Angiotensin Converting Enzyme Inhibition Modulates Endogenous Endothelin in Chronic Canine Thoracic Inferior Vena Caval Constriction

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Abstract

Endothelin (ET) is a potent vasoconstrictor peptide which is elevated in plasma in congestive heart failure. Recent studies suggest an important role for angiotensin II (AII) in the activation of ET in cultured cardiomyocytes. Chronic thoracic inferior vena caval constriction (TIVCC) is a model of reduced cardiac output that mimics the neurohumoral activation observed in congestive heart failure. We hypothesized that activation of the renin-angiotensin system in TIVCC plays a role in the activation of ET and that the elevation of endogenous ET contributes to the systemic and renal vasoconstriction that characterizes this model of venous congestion. We studied conscious dogs after 7 d of TIVCC in the presence or absence of chronic angiotensin converting enzyme inhibition with enalapril. TIVCC resulted in marked activation of plasma AII and ET in plasma, right atrium, lung, and renal medulla which was further localized to cardiomyocytes, pulmonary, and renal epithelial cells. Chronic angiotensin converting enzyme inhibition abolished the increases in plasma AII and ET during TIVCC. Acute endothelin A receptor blockade with FR-139317 resulted in significant decreases in mean arterial pressure and systemic vascular resistance in TIVCC. We conclude that activation of the renin-angiotensin system contributes to the activation of circulating and local ET in TIVCC and that this activation plays an important role in the regulation of arterial pressure and systemic vascular resistance in this model of congestive failure. (*J. Clin. Invest.* 1996. 97:1286–1292.) Key words: endothelin • systemic hemodynamics • receptor antagonism • converting enzyme inhibition

Introduction

Endothelin (ET)[1] is a potent vasoconstrictor peptide originally isolated from endothelial cells, which is also produced by a variety of cells including renal epithelial cells and cardiomyocytes (1–3). In addition, ET is present in plasma and is elevated in the circulation in various cardiovascular pathophysiological states. Specifically, plasma ET has been reported to be elevated in congestive heart failure (CHF), hypertension, atherosclerosis and acute myocardial infarction (4–7).

The endothelins which are comprised of three distinct isoforms, ET-1, ET-2, and ET-3, mediate their biological actions by interacting with at least two receptors which have been cloned and well characterized (8, 9). The endothelin A (ET_A) receptor binds preferentially the ET-1 isoform and mediates potent vasoconstriction (10). The endothelin B (ET_B) receptor binds all three identified ET isoforms, ET-1, ET-2, and ET-3, and when activated releases nitric oxide and prostacyclin (11). However, recent studies have shown that the ET_B receptor may mediate vasoconstriction depending upon the species and regional vasculatures studied (12–14).

ET has been suggested to be an important mediator in disease states characterized by vasoconstriction due to the wide distribution of its receptors and its potent vasoconstrictor actions. In recent studies, specific ET antagonists have been shown to have potent hemodynamic actions in models of subarachnoid hemorrhage, hypertensive rats, and rats with chronic heart failure (15, 16). However, the mechanism of ET activation and its tissue and plasma distribution in these states remain poorly defined. Specifically, the role of angiotensin II (AII) a potent in vitro stimulant of ET production and release has not been defined in vivo.

Chronic thoracic inferior vena caval constriction (TIVCC) is a model of congestive failure characterized by a low cardiac output (CO), systemic and renal vasoconstriction, ascites, sodium retention, and increased circulating ET (17). Indeed, this model was used successfully to define the pathophysiologic role of the renin-angiotensin system (RAS) in the control of vascular tone and sodium excretion (18–20). TIVCC like severe heart failure is characterized by marked activation of the RAS and the sympathetic nervous system but without elevation of cardiac filling pressures or activation of the cardiac natriuretic peptide system.

This study was designed to extend our understanding of both the circulating and tissue ET during the evolution of

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1. *Abbreviations used in this paper:* ACE-I, angiotensin converting enzyme inhibitor; AII, angiotensin II; ANP, atrial natriuretic peptide; CHF, congestive heart failure; CO, cardiac output; ET, endothelin; ET_A, endothelin A receptor; ET_B, endothelin B receptor; HR, heart rate; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PA, plasma aldosterone; PAH, sodium para-aminohippurate; PCWP, pulmonary capillary wedge pressure; PRA, plasma renin activity; PVR, pulmonary vascular resistance; RAP, right atrial pressure; RAS, renin-angiotensin system; RPF, renal plasma flow; RVR, renal vascular resistance; SVR, systemic vascular resistance; TIVCC, thoracic inferior vena caval constriction.

TIVCC and to address the potential role of the RAS in the activation of this potent vasoconstrictor. In addition, we hypothesized that activation of endogenous ET contributes to the systemic and renal vasoconstriction observed in TIVCC. To accomplish our goals we used immunohistochemistry and radioimmunoassay techniques to address the activity of the circulating and local tissue ET systems in TIVCC in the presence or absence of chronic angiotensin converting enzyme inhibition (ACE-I) with enalapril. Furthermore, we used acute ET_A receptor blockade with FR-139317, a highly selective ET_A receptor antagonist (21), in chronically instrumented conscious TIVCC and normal dogs to address the contribution of endogenous ET in the regulation of systemic and renal hemodynamics.

Methods

Experiments were performed in 21 male mongrel dogs divided in four groups weighing between 18 and 22 kg, fed normal dog chow (Lab Canine Diet 5006; Purina Mills, St. Louis, MO), and allowed free access to tap water. Groups I, II, and IV consisted of 15 TIVCC dogs which underwent thoracotomy for placement of a constricting band around the thoracic inferior vena cava to produce experimental congestive failure. Group III consisted of six normal dogs.

Surgical preparation. Under sodium pentobarbital anesthesia (30 mg/kg) via a right thoracotomy and after adequate exposure, a band was placed about the thoracic inferior vena cava to create $\sim$ 50% reduction in diameter in groups I, II, and IV, as described previously (17). In all groups, animals underwent implantation of a subcutaneously placed chronic arterial catheter (Access Technologies, Skokie, IL). This was implanted via the left femoral artery with the access well tunneled subcutaneously and placed on the left upper hind limb. The catheter was flushed biweekly with heparinized saline. All surgical procedures were preceded by prophylactic antibiotic treatment with clindamycin and Combiotic (Pfizer, New York) preoperatively and on the first 2 d postoperatively. All dogs were preacclimated to the sling in which the conscious studies were performed.

Evolution studies (group I). Five dogs underwent arterial catheter placement and were placed in metabolic cages and were fed two cans of Hill's canine i/d food (Hills Pet Nutrition, Topeka, KS) daily to provide a fixed sodium intake of 58 meq/d. After 3 d of equilibration, dogs were briefly anesthetized with sodium pentothal to allow the percutaneous placement of a cardiac thermodilution catheter through the external jugular vein. Dogs were then placed in the study sling and allowed to recover. Baseline hemodynamics including heart rate (HR), mean arterial pressure (MAP), right atrial (RAP) and pulmonary capillary wedge pressures (PCWP), mean pulmonary arterial pressure (MPAP), and CO were obtained. Blood samples were obtained for baseline plasma renin activity (PRA), plasma aldosterone (PA), plasma atrial natriuretic peptide (ANP), and plasma ET. Dogs then underwent TIVCC and were returned to their metabolic cages. Daily blood samples were obtained for plasma ET and PRA, and on the seventh day hemodynamics and hormonal measurements were repeated as outlined for the baseline measurements. Dogs were then killed with 10 cm³ of a 26% sodium pentobarbital, 10% isopropyl alcohol solution, Sleep-Away (Fort Dodge Laboratories Inc., Fort Dodge, IA), and tissue was harvested from the right atrial and ventricular cardiac chambers, lung, and renal medulla and immediately frozen in liquid nitrogen. Tissue was stored at $-80°C$ until the time of tissue ET determination.

Chronic angiotensin converting enzyme inhibition studies (group II). Five dogs underwent TIVCC and arterial catheter placement and were allowed to recover. On the first day after TIVCC, enalapril (Merck Sharp & Dohme, West Point, PA) was begun at a dose of 5 mg twice a day orally. Daily blood samples for plasma ET and PRA were obtained, and after 7 d hemodynamic measurements were ob-

tained in the conscious state. Dogs were then killed and the tissues were harvested as described for group I.

Acute ET blockade studies (groups III and IV). Six normal dogs and five chronic TIVCC dogs were briefly anesthetized with sodium pentothal to allow the placement of a cardiac thermodilution catheter via the external jugular vein. Dogs were then allowed to equilibrate in the study sling while standing on their four extremities and the experimental protocol was conducted in the conscious state. During the 1-h equilibration period, a bolus of sodium para-aminohippurate (PAH) (10 mg/kg) (Merck Sharp & Dohme) was given intravenously and a maintenance infusion at 0.25 mg/kg/min was begun to permit the estimation of renal plasma flow (RPF) and the calculation of renal vascular resistance (RVR). After equilibration, a 30-min baseline clearance was obtained. During this and subsequent experimental clearances, HR, CO in triplicate, MAP, RAP, PCWP, and MPAP were obtained at the beginning and end of each clearance, and urine was collected at the end of each clearance in a calibrated cylinder to quantify flow and later measurement of PAH. Arterial blood samples were obtained mid-clearance for the determination of PAH, plasma ET, PRA, PA, plasma ANP, and hematocrit. After baseline, an intravenous infusion of FR-139317 (5 μg/kg/min) was begun at a rate of 1 ml/min and the first experimental clearance was obtained after allowing a 15-min lead-in period. Subsequently the FR-139317 infusion was increased to (10 μg/kg/min), and after allowing a 15-min lead-in period a final experimental clearance was obtained.

Plasma AII studies. 5-ml blood samples from four normal dogs, three TIVCC dogs, and four TIVCC+ACE-I dogs were collected in prechilled tubes containing 0.25 mM EDTA, 0.1 mM PMSF, and 5 mM sodium tetrathionate which were used to inhibit intrinsic renin activity. Blood was centrifuged at 2,500 rpm at 4°C and the plasma was separated and snap frozen in liquid nitrogen. Samples were then stored at $-70°C$ until the time of radioimmunoassay. AII was extracted from plasma using Bond Elut C18 cartridges (Analytichem International, Inc., Harbor City, CA) with a recovery rate of 77% and an interassay and intraassay variability of 8 and 9%, respectively. Extracted samples were incubated overnight with the primary rabbit polyclonal antibody for AII (Peninsula Laboratories, Inc., Belmont, CA). Labeled antigen was added the next day and incubated overnight. Lastly, goat anti–rabbit antibody was applied and the antibody–antigen complex was counted on an automated gamma counter. The assay has a sensitivity of 7.5 pg/ml and a range of 7.5–500 pg/ml. Cross-reactivity to AI and AIII was 1.4 and 31%, respectively.

Analysis. CO was determined by thermodilution (Cardiac Output model 9510-A computer; American Edwards Laboratories, Irvine, CA). The following formulas were used: $RPF = (Urinary_{PAH} \times Urine_{Flow}/Plasma_{PAH})$; renal blood flow $(RBF) = (RPF/[1 - Hct])$; $RVR = (MAP - RAP/RBF)$; systemic vascular resistance $(SVR) = (MAP - RAP/CO)$; and pulmonary vascular resistance $(PVR) = (MPAP - PCWP/CO)$.

Blood for hormone analysis was collected into sodium EDTA tubes, immediately placed on ice, and centrifuged at 2,500 rpm at 4°C. Plasma was separated and stored at $-20°C$ until the assay. Plasma and urinary PAH were measured with a colorimetric assay (22). Plasma ANP was determined by a sensitive radioimmunoassay as described previously (23). PA levels were determined by radioimmunoassay using a Coat-a-Count (Diagnostic Products Corp., Los Angeles, CA). PRA was determined by radioimmunoassay using the method of Haber et al. (24).

Plasma and tissue ET determination. The tissues from right atria, right ventricle, lung, and renal medulla were pulverized, boiled for 5 min in 10 vol of 1 M acetic acid/20 mM hydrochloric acid solution to abolish intrinsic proteolytic activity, and then homogenized. Samples were then centrifuged at 15,000 rpm for 30 min and the supernatant was stored at $-20°C$ until the time of radioimmunoassay. Plasma and tissue ET were determined using an ET-1 assay system (Amersham International, Buckinghamshire, United Kingdom) as described previously (25).

Immunohistochemistry. Immunohistochemical staining for ET in cardiac atria, lung parenchyma, and renal medulla was performed as described previously (6). Briefly, paraffin embedded tissue was cut to 6 μm and placed on silinized slides. The slides were incubated with 0.6% hydrogen peroxide to block endogenous peroxidase activity. 5% normal goat serum was then added to block nonspecific binding sites before antibody was applied. Sections were incubated for 24 h with rabbit anti–ET-1 antibody (Peninsula Laboratories, Inc.) at a dilution of 1:1,600. Control slides were treated with normal diluted rabbit serum. Sections were then incubated for 30 min with the secondary antibody goat anti–rabbit antiserum at a dilution of 1:100 and counter-stained with hematoxylin. Slides were then reviewed blindly by two observers and graded according to intensity and percent coverage of immunostaining and compared with its respective control slide to exclude nonspecific staining. Representative slides of each group were then photographed and are illustrated in Fig. 3.

Statistical analysis. All data are presented as mean±standard error. Data from normals group I + group III ($n = 11$) at baseline were pooled and compared with pooled data from group I + group IV ($n = 10$) after 7 d of TIVCC and compared with data from group II TIVCC + ACE-I ($n = 5$) after 7 d of TIVCC in the presence of chronic oral ACE-I by one-way ANOVA. For groups III and IV, within the group data were compared using ANOVA for repeated measures followed by Fisher's least significant difference when appropriate, between group data were compared by one-way ANOVA. Statistical significance was accepted at $P < 0.05$.

Results

Table I reports the hemodynamic and hormonal characteristics in normal dogs and after 7 d of TIVCC with or without chronic angiotensin converting enzyme inhibition. 7 d of TIVCC resulted in significant decreases in CO and RAP and significant increases in SVR and PVR without altering HR, MAP, or PCWP. In the presence of chronic ACE-I, the increases in SVR tended to be attenuated, the increases in PVR and the decreases in RAP were abolished, and MAP was reduced significantly when compared with normal dogs. 7 d of TIVCC also resulted in significant changes in hormonal parameters with increases in PRA, PA, and plasma ET, while ANP significantly decreased. Chronic ACE-I during the evolution of TIVCC abolished the observed increases in plasma ET during TIVCC alone without significantly affecting the other hormonal parameters. In addition, in the subset of animals where AII was measured, 7 d of TIVCC resulted in marked increases in plasma AII (732±310 pg/ml) when compared with normal animals (80±10 pg/ml) ($P < 0.05$). These increases in plasma AII in TIVCC were completely abolished by chronic ACE-I to levels not different than in normal animals (81±20 pg/ml) (NS).

Fig. 1 illustrates the daily PRA and plasma ET values during the evolution of TIVCC in the presence or absence of chronic ACE-I. PRA significantly increased on the first day after TIVCC, progressively increased throughout the remainder of the study, and was not affected by the addition of enalapril. Plasma ET significantly increased on the fourth day after TIVCC and remained elevated throughout the remainder of the study. In contrast, chronic ACE-I during the evolution of TIVCC resulted in no significant increases in plasma ET compared with normal values.

Fig. 2 illustrates the tissue ET concentrations in normal and chronic TIVCC dogs in the presence or absence of chronic ACE-I. Right atrial, pulmonary, and renal medullary ET concentrations were significantly increased in TIVCC when compared with normal dogs. This tissue ET response was significantly abolished with the addition of enalapril during the evolution of TIVCC. In contrast, right ventricular concentrations were not significantly different between the three groups.

Fig. 3 illustrates representative ET immunohistochemical staining of normal, TIVCC, and TIVCC + ACE-I in right atrium, lung parenchyma, and renal medulla. Normal cardiomyocytes and inner medullary renal epithelial cells had modest staining for ET, and this was markedly enhanced after 7 d of TIVCC. Chronic ACE-I during the evolution of TIVCC abolished the increases in ET immunostaining in cardiomyocytes and renal epithelial cells observed in TIVCC alone. Normal pulmonary epithelial cells had modest staining for ET when compared with control slides. After 7 d of TIVCC, there was no detectable change in ET staining in epithelial cells. However, pulmonary epithelial cell ET staining was reduced in the ACE-I–treated TIVCC group. Although not illustrated, normal rabbit serum–treated control slides had no significant nonspecific staining.

Table II reports the systemic and renal hemodynamic response to acute ET$_A$ receptor blockade in normal and TIVCC dogs. FR-139317 in normal dogs resulted in modest but signifi-

Table I. Systemic Hemodynamics and Hormonal Parameters in Normal and TIVCC Dogs with or without ACE-I

	Normal ($n = 11$) (Groups I and III)	TIVCC ($n = 10$) (Groups I and IV)	TIVCC + ACE-I ($n = 5$) (Group II)
HR (bpm)	136±3	143±4	132±6
MAP (mmHg)	118±4	105±5	96±9*
CO (liters/min)	4.78±0.32	2.10±0.24*	2.45±0.44*
RAP (mmHg)	5±1	3±1*	4±1
PCWP (mmHg)	8±1	6±1	7±1
SVR (mmHg/liter/min)	24.6±1.7	54.4±6.1*	40.8±5.0*
PVR (mmHg/liter/min)	2.48±0.37	4.39±0.55*	2.96±0.71
PRA (ng/ml/h)	0.7±0.2	9.4±2.4*	10.7±1.1*
ALDO (ng/dl)	3.6±0.6	247.2±49.6*	208.1±87.9*
ET (pg/ml)	9.9±0.5	15.3±1.2*	11.4±1.8‡
ANP (pg/ml)	36.7±8.6	13.7±3.4*	7.8±2.6*

Data are mean±SE. * $P < 0.05$ vs. Normal, ‡ $P < 0.05$ vs. TIVCC. *ALDO*, plasma aldosterone.
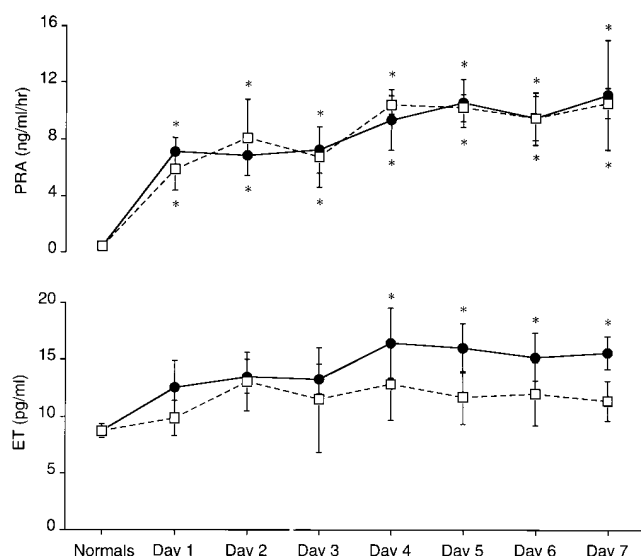
Figure 1. Daily PRA and plasma ET values during the evolution of TIVCC in the presence or absence of ACE-I. *Solid circles*, TIVCC group 1; *open squares*, TIVCC + ACE-I group 2. *$P < 0.05$ vs. Normals.

cant decreases in SVR and RVR without changes in HR, MAP, CO, RAP, PCWP, or PVR. FR-139317 in TIVCC resulted in significant decreases in MAP and SVR without changes in HR, CO, RAP, PCWP, PVR, or RVR.

Fig. 4 illustrates the absolute changes from baseline in MAP, SVR, and RVR to acute ET_A receptor blockade in normal and TIVCC dogs. FR-139317 resulted in a significantly greater decrease in MAP at the $10 \mu g/kg/min$ dose (-21.5 ± 3.67
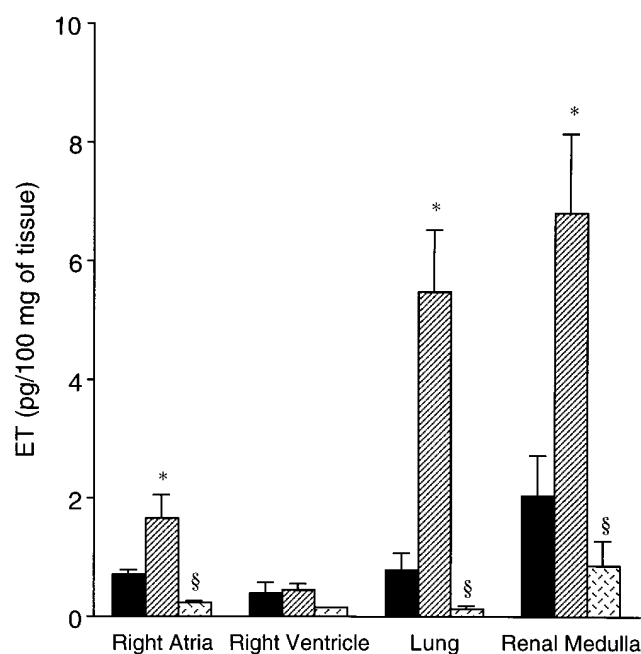

Figure 2. Tissue ET concentrations in normal dogs ($n = 4$) (*solid bars*), after 7 d of TIVCC ($n = 4$) (*hatched bars*), and after 7 d of TIVCC in the presence of ACE-I ($n = 4$) (*gridded bars*). *$P < 0.05$ vs. Normals; $^{\S}P < 0.05$ vs. TIVCC.

vs. -5.42 ± 3.62 mmHg) and SVR (-13.7 ± 4.4 vs. -3.3 ± 1.2 mmHg/liter/min). In contrast, FR-139317 resulted in a significant decrease in RVR in normal dogs while no change was observed in the TIVCC group (-0.16 ± 0.06 vs. 0.05 ± 0.08 mmHg/ml/min).

Discussion

This study demonstrates the activation of the circulating and local ET systems in a model of reduced CO and marked systemic, pulmonary, and renal vasoconstriction that neurohumorally mimics CHF but in the absence of elevated cardiac filling pressures or left ventricular dysfunction. Furthermore, immunohistochemical staining demonstrates enhanced cardiomyocyte and renal epithelial cell ET staining in association with increased tissue concentrations. Secondly, this study demonstrates for the first time in vivo that chronic angiotensin converting enzyme inhibition with low dose enalapril during the evolution of TIVCC inhibits the activation of circulating and tissue ET and plasma AII. Lastly, this study also reports a pathophysiological role for endogenous ET, during acute ET_A receptor blockade, in the maintenance of arterial pressure and systemic vasoconstriction in this model of reduced preload and systemic vasoconstriction.

This study confirms that 7 d of TIVCC result in significant elevation of plasma ET, as is observed in human and experimental CHF (4, 17). Furthermore, the studies of evolving TIVCC demonstrate that unlike the early activation of the RAS in this model, circulating ET is activated later during the evolution of TIVCC. This elevation of circulating ET may represent endothelial cell spillover or local tissue ET production. Nonetheless, this study extends previous studies by demonstrating selective local increases in ET in cardiac, pulmonary, and renal tissues which were further localized by immunohistochemical staining to atrial cardiomyocytes and renal medullary epithelial cells. This observation is supported by recent in vitro studies which have demonstrated ET production by cardiomyocytes and renal epithelial cells (2, 3).

Although pulmonary epithelial cell ET immunostaining was observed in the normal dog, immunohistochemical staining did not detectably change in the TIVCC lung as was observed in cardiac and renal tissues. Nevertheless, increased tissue ET immunoreactivity in TIVCC, preliminary reports of increased ET mRNA in the lung in this model (26), and decreased ET immunostaining in the lung of the ACE-I–treated TIVCC group support local pulmonary ET production in this model. Indeed, pulmonary endothelial cells may be involved in the production and release of ET into the pulmonary circulation, accounting for our findings of increased ET immunoreactivity. Further studies will be helpful in determining the source of increased pulmonary ET in this model.

Recent studies have shown that ET mediates myocardial cell hypertrophy, is a potent pulmonary vasoconstrictor, and modulates proximal tubular sodium reabsorption and osmolar regulation in the inner medullary collecting duct (2, 27–29). The local activation of ET in cardiac, pulmonary, and renal tissues in TIVCC suggests a potential role for ET in the cardiac, pulmonary, and renal adaptations to chronic reductions in CO and venous congestion. Further studies investigating the potential role of chronic ET blockade may provide insight into the pathophysiologic role of locally activated ET.
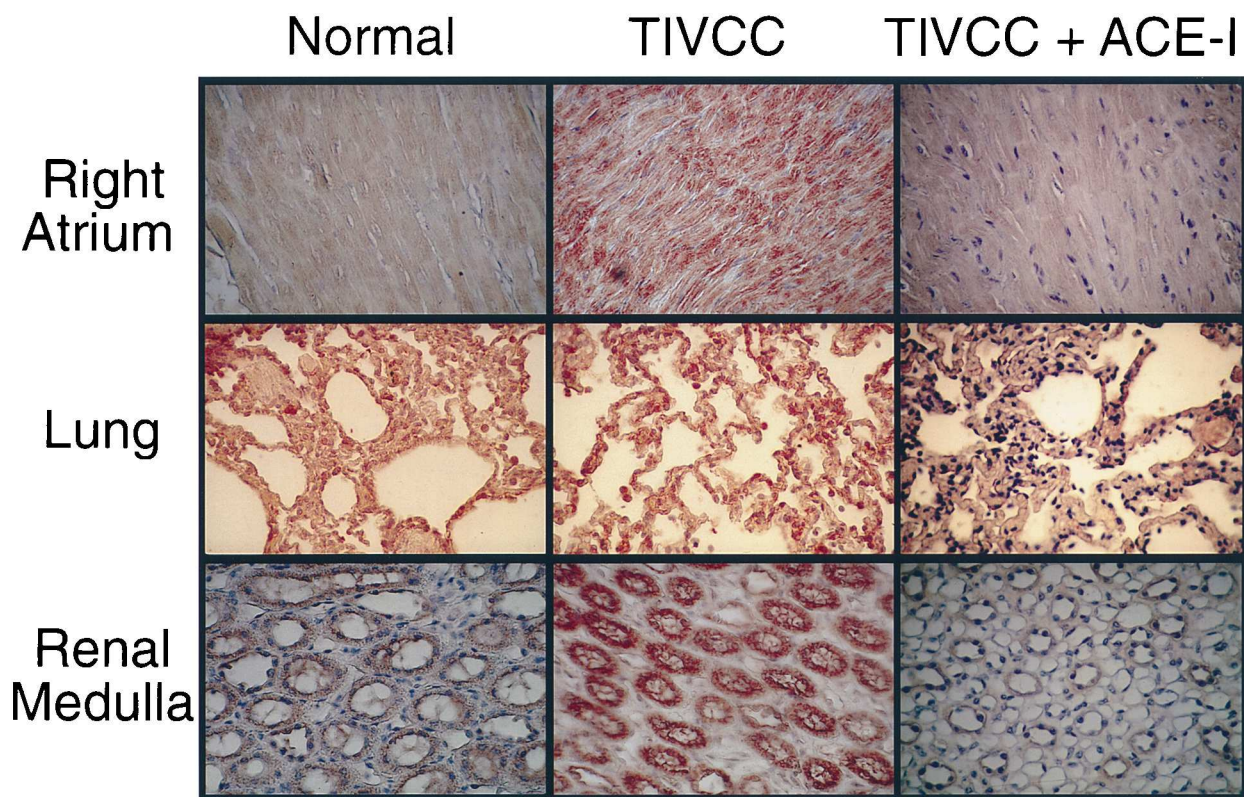
The mechanism of local ET activation in this model re-

Figure 3. Representative immunohistochemical staining of right atrium, lung parenchyma, and renal medulla in normal dogs (*left*), in TIVCC (*middle*), and in TIVCC during chronic ACE-I (*right*).

mains unclear but may involve the activation of the RAS via AII which has been reported to directly enhance ET production in vitro (30). This mechanism is supported by our studies in group II in which chronic ACE-I abolished the increases in both the circulating and tissue ET in this model and by the plasma AII concentrations which decreased to levels no different than in normal animals in the presence of chronic ACE-I. However, this observation should be interpreted with caution since other mechanisms other than AII inhibition may be involved, including the potentiation of bradykinin which via nitric oxide and cGMP could also suppress ET production as suggested by recent studies in cultured endothelial cells (31, 32). Nevertheless, modulation of circulating and tissue ET may

play an important role in the therapeutic actions of ACE-I agents which are commonly used in patients with heart failure. This is underscored by the favorable hemodynamic effects of chronic ACE-I in our model in group II with reduction in arterial pressure and attenuation of systemic and pulmonary vasoconstriction resulting in a hemodynamic profile which is similar to that observed in group IV with TIVCC during acute ET_A receptor blockade. Further studies using selective AII antagonists and bradykinin antagonists may help clarify the exact mechanism of ACE-I–mediated ET inhibition although it is likely that a multifactorial mechanism is invoked.

Another potential mechanism for ET activation in this model may involve the absence of ANP activation in the ab-

Table II. Systemic and Renal Hemodynamic Response to FR-139317 in TIVCC and Controls

	Group III: Control (*n* = 6)			Group IV: TIVCC (*n* = 5)		
	Baseline	FR (5 μg/kg)	Fr (10 μg/kg)	Baseline	FR (5 μg/kg)	FR (10 μg/kg)
HR (bpm)	131±3	138±4	137±6	142±5	148±6	144±5
MAP (mmHg)	120±4	114±3	115±3	116±6	104±6*	95±9*‡
CO (liters/min)	4.71±0.42	4.96±0.40	5.11±0.39	2.14±0.27‡	2.12±0.30‡	2.21±0.29‡
RAP (mmHg)	5±1	5±1	5±1	2±1‡	3±1‡	3±1‡
PCWP (mmHg)	9±1	9±1	9±1	5±1‡	5±1‡	5±1‡
SVR (mmHg/liter/min)	25.4±2.5	22.6±1.7*	22.1±1.9*	56.5±7.0‡	50.5±5‡	42.8±3*‡
PVR (mmHg/liter/min)	2.6±0.4	2.6±0.4	2.6±0.4	3.6±0.4	3.7±0.6	3.8±0.8
RVR (mmHg/ml/min)	0.32±0.06	0.21±0.05	0.16±0.01*	0.55±0.09	0.60±0.13‡	0.59±0.15‡

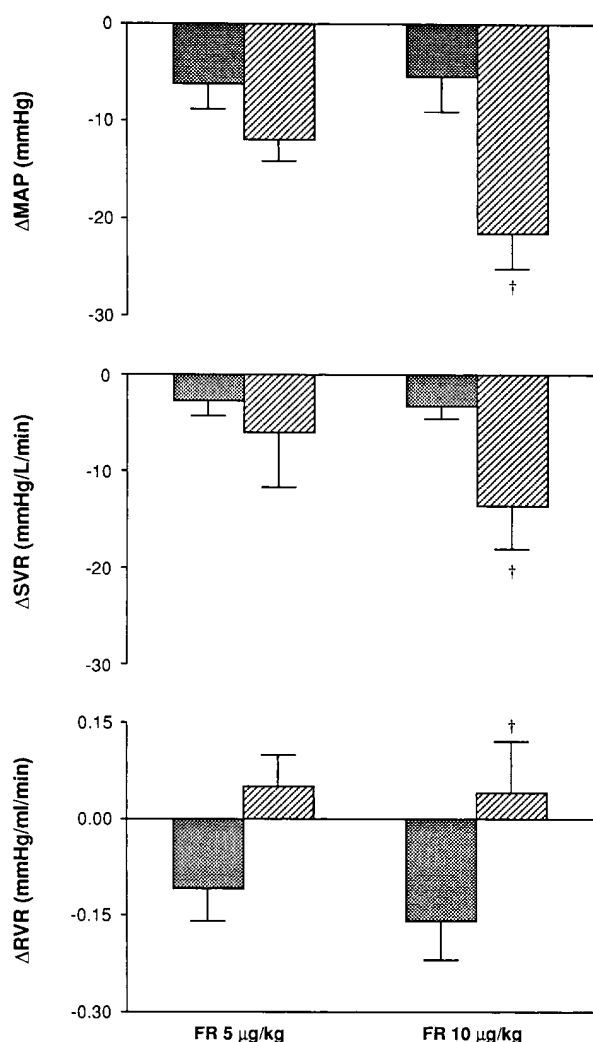Data are mean±SE. *P < 0.05 vs. Baseline; ‡P < 0.05 vs. Controls.

Figure 4. Absolute changes from baseline in MAP (ΔMAP), SVR (ΔSVR), and RVR (ΔRVR) in controls (group II) (*solid bars*) and after 7 d of TIVCC (group III) (*hatched bars*) during acute intravenous FR-139317 administration at 5 and 10 μg/kg/min. †$P < 0.05$ vs. controls.

sence of elevated atrial pressures. Recent studies have demonstrated that ANP inhibits AII-stimulated ET production by endothelial cells via activation of second messenger cGMP (30) and in pathophysiological states of ET activation such as in postischemic acute renal dysfunction (32, 33). Lastly, tissue hypoxia which is characteristic of low CO states may also be a stimulus for ET production in TIVCC (34). This is supported by recent studies in the dog and the rat during acute moderate hypoxia and ischemia which were associated with marked renal ET activation and gene expression (35–37). Moreover, the peripheral and pulmonary vasodilatation with improved tissue perfusion during ACE-I could also explain the subsequent decrease in circulating and local ET.

Although several studies have demonstrated circulating ET activation in experimental and human CHF, the pathophysiologic significance of this elevation remains unclear. However, recent studies in a model of acute renal dysfunction and ET activation during ET$_A$ receptor blockade suggested a role for ET in the regulation of acute systemic and renal hemodynamics

(31). The present study extends those observations to a state of chronic ET activation and marked vasoconstriction and reports an important role for endogenous ET in the maintenance of arterial pressure and in the regulation of SVR in this state of a chronically reduced CO. Acute ET$_A$ receptor blockade with FR-139317 in TIVCC resulted in dose-dependent decreases in MAP. The physiological mechanism of this decrease in arterial pressure is via arterial vasodilatation since neither HR, CO, nor cardiac filling pressures significantly changed. However, a reduction in cardiac contractility which may have contributed to the decrease in arterial pressure cannot be excluded. This study also demonstrates that the renal circulation did not respond to acute ET$_A$ blockade unlike the renal vasodilatation observed in normal animals. Several potential mechanisms may explain this observation. Recent studies have shown that the ET$_B$ receptor can mediate vasoconstriction in the renal circulation in the rat and therefore can be unaffected by ET$_A$ receptor blockade. However, we have demonstrated recently that in the dog the renal vasoconstrictor response is ET$_A$ dependent (38). In addition other vasoconstrictors such as AII, which is activated in this model, may be the dominant vasoconstrictor in the renal circulation.

In this study, PVRs were unaffected by the ET$_A$ antagonist despite our findings of increased tissue ET immunoreactivity in the lung. However, recent studies may suggest that the dominant vasoconstrictor as well as most abundant receptor in the lung is the ET$_B$ receptor. This would explain the lack of vasodilatation with acute ET$_A$ receptor antagonism despite the observed decreases in PVR during chronic ACE-I and decreased pulmonary ET production. Alternatively, the inhibition of plasma AII may account for the pulmonary vasodilatation in the chronic ACE-I group, but additional studies with specific AII antagonism or ET$_B$ antagonism may help to elucidate these issues.

This study also provides insight into the basal regulation of systemic and renal resistances by endogenous ET. Specifically, acute ET$_A$ receptor blockade in normal animals resulted in a small but significant decrease in SVR and RVR, suggesting a role for basal circulating and/or local ET in the regulation of vascular tone. These vascular actions are clearly enhanced in a state like TIVCC where there is marked activation of the ET systems.

In conclusion, our studies report circulating and local ET activation in a model of congestive failure produced by chronic TIVCC. Chronic angiotensin converting enzyme inhibition abolishes both the circulating and local ET activation, and acute ET blockade studies suggest an important role for endogenous ET in the regulation of vascular tone and arterial pressure in this state characterized by marked systemic and renal vasoconstriction. One may speculate that intense activation of the RAS which also characterizes severe CHF may play an important role in the activation of ET. Together, these two neurohumoral systems may mediate an important vasoconstrictor response for the maintenance of arterial pressure in states of chronic reductions in CO.

Acknowledgments

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