TEMPORAL ENDOTHELIN DYNAMICS OF THE MYOCARDIAL INTERSTITIUM AND SYSTEMIC CIRCULATION IN CARDIOPULMONARY BYPASS

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Objective: Increased systemic levels of the bioactive peptide endothelin 1 during and after cardioplegic arrest and cardiopulmonary bypass have been well documented. However, endothelin 1 is synthesized locally, and therefore myocardial endothelin 1 production during and after cardiopulmonary bypass remains unknown.

Methods: Pigs ($n = 11$) were instrumented for cardiopulmonary bypass, and cardioplegic arrest was initiated. Myocardial interstitial and systemic arterial levels of endothelin 1 were measured before cardiopulmonary bypass, throughout bypass and cardioplegic arrest (90 minutes), and up to 90 minutes after separation from bypass. Myocardial interstitial endothelin 1 was determined by microdialysis and radioimmunoassay.

Results: Baseline myocardial endothelin 1 levels were higher than systemic endothelin 1 levels (25.6 ± 6.7 vs 8.3 ± 1.1 fmol/mL, $P < .05$). With the onset of bypass, myocardial endothelin 1 increased by $327\% \pm 92\%$ from baseline ($P < .05$), which preceded the increase in systemic endothelin 1 levels.

Conclusion: Myocardial compartmentalization of endothelin 1 exists in vivo. Cardiopulmonary bypass and cardioplegic arrest induce temporal differences in endothelin 1 levels within the myocardial interstitium and systemic circulation, which, in turn, may influence left ventricular function in the postbypass period. (J Thorac Cardiovasc Surg 2000;120:864-71)

Endothelin 1 (ET-1) is a potent bioactive peptide that has been clearly demonstrated to influence vascular resistance properties in the systemic, pulmonary, and coronary circulation.¹⁻⁴ Moreover, exposure of myocardial and isolated cardiac myocyte preparations to ET-1 has been shown to influence contractile performance.⁵⁻⁷ Increased ET-1 production has been reported in a number of cardiovascular disease states,⁸ including pulmonary hypertension,^{9,10} acute myocardial infar-

tion,^{11,12} and congestive heart failure.^{13,14} Increased ET-1 levels in the systemic circulation have also been identified during cardiac surgery requiring cardiopulmonary bypass (CPB) and cardioplegic arrest, which persists into the postoperative period.¹⁵⁻²¹ Thus, increased production, release, or both, of ET-1 after cardioplegic arrest and CPB may induce deleterious effects on vascular resistance properties in a number of circulatory beds and modify myocardial contractile performance.²²⁻²⁶

A number of cell types, including the cardiac myocyte, have been shown to synthesize and release ET-1.²⁷⁻²⁹ The synthesis and release of ET-1 into the local extracellular environment results in the activation of the ET-1 receptor in an autocrine-paracrine manner.³⁰⁻³² Increased systemic levels of ET-1 that occur with CPB likely reflect spillover from local tissue compartments.^{33,34} However, the actual local tissue levels of ET-1, particularly in the myocardial compartment, remain to be defined. Moreover, whether and to what degree myocardial ET-1 synthesis and release is influenced by CPB and cardioplegic arrest remains unknown. With the use of microdialysis techniques, it has been demonstrated that interrogation of the myocar-

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dial compartment is possible.³⁵⁻³⁷ Thus, the goals of this study were 2-fold: (1) to investigate the temporal production of circulating and local myocardial ET-1 during and after CPB and cardioplegic arrest and (2) to relate systemic circulating and myocardial interstitial ET-1 levels to left ventricular (LV) function and hemodynamic parameters after CPB and cardioplegic arrest.

Methods

Instrumentation. Pigs ($n = 11$, 45 kg; Hambone Farms) were instrumented to assess LV function and systemic hemodynamics.³⁸ The pigs were anesthetized with a bolus of sufentanil (1 $\mu\text{g}/\text{kg}$, ESI-Elkins-Sinn Inc), and anesthesia was maintained with an intravenous infusion of sufentanil (0.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). After intubation, vecuronium bromide (0.1 mg/kg, Organon Inc, West Orange, NJ) was administered, and this dose was repeated every 45 minutes. An arterial line (7.5F) was placed in the carotid artery. A multilumen thermodilution catheter (7.5F, Baxter Healthcare Corp, Irvine, Calif) was positioned in the pulmonary artery. The aortic and pulmonary artery catheters were connected to externally calibrated transducers (Statham P231D, Gould, Inc, Oxnard, Calif). A sternotomy was performed, the great vessels were isolated, and a vascular ligature was placed around the inferior vena cava to perform transient caval occlusion.³⁸ A precalibrated microtipped transducer (7.5F, Millar Instruments Inc, Houston, Tex) was placed through the LV apex and sutured in place. A flow probe was placed around the ascending aorta and connected to a digital flowmeter (HT107; Transonic Systems, Inc, Ithaca, NY) for the continuous measurement of LV stroke volume and cardiac output. The electrocardiograms, pressure waveforms, flow probes, and crystal signals were digitized for subsequent analysis at a sampling frequency of 250 Hz (80386 processor; Zenith Data Systems, St Joseph, Mo).

A microdialysis probe was placed into an anterolateral section of LV myocardium and prepared as described in the following section. After a 20-minute equilibration period, baseline hemodynamics were recorded.

CPB. After collection of baseline hemodynamics and LV function, the pigs were anticoagulated with sodium heparin (300 U/kg) to achieve an extended clotting time of greater than 400 seconds (ACTII, Medtronic Hemotec, Inc, Parker, Colo). A purse-string suture was placed around the right atrial appendage, and a venous return cannula (34F, CR Bard Inc, Santa Ana, Calif) was placed in the inferior vena cava. An aortic cannula (3.8 mm, Sarns Inc, Ann Arbor, Mich) was placed in the ascending aorta and sutured in place. The aortic and venous cannulas were connected to a previously primed CPB circuit containing a membrane oxygenator (Bentley Univox Spiral Gold; Bentley Laboratories, Irvine, Calif) and driven by a modular roller pump (Sarns 5000; 3M Healthcare, Ann Arbor, Mich). A 12-gauge catheter (DLP Inc, Grand Rapids, Mich) was placed at the root of the aorta for infusion of cardioplegic solutions. After cannulation, CPB was initiated, the aorta was crossclamped, and an oxygenated, hypothermic, crystalloid cardioplegic solution (4°C; 500 mL; Na^+ , 130

mmol/L; Cl^- , 109 mmol/L; K^+ , 24 mmol/L; Ca^{2+} , 1.8 mmol/L; HCO_3^- , 30 mEq/L) was delivered through the aortic root cannula. CPB continued for 90 minutes, and 500 mL of cardioplegic solution was administered every 30 minutes after the initial cardioplegic dose. During CPB, systemic hypothermia was not used, and total flow was maintained within 2.5 to 3.0 L/min. At the completion of the cardioplegic arrest period, the crossclamp was removed, and reperfusion of the myocardium was initiated. Lidocaine (2 mg/kg) was administered at reperfusion, and if ventricular fibrillation occurred during the initial reperfusion period, defibrillation was performed at 20 joules with internal paddles. After a 10-minute period to allow for resumption of adequate LV function, CPB was discontinued. No inotropic or vasoactive agents were used at any time during the protocol, and protamine was not administered. None of the animals included in the present study required cardioversion after aortic cross-clamp release and CPB cessation. LV function and systemic hemodynamics were recorded at 30, 60, and 90 minutes after crossclamp removal and separation from CPB.

Myocardial microdialysis and plasma collection.

Myocardial microdialysis studies have been performed previously for angiotensin II, and the approach was adapted for this study.^{39,40} A microdialysis probe containing a 4-mm long membrane was used (20 kD; outer diameter of probe shaft, 0.77 mm; outer diameter of probe membrane, 0.5 mm; CMA/Microdialysis, North Chelmsford, Mass). The probe was immersed in Krebs buffer containing 0.5% bovine serum albumin and various concentrations of iodine 125 (¹²⁵I)-labeled ET-1 (40-100 fmol/mL). The probe was then perfused with the same buffer by a precision infusion syringe pump (Bioanalytical Systems, West Lafayette, Ind) at various flow rates (1, 2.5, and 5.0 $\mu\text{L}/\text{min}$). The effluent was collected from the outflow tube at 30-minute sample periods. The relative recovery of ¹²⁵I-labeled ET-1 was calculated as the percentage of radioactivity (in counts per minute) in the perfusate to the radioactivity in the buffer. On the basis of these in vitro calibration experiments, the relative recovery of ET-1 at 2.5 $\mu\text{L}/\text{min}$ flow rate was calculated to be $15\% \pm 1\%$ at various concentrations of ¹²⁵I-labeled ET-1 (40-100 fmol/mL), and this value was used as the correction factor for recovery of microdialysis samples.

The microdialysis probe was connected to the microdialysis perfusion pump and flushed with Krebs buffer solution containing 0.5% bovine serum albumin for 30 minutes to allow for equilibration. This equilibration period was determined through repeated measurements taken at 30-minute time intervals after probe insertion. The mean ET-1 values for these collection time points were not statistically different, and thus it was determined that an equilibration period of 30 minutes was sufficient. A microdialysis sample was collected before CPB and cardioplegic arrest to establish baseline myocardial interstitial ET-1 levels. Microdialysis samples were collected at 30-minute intervals throughout CPB and up to 90 minutes after crossclamp removal and separation from CPB. The samples were collected into chilled microcentrifuge tubes and stored at -70°C until time of assay.

Fig 1. A, Linear regression analysis for microlevels of ET-1 (0.003-1 fmol/mL) using an optimized radioimmunoassay technique. This analysis allowed for the accurate measurement of ET-1 from myocardial microdialysis samples. The regression analysis was constructed to encompass the levels of ET-1 within the myocardial dialysate samples. The x-axis denotes the quotient of bound and total ligand (ET-1), and the y-axis denotes the logarithmic transformation for known ET-1 concentrations. **B,** Representative photomicrograph of a histologic cross section of the myocardium with the microdialysis probe in situ. No major blood vessels were observed in the proximity of the probe. Placement of the probe caused minimal trauma to the surrounding parenchyma, as evidenced by no extravasation of red blood cells and minimal inflammatory response. (Final magnification 60 $\times$.)

Plasma ET-1 samples were obtained at the end of corresponding microdialysis collection time points.

ET-1 measurement. Plasma and dialysate were first eluted over a cation exchange column (C-18 Sep-Pak; Waters Associates, Milford, Mass) and then dried by vacuum centrifugation. Recovery from the extraction procedure was 70% $\pm$ 5% on the basis of spiked plasma and microdialysis standards (0.003-32 fmol/mL). The samples were reconstituted in 0.02 mol/L borate buffer, and a high-sensitivity radioimmunoassay was performed (RPA 545, Amersham, Arlington Heights, Ill). After incubation of samples with 125 I-labeled ET-1 and an ET-1-specific antibody, a charged secondary antibody was added. The final solution was placed in a magnetic field, and bound and free labels were separated by means of magnetic separation. To support the range of ET-1 within the two samples, we constructed two sets of standards: a low range (0.003-1 fmol/mL) for the microdialysis samples and a high range (2-32 fmol/mL) for the plasma samples. A standard linear curve was generated by using regression analysis (Fig 1, A). The myocardial interstitial ET-1 levels were corrected for both the microdialysis and extraction procedures. The interassay variation was 10% and 9% for the plasma and microdialysis ET-1 measurements, respectively.

Data analysis. Indices of LV function, hemodynamics, and ET-1 levels after CPB and cardioplegic arrest were initially compared with baseline values by analysis of variance. Specific comparisons between pre-CPB and post-CPB values were performed by a Bonferroni adjusted *t* test. All statistical analyses were performed by means of statistical software programs (BMDP Statistical Software Inc, University of California Press, Los Angeles, Calif). Results are presented as means $\pm$ SEM.

Results

Microdialysis probe storage. After the final set of sample collections and hemodynamic measurements, the LV region containing the microdialysis probe was placed in a buffered formalin solution and maintained in situ for histologic analysis. The microdialysis probe was not located in the proximity of any major coronary vessels and caused minimal trauma to the surrounding tissue (Fig 1, B).

LV function and hemodynamics. Systemic hemodynamics and LV function at baseline and after CPB are presented in Table I. Mean arterial pressure, stroke volume, and systemic vascular resistance were reduced

Fig 2. Pulmonary vascular resistance was increased from baseline throughout the post-CPB period. Pulmonary vascular resistance increased further at 60 minutes after CPB (* $P < .05$ vs baseline; + $P < .05$ vs 30 minutes after CPB).

Table I. Systemic hemodynamics and cardiac performance before and after cardioplegic arrest and CPB

	Steady-state baseline	Time after CPB and cardioplegic arrest (min)		
		30	60	90
Heart rate (beat/min)	78 ± 4	119 ± 11*	127 ± 10*	129 ± 9*
Cardiac output (L/min)	4.2 ± 0.3	3.7 ± 0.4	3.6 ± 0.4	3.3 ± 0.4
Mean arterial pressure (mm Hg)	100 ± 3	61 ± 3*	63 ± 3*	63 ± 3*
Stroke volume (mL/beat)	39.8 ± 3.0	24.5 ± 2.9*	22.2 ± 2.6*	19.5 ± 2.6*
Mean pulmonary arterial pressure (mm Hg)	11.7 ± 1.4	17.7 ± 1.5*	22.1 ± 1.3*†	24.3 ± 1.1*†
Systemic vascular resistance (mm Hg · min ⁻¹ · mL ⁻¹)	1849 ± 167	1149 ± 121*	1231 ± 177*	1313 ± 215*
Sample size (n)	11	11	11	11

* $P < .05$ versus baseline.

† $P < .05$ versus 30 minutes after CPB and cardioplegic arrest.

after CPB. Heart rate and mean pulmonary arterial pressure were increased after separation from CPB. Pulmonary vascular resistance (Fig 2) was measured before and after CPB and cardioplegic arrest. Pulmonary vascular resistance increased from baseline at both 30 and 60 minutes after separation from CPB.

Arterial and myocardial interstitial ET-1. Baseline myocardial dialysate ET-1 levels were 3-fold higher than systemic ET-1 levels (Fig 3). Systemic ET-1 levels were increased at 60 minutes and reached a plateau at 90 minutes during CPB and cardioplegic arrest. Myocardial dialysate ET-1 levels also appeared to increase during CPB; however, the high degree of variability in these values failed to reflect the intrinsic changes in ET-1 from preparation to preparation. To address this issue, we computed the percentage change in systemic and myocardial dialysate ET-1 levels with respect to baseline values (Fig 3). The relative change

in myocardial dialysate ET-1 levels increased by over 300% from baseline at 30 minutes after CPB and induction of cardioplegic arrest. This increase in myocardial dialysate ET-1 appeared to plateau with a longer duration of CPB. Systemic arterial ET-1 levels increased by over 200% at 60 minutes after cardioplegic arrest and CPB. The robust and early increase in myocardial dialysate ET-1 levels at the onset of CPB occurred before a change in plasma arterial ET-1 levels.

Discussion

Postoperative LV pump dysfunction and hemodynamic instability can occur after cardioplegic arrest and CPB. One potential contributory factor for this phenomenon is the increased neurohormonal system activity that has been documented after CPB.⁴¹ Heightened levels of the bioactive peptide ET-1 have been reported to occur within the systemic circulation.¹⁵⁻²¹ Increased

Fig 3. *Top*, Arterial plasma ET-1 levels increased from baseline during cardioplegic arrest and CPB. Plasma ET-1 levels remained elevated after CPB. Myocardial interstitial ET-1 levels were higher than plasma arterial ET-1 levels at baseline and 30 minutes during CPB and appeared to increase further during and after CPB, but this did not reach statistical significance. *Bottom*, The percentage change in arterial plasma ET-1 from baseline increased at 60 minutes of CPB and reached a plateau at further time points. Myocardial ET-1 levels increased at 30 minutes of CPB, and this was higher than systemic arterial ET-1 levels. Moreover, the increase in myocardial ET-1 levels occurred earlier than that of systemic ET-1 levels (* $P < .05$ vs baseline; + $P < .05$ vs 60 minutes of CPB; # $P < .05$ vs arterial).

levels of ET-1 can produce a number of cardiovascular effects, which include increased vascular resistance and the modulation of myocardial contractility.²²⁻²⁶ However, the temporal dynamics of ET-1 release into the myocardial interstitium remains unknown. By using a porcine model of CPB and a microdialysis approach, the present study yielded two important find-

ings. First, this study clearly demonstrates that compartmentalization of ET-1 within the myocardium occurs and that these levels differ from those in the systemic circulation. Second, differences in temporal ET-1 dynamics occurred with the onset of CPB between the myocardial interstitium and systemic circulation. The changes in ET-1 that occur in these compartments

during and after CPB may have important effects on vascular resistance properties and myocardial contractility, respectively.

Several studies have reported increased circulating ET-1 levels in the CPB setting.¹⁵⁻²¹ For example, Knothe and associates²⁰ reported a 2-fold increase in ET-1 levels after CPB when compared with baseline values. In addition, Petrossian and coworkers²³ reported an association between pulmonary vascular resistance and increased ET-1 levels after CPB. Pulmonary vascular resistance increased after CPB and was accompanied by an increase in systemic ET-1 levels. Although ET-1 causes potent vasoconstriction,²⁹ computed systemic vascular resistance was reduced after CPB. This reduction in systemic vascular resistance was likely caused by several computational factors. First, despite a 50% reduction in stroke volume after CPB, cardiac output was maintained by a compensatory increase in heart rate. Second, mean arterial pressure significantly fell after CPB, which was likely caused by the absolute reduction in LV stroke volume. Thus the denominator in the vascular resistance computation remained unchanged, and the numerator fell. Therefore, it is likely that the change in the intrinsic compliance of the systemic vasculature after CPB was masked by these confounding factors.

ET-1 has been shown to directly influence myocardial contractile processes.^{6,7} Therefore, determining the levels of ET-1 within the myocardium during and after CPB may be of particular relevance. Past studies have attempted to address this issue by measuring coronary sinus levels in the setting of CPB.^{16,42} For example, Hasdai and coworkers¹⁶ reported a transient fall in coronary sinus levels during CPB, suggesting that increased myocardial ET-1 uptake may have occurred. The present study builds on these past reports through direct interrogation of the myocardial compartment through microdialysis to determine ET-1 dynamics during and after CPB. An early and robust increase in ET-1 levels occurred in the myocardial compartment with the onset of CPB, which persisted into the post-CPB period. These results suggest that increased ET-1 levels within the myocardium after CPB may directly influence myocardial contractile function.

Under normal resting conditions, circulating levels of ET-1 are very low. This is primarily the result of local ET-1 production, which is sequestered in the intracellular space, as well as being bound to ET-1 receptor systems.²⁹ Thus, increased detectable levels of ET-1 within the systemic circulation most likely reflect a *spillover* phenomenon from local tissue compartments. The spillover of ET-1 into the systemic circulation with the onset of CPB is likely caused by several factors.

First, increased synthesis and release of ET-1 can occur, which overwhelms local ET-1-binding sites and egresses into the systemic circulation. Second, CPB may induce release of endogenous stores of ET-1, which, in turn, would result in increased levels in the systemic circulation. The present study also demonstrated a rapid and robust increase of ET-1 within the myocardial interstitial compartment during and after CPB. This study did not determine the precise mechanism of increased myocardial interstitial ET-1; however, this increase is most likely multifactorial. Although the myocyte can produce and secrete ET-1,⁵ the abrupt and early increase in ET-1 within the myocardial interstitium after induction of cardioplegic arrest was likely caused by the induction of a cold hyperpolarizing solution. This would cause the disassociation of ET-1 from endogenous receptor sites. For example, Franco-Cereceda and associates⁴³ provided evidence that hypothermia induced release of ET-1 from endogenous receptors. In addition, myocardial ET-1 uptake and binding has been reported in the bypass setting.¹⁶ In the present study ET-1 levels remained elevated within the myocardial interstitium throughout and after CPB, which suggests *de novo* myocardial ET-1 synthesis in the CPB setting.

A microdialysis technique to interrogate the myocardial interstitial compartment has been successfully used previously to measure bioactive peptides, as well as determinants of high-energy phosphate production.^{35,39} For example, Dell'Italia and coworkers³⁹ used this microdialysis technique to measure angiotensin II levels in the myocardial interstitial compartment. More important, the study documented a 100-fold greater concentration of myocardial interstitial angiotensin II versus that of the systemic circulation. In addition, temporal angiotensin II dynamics of the systemic circulation did not reflect that of the myocardium interstitium. Currently, this technique is limited to the measurement of low-molecular-weight molecules.⁴⁴ This approach is amendable to the measurement of ET-1 because the molecular weight of this peptide is approximately 2500 d. Another important consideration of this technique is proper placement to ensure minimal tissue trauma. These issues were considered in the present study through postmortem examination of the LV region containing the microdialysis probe.

ET-1 induces biologic effects through two receptor subtypes, ET_A and ET_B, both of which exist in the myocardium.²⁹ Stimulation of the ET_A receptor results in vasoconstriction, as well as modulation of myocardial contractile properties. ET_B stimulation results in vasodilation, primarily through the production of nitric oxide. Although the present study demonstrated a

robust increase in ET-1 within both the myocardial interstitial compartment and the systemic circulation, the downstream effects of these levels on the two receptor subtypes remains to be defined. One approach would be through the use of ET-1 receptor antagonists, which can be a useful tool to elucidate the potential role of the ET-1 receptor subtypes. There are several studies that have demonstrated beneficial hemodynamic effects through the use of ET-1 receptor antagonists in the setting of CPB^{23,26}; however, the effects of such blockade on myocardial contractility remain to be defined. The present study did not precisely determine the mechanism of increased ET-1 during and after CPB. The biosynthesis of mature ET-1 requires cleavage from the proform or big ET-1.²⁹ This enzymatic process results in the liberation of the C-terminal peptide, which may be possible to measure in the interstitial space. A limitation of the present study is the lack of information on the regional differences in ET-1 levels in the CPB setting and as such warrants further investigation. Furthermore, future studies are necessary to more carefully define the mechanistic role that ET-1 plays in the CPB setting. However, the results of this study clearly demonstrated the compartmentalization of this potent bioactive peptide in the myocardium.

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