Inhibition by calcium antagonism of circulating and renal endothelin in experimental congestive heart failure

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Wei, Chiming, and John C. Burnett, Jr. Inhibition by calcium antagonism of circulating and renal endothelin in experimental congestive heart failure. Am. J. Physiol. Heart Circ. Physiol. 278: H263–H268, 2000.—Endothelin (ET) is a potent vasoconstrictor and sodium-regulating peptide whose tissue and plasma concentrations are increased in congestive heart failure (CHF). ET may mediate its vasoconstrictor and sodium-regulatory actions secondary to an increase in intracellular calcium. Calcium influx may augment ET synthesis. Although felodipine, a dihydropyridine calcium-channel antagonist, is effective in reducing vascular resistance in states of generalized vasoconstriction, its actions in CHF on circulating and local tissue ET remain undefined. The current studies were designed to determine the modulating actions of felodipine (oral, 40 mg/day for 7 days; n = 6) in an experimental canine model of CHF produced by chronic thoracic inferior vena caval constriction (TIVCC) compared with normal (n = 7) and TIVCC-alone (n = 7) dogs. We hypothesized that felodipine would decrease circulating and renal ET. Plasma ET was significantly increased in TIVCC compared with normal dogs (26 ± 0.5 vs. 12 ± 0.7 pg/ml, P < 0.05) and was markedly decreased by felodipine compared with TIVCC alone (14 ± 3 vs. 26 ± 0.5 pg/ml, P < 0.05). Renal ET immunohistochemical staining demonstrated the presence of ET in normal kidney, which was markedly increased in renal cortex and medulla in TIVCC dogs. Renal cortical and medullary ET staining densities were markedly decreased with felodipine compared with those with TIVCC alone. In the TIVCC + felodipine group, cardiovascular hemodynamics also was markedly improved compared with the TIVCC-alone group (systemic vascular resistance: 27 ± 2 vs. 44 ± 3 resistance units (RU); P < 0.05; pulmonary vascular resistance: 3.3 ± 0.1 vs. 5.7 ± 0.4 RU, P < 0.05; cardiac output: 2.9 ± 0.2 vs. 1.7 ± 0.1 l/min, P < 0.05). This study demonstrates important modulating inhibitory actions of felodipine on renal and plasma ET in an experimental model of CHF.

Endothelin (ET) is an endothelium-derived potent vasoconstrictor and sodium-regulating peptide (26). Tissue and plasma concentrations of ET are increased in human and animal congestive heart failure (CHF) (4, 14, 24). Studies reported that increased plasma ET in CHF in part may be of renal origin (3, 21). ET may mediate its biological actions secondary to an increase in intracellular calcium, and calcium influx may augment ET synthesis (12). Although felodipine, a dihydropyridine calcium-channel antagonist, is effective in reducing vascular resistance in states of generalized vasoconstriction (16), the actions of felodipine on circulating ET and local tissue ET in CHF remain undefined.

Chronic thoracic inferior vena caval constriction (TIVCC) is a model of low cardiac output (CO) and congestive failure that results in marked systemic and pulmonary vasoconstriction and sodium retention with edema in association with activation of the plasma and tissue ET system (3, 23). This model is unique in mimicking CHF, in that the chronic reduction in venous return produced by TIVCC reduces CO in the absence of ventricular volume overload and therefore is not associated with ventricular dilatation and hypertrophy or increased atrial natriuretic peptide. Several studies utilizing this model reported initial insights into the temporal activation and functional importance of the renin-angiotensin system (RAS) in CHF (3).

The current studies were designed to determine the modulating actions of felodipine in an experimental model of CHF produced by TIVCC compared with normal and TIVCC-alone dogs. We hypothesized that felodipine would decrease circulating and local renal ET and improve cardiovascular hemodynamics in TIVCC.

METHODS

Experimental protocol. Studies were conducted on mongrel dogs (weighing between 18 and 23 kg) fed normal chow (Lab Canine Diet 5006, Purina Mills, St. Louis, MO) and allowed free access to tap water. The dogs were divided into three groups: normal dogs (n = 7); dogs that underwent surgery for placement of a chronic TIVCC to create a low-CO model of congestive failure (n = 7) (3, 5, 23); and dogs that were subjected to TIVCC + felodipine administration (oral, 40 mg/day for 7 days; n = 6). Felodipine was given orally at 20 mg every 12 h (40 mg/day) starting the day before the TIVCC procedure and continuing for 7 days after the TIVCC procedure. On the basis of our preliminary dose-response studies in dogs (oral felodipine 20, 40, and 60 mg daily), we selected the current dose of felodipine because plasma level of felodipine was stable and no hypotensive effect of felodipine was found. Dogs received prophylactic antibiotic treatment with cindamyicin (Pfizer) preoperatively and on the first 2 days postoperatively. In TIVCC dogs, surgery was performed via a right thoracotomy under pentobarbital sodium anesthesia (30 mg/
kg). After adequate exposure was achieved, a band was placed about the thoracic inferior vena cava to create an ~50% reduction in diameter, as previously described (3, 5, 23). The dogs were then allowed to recover. The acute experiment was performed on postoperative day 8. On the night before the acute experiment, all dogs were fasted but allowed continued access to tap water. On the day of the acute experiment, all dogs were anesthetized with pentobarbital (10–30 mg/kg iv), with supplemental doses given as needed during the experiment. The dogs were intubated and mechanically ventilated (Harvard Respirator, Harvard Apparatus, Millis, MA) with supplemental oxygen at 4 l/min. The right external jugular vein was exposed, and a flow-directed, balloon-tipped 7-F thermodilution catheter (model 93, 121A, American Edwards Laboratories, Santa Ana, CA) was advanced into the pulmonary artery. A femoral artery was cannulated with a polyethylene catheter (PE-240) for measurement of arterial pressure and blood sampling. Mean arterial pressure (MAP), CO, right atrial pressure (RAP), pulmonary capillary wedge pressure (PCWP), and main pulmonary arterial pressure (MPAP) were measured. Systemic (SVR) and pulmonary (PVR) vascular resistance were calculated by the following formulas: 

$$SVR = \frac{MAP - RAP}{CO}$$

$$PVR = \frac{MPAP - PCWP}{CO}$$

After the dogs were killed, the kidneys were removed and stored in 10% Formalin for immunohistochemical staining studies.

Quantification of plasma ET. Plasma ET was determined by 125I-ET1,2 assay (Amersham International, Amersham, UK) as previously described (3, 14, 24). Briefly, arterial blood was collected in EDTA tubes and immediately placed on ice. After centrifugation at 2,500 rpm at 4°C, the plasma was decanted in glass tube and stored at −20°C until assay. Before the radioimmunossay, plasma was acidified with 0.5% trifluoroacetic acid (TFA). C8 Bond Elut cartridges were washed with 4 ml of methanol and 4 ml of water to extract the plasma. After the plasma was applied, cartridges were washed with 2 ml of normal saline and 6 ml of water. ET was eluted from the cartridges with 2 ml of 90% methanol in 1% TFA and then dried and reconstituted for the radioimmunossay. Interassay and intra-assay variations in recovery for the extraction procedure were 9 and 5%, respectively. The minimal level of detection was 0.5 pg/tube. The cross-reactivity of ET-2, ET-3, and pro-ET in this assay was <5%, <3%, and <37%, respectively.

Immunohistochemical staining. The presence of ET was documented utilizing a specific immunohistochemical stain-

Fig. 1. Mean arterial pressure (A) and cardiac output (B) in normal, thoracic inferior vena caval constriction (TIVCC), and TIVCC + felodipine groups.

Fig. 2. Systemic (A) and pulmonary (B) vascular resistances (in resistance units) in normal, TIVCC, and TIVCC + felodipine groups.
ing technique we described previously (3, 24). Briefly, immunochemistry staining (IHCS) for ET was performed in renal tissue from the normal dog group, the TIVCC group, and the TIVCC + felodipine group. Renal sections were taken from the renal cortex and renal medulla. Tissues were immediately fixed in 10% buffered Formalin. After fixation, the tissue was dehydrated and embedded in paraffin. Serial sections were cut at a thickness of 5 µm and mounted on glass slides treated with silica. The slides were incubated at 60°C and deparaffinized with graded concentrations of xylene and ethanol. To block the activity of endogenous peroxidase, the slides were incubated with 0.6% hydrogen peroxide in methanol for 20 min at room temperature. After being washed, the slides were incubated with 5% goat serum (Dako) for 10 min at room temperature to reduce nonspecific background staining and were then incubated with rabbit polyclonal anti-ET antiserum (Peninsula Laboratories) at a dilution of 1:800 in humidified chambers for 24 h at room temperature. All the treated slides were incubated for 30 min with secondary antibody-horseradish peroxidase conjugate (Tago) at a dilution of 1:100. The final reaction was achieved by incubating the sections with freshly prepared reagent containing 3-amino-9-ethylcarbazole (Sigma) dissolved in dimethylformamide and sodium acetate. Slides from renal tissues were counterstained with hematoxylin to enhance nuclear detail. The sections were mounted and reviewed with an Olympus microscope. Two trained observers reviewed the sections without knowledge of the respective group(s) from which the tissue was harvested. The presence of ET immunohistochemical staining was assessed by microscopic examination of the final slides and evaluated to quantify the degree of staining of ET (0: no staining; 0.5: minimal staining; 1.0: mild-density staining; 1.5: moderate-density staining; 2.0: maximal-density staining).

Statistics. Results of the quantitative studies are expressed as means ± SE. Statistical comparisons within each group were performed by ANOVA for repeated measures followed by Fisher’s least significant difference test of repeated measures. Statistical comparisons between groups were performed by factorial ANOVA followed by Fisher’s least significant difference test of repeated measures. Statistical significance was accepted for \( P < 0.05 \).

RESULTS

Hemodynamics. Figures 1 and 2 report the hemodynamic characteristics of normal, TIVCC-alone, and TIVCC + felodipine groups. MAP and CO were significantly decreased in the TIVCC-alone group compared with the normal group. In the TIVCC-alone group, although MAP was similar to that in the TIVCC-alone group, CO was markedly increased compared with the normal group (Fig. 1). Both RAP and PCWP were decreased in TIVCC compared with control dogs (RAP: 4 ± 1 to 1.2 ± 0.4 mmHg, \( P < 0.05 \); PCWP: 6 ± 1 to 1.8 ± 0.6 mmHg, \( P < 0.05 \)), and no significant difference was found between the TIVCC-alone group and the TIVCC + felodipine group. SVR and PVR were significantly increased in the TIVCC-alone group compared with the normal group. Both SVR and PVR were significantly decreased in the TIVCC + felodipine group compared with the TIVCC-alone group (Fig. 2).

Plasma ET concentration. Plasma ET concentration was significantly increased in the TIVCC-alone group compared with the normal group. In contrast, plasma ET concentration was markedly decreased in the TIVCC + felodipine group compared with the TIVCC-alone group (Fig. 3).
ET immunohistochemical staining. ET immunohistochemical staining in renal tissue from representative normal, TIVCC-alone, and TIVCC + felodipine groups is illustrated in Figs. 4 and 5. As shown in these figures, ET is present in renal cortex and medulla and localized in the cytoplasm of tubular cells. ET immunoreactivity was markedly increased in the TIVCC-alone group both in renal cortex and renal medulla tissues. In contrast, ET IHCS was markedly decreased in the TIVCC + felodipine group in both renal cortex and medullar tissues. A negative control, in which normal rabbit serum was substituted for the primary antibody, did not stain.

Table 1 illustrates the staining scores from three groups. The ET immunohistochemical staining score was significantly increased in the TIVCC-alone group both in renal cortex and medulla tissue compared with the normal group. In the TIVCC + felodipine group, the ET staining score was markedly decreased compared with the TIVCC-alone group.

**DISCUSSION**

The present study demonstrates that calcium antagonism with felodipine has important inhibitory actions on circulating and local renal ET, with significantly improved cardiovascular hemodynamics in the canine TIVCC model.

Previous studies demonstrated that plasma ET is increased in patients with myocardial infarction (17), advanced atherosclerosis (8), cardiogenic shock (2), subarachnoid hemorrhage (15), hypertension (19), and Raynaud's phenomenon (1). Circulating ET concentration was reported to be significantly increased in humans with CHF (4, 24) and in animal models of CHF (6, 13, 14, 20). Plasma ET correlated with New York Heart Association functional classifications (6, 24), left ventricular ejection fraction (24), cardiac index (24), left ventricular end-diastolic volume index (24), pulmonary artery pressures (4), and PVR (4). Thus the increased plasma ET level suggests that ET is an important mediator of control cardiovascular function and pulmonary circulation in patients and animal models with CHF.

The production of ET may be regulated mainly at the level of transcription or translation of mRNA (27). The expression of prepro-ET-1 mRNA is markedly upregulated in cultured endothelial cells by various chemical and mechanical stimuli, including Ca^2+ ionophore (26), thrombin, phorbol ester (25), transforming growth factor-β, and fluid-mechanical shear stress (28). Because the Ca^2+ ionophores can induce an increase in prepro-ET-1 mRNA, Ca^2+ may be involved in ET-1 synthesis. The vasodilator effects of the calcium antagonists nifedipine, nisoldipine, nitrendipine, isradipine, and amlodipine on human internal mammary artery precontracted with ET-1 were reported previously (11). These studies demonstrated that calcium antagonists had potent vasodilator actions on the human internal mammary artery.

The previous in vitro study demonstrated that incubation of human umbilical artery endothelial cells and human umbilical vein endothelial cells with the calcium antagonist nisoldipine for either 7 or 24 h resulted in a dose-dependent reduction in ET levels in the conditioned media (12). On the other hand, a recent in vivo study demonstrated that chronic administration of another dihydropyridine subclass of calcium-channel antagonists, amlodipine, improves hemodynamics in conscious animals and reduces circulating levels of ET to control levels in the pacing model of heart failure (7).

Table 1. Immunohistochemical staining scores for endothelin in normal, TIVCC, and TIVCC + felodipine groups

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<th></th>
<th>Normal</th>
<th>TIVCC</th>
<th>TIVCC + Felodipine</th>
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<tr>
<td>Renal cortex</td>
<td>0.4 ± 0.1</td>
<td>1.4 ± 0.4*</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Renal medulla</td>
<td>0.3 ± 0.1</td>
<td>1.7 ± 0.5*</td>
<td>0.6 ± 0.2</td>
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Values are means ± SE. TIVCC, thoracic inferior vena caval constriction. Endothelin staining scores: 0, no staining; 0.5, minimal staining; 1.0, mild-density staining; 1.5, moderate-density staining; 2.0, maximal-density staining. *P < 0.05 vs. normal group.
These data suggest that ET concentrations can be reduced by calcium antagonism. It was reported that ET-1 can stimulate its own synthesis in cultured human umbilical vein endothelial cells (18). This study suggests a positive paracrine feedback action of ET on its own synthesis (18). Because circulating ET concentrations in normal human are very low, this feedback action of ET may occur only in abnormally high ET concentration states. The positive feedback mechanism might play a role in or be a contributor to the time-dependent increase in ET concentration in cell culture studies. The calcium antagonist-reduced ET levels in cultured cells suggest that a calcium antagonist may inhibit the positive feedback action of ET (10, 18).

The source of increased plasma ET in CHF remains controversial. In the current studies, we only investigated ET expression in the kidney. We did not measure ET levels in other tissues. However, previous studies by us (21) and by others (22) demonstrated that the kidney is an important source of elevated circulating ET in CHF. The current studies suggest that the kidney is one of the major sources of increased circulating ET in CHF. However, we do not exclude other tissue that may contribute to the elevated circulating concentration of ET in CHF. We and others reported that with renal hypoperfusion during supraaortal aortic cross-clamping in dogs (21) or in a rat model of acute ischemic renal failure (22), plasma ET concentrations were significantly increased. Simulated nephrectomy in a canine model of supraaortal aortic cross-clamping abolished the increase in plasma ET (21). These studies suggest that, in response to renal hypoperfusion, increased circulating ET may be of renal origin and is not caused by decreased renal clearance. In the current study, which is a TIVCC model of CHF, significantly decreased CO can cause marked renal ischemia, which may contribute to stimulate the kidney to produce ET. The mechanism(s) by which decreased renal perfusion pressure may further stimulate ET production or release may be multifactorial and include the mechanical stimulus of decreased wall stress and/or the activation of chemical stimuli such as angiotensin II and/or hypoxia (21). In the current studies, we did not measure renal blood flow and plasma renin activity (PRA) level.

However, in previous studies, we reported that PRA level and renal vascular resistance were significantly increased in the TIVCC model. These studies suggest that the RAS and renal hemodynamics may contribute the renal ET production in the TIVCC model. Therefore, the effects of felodipine on decreasing renal and plasma ET may also through improvement of renal hemodynamics and suppression of the RAS. Further studies will be needed to address these points. On the other hand, because anesthetia and surgical stress may also lead to alterations in neurohormonal activity and hemodynamics, further study in conscious animals may be required.

TIVCC is a model of low CO and CHF that results in marked systemic and pulmonary vasoconstriction and sodium retention with edema in association with activation of the plasma and tissue ET system as we described previously (3, 23). This model is unique in mimicking CHF, in that the chronic reduction in venous return produced by TIVCC reduces CO in the absence of ventricular volume overload and therefore is unassociated with ventricular dilatation and hypertrophy or increased atrial natriuretic peptide. In contrast, in the pacing model of CHF, plasma and tissue ET only increased in the late stage of severe CHF. Therefore, the current studies were designed to determine the modulating actions of felodipine in an experimental model of CHF produced by TIVCC compared with normal and TIVCC-alone dogs. In the TIVCC model of heart failure, local tissue ET concentration was increased in cardiac, pulmonary, and renal tissues with increasing circulating ET level (3). The elevation of tissue and plasma concentrations of ET in this low-CO model is consistent with a compensatory role for ET as a vasoconstrictor peptide activated in low-CO states in an attempt to maintain blood pressure (3, 23).

In summary, we report important modulating inhibitory actions of calcium antagonism with felodipine in TIVCC on circulating and local renal ET. On the basis of the current findings, we conclude that plasma ET concentrations and renal ET immunohistochemical staining are significantly increased in TIVCC dogs compared with normal dogs. With calcium antagonism by felodipine treatment, circulating ET and local renal ET staining were significantly decreased with markedly improved hemodynamics in TIVCC. These results suggest that the calcium antagonist felodipine may play an important therapeutic role in modulating renal and plasma ET concentrations in heart failure.

This work was supported in part by grants from the American Heart Association, Mid-Atlantic Affiliate (C. Wei), Astra/Merck Group (C. Wei), National Heart, Lung, and Blood Institute Grants HL-03174 and HL-61299 (C. Wei) and HL-36634 (J. C. Burnett, J. r.).

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Received 7 April 1998; accepted in final form 12 July 1999.

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