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Cardiac effects of endothelin receptor antagonism in endotoxemic pigs

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Konrad D, Haney M, Johansson G, Wanecek M, Weitzberg E, Oldner A. Cardiac effects of endothelin receptor antagonism in endotoxemic pigs. *Am J Physiol Heart Circ Physiol* 293: H988–H996, 2007. First published March 30, 2007; doi:10.1152/ajpheart.01023.2006.—Myocardial depression in sepsis is frequently encountered clinically and contributes to morbidity and mortality. Increased plasma levels of endothelin-1 (ET-1) have been described in septic shock, and previous reports have shown beneficial effects on cardiovascular performance and survival in septic models using ET receptor antagonists. The aim of the current study was to investigate specific cardiac effects of ET receptor antagonism in endotoxemia. Sixteen domestic pigs were anesthetized and subjected to endotoxin for 5 h. Eight of these pigs were given tezosentan (dual ET receptor antagonist) after 3 h. Cardiac effects were evaluated using the left ventricular (LV) pressure-volume relationship. Endotoxin was not associated with any effects on parameters of LV contractile function [end-systolic elastance (Ees), preload recruitable stroke work (PRSW), power_{max}/end-diastolic volume (PWR_{max}/EDV) and dP/dt_{max}/end-diastolic volume (dP/dt_{max}/EDV)] but with impairments in isovolumic relaxation (time constant for pressure decay, tau) and mechanical efficiency. Tezosentan administration decreased Ees, PWR_{max}/EDV, and dP/dt_{max}/EDV, while improving tau and LV stiffness. Thus, dual ET receptor antagonism was associated with a decline in contractile function but, in contrast, improved diastolic function. Positive hemodynamic effects from ET receptor antagonism in acute endotoxemia may be due to changes in cardiac load and enhanced diastolic function rather than improved contractile function.

sepsis; endotoxin; inotropy; end-systolic elastance; diastolic

SEPTIC MYOCARDIAL DEPRESSION is a dire manifestation of sepsis, enhancing mortality in an already devastating disease (13). Diastolic and systolic dysfunction have been described (40, 45, 48) as potentially causing deterioration of both right and left ventricular function (42, 44). The causative mechanisms are far from clear, although a number of cytokines and nitric oxide have been proposed and investigated (31, and references therein). The endothelins (ETs), also proposed in this context, are a family of peptides with powerful vasoactive properties first described in 1988 (64). Endothelin-1 (ET-1), which is probably the most important of the ETs, as far as cardiovascular effects in humans are concerned, is mainly produced by the vascular endothelium and acts on two groups of receptors: ET_A and ET_B receptors, which are located on vascular smooth muscle cells, mediating contraction. The ET_B receptor is also found in the endothelium, mediating vasodilation by release of nitric oxide or prostacycline (15, 17).

In the heart, the predominant ET isopeptide is ET-1 (47), and both ET_A and ET_B receptors are found in the myocar-

dium, endocardium, conducting system, and coronary vessels (4, 39). The ET system and ET-1 binding properties on cardiomyocytes are largely similar in pigs and humans (38).

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 (59). In human sepsis, ET-1 plasma levels are increased fivefold (61) and correlates to severity of illness, as well as outcome (7). Previous reports, including those from our own group, have shown positive cardiovascular effects using ET-receptor antagonists in septic settings (10, 29, 43, 56). However, none of these studies was designed to evaluate specific cardiac effects, and the positive results seen may merely constitute alterations in cardiac pre- or afterload. Interestingly, other investigators have reported conflicting inotropic effects of ET-1 in normal vs. pathological conditions (33, 53). By analogy, we were recently able to demonstrate positive inotropic effects of intracoronary administration of ET-1 in normal, anesthetized pigs (30).

The aim of the present study was to investigate the cardiac effects of ET-receptor antagonism in endotoxemic pigs. Left ventricular (LV) pressure-volume relations (LVPVR) were examined by means of conductance volumetry in an in vivo model of porcine endotoxemia. On the basis of our previous results, we postulated that dual ET-1 receptor antagonism by administration of tezosentan would improve myocardial contractile and diastolic function in a septic state.

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, 1996).

Sixteen female domestic land race pigs weighing between 38 and 55 kg were anesthetized after fasting overnight with free access to water. After intramuscular injections of ketamine 10 mg/kg, azaperone 4 mg/kg, and atropine 50 µg/kg, anesthesia was induced with pentobarbital sodium 12 mg/kg iv and maintained by a continuous infusion of pentobarbital sodium 5 mg·kg⁻¹·h⁻¹, midazolam 0.3 mg·kg⁻¹·h⁻¹ and fentanyl 20 µg·kg⁻¹·h⁻¹. Intravenous fluids were administered as Ringer acetate at a rate of 20 ml·kg⁻¹·h⁻¹ throughout the study period. After tracheotomy, the animals were mechanically ventilated (Evita 4 ventilator; Draeger Medical, Lubeck,

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Germany) with an FI_{O_2} of 0.4, peak end-expiratory pressure of 5 cmH₂O (Artema; Artema Medical, Stockholm, Sweden) with tidal volumes less than 10 ml/kg. If arterial oxygen tension (Pa_{O_2}) dropped below 6.5 kPa, FI_{O_2} was increased with increments of 0.10. Blood gas measurements were performed hourly (ABL 5, Radiometer, Copenhagen, Denmark). Body temperature was measured and maintained between 38° and 39°C with the help of heating pads and a warming blanket.

All vascular catheters were placed through direct cutdowns onto the jugular or carotid vessels. A three-lumen central venous catheter (Arrow International, Reading, PA) and a thermistor-tipped pulmonary artery catheter (Optimetrix, Abbott, IL) were placed. An arterial catheter was placed with the tip in the descending aorta. A 7.5 French (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 using a fluid-filled catheter system and transducers (Gabarrith PMSET, Becton Dickinson, Franklin Lakes, NJ). A 7 Fr left ventricular (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 left ventricle using fluoroscopic guidance. A dual thermistor-tipped coronary sinus catheter (Webster, CA) was placed in the great cardiac vein. Catheter position was checked and rechecked using fluoroscopy, and minimal amounts of intravascular radiographic contrast (Visipaque; Amersham, Solna, Sweden). An intravenous heparin

infusion, 1,000 IE per hour, was started when the cardiac catheters were in place to minimize the risk of catheter-related thrombosis. At termination of the experiment, the pigs were euthanized using a combination of pentobarbital sodium bolus intravenously followed by a bolus of potassium intravenously.

Measurements and calculations. The conductance volumetry technique is well described elsewhere (52) and we have previously described this method in depth (8, 21). LV volume was measured using the 12-electrode dual-field conductance catheter with 8-mm spacing between electrodes, and a signal conditioning-amplifier (Leycom Sigma 5DF, Cardiodynamics, Zoetermeer, Holland). The volume signal was calibrated using a stroke volume and flow reference ratio derived from thermodilution cardiac output measurements obtained using the pulmonary artery catheter and a thermodilution computer (Wetenschappelijk Technische Instituut, Rotterdam, Holland). Parallel conductance for LV volume signal was measured using the hypertonic saline method (51). Left ventricular pressure and conductance data were recorded with a sampling rate of 250 Hz using a software package (PC Conduct, Cardiodynamics). All circulatory measurements were recorded and analyzed using a digital signal acquisition and analysis software package (Acqknowledge, Biopac Systems, Santa Barbara, CA).

Great cardiac vein flow (Q_{GCV}) was measured by thermodilution. Coronary oxygen (O_2) kinetics were calculated as follows: arterial O_2 content = [(arterial partial pressure $\text{O}_2 \times 0.23$) + [hemoglobin concentration](1.39 $\times$ arterial O_2 saturation)]; great cardiac vein (GCV) O_2 content = {(GCV partial pressure $\text{O}_2 \times 0.23$) + [hemo-

Fig. 1. Hemodynamic variables. General hemodynamics were studied following endotoxin administration ($0.25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) for 5 h. After 3 h of endotoxemia, tezoesentan administration (Tezo; $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was started ($n = 8$, $\blacktriangle$) and compared with animals receiving endotoxin alone (controls, $n = 8$, $\circ$). Data are presented as means $\pm$ SE, with relative changes from baseline. Effects of endotoxin before intervention are displayed as $\#P < 0.01$, $\#\#\#P < 0.001$. Significant differences between groups postintervention are displayed as $**P < 0.01$ and $***P < 0.001$.

globin concentration] [$1.39 \times \text{GCV O}_2 \text{ saturation (S}_{\text{GCV O}_2})$]; myocardial O_2 delivery (MDO_2) = $\text{Q}_{\text{GCV}} \times \text{arterial O}_2 \text{ content}$; myocardial O_2 consumption (MVO_2) = $(\text{arterial O}_2 \text{ content} - \text{GCV O}_2 \text{ content}) \times \text{Q}_{\text{GCV}}$; and myocardial O_2 extraction ratio (MOER) = $100 \times \text{MVO}_2 / \text{DO}_2$. The units used for O_2 content is milliliters per liter and for partial pressure in kilopascals.

General hemodynamic parameters for each point in the protocol were measured: heart rate (HR), mean arterial blood pressure (MAP), cardiac output, stroke volume (SV), central venous pressure (CVP), mean pulmonary artery pressure (MPAP), LV end-systolic volume, LV end-diastolic volume (LVEDV), LV end-systolic pressure, LV end-diastolic pressure, LV maximal rate of change in pressure ($\text{dP/dt}_{\text{max}}$), and maximum negative rate of pressure change ($\text{dP/dt}_{\text{min}}$). End diastole was identified as the maximum LV volume before isovolumic pressure increase, which was timed for the purpose of analysis of sequences with multiple heart cycles to 8–16 ms before measured $\text{dP/dt}_{\text{max}}$ or 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. Power max ($\text{Power}_{\text{max}}$) was calculated for each beat as the maximal instantaneous pressure-flow product during systole (41). The analysis of contractile parameters was made from a selection of contiguous beats within physiological pressure ranges and also based on strong linearity in the end-systolic pressure-volume (P/V) relation. The end-systolic points were initially estimated as maximal pressure/volume for each cycle, and these beats were used to establish an end-systolic pressure-volume relation (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 (24). Total potential energy (PVA) was calculated for a single resting beat at the onset of a preload reduction sequence using the ESPVR and then $(0.5)P_{\text{es}}(V_{\text{es}} - V_0)$, where P_{es} was LV end-systolic pressure, V_{es} was LV end-systolic volume, and V_0 was 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 . For diastolic parameters, tau is the time constant for pressure decay during the isovolumic relaxation phase assuming a nonzero asymptote (9). Additionally, the half-time for pressure decay during isovolumic relaxation ($t_{1/2}$) was measured (37).

Also, for each measurement point in the protocol, a controlled preload alteration was performed during a brief period of apnea using 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, based on a progressive beat-to-beat reduction in end-diastolic and end-systolic LV volumes. This sequence was analyzed for end-systolic elastance (E_{es}) (24) and preload recruitable stroke work (PRSW) (18). All myocardial function parameters were calculated using custom software.

Biochemical analyses. Plasma levels of ET-1-like immunoreactivity (ET-1 LI) were analyzed with radioimmunoassay, as described by Hemsén (22). Troponin I in plasma was analyzed by a two-position immunoenzymatic assay (Beckman Coulter, Fullerton, CA).

Experimental protocol. Upon completion of the preparation, a 45-min stabilization period was allowed. After baseline measurements, an intravenous infusion of endotoxin (*Escherichia coli* B0111: B4; Sigma, St. Louis, MO) was started in all animals beginning at $0.05 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and gradually increased to reach $0.25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ within 45 min. After 3 h, eight animals received a short infusion of tezosentan (1 mg/kg in 10 min) followed by a continuous infusion of tezosentan at $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. General hemodynamics, blood gases, and cardiac function were assessed every hour, and plasma samples for analyses of ET-1 LI and troponin I were drawn at baseline, after 3 and 5 h.

Statistical analysis. Data are presented as means $\pm$ SE. A univariate repeated-measures ANOVA was used for analyzing changes over time from baseline until 3 h for evaluating effects of endotoxin administration and for differences between groups before intervention. A repeated-measures ANOVA using the time point 3 h as a covariate was used for evaluating effects of tezosentan administration from 4 to 5 h. Regarding ET-1 LI and troponin I, differences between groups postintervention were evaluated by ANOVA with analysis of the time-treatment interaction. Differences were considered significant at $P < 0.05$. A computer software program (STATISTICA 7.0 StatSoft, Tulsa, OK) was utilized for statistical calculations.

Table 1. Hemodynamic and blood gas-derived parameters

Parameter	Group	0 h	1 h	2 h	3 h	4 h	5 h	P Values
SVI, $\text{ml} \cdot \text{kg}^{-1} \cdot \text{beat}^{-1}$	ctrl	1.59 ± 0.08	1.42 ± 0.12	1.36 ± 0.09	1.15 ± 0.11	1.00 ± 0.10	1.05 ± 0.10	###
	tezo	1.88 ± 0.16	1.730 ± 0.16	1.67 ± 0.21	1.35 ± 0.15	1.45 ± 0.12	1.58 ± 0.13	*
CVP, mmHg	ctrl	7.5 ± 0.5	9.3 ± 0.4	10.8 ± 0.8	9.3 ± 0.4	9.9 ± 0.8	10.1 ± 0.8	###
	tezo	7.2 ± 0.5	8.5 ± 0.4	10.1 ± 0.4	9.3 ± 0.6	7.5 ± 0.2	8.0 ± 0.3	*
SVRI, $\text{mmHg} \cdot \text{kg} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$	ctrl	668 ± 41	592 ± 48	804 ± 84	786 ± 86	677 ± 54	665 ± 47	###
	tezo	697 ± 51	572 ± 45	757 ± 61	803 ± 86	511 ± 52	473 ± 54	**
PVRI, $\text{mmHg} \cdot \text{kg} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$	ctrl	49 ± 9	228 ± 36	233 ± 14	306 ± 26	276 ± 29	273 ± 35	
	tezo	36 ± 7	189 ± 18	204 ± 37	266 ± 35	111 ± 18	79 ± 11	***
SvO_2 , %	ctrl	69.3 ± 3.1	67.7 ± 4.5	60.3 ± 4.8	48.2 ± 6.8	48.9 ± 6.3	53.7 ± 5.1	###
	tezo	74.5 ± 2.5	72.9 ± 3.4	62.7 ± 5.9	58.3 ± 6.1	64.3 ± 5.0	65.8 ± 4.2	$P = 0.051$
Hemoglobin, g/l	ctrl	85 ± 3	90 ± 3	98 ± 4	103 ± 4	101 ± 3	99 ± 4	###
	tezo	86 ± 1	92 ± 2	102 ± 2	107 ± 2	94 ± 1	90 ± 1	***
pH	ctrl	7.49 ± 0.02	7.47 ± 0.01	7.44 ± 0.03	7.41 ± 0.03	7.41 ± 0.03	7.42 ± 0.03	##
	tezo	7.50 ± 0.02	7.49 ± 0.02	7.45 ± 0.04	7.45 ± 0.03	7.49 ± 0.02	7.48 ± 0.02	NS
Base excess, mM	ctrl	5.1 ± 0.9	3.3 ± 0.7	3.1 ± 0.9	2.1 ± 0.9	2.0 ± 1.3	2.1 ± 1.3	###
	tezo	4.9 ± 0.9	4.0 ± 0.8	3.7 ± 1.0	2.9 ± 0.9	5.3 ± 0.9	4.1 ± 0.9	$P = 0.069$
Arterial PO_2 , kPa	ctrl	25.3 ± 1.4	24.5 ± 1.6	22.7 ± 2.5	13.5 ± 3.0	13.7 ± 3.1	17.9 ± 4.3	###
	tezo	27.4 ± 1.2	24.5 ± 1.8	19.7 ± 3.0	16.8 ± 3.5	18.5 ± 2.8	18.1 ± 2.5	NS
Arterial PCO_2 , kPa	ctrl	5.0 ± 0.2	5.0 ± 0.1	5.0 ± 0.3	5.7 ± 0.3	5.7 ± 0.3	5.6 ± 0.4	#
	tezo	4.7 ± 0.2	4.8 ± 0.2	5.0 ± 0.4	5.2 ± 0.3	5.0 ± 0.2	5.0 ± 0.2	NS

Data are presented as means $\pm$ SE. Hemodynamics and blood gases were studied following endotoxin administration ($0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) for 5 h. After 3 h of endotoxemia, tezosentan ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was started ($n = 8$) and compared with animals receiving endotoxin alone (ctrl, $n = 8$). SVI, stroke volume index; CVP, central venous pressure; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index. Effects of endotoxin prior to intervention (both groups) are displayed as # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$. Differences between groups postintervention are displayed as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

RESULTS

Effects on general circulation and metabolic parameters. Endotoxemia evoked a hypodynamic response with a prominent pulmonary hypertension seen as a decreases in cardiac index (CI), stroke volume, MAP and SvO_2 , increases in HR, systemic vascular resistance index (SVRI), CVP, as well as MPAP and PVRI. Gas exchange deteriorated and a metabolic acidosis was noted. Tezosentan improved CI and stroke volume, as well as reduced MAP, CVP and SVRI (Fig. 1 and Table 1). Pulmonary hypertension was abolished without further effects on gas exchange. A tendency to an increase in base excess and SvO_2 was also seen in response to tezosentan.

Effects on left ventricular systolic performance. The first 3 h of endotoxemia were not associated with detectable changes in parameters of systolic function. A trend ($P = 0.052$) for differences between groups prior to intervention were noticed regarding E_{es} . Tezosentan administration had negative effects on E_{es} , $Power_{max}/LVEDV$, and $dP/dt_{max}/LVEDV$ (Fig. 2 and Table 2). A tendency toward decreased PRSW ($P = 0.067$) and ejection fraction ($P = 0.060$) was also seen in response to tezosentan. SW/PVA was slightly reduced by endotoxin, and tezosentan had no effect on this parameter.

Effects on diastole/isovolumic relaxation. Endotoxin infusion had negative effects on the time constant for pressure

decay, tau, whereas no significant effects regarding $t_{1/2}$ ($P = 0.08$) or LV stiffness were seen. Upon tezosentan administration, tau and $t_{1/2}$ were improved, and left ventricular stiffness decreased (Fig. 3).

Coronary blood flow and oxygen utilization. Neither Q_{GCV} nor $S_{GCV}O_2$ were affected by either endotoxin or tezosentan, whereas coronary perfusion pressure was modestly increased by endotoxin and likewise modestly decreased by tezosentan. MDO_2 and $M\dot{V}O_2$ were not affected by endotoxin, but there were significant differences between groups before intervention. MDO_2 was slightly decreased in response to tezosentan. MOER was slightly increased in the tezosentan group compared with controls.

Biochemical parameters. Endotoxemia caused a twofold increase in plasma ET-1 LI immunoreactivity. Tezosentan further increased ET-1 LI and resulted in a fourfold increase compared with controls at 5 h. Troponin levels were elevated in response to endotoxin by 57%. Tezosentan did not influence this parameter (Fig. 4).

DISCUSSION

In this study we have demonstrated opposite inotropic and lusitropic cardiac effects of dual endothelin-receptor antagonism in endotoxemic pigs. First, tezosentan resulted in deterioration of left ventricular contractile performance. Second,

Fig. 2. Myocardial contractile function parameters: end-systolic elastance (E_{es}), preload-recruitable stroke work (PRSW), $power_{max}/end-diastolic\ volume$ ($Power_{max}/EDV$) and $dP/dt_{max}/EDV$ were studied following endotoxin administration ($0.25\ \mu g \cdot kg^{-1} \cdot h^{-1}$) for 5 h. After 3 h of endotoxemia, tezosentan ($1\ mg \cdot kg^{-1} \cdot h^{-1}$) was started ($n = 8$, ▲) and compared with animals receiving endotoxin alone (controls, $n = 8$, ○). Data are presented as means $\pm$ SE, with relative changes from baseline. Differences between groups postintervention are displayed as: * $P < 0.05$ and ** $P < 0.01$.

Table 2. Cardiac effects

Parameter	Group	0 h	1 h	2 h	3 h	4 h	5 h	P Values
SW, mmHg/ml	ctrl	6105±791	5061±789	5338±665	4231±491	3640±491	3657±337	###
	tezo	6815±631	5903±774	6313±922	5230±665	4416±535	4631±554	*
LVEDP, mmHg	ctrl	14.4±1.5	11.8±1.8	12.8±1.3	9.0±1.3	10.7±1.4	10.5±1.5	###
	tezo	14.9±1.4	12.5±1.1	15.1±1.3	11.3±1.3	9.8±1.9	11.9±1.3	<i>P</i> = 0.053
LVEDV, ml	ctrl	114.5±9.8	98.1±11.0	99.7±7.6	83.2±6.0	74.9±9.0	79.4±7.7	#
	tezo	111.6±9.4	98.7±8.9	107.5±10.2	101.3±8.4	108.5±7.4	122.0±6.8	**
LVESP, mmHg	ctrl	98.7±4.4	84.7±3.0	100.3±4.0	90.1±5.0	85.2±5.6	85.8±3.6	###
	tezo	105.9±2.7	90.1±1.8	106.0±5.5	97.8±5.3	75.8±3.7	75.9±4.1	***
LVESV, ml	ctrl	52.1±6.2	40.0±4.8	44.3±4.8	36.2±6.1	32.2±6.0	35.3±5.3	
	tezo	42.7±4.6	34.9±5.3	43.5±5.0	46.8±5.9	53.3±5.9	62.8±5.2	**
SW/PVA	ctrl	0.60±0.03	0.60±0.03	0.59±0.04	0.54±0.06	0.60±0.03	0.59±0.05	#
	tezo	0.55±0.03	0.60±0.04	0.54±0.05	0.51±0.04	0.56±0.03	0.54±0.02	*
EF, %	ctrl	58.3±2.4	61.5±2.8	58.2±3.8	60.5±6.0	61.4±4.3	60.1±3.9	
	tezo	64.7±2.3	69.1±5.8	61.7±3.8	57.8±4.9	54.0±4.3	51.8±3.7	<i>P</i> = 0.06
Q _{GCV} , ml/min	ctrl	143±15	132±16	131±19	134±12	119±9	128±17	
	tezo	115±24	134±38	170±51	145±38	108±26	134±36	NS
S _{GCV} O ₂ , %	ctrl	25.1±1.6	26.5±2.4	28.6±1.6	23.4±2.5	25.1±1.9	29.6±2.8	
	tezo	23.2±1.0	22.0±1.2	24.2±1.9	24.0±3.0	24.3±2.0	25.5±1.3	NS
MDO ₂ , ml/min	ctrl	17.6±2.1	17.1±2.2	17.4±2.5	16.3±1.8	15.2±1.8	16.5±2.4	§
	tezo	14.2±2.9	17.6±5.0	24.6±7.6	20.9±5.8	14.2±3.4	16.9±4.4	*
MVO ₂ , ml/min	ctrl	12.2±1.9	11.3±1.4	10.8±1.2	10.7±1.3	9.7±1.3	10.1±1.7	§
	tezo	11.0±2.2	13.6±3.7	18.0±5.2	14.8±3.4	10.5±2.3	12.4±3.0	NS
MOER	ctrl	69.7±5.7	68.4±5.1	65.8±5.6	67.4±5.8	65.5±4.6	63.0±5.4	#
	tezo	77.7±1.1	78.4±1.2	75.4±1.5	74.5±2.8	75.7±1.7	75.1±1.0	*

Data are presented as means ± SE. Cardiac effects were studied using LVPVR following endotoxin administration (0.25 mg·kg⁻¹·h⁻¹) for 5 h. After 3 h of endotoxemia, tezo (1 mg·kg⁻¹·h⁻¹) was started (*n* = 8) and compared with animals receiving endotoxin alone (ctrl, *n* = 8). SW, stroke work; LVEDP, left ventricular end-diastolic pressure; LVEDV, LV end-diastolic volume; LVESP, LV end-systolic pressure; PVA, total potential energy; EF, ejection fraction; Q_{GCV}, great cardiac vein flow; S_{GCV}O₂, GCV O₂ saturation; MDO₂, myocardial O₂ delivery; MVO₂, myocardial O₂ consumption; MOER, myocardial O₂ extraction ratio. Effects of endotoxin prior to intervention are displayed as #*P* < 0.05 and ###*P* < 0.001. Differences between groups prior to intervention are displayed as §*P* < 0.05. Differences between groups postintervention are displayed as **P* < 0.05 and ***P* < 0.01.

tezosentan significantly improved diastolic performance. These findings are somewhat surprising and in contrast to previously published findings where heart function was assessed with less sensitive methods (29).

Endotoxemia per se was not associated with detectable changes in myocardial contractile function in the present study. Similar in vivo findings have been described by others (3, 46), whereas some authors have reported increased (12, 23) or decreased (1, 32, 62) contractile function in early endotoxemia. The abovementioned investigators have all used load-independent measures of contractility and endotoxin, although in different doses and serotypes and in various species. Therein lies possibly the explanation for the diverging reports. The manner by which endotoxin is infused is also important, where a continuous infusion of endotoxin is preferable to bolus infusions as a model of human sepsis because it generally produces a more persistent pathophysiological response (20). Our current results suggest that endotoxin did not cause detectable LV contractile impairment, but sympathetic activation in response to endotoxin may very well have compensated for a negative inotropic effect of endotoxin, as suggested by Smith et al. (12, 50). The increase in HR seen following endotoxemia would support this concept but when blocking baroreceptor reflexes, Aghajani et al. (2) still could not see evidence of impaired contractility in endotoxemic pigs. Intriguingly, Ishihara et al. (23) reported biphasic, time-dependent changes in LV systolic performance in awake pigs receiving continuous infusion of endotoxin for 24 h. They reported an initial increase in *E_{es}* in the first hours followed by a significant sustained decrease in *E_{es}* after 7 h and onward. Studies in humans of myocardial depression are invariably not done within the

first few hours of sepsis debut, but investigators often find depressed systolic function upon presentation in the intensive care unit and days thereafter (48). Our findings, within the limited time frame under which they are conducted, do not rule out the possibility that ET-1 may play a significant role in the clinical presentation of depressed systolic function at a later stage of sepsis as well. Interestingly, ET plasma levels have been shown to remain elevated in up to 28 days after onset of severe sepsis (55).

Although many studies performed in vitro in various species show conflicting results regarding myocardial effects of ET-1 (49, 65), there are several reports done in larger animals and humans that seem to indicate positive inotropic effects of ET-1 under nondisease conditions (33, 60). We recently reported that exogenous ET-1 administered into the coronary circulation had positive, dose-related effects on LV systolic performance in a nonseptic setting (30).

Interestingly, in pathological states, such as congestive heart failure, ET receptor antagonism has shown positive effects in some clinical trials (54) but with increasing doses, the overall effect may be negative. Similarly, there are reports on ET-1 exerting negative inotropic effects during pathological conditions such as congestive heart failure (33, 53).

Despite an improved CI, tezosentan administration was associated with impairment of LV contractile status. In our previous studies, we have shown beneficial effects on CI, SV, SW, and survival using dual ET receptor antagonists (29, 58). However, load-independent measures of LV contractile performance were not used in those studies. The positive effects seen may well have been due to reductions in afterload. In the present study, we therefore utilized LVPVR to minimize load-

ing confounders, a method previously validated by others (52). Sepsis and endotoxemia are associated with marked alterations in both pre- and afterload, making the choice of method crucial for analyzing myocardial effects in vivo.

In the current paper Ees, $PWR_{max}/LVEDV$, and $dP/dt_{max}/LVEDV$ all decreased in response to tezosentan, and there was a tendency for PRSW to move in the same direction. Because all of these load-independent parameters show congruent results, the conclusion that tezosentan had negative inotropic effects in this setting is fair. This is also in agreement with our recent study in which intracoronary ET-1 administration was associated with increased myocardial contractile function in “nonseptic” pigs (30), an effect likely mediated by ET_A receptors. The inotropic effect of ET_A -receptor activation has previously been described (25, 33), and this activation is thought to lead to increased sensitivity of the myofilaments for Ca^{2+} via the Na^+/H^+ exchanger, thus increasing cytoplasmic pH, increase in the inward Ca^{2+} current during depolarization and posttranslational modification of myofibrillar proteins (63). Few investigators have proposed the ET_B receptor as primarily responsible for the inotropic effects of ET-1 (5). Our current data implicate that the increase in ET-1 levels seen in endotoxemia may provide a response to uphold LV contractile function.

The effects of endotoxemia on general hemodynamics were primarily hypodynamic, and pulmonary hypertension was prominent. Gas exchange was impaired, seen as decrease in PaO_2 and increase in $PaCO_2$. Tezosentan administration was associated with increases in CI and stroke volume index, as well as decreases in SVRI, MPAP, PVRI, and CVP, whereas MAP was further decreased and HR was unaffected. The beneficial findings on global hemodynamics are possibly related to the vasodilatory effects of dual ET-receptor antagonism, more pronounced in a state of sepsis in which the ET system is markedly activated (fourfold increase in plasma ET-1 LI levels) than in a state of nonseptic anesthetized pigs (35).

Mechanical efficiency (SW/PVA) was also studied, and there was a modest decrease in response to endotoxin before intervention. In another pig model of endotoxemia, mechanical efficiency was also impaired (3), and similar findings have been shown in septic models in rats (26) and dogs (28). Contrarily, Constable et al. (12) demonstrated increased SW/PVA in endotoxemic neonatal calves. SW/PVA is most reliable as a measure of in vivo mechanical efficiency if ventricular load and heart rate are maintained relatively constant during serial mechanical efficiency measures. These were not experimentally controlled in this model.

In the literature, there is some evidence that ET-1 improves contractile efficiency in vitro (63, and references therein). This means that antagonizing the ET system would impair myocardial efficiency but, in the current study, tezosentan administration was not associated with further effects on SW/PVA.

Fig. 3. Diastolic parameters. Isovolumic relaxation parameters, tau and pressure half-time, as well as left ventricular stiffness were studied following endotoxin administration ($0.25 \mu g \cdot kg^{-1} \cdot h^{-1}$) for 5 h. After 3 h of endotoxemia, tezosentan ($1 mg \cdot kg^{-1} \cdot h^{-1}$) was started ($n = 8$, Δ) and compared with animals receiving endotoxin alone (controls, $n = 8$, $\circ$). Data are presented as means $\pm$ SE, with relative changes from baseline. Effects of endotoxin before intervention are displayed as $\#P < 0.05$. Differences between groups post-intervention are displayed as $*P < 0.05$ and $**P < 0.01$.

Fig. 4. Endothelin-1-like immunoreactivity (ET-1 LI; A) and troponin I (B) in arterial plasma were studied following endotoxin administration ($0.25 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) for 5 h. After 3 h of endotoxemia, tezoesentan ($1 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) was started ($n = 8$, ▲) and was compared with animals receiving endotoxin alone (controls, $n = 8$, ○). Data are presented as means $\pm$ SE. Effects of endotoxin are displayed as $##P < 0.01$ and $###P < 0.001$. Differences between groups postintervention are displayed as $***P < 0.001$.

Endotoxemia was associated with deterioration of isovolumic relaxation, seen as prolongation of tau, but had no evident effects on LV stiffness. Several investigators have reported similar findings (1, 62). In healthy volunteers, Kiely et al. (27) infused ET-1 intravenously and found impaired LV relaxation using echocardiographic parameters. In a cecal inoculation model in rats, Brahmabhatt and coworkers (6) could show

prolongation of tau at 12 and 24 h postinoculation, which was further prolonged by infusing BigET-1, a precursor of ET-1. This suggests that sepsis per se as well as the ET system impairs LV isovolumic relaxation. Diastolic dysfunction is also seen in septic patients either as a sole manifestation of septic myocardial depression or in conjunction with systolic dysfunction (48).

Tezosentan improved isovolumic relaxation (tau and $t_{1/2}$) and decreased LV stiffness. These findings are in line with our previous work (29) in which high-volume resuscitated endotoxemic pigs improved measures of LV stiffness when treated with tezoesentan in the same dosage. In a recent study from our group, we administered either ET-1 or sarafotoxin 6c, a selective ET_B -receptor agonist, into the coronary circulation (30). Both of these peptides were associated with deteriorated isovolumic relaxation (tau and $t_{1/2}$) which suggest that the ET_B -receptor is strongly involved. ET receptor antagonism has also been beneficial in this regard in other models. Goldberg et al. (19) reported impairment in human myocyte relaxation upon ET-1 administration, which was attenuated by an ET_A -receptor antagonist, and Mebazaa et al. (34) reported that papillary muscles from rabbits exposed to endotoxin in vivo could show prolonged time to half relaxation, which was counteracted by an ET_A -receptor antagonist. These authors found the ET_A -receptor responsible for the negative lusitropic effects, whereas our previous results strongly suggest the ET_B -receptor (30). The present data do not discriminate which of the ET receptors are responsible for the effects seen. However, while being a dual ET receptor antagonist, tezoesentan has a high ET_A/ET_B antagonizing effect ratio (11). Therefore, a high degree of ET_A -receptor antagonism would be expected in this model, suggesting that the results possibly were mainly due to ET_A -receptor antagonism. On the other hand, there is clear evidence of ET_B -receptor antagonizing effects by tezoesentan seen as increased levels of plasma ET-1 LI. The elevation of plasma ET-1 upon ET receptor antagonism depends upon blocking pulmonary endothelial ET_B -receptors, which are responsible for the clearing function of circulating ET-1 (16).

These results suggest that specific ET_B receptor antagonism could be preferable, improving diastolic function without negatively affecting systole. However, in a previous study from our group, selective ET_B -receptor antagonism proved detrimental during endotoxemia, probably due to unopposed vasoconstriction mediated by ET_A receptors and decreased ET clearance (57).

Endotoxemia was not associated with any effects on cardiac oxygen utilization parameters or Q_{GCV} . MOER decreased somewhat in response to endotoxin, an effect also seen in human sepsis (14). As previously reported in both human sepsis and animal endotoxemia (29) cardiac troponin I was increased in response to endotoxin. The mechanisms behind this phenomenon are yet unclear, but mere ischemia is unlikely since neither in this model nor in human sepsis is myocardial hypoperfusion evident (14). Tezosentan administration was coupled to a modest decrease in MDO_2 , possibly related to the concomitant decrease in hemoglobin, and a modest increase in MOER. These effects were quite small and are less likely to have had an impact on the results of cardiac function. Interestingly, a recent report from Merkus et al. (36) suggests a local regulating factor responsible for abolished ET-1-medi-

ated constrictor effect on coronary resistance vessels. Cardiac troponin I was not affected by tezosentan.

In conclusion, in this porcine model of early endotoxemia dual ET receptor antagonism with tezosentan was associated with a reduction in contractile function, despite improved global hemodynamic parameters. In contrast, ET receptor antagonism seemed to improve diastolic function. Positive hemodynamic effects from ET receptor antagonism in acute endotoxemia may be due to changes in cardiac load and enhanced diastolic function rather than improved contractile function.

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