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Positive inotropic and negative lusitropic effects of endothelin receptor agonism in vivo

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Konrad, D., A. Oldner, M. Wanecek, A. Rudehill, E. Weitzberg, B. Biber, G. Johansson, S. Häggmark, and M. Haney. Positive inotropic and negative lusitropic effects of endothelin receptor agonism in vivo. *Am J Physiol Heart Circ Physiol* 289: H1702–H1709, 2005. First published June 10, 2005; doi:10.1152/ajpheart.00892.2004.—The endothelin (ET) system is involved in the regulation of myocardial function in health as well as in several diseases, such as congestive heart failure, myocardial infarction, and septic myocardial depression. Conflicting results have been reported regarding the acute contractile properties of ET-1. We therefore investigated the effects of intracoronary infusions of ET-1 and of the selective ET_B receptor-selective agonist sarafotoxin 6c with increasing doses in anesthetized pigs. Myocardial effects were measured through analysis of the left ventricular pressure-volume relationship. ET-1 elicited increases in the myocardial contractile status (end-systolic elastance value of 0.94 ± 0.11 to 1.48 ± 0.23 and preload recruitable stroke work value of 68.7 ± 4.7 to 83.4 ± 7.2) that appear to be mediated through ET_A receptors, whereas impairment in left ventricular isovolumic relaxation ($\tau = 41.5 \pm 1.4$ to 58.1 ± 5.0 and $t_{1/2} = 23.0 \pm 0.7$ to 30.9 ± 2.6 , where τ is the time constant for pressure decay and $t_{1/2}$ is the half-time for pressure decay) was ET_B receptor dependent. In addition, intravenous administration of ET-1 impaired ventricular relaxation but had no effect on contractility. Intracoronary sarafotoxin 6c administration caused impairments in left ventricular relaxation (τ from 43.3 ± 1.8 to 54.4 ± 3.4) as well as coronary vasoconstriction. In conclusion, ET-1 elicits positive inotropic and negative lusitropic myocardial effects in a pig model, possibly resulting from ET_A and ET_B receptor activation, respectively.

cardiac; inotropy; end-systolic elastance; diastolic; porcine; sarafotoxin

THE ENDOTHELINS ARE A FAMILY of 21 amino acid peptides with powerful vasoactive properties, which were first described in 1988 (48). Endothelin-1 (ET-1), which is probably the most important of the endothelins as far as effects in humans are concerned, is mainly produced by the vascular endothelium. This vasoconstrictive peptide acts on two groups of receptors: ET_A and ET_B receptors, located on vascular smooth muscle cells mediating contraction, and ET_B receptors, located on the endothelium mediating vasodilation by release of nitric oxide and prostacyclin (1, 10, 31). In the heart, the predominant ET isopeptide is ET-1 (30), and both ET_A and ET_B receptor mRNA are found in atrial and ventricular myocardium, the conducting system, and endocardial cells as well as in coronary arteries (3, 26). The ET system and ET-1 binding properties on cardiomyocytes are largely similar in pigs and humans (25).

The ET system is involved in the cardiovascular response to several disease processes. Increased plasma levels of ET-1 have been noted in association with acute myocardial infarction, congestive heart failure, pulmonary hypertension, and septic shock (44). There are numerous reports regarding the cardiac effects of ET-1. The majority of studies done in larger animals or humans seems to indicate positive inotropic effects of ET-1 under nondisease conditions. Several studies have been performed in vitro in various species with conflicting results regarding myocardial effects (16, 21, 32, 34, 49). In these previous in vivo animal studies on cardiac effects of ET-1, the peptide has been administered intravenously. The potent vasoconstrictor properties of ET-1 may then have been confounding when myocardial effects have been assessed with load-dependent parameters.

The aim of the present study was to investigate the effects of cardiac ET receptor activation on left ventricular (LV) myocardial function. ET-1, which possesses a similar affinity for the ET_A and ET_B receptors, and sarafotoxin 6c (S6c), a selective ET_B receptor agonist (46), were used for this purpose. These peptides were administered in low doses directly into the coronary circulation, and an intravenous dose was used as a reference. Furthermore, we assessed myocardial function through analysis of the LV pressure-volume relationship (LVPVR) to minimize loading confounders.

MATERIALS AND METHODS

The Research Ethical Committee at Umeå University approved the experimental protocol for this study, which was conducted in conformity with the European Convention for the protection of vertebrate animals used for experimentation and other scientific purposes (Council of Europe No. 123, Strasbourg 1985) and with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publ. No. 85-23, Revised 1996).

Eleven female domestic land race pigs weighing between 33 and 44 kg were anesthetized after being fasted overnight with free access to water. After intramuscular injections of ketamine (10 mg/kg), azaperone (4 mg/kg), and atropine (50 μ g/kg) were administered, anesthesia was induced with pentobarbital sodium (12 mg/kg iv) and maintained by a continuous infusion of pentobarbital sodium (5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), midazolam (0.3 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), and fentanyl (20 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Intravenous fluids were administered with the goal of maintaining normovolemia: Ringer acetate solution (25 ml/kg) during the first hour and thereafter (15 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) throughout the study period. After a tracheotomy was performed, the animals were mechanically ventilated (Servo 900B ventilator; Siemens Elema) to normoxia and normocapnea (Artema Medical, Stockholm, Sweden)

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with tidal volumes <10 ml/kg. Blood gas measurements were performed intermittently (ABL 5; Radiometer Copenhagen) to confirm capnography and pulse oximeter observations. Body temperature was measured and maintained between 38° and 39°C with the help of warmed intravenous fluids and a warming blanket.

All vascular catheters were placed through direct cutdowns onto the jugular or carotid vessels. A triple-lumen central venous catheter (Arrow International) and a thermistor-tipped pulmonary artery catheter (Optimetrix, Abbott) were placed as well as an arterial catheter with the tip in the descending aorta. A 7.5-Fr balloon occlusion catheter (Vascular Technologies, Solna, Sweden) was positioned in the inferior vena cava directly adjacent to the right atrium to provide a controlled transient restriction of venous return. Arterial, central venous, and pulmonary artery pressures were measured with the use of a fluid-filled catheter system and transducers (Gabarith PMSET; Becton Dickinson). A 7-Fr LV pigtail combination tip manometer and conductance catheter (CA-71083-PN; CD Leycom, Zoetermeer, Holland) was placed through an 8.5-Fr introducer in the carotid artery system into the LV with the use of fluoroscopic guidance. A dual thermistor-tipped coronary sinus catheter (Webster) was placed in the great cardiac vein (GCV). A guiding catheter (Guidant) was placed with the tip in the left main coronary artery for infusion of the drugs. The catheter position was checked and rechecked with the use of fluoroscopy and minimal amounts of intravascular radiographic contrast (Visipaque; Amersham, Solna, Sweden). An intravenous heparin infusion (1,000 IE/h) was started when the cardiac catheters were in place to minimize the risk of catheter-related thrombosis. At the conclusion of the experiment, the pigs were euthanized with an intravenous overdose combination of pentobarbital sodium bolus followed by a bolus of potassium.

Measurements and calculations. The conductance volumetry technique is well described elsewhere (37), and we have previously described this method in depth (7, 14). The LV volume was measured with a 12-electrode, dual-field conductance catheter with 8-mm spacing between electrodes and a signal conditioning amplifier (Leycom Sigma 5-DF; Cardiodynamics, Zoetermeer, Holland). The volume signal was calibrated with a stroke volume (SV) and flow reference ratio derived from thermodilution cardiac output (CO) measurements obtained with the use of the pulmonary artery catheter and a thermodilution computer (Wetenschappelijk Technische Instituut, Rotterdam, Holland). Parallel conductance for the LV volume signal was measured with the hypertonic saline method (36). The LV pressure and conductance data were recorded with a sampling rate of 250 Hz with the use of a software package (PC Conduct, Cardiodynamics). All circulatory measurements were recorded and analyzed with the use of a digital signal acquisition and analysis software package (Acqknowledge; Biopac Systems, Santa Barbara, CA).

GCV flow (Q_{GCV}) was measured by thermodilution (4). Coronary O_2 kinetics were calculated as follows: arterial O_2 content = (arterial partial pressure $O_2 \times 0.23$) + hemoglobin concentration ($1.39 \times$ arterial O_2 saturation); GCV O_2 content = (GCV partial pressure $O_2 \times 0.23$) + hemoglobin concentration [$1.39 \times$ GCV O_2 saturation ($S_{GCV O_2}$)]]; LV O_2 delivery (MD_{O_2}) = $Q_{GCV} \times$ arterial O_2 content; LV myocardial O_2 consumption ($M\dot{V}_{O_2}$) = (arterial O_2 content – GCV O_2 content) $\times Q_{GCV}$; myocardial O_2 extraction ratio = $100 \times M\dot{V}_{O_2}/DO_2$. The unit for O_2 content is milliliters per liter, and the unit for partial pressure is kilopascal.

The following general hemodynamic parameters for each point in the protocol were measured: heart rate (HR), mean arterial blood pressure (MAP), CO, SV, central venous pressure (CVP), mean pulmonary artery pressure (MPAP), LV end-systolic volume (LVEDV), LV end-diastolic pressure (LVEDP), LV maximal rate of change in pressure (dp/dt_{max}), and maximum negative rate of pressure change (dp/dt_{min}). End diastole was identified as the maximum LV volume before the isovolumic pressure increase, which was timed for the purpose of analysis of sequences with multiple heart cycles to 8–16 ms before the measured dp/dt_{max} or to 8–16 ms after the

intracardiac ECG R wave. LV stroke work (SW) was measured from the integral of the pressure-volume area for each heart cycle. For each beat, the maximal instantaneous pressure-flow product during systole ($Power_{max}$) was calculated. The end-systolic points were initially estimated as maximal pressure-volume for each cycle, and these beats were used to establish an end-systolic PVR (ESPVR) for all beats with an x -intercept. A tangent to this x -intercept was then used to find a new end-systolic P-V point for all beats and a final ESPVR (18). End-systolic aortic elastance (E_a) was measured as end-systolic aortic pressure divided by SV. Ventricular-arterial coupling was calculated as end-systolic elastance (E_{es}) divided by E_a . Total potential energy [i.e., pressure-volume area (PVA)] was calculated for a single resting beat at the onset of a preload reduction sequence with the ESPVR and then $(0.5)P_{es}(V_{es} - V_o)$, where P_{es} is LV end-systolic pressure, V_{es} is LV end-systolic volume, and V_o is the LV volume at the x -intercept for the ESPVR. SW was calculated for the same beat, and myocardial efficiency was expressed as SW/PVA , as well as single beat $PVA/M\dot{V}_{O_2}$ (38). For diastolic parameters, τ is the time constant for pressure decay during the isovolumic relaxation phase assuming a nonzero asymptote (8). Additionally, the half-time for pressure decay during isovolumic relaxation ($t_{1/2}$) was measured (24). Also, for each measurement point in the protocol, a controlled preload alteration was performed during a brief period of apnea with transient inflation of the balloon-tipped catheter to occlude the inferior vena cava for a short period (6–8 s). A sequence of 6–12 contiguous heart cycles was later selected from this sequence for analysis on the basis of a progressive beat-to-beat reduction in the end-diastolic and end-systolic LV volumes. This sequence was analyzed for E_{es} (18) and preload recruitable SW (PRSW) (12). All myocardial function parameters were calculated with custom software.

Biochemical analyses. Plasma levels of ET-1-like immunoreactivity (ET-1 LI) were analyzed with radioimmunoassay as described by Hemsén (15). The cross-reactivity of the E1 antiserum used with other endothelins was 27% with ET-2, 8% with ET-3, and 0.03% with porcine Big ET-1. The cross-reactivity with S6c was 94%.

Experimental protocol. After completion of the preparation, a 45-min stabilization period was observed. After the baseline measurements were taken, an intracoronary infusion of ET-1 (Sigma) was started ($n = 9$ pigs). The dosage was increased at 30-min intervals (1, 2, 4, and 8 $pmol \cdot kg^{-1} \cdot min^{-1}$). All dosages were diluted to a volume of 50 ml with sodium chloride and infused into the left main coronary artery at a rate of 1.7 ml/min. Measurements were made after each dose during the last 10 min of the ongoing infusion. After a recovery period of 45 min, a 30-min intravenous infusion of ET-1 (10 $pmol \cdot kg^{-1} \cdot min^{-1}$) was followed with measurements made in the same fashion ($n = 10$ pigs). After another recovery period, an intracoronary infusion of S6c (Sigma) of 1 and 2 $pmol \cdot kg^{-1} \cdot min^{-1}$ took place in the same manner as described above ($n = 10$ pigs).

Statistical analysis. Data are presented as means $\pm$ SE. ANOVA was used to analyze changes over time from each control point to the last dose administered. For differences between doses compared with each control point, post hoc comparisons were made with the use of the Holm-Sidak method. The paired t -test was used for analysis of ET-1 given intravenously. Differences were considered significant at $P < 0.05$. A computer software program (SigmaStat 3.0; SPSS, Chicago, IL) was used for statistical calculations.

RESULTS

LV contractile function. Intracoronary infusions of ET-1 evoked positive inotropic effects as demonstrated by increases in PRSW and E_{es} as well as $dp/dt_{max}/EDV$ (Fig. 1 and Table 1). No changes in systolic function (E_{es} , PRSW, and $dp/dt_{max}/EDV$) were observed during intravenous ET-1, although a tendency toward a decrease in $Power_{max}/EDV$ ($P = 0.059$) was noted (Table 2). S6c was associated with no change in E_{es} , a

Fig. 1. Myocardial contractile function parameters: end-systolic elastance (E_{es}), preload recruitable stroke work (PRSW), maximal instantaneous pressure-flow product during systole/end-diastolic volume ($Power_{max}/EDV$), and left ventricular maximal rate of change in pressure (dP/dt_{max})/EDV were studied after intracoronary endothelin-1 (ET-1; solid circles) infusion in nine pigs in increasing doses (1, 2, 4, and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). After recovery period, sarafotoxin 6c (S6c; open squares) was infused in 10 pigs in same manner with doses of 1 and 2 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Data are presented as means $\pm$ SE. Each dose was compared with its corresponding baseline value, and significant differences are displayed as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tendency toward a decrease in PRSW ($P = 0.061$), and decreases in $Power_{max}/EDV$ as well as $dP/dt_{max}/EDV$ during both doses (1 and 2 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Myocardial efficiency, expressed as SW/PVA, and $M\dot{V}O_2$ both increased in response to intracoronary ET-1. PVA/ $M\dot{V}O_2$, the parameter for myocardial efficiency that considers oxygen consumption, did not change (tendency was toward a decrease; $P = 0.056$). Ventricular-arterial coupling (E_{es}/E_a) changed in the same direction. Myocardial efficiency (but not E_{es}/E_a) appeared to decrease a small amount during intravenous ET-1 (decrease in SW/PVA; no change in PVA/ $M\dot{V}O_2$). $M\dot{V}O_2$ decreased during S6c infusion, although mechanical efficiency and E_{es}/E_a showed no consistent change.

Diastole/isovolumic relaxation. Negative effects on isovolumic relaxation were observed with significant prolongations in τ and $t_{1/2}$ at doses of 2, 4, and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of ET-1 in the coronary circulation (Fig. 2). Both the doses of S6c as well as the systemic administration of ET-1 were associated with prolongations in τ and $t_{1/2}$ (Table 2).

Descriptive circulatory parameters. Administration of ET-1 in the coronary circulation did not affect CO, HR, SV, or SW but caused an increase in CVP, MAP (4 and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and MPAP (8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Table 1). Increases in dP/dt_{max} were seen for 4 and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of ET-1, whereas $Power_{max}$ increased for 4 and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of ET-1. With regard to diastolic effects, dP/dt_{min} was increased by 2, 4, and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of ET-1. LVEDV was slightly increased at all doses, whereas LVEDP was unchanged.

Systemic administration of ET-1 caused decreases in CO, SV, and HR along with increases in MAP and CVP. MPAP and SW were not affected (Table 2). No effects were seen with regard to dP/dt_{max} or $Power_{max}$, whereas dP/dt_{min} decreased. An increase in LVEDP was also seen; however, LVEDV was unchanged.

Both doses of S6c in the coronary circulation were associated with decreases in CO, SV, and SW but had no effect on HR, MAP, MPAP, LVEDV, or LVEDP. CVP increased slightly by both 1 and 2 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, whereas dP/dt_{max} and $Power_{max}$ were decreased and dP/dt_{min} was increased.

Coronary blood flow and oxygen utilization. Q_{GCV} was not affected during intracoronary ET-1 infusion (Table 1). However, $M\dot{V}O_2$ increased with 2, 4, and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of ET-1. There was no effect on Q_{GCV} by intravenous ET-1. The myocardial O_2 extraction ratio increased in response to intravenous ET-1, although no statistical effects were seen regarding MDO_2 or $M\dot{V}O_2$. Q_{GCV} was decreased by S6c at both doses, with a more limited decrease by S6c in $M\dot{V}O_2$ (2 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$).

Biochemical parameters. Increases in both arterial and GCV ET-1 LI plasma levels were seen in response to intracoronarily administered ET-1 at doses of 4 and 8 $\text{pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (Fig. 3). Intravenously administered ET-1 rendered equally high levels in arterial and GCV plasma. S6c caused increases in ET-1 LI plasma levels in the GCV; however, the cross-reactivity for S6c with ET-1 was 94%.

Table 1. Descriptive circulatory parameters

Parameter	Baseline	Drug Dose, pmol·kg ⁻¹ ·min ⁻¹			
		1	2	4	8
HR, beats/min					
ET-1	79±3	77±3	76±4	76±4	79±4
S6c	88±6	88±6	85±6		
MAP, mmHg					
ET-1	98±5	101±5	103±5	104±5*	109±5‡
S6c	94±4	92±4	92±4		
SVR, dyn·s·cm ⁻⁵					
ET-1	1,475±107	1,523±96	1,547±85	1,600±83†	1,658±104‡
S6c	1,311±63	1,373±75	1,515±73‡		
CO, l/min					
ET-1	5.0±0.2	5.0±0.2	4.9±0.2	4.8±0.2	4.9±0.2
S6c	5.4±0.4	5.0±0.4*	4.5±0.3‡		
SV, ml					
ET-1	65.2±5.3	65.9±5.3	66.5±5.6	65.2±5.8	63.5±4.9
S6c	64.5±7.0	59.4±6.7†	54.9±5.4‡		
SW, mmHg/ml					
ET-1	6,140±607	6,306±599	6,382±623	6,405±689	6,311±629
S6c	5,748±822	4,965±742‡	4,462±589‡		
CVP, mmHg					
ET-1	6.8±0.3	7.6±0.3*	7.8±0.4†	8.4±0.5‡	8.4±0.7‡
S6c	7.4±0.6	8.0±0.6*	8.6±0.6‡		
MPAP, mmHg					
ET-1	24.5±1.0	23.7±1.1	24.0±1.1	24.1±1.1	25.1±1.1*
S6c	26.7±1.3	27.0±1.3	26.7±1.5		
LVEDP, mmHg					
ET-1	15.6±1.2	17.3±1.6	16.8±1.5	16.8±1.6	18.4±1.6
S6c	11.2±1.0	12.9±1.2	13.1±1.0		
LVEDV, ml					
ET-1	98±9	104±9‡	107±10‡	108±10‡	106±9‡
S6c	111±6	115±6	116±6*		
dP/dt _{min} , mmHg/s					
ET-1	-1,913±79	-1,881±94*	-1,819±107*	-1,776±112‡	-1,696±129‡
S6c	-1,732±94	-1,484±83‡	-1,391±92‡		
Q _{GCV} , ml/min					
ET-1	82±13	78±12	82±14	82±14	81±12
S6c	92±16	72±10†	70±10‡		
S _{GCV} O ₂ , %					
ET-1	25.4±2.2	20.4±1.4‡	18.5±1.6‡	16.0±1.3‡	16.2±1.2‡
S6c	25.7±1.7	17.1±2.3‡	15.5±1.6‡		
MDO ₂ , ml/min					
ET-1	9.5±1.6	9.0±1.4	9.5±1.8	9.7±1.7	9.9±1.5
S6c	11.1±2.1	8.5±1.4†	8.3±1.4‡		
MVO ₂ , ml/min					
ET-1	7.4±1.4	7.3±1.2	7.9±1.6*	8.3±1.6*	8.4±1.4†
S6c	8.4±1.6	7.2±1.2	7.1±1.2†		
SW/PVA					
ET-1	0.79±0.01	0.81±0.01*	0.82±0.01*	0.83±0.01‡	0.83±0.01†
S6c	0.79±0.01	0.77±0.01	0.76±0.02		
PVA/MVO ₂					
ET-1	0.87±0.19	0.73±0.13	0.72±0.13	0.64±0.10	0.64±0.10
S6c	0.76±0.20	0.74±0.18	0.67±0.17		
E _{es} /E _a					
ET-1	0.57±0.03	0.59±0.04	0.66±0.04†	0.69±0.05‡	0.78±0.05‡
S6c	0.58±0.03	0.63±0.06	0.57±0.05		

Values are means ± SE. Endothelin-1 (ET-1) was infused intracoronarily in nine pigs in increasing doses (1, 2, 4, and 8 pmol·kg⁻¹·min⁻¹). After recovery period, sarafotoxin 6c (S6c) was infused in 10 pigs in the same manner with doses of 1 and 2 pmol·kg⁻¹·min⁻¹. HR, heart rate; MAP, mean arterial blood pressure; SVR, stroke volume (SV) resistance; CO, cardiac output; SW, left ventricular (LV) stroke work; CVP, central venous pressure; MPAP, mean pulmonary artery pressure; LVEDP, LV end-diastolic pressure; LVEDV, LVED volume; dP/dt_{min}, maximum negative rate of pressure change; Q_{GCV}, great cardiac vein flow; S_{GCV}, GCV O₂ saturation; MDO₂, LV O₂ delivery; MVO₂, LV O₂ consumption; PVA, pressure-volume area; E_{es}, end-systolic elastance; E_a, end-systolic aortic elastance; E_{es}/E_a, ventricular-arterial coupling. Each dose was compared with its corresponding baseline value, and significant differences are displayed as follows: **P* < 0.05; †*P* < 0.01; ‡*P* < 0.001.

DISCUSSION

The main effects seen in this study were enhanced myocardial contractile function by ET-1 and negative lusitropic effects by both ET-1 and S6c. The increases in myocardial contrac-

tility and measured ventricular performance with intracoronary ET-1 administration were accompanied by an increase in MVO₂. ET-1-related increases in the parameters of systolic function were derived from serial assessments of ventricular

Table 2. Effects of intravenous infusion of ET-1

Parameter	Baseline	Intravenous ET-1
HR, beats/min	86 ± 7	77 ± 5*
MAP, mmHg	97 ± 3	108 ± 4‡
SVR, dynes·cm ⁻⁵	1,290 ± 68	1,737 ± 104‡
CO, l/min	5.6 ± 0.4	4.7 ± 0.4‡
SV, ml	68.1 ± 6.6	62.8 ± 6.7‡
SW, mmHg/ml	6,260 ± 791	6,146 ± 835
CVP, mmHg	7.6 ± 0.5	9.6 ± 0.5‡
MPAP, mmHg	25.7 ± 1.1	25.7 ± 1.1
LVEDP, mmHg	14.4 ± 1.4	16.7 ± 1.2†
LVEDV, ml	108 ± 5.6	109 ± 5.3
Q _{GCV} , ml/min	91.0 ± 12.7	84.5 ± 11.8
S _{GCV} O ₂ , %	24.2 ± 2.0	17.8 ± 1.6†
MDO ₂ , ml·kg ⁻¹ ·min ⁻¹	10.7 ± 1.7	10.3 ± 1.5
MVO ₂ , ml·kg ⁻¹ ·min ⁻¹	8.3 ± 1.4	8.5 ± 1.3
E _{es} , mmHg/ml	0.88 ± 0.09	0.93 ± 0.09
PRSW, mmHg	63.8 ± 2.9	61.3 ± 4.2
Power _{max} /EDV, mmHg/s	341.6 ± 25.0	322.8 ± 32.4
dP/dt _{max} /EDV, mmHg·ml ⁻¹ ·s	17.1 ± 1.0	16.7 ± 1.2
dP/dt _{max} , mmHg/s	1,821 ± 87	1,779 ± 103
τ, ms	42.3 ± 1.2	47.5 ± 2.0‡
t _{1/2} , ms	23.3 ± 1.0	26.5 ± 1.3‡
dP/dt _{min} , mmHg/s	-1,798 ± 94	-1,902 ± 100‡
SW/PVA	0.80 ± 0.01	0.77 ± 0.01*
PVA/MVO ₂	0.78 ± 0.21	0.67 ± 0.15
E _{es} /E _a	0.59 ± 0.04	0.51 ± 0.05

Values are means ± SE. ET-1 at 10 pmol·kg⁻¹·min⁻¹ was infused intravenously in 10 pigs. PRSW, preload recruitable SW; Power_{max}, maximal instantaneous pressure-flow product during systole; dP/dt_{max}, LV maximal rate of change; τ, time constant for pressure decay; t_{1/2}, half-time for pressure decay. Each dose was compared with its corresponding baseline value, and significant differences are displayed as follows: **P* < 0.05; †*P* < 0.01; ‡*P* < 0.001.

pressure-volume relations, allowing the detection of changes in myocardial contractile status independent of the prevailing loading conditions (17). The increases in *E_{es}* and PRSW induced by ET-1 were not seen when S6c was administered, suggesting that the enhanced contractile effects were related to ET_A receptor activation.

It is currently believed that ET_A receptor agonism subsequently results in the activation of protein kinase C that in turn increases the mobilization and reuptake of cytosolic Ca²⁺ and alters Ca²⁺ channel activity as well as enhances myofibrillar Ca²⁺ sensitivity via alkalization of the myocytes by Na⁺/H⁺ exchange (5, 47). In the present study, intracoronary ET-1 elicited positive effects on myocardial contractile status, seen

Fig. 2. Isovolumic relaxation parameters: time constant for pressure decay (τ) and pressure half-time were studied after intracoronary ET-1 (solid circles) infusion in nine pigs in increasing doses (1, 2, 4, and 8 pmol·kg⁻¹·min⁻¹). After recovery period, S6c (open squares) was infused in 10 pigs in same manner with doses of 1 and 2 pmol·kg⁻¹·min⁻¹. Data are presented as means ± SE. Each dose was compared with its corresponding baseline value, and significant differences are displayed as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Fig. 3. ET-1-like immunoreactivity (ET-1 LI) in arterial (solid circles) and great cardiac venous plasma (open squares) in nine pigs receiving ET-1 infusion of increasing doses (1, 2, 4, and 8 pmol·kg⁻¹·min⁻¹). After recovery period, ET-1 was administered intravenously to 10 pigs at 10 pmol·kg⁻¹·min⁻¹. After yet another recovery period, S6c was infused in 10 pigs in same manner with doses of 1 and 2 pmol·kg⁻¹·min⁻¹. C, control point. Data are presented as means ± SE. Each dose was compared with its corresponding baseline value, and significant differences for arterial ET-1 LI are displayed as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; significant differences for great cardiac vein ET-1-LI are displayed as follows: #*P* < 0.05; ###*P* < 0.001.

as increases in *E_{es}* and PRSW. In healthy humans, ET_A receptor agonism seems to provide a tonic positive contractile effect (23), and similar findings have been noted in an ex vivo model of perfused rat hearts where ET-1-induced positive LV contractile effects were abolished by an ET_A receptor antagonist (40). In anesthetized pigs, ET-1 in combination with an ET_B receptor antagonist caused similar positive inotropic effects as ET-1 infusion alone (9). The literature is, however, not uniform in this regard. Beyer et al. (6) used a selective ET_B receptor agonist as well as ET-1 in combination with an ET_A receptor antagonist in rats to demonstrate that ET_B receptor activation was responsible for the myocardial contractile effects seen. The dosage used was, however, ~10 times higher than that in our study and was also administered intravenously, causing major vascular effects as well.

A rather recent study on an isolated rat heart model (40) demonstrated positive effects of ET-1 on myocardial efficiency with the use of PVA/MVO₂. We were unable to confirm these results with intracoronary ET-1 in the current study where the

positive effects on contractility were accompanied by increased oxygen consumption. However, when expressing myocardial efficiency as SW/PVA and taking mechanical work into account, we see an increase in response to intracoronary ET-1.

In the current study, ET_B receptor activation via intracoronary infusion of S6c did not cause significant changes in E_{es} or PRSW but decreased $Power_{max}/EDV$ and $dP/dt_{max}/EDV$ with unaltered MAP. This would suggest a mild negative effect on myocardial contractility related to ET_B receptor activation. These findings are well in line with the effects seen in human LV myocytes (13). When administering S6c intravenously in anesthetized pigs, Cirino et al. (9) reported negative effects on myocardial contractile function (dP/dt_{max} and dP/dt_{min}); however, those findings were accompanied by a reduction in MAP of 60% (9).

S6c had profound negative lusitropic effects as shown by the changes in τ and $t_{1/2}$ findings. These findings were accompanied by an attenuation of dP/dt_{min} . ET-1 intracoronary infusion caused an identical pattern in diastolic parameters, implying ET_B receptor agonism as the mediator of the negative effect on LV isovolumic relaxation, as shown by Goldberg et al. (13) on human LV myocytes. In our study, S6c decreased Q_{GCV} and $M\dot{V}O_2$ in connection with the negative diastolic effects, which for us awakened concern that severe coronary flow restriction could lead to an ischemic state with related myocardial dysfunction. The study design did not include methods to confirm or refute myocardial ischemia. However, ET-1 infusion did not affect Q_{GCV} and increased $M\dot{V}O_2$ while causing the same diastolic effects as S6c, suggesting that mechanisms other than ischemia may be involved. Teerlink et al. (41) reported that intracoronary S6c had a transient vasodilatory effect in lower doses (up to 120 pmol) and a marked vasoconstricting effect in higher doses (up to 1,200 pmol) in open-chest dogs. These results agree with the findings where the lower dose of S6c (total of ~800 pmol) induced a >20% reduction in Q_{GCV} . This is likely explained by the activation of vascular smooth muscle ET_B receptors (45).

Most in vivo studies on cardiac effects of ET-1 have used the intravenous route of administration. The vascular effects seen in response to systemic administration can affect the cardiac variables studied due to alterations in loading conditions. In the current study, the intravenous dose was used as a reference, where no effects were seen on the contractile function parameters (see Table 2), despite significant changes in MAP, CVP, and CO. This may suggest an increase in load and particularly afterload that could have elicited a baroreflex response, though no change in measured myocardial contractile status was noted. The differences in myocardial contractile effects for intravenous and intracoronary administration of ET-1 demonstrate the difficulty in assessing intrinsic myocardial function when using a highly potent vasoactive substance systemically. Moreover, the current study illustrates a clear advantage when using load-indexed measures of myocardial contractile status (E_{es} , PRSW, and $Power_{max}/EDV$) for interpreting direct myocardial effects in response to systemic administration.

Diastolic function parameters did not change during load alterations; both routes of administration produced similar results regarding τ and $t_{1/2}$. Q_{GCV} was unchanged in response to intravenous ET-1. In a model with anesthetized pigs, Cirino et al. (9) infused 400 pmol/kg of ET-1 intravenously and noted transient decreases in MAP, dP/dt_{max} , and relaxation followed

by increases in MAP, LVEDP, and dP/dt_{max} as well as a decrease in CO. Apart from the early transient effects, which we did not observe in our experiments, our observations agree with theirs, apart from dP/dt_{max} . They conclude after combining ET-1 with an ET_B receptor antagonist that the positive inotropic effects were ET_A receptor dependent. Our results, however, suggest that the major cardiac effects of intravenous ET-1 were mainly ET_B receptor mediated. Furthermore, our findings are supported by a report from Kiely et al. (19), who, in a model of human volunteers, infused ET-1 intravenously at low doses and found an increase in LV isovolumic relaxation time or, in other words, negative lusitropy. They also reported negative effects on myocardial contractility of ET-1 in terms of reduced CO along with changes in electromechanical and Doppler acceleration indexes, which they admitted are highly loading condition dependent and were not taken into account. Those systolic function observations could be explained by changes in vascular tone and shifts in intravascular volumes, quite independent of myocardial contractile status. This, again, emphasizes the novelty of the results we have obtained in this context by using an invasive load-independent method of assessment of myocardial contractile status that is not readily available in healthy human volunteers. An alternative interpretation for the divergence of our systolic function results from those of Kiely et al. (19) is that pigs possibly respond differently to ET-1 than humans, though we know of no evidence to support this.

As expected, the intracoronary ET-1 infusion rendered a more pronounced increase in GCV plasma ET-1 LI than in arterial plasma. This difference was not noted with the intravenous infusion. An increase in ET-1 LI was also seen in response to S6c, a phenomenon explained by the high cross-reactivity of S6c to ET-1 (94%) in the radioimmunoassay used. The plasma levels noted in the GCV in the present study are high and by far exceed those observed in several other studies on failing hearts (4.7–15 pmol/l; Refs. 11 and 22). However, the relation between plasma levels and tissue concentrations or tissue activity of ET is complex. Several reports have described a preferential abluminal secretion of ET rather than intravascular by the endothelial cells (43). In a pathophysiological process where there is an increased local production of ET, the plasma levels constitute merely an indirect reflection of the local activity. This relation is confirmed by other investigators who assessed both plasma and tissue concentrations of ET (11). In fact, in a recent study on severely failing human hearts (33), cardiac tissue levels were approximately >500 pmol/kg. These reports are, however, limited to studies on endogenous ET. To our knowledge, no studies have been performed that look at the effects of exogenous ET administration on subsequent tissue concentrations. Therefore, it is difficult to anticipate the local ET receptor activity achieved in the myocardium in response to an infusion. Thus, although it could be argued that the concentrations achieved in the present study are pharmacological, their pathophysiological relevance may nonetheless be valid.

The data from the current study would suggest that ET_A receptor agonism may be beneficial from a myocardial contractile function point of view and that ET_B receptor antagonism may be useful for improving diastolic function. However, in pathophysiological states the situation seems to be far more complex. In induced congestive heart failure models, ET-1 seems to exert negative contractile effects (29, 42), hence the

interest in using selective or nonselective ET receptor antagonists in clinical trials (35, 39). In healthy humans, ET_A receptor agonism seems to provide enhanced myocardial contractile function, which does not occur in patients with dilated cardiomyopathy (23). Previous results from our own laboratory in which selective ET_B receptor antagonism in septic pigs was used proved to be fatal, whereas dual ET receptor antagonism seems beneficial (20, 28). The mechanisms behind these apparent diverging results are not fully elucidated. Changes in receptor density as well as alterations in ET_A-to-ET_B receptor ratio may be involved (2). Furthermore, the cardiac unloading effects from vasodilation may result in overall beneficial hemodynamic responses, masking the potential negative inotropic effects seen in response to ET receptor antagonism in various conditions, such as sepsis (28).

Limitations. This protocol was determined after pilot studies where the S6c-elicited effects on Q_{GCV} were evident. To avoid ischemic events potentially clouding the myocardial effects of ET-1, we decided not to perform the infusions in random order. Nonetheless, the recovery periods seem to have been adequate in the sense that plasma ET-1 LI concentrations returned to baseline levels before commencement of the postrecovery period infusion (Fig. 3). In addition, key cardiac parameters also returned to baseline values after each recovery period (Figs. 1 and 2).

The intracoronary infusions evoked minimal effects on systemic parameters; however, the doses chosen (based on the pilot study) rendered supranormal plasma concentrations of ET-1 LI in the GCV. However, ET-1 is normally secreted abluminally and acts from the interstitial side of the myocytes (43). Thus high doses may need to be administered to achieve relevant interstitial concentrations.

In conclusion, positive contractile effects of intracoronary ET-1 were observed through analysis of the LVPVR in this model, most probably mediated via ET_A receptor activation. ET-1 also demonstrated negative lusitropic effects, as did S6c, suggesting an ET_B-receptor-mediated negative diastolic function. These findings are strengthened by the use of this method of assessment of myocardial contractile function that is strongly resistant to changes in prevailing loading conditions, including ventricular loading changes brought about by vasoactive effects of ET-1. We conclude that ET-1 has acute positive inotropic effects in healthy in vivo myocardium. Further studies are warranted to understand the role of ET-1 in the regulation of myocardial function in pathophysiological states.

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