16 ± 1</td>
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<td>17 ± 1</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
<td>17 ± 1</td>
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<tr>
<td><strong>EDPVR, mm Hg/mL</strong></td>
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<tr>
<td>Control</td>
<td>0.40 ± 0.09</td>
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<td>EMD 87580</td>
<td>0.26 ± 0.02</td>
<td>0.28 ± 0.04</td>
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<td>0.28 ± 0.03</td>
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<tr>
<td><strong>τ, ms</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
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<td>32 ± 3</td>
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<td>38 ± 7</td>
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<tr>
<td>EMD 87580</td>
<td>26 ± 1</td>
<td>29 ± 2</td>
<td>35 ± 1</td>
<td>35 ± 3</td>
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</table>

*Data are presented as mean ± SEM (control, n = 9; EMD 87580, n = 5).
†p < 0.05 vs baseline.
‡p < 0.05 between groups.
RESULTS

Hemodynamic parameters are shown in Table 1. Cardiac index decreased in both groups after CPB/CPA. Cardiac index was significantly lower at all time points after CPB/CPA in control animals, and lower at 120 min after CPB/CPA in EMD 87580-treated animals. Cardiac index was significantly lower in control animals compared to EMD 87580-treated animals at 60 min after CPB/CPA. Systolic function as measured by PRSW significantly decreased after CPB/CPA in control animals and was significantly lower than EMD 87580-treated animals at 120 min after CPB/CPA (Fig 1). Systolic function was also measured by plotting LVEDV vs. +dp/dtmax (Figs 2, 3). At a constant LVEDV, +dp/dtmax significantly decreased after CPB/CPA in control animals. At a constant LVEDV, there was no decrease in +dp/dtmax after CPB/CPA in EMD 87580-treated animals. Diastolic function as measured by the slope of the EDPVR was not significantly altered by CPB/CPA in either group (Table 1). τ was not significantly different between groups. Myocardial tissue water increased with the institution of CPB/CPA and was statistically greater than baseline in both groups (Fig 4). There were no differences between groups.

DISCUSSION

Our study shows that Na\(^+/\)H\(^+\) exchange inhibition with EMD 87580 prevents the post-CPB/CPA reduction in myocardial contractility. This effect is not due to a reduction in post-CPB/CPA myocardial edema.

The Na\(^+/\)H\(^+\) exchanger normalizes intracellular pH during intracellular lactate accumulation that occurs with myocardial ischemia. Na\(^+/\)H\(^+\) exchanger activity during ischemia results in Na\(^+\) influx into the myocardium.
cell. A secondary effect of this phenomenon is a reversal of the Na⁺/Ca²⁺ exchanger resulting in an increase in intracellular Ca²⁺. Increased intracellular Ca²⁺ within the myocyte may alter Ca²⁺ kinetics of excitation-contraction coupling or Ca²⁺ cycling between the contractile apparatus and the sarcoplasmic reticulum leading to myocardial contracture ("stone heart"). Numerous studies have demonstrated attenuation in the expected rise in LV end-diastolic pressure after myocardial I/R using Na⁺/H⁺ exchange inhibition. Increased intracellular Ca²⁺ may initiate reperfusion-induced arrhythmias through a mechanism involving oscillatory release of Ca²⁺ from the sarcoplasmic reticulum, thus inducing delayed afterpolarizations. During reperfusion, lactate is removed and there is an intracellular "alkaline overshoot." Intracellular alkalization has a potent sensitizing effect on myocardial contractile proteins to Ca²⁺ resulting in increasing myocardial resting tension and contractile dysfunction. Therefore, Na⁺/H⁺ exchanger inhibition may improve post-CPB/CPA myocardial contractility and function in four ways: (1) limiting intracellular Na⁺ gain (and water) during myocardial ischemia, (2) suppression of postreperfusion contractile and diastolic dysfunction caused by myofilament sensitization by the alkaline overshoot, (3) limiting intracellular Ca²⁺ overload with secondary hypercontracture and/or progression to infarction, and (4) prevention of Ca²⁺ overload-mediated reperfusion arrhythmias.

We demonstrated a preservation of systolic func-

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**Figure 2.** Ventricular pressure-volume relationship, control animals. + dp/dtMAX is plotted vs LVEDV to examine within-group contractility comparing baseline to post-CPB/CPA time periods. At a constant LVEDV, there is significant reduction of + dp/dtMAX after CPB/CPA. This parallels the PRSW data demonstrating a reduction of LV systolic function. dp/dt = rate of rise of LV pressure; see Figure 1 legend for expansion of abbreviations.
tion after CPB/CPA using two measures of contractility. We have previously demonstrated a decrease in PRSW after CPB/CPA, and our current results are consistent with previous reports.\textsuperscript{2-5} In this study, PRSW was preserved in the EMD 87580-treated animals and decreased after CPB/CPA in control animals. We also examined the within-group +\( \frac{dp}{dt} \)\textsubscript{max}/LVEDV relationship before and after CPB/CPA. +\( \frac{dp}{dt} \)\textsubscript{max} was preserved at a similar LVEDV in EMD 87580-treated animals with depression of +\( \frac{dp}{dt} \)\textsubscript{max} at similar LVEDV in control animals. Unlike isolated heart preparations, LVEDV cannot be directly controlled in this model. Moreover, the LVEDV data are dependent on the ultrasonic crystal placement that may vary between dogs due to anatomic differences.\textsuperscript{21} Therefore, the approximate 20\% between-group difference in the absolute value for LVEDV is not unexpected. While the baseline +\( \frac{dp}{dt} \)\textsubscript{max} is lower in EMD 87580-treated animals when compared to control animals, the change in +\( \frac{dp}{dt} \)\textsubscript{max} relative to baseline parallels the other myocardial performance data. Specifically, these contractility data correspond to the findings that cardiac index was significantly higher in EMD 87580-treated animals at 60 min after CPB/CPA.

Because both groups acquired myocardial edema, we anticipated a loss of myocardial compliance and diastolic dysfunction\textsuperscript{22}; however, there was no difference in the isovolumic relaxation constant or the EDPVR. While we did not demonstrate any difference in total myocardial edema, we did not differentiate between intracellular and interstitial edema. An imbalance in Starling forces with CPB/CPA (reduction in capillary oncotic pressure, decreased

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Ventricular pressure-volume relationship, EMD 87580-treated animals. +\( \frac{dp}{dt} \)\textsubscript{max} is plotted vs LVEDV to examine within-group contractility comparing baseline to post-CPB/CPA time periods. + denotes a significant difference compared to baseline. In contrast to control animals, at a constant LVEDV there is preservation in +\( \frac{dp}{dt} \)\textsubscript{max} after CPB/CPA. This parallels the PRSW data demonstrating preservation of LV systolic function. See Figure 1 legend for expansion of abbreviations.}
\end{figure}
myocardial lymph flow, and reduced interstitial pressure from diastolic relaxation) results primarily in interstitial myocardial edema. Na\(^+/\)H\(^+\) exchange inhibition would be expected to primarily decrease intracellular edema. Thus, we may not have been able to differentiate between changes in interstitial vs intracellular myocardial edema. Other techniques, such as morphometric analyses and tracer dilution methods, could possibly help differentiate interstitial from intracellular myocardial edema. Morphometric analyses are prone to artifacts that alter the interstitium, and can yield more qualitative data regarding edema. Although radiolabeled tracer methods can be used to determine cellular compartment volumes, we chose not to pursue this due to the volume of perfusion apparatus (among other items) that would be contaminated. Of note, in a porcine model of CPB/CPA, Goldberg et al\(^{23}\) showed that Na\(^+/\)H\(^+\) exchanger inhibition decreased intracellular myocardial water while total myocardial water remained unchanged.

A component of any model using CPB/CPA requires the use of anticoagulation—typically heparin sodium. Heparin has diverse effects on cardiovascular function after hemorrhagic and endotoxic shock, two models that share similar characteristics with CPB. Heparin has been shown to improve cardiac output, primarily by reducing afterload during hyperdynamic endotoxic shock,\(^{24}\) and improve organ function after hemorrhage/resuscitation.\(^{25}\) However, in clinical doses, there is a lesser effect in terms of endothelial inflammation/function.\(^{26}\) While there may be some heparin effect in this study, it was not specifically tested.

In summary, we demonstrated that Na\(^+/\)H\(^+\) exchange inhibition with EMD 87580 improved post-CPB/CPA systolic, but not diastolic function. The preservation of myocardial function was not due to a prevention of post-CPB/CPA myocardial edema. Na\(^+/\)H\(^+\) exchange inhibitors may be a useful adjunct to myocardial protection strategies to prevent post-CPB/CPA myocardial dysfunction.

Figure 4. MWC vs time. Myocardial water increases with the initiation of CPB/CPA. + denotes a significant difference compared to baseline. These differences are significant in both groups, comparing to baseline. There are no between-group differences. 10' CP = 10 min of cardioplegia; 60' CP = 60 min of cardioplegia; 110' CP = 110 min of cardioplegia; 15' REP = 15 min of reperfusion; see Figure 1 legend for expansion of abbreviations.
ACKNOWLEDGMENT: The authors thank Cobe Cardiovascular, Inc., for their donation of perfusion equipment and oxygenators. Merck KGaA provided EMD 87580 and research support. The authors also appreciate the technical assistance of Matthew Murray.

REFERENCES