Endothelin Has Biological Actions at Pathophysiological Concentrations

Amir Lerman, MD; Fredric L. Hildebrand Jr., MD; Lawrence L. Aarhus; and John C. Burnett Jr., MD

**Background.** Endothelin is an endothelium-derived peptide that produces sustained contraction of arterial and venous smooth muscle in vitro. Several studies have established endothelin as a systemic, renal, and coronary vasoconstrictor in vivo at pharmacological concentrations. Such concentrations of endothelin were antinatriuretic in association with activation of the renin-angiotensin-aldosterone system. Recent studies have demonstrated that endothelin is present in the plasma and that its plasma concentrations are increased in various pathophysiological states associated with systemic and renal vasoconstriction. To date, it remains unclear if such increases in circulating endothelin are associated with biological activity. Thus, the objective of this study was to determine the biological action of endothelin on cardiorenal and endocrine function through administration of exogenous endothelin, which achieves plasma concentrations that have been reported in various pathophysiological conditions.

**Methods and Results.** Experiments were conducted in two groups of anesthetized dogs. In group 1, endothelin-1 was infused intravenously at 2.5 mg/kg/ml (n=6), which produced a doubling of circulating concentrations. Group 2 (n=8) received saline vehicle to serve as a time control. The current studies demonstrate that a twofold increase in plasma endothelin concentrations did not affect mean blood pressure or coronary blood flow. Heart rate and cardiac output decreased in association with increased renal and systemic vascular resistances and antinatriuresis.

**Conclusions.** The present studies demonstrate that endothelin at pathophysiological plasma concentrations produced by exogenous endothelin has biological action. These studies support a functional role for endogenous endothelin as a potentially pathophysiological vasoconstrictor peptide hormone in the regulation of cardiovascular, renal, and endocrine function. (Circulation 1991;83:1808–1814)

Endothelin (ET) is an endothelium-derived peptide that produces sustained contraction of arterial and venous vascular smooth muscle in vitro. Recent in vivo studies have demonstrated that at pharmacological concentrations ET is also a potent coronary, renal, and systemic vasoconstrictor, which may decrease sodium excretion and activate the renin-angiotensin-aldosterone system. These actions are consistent with a role for ET as an important cardiovascular regulatory peptide.

Increasing evidence indicates that, in addition to its known role as an autacoid, ET is present in the plasma of normal humans and animals. The importance of its presence in the circulation is underscored by increased plasma levels of ET in disorders of cardiovascular and renal dysfunction. Specifically, plasma ET concentrations have been reported to be increased threefold in congestive heart failure, twofold in essential hypertension, threefold in cardiogenic shock, and twofold in transplantation-associated hypertension. At present, multiple investigators have stated that no evidence exists to date to indicate that any biological activity is produced by such concentrations of elevated ET in these pathophysiological states.

The current study was designed, therefore, to address the functional significance of a twofold increase in circulating ET on cardiovascular and renal function as well as on the renin-angiotensin-aldosterone system. To accomplish this objective, exogenous ET was administered to anesthetized dogs at a
concentration to increase plasma ET twofold as determined by sensitive radioimmunoassay to endothelin 1 (ET-1). To exclude the effects of time, a saline control group was also investigated.

Methods

Acute experiments were conducted in two groups of anesthetized dogs (18–25 kg). Each dog was given an oral dose of lithium carbonate (300 mg) and fasted overnight. Tap water was available ad libitum. Dogs were anesthetized with sodium pentobarbital (30 mg/kg), with additional anesthetic given as needed to maintain a constant level of anesthesia. An endotracheal tube was inserted, and the dog was ventilated with a Harvard respirator (Harvard Apparatus, South Natick, Mass.), using room air supplemented with 100% oxygen, throughout the experiment. Respiratory rate and tidal volume were adjusted to body weight to maintain an arterial pH greater than 7.35, P02 greater than 100 mm Hg, and Pco2 less than 50 mm Hg. Femoral arteries and veins were cannulated with polyethylene catheters for monitoring mean arterial pressure and intravenous infusions of inulin, saline, and ET respectively. Inulin was infused in isotonic saline at 1 ml/min in a concentration sufficient to establish a plasma level of approximately 50 mg/dl.

The heart was exposed through a left thoracotomy along the fourth intercostal space. The pericardium was opened, and a 1-cm segment of the proximal left circumflex coronary artery was dissected free from the surrounding tissue. A calibrated electromagnetic flow probe was positioned on the proximal circumflex coronary artery and connected to a flowmeter (model FM 5010, Carolina Medical Electronics, Inc., King, N.C.) for measurement of coronary blood flow. A flow-directed, balloon-tipped 7F thermodilution catheter (model 93A-131, American Edwards Laboratories, Anaso, Puerto Rico) was advanced from the isolated right external jugular vein through the right cardiac chamber and positioned in the pulmonary artery for measurement of right atrial pressure, pulmonary artery pressure, and pulmonary wedge pressure. The left kidney was exposed through a flank incision, permitting cannulation of the ureter for urine collection and placement of a calibrated electromagnetic flow probe around the left renal artery for measurement of renal blood flow. The flow probe was connected to a flowmeter as described above for measurement of coronary blood flow.

Cardiac output was determined as an average of four measurements by the thermodilution technique with a cardiac output computer (model 9510-A, American Edwards Laboratories). Continuous electrocardiographic monitoring was performed throughout the experiment for measurement of heart rate. Blood flows and arterial blood pressures were recorded on a strip recorder (model 2200, Gould, Cleveland, Ohio).

Experimental Protocol

Synthetic endothelin administration. After an equilibration period of 1 hour after completion of surgical procedures, and during which inulin and saline infusions at 1 ml/min were started, two consecutive 20-minute baseline control clearances were performed. At the midpoint of each clearance period, hemodynamic parameters were measured, and arterial blood was collected for subsequent measurement of plasma atrial natriuretic factor (ANF), plasma renin activity, ET, aldosterone, and chemistries (inulin, lithium, and sodium). Urine was collected during the entire 20-minute clearance period for measurement of electrolytes.

After completion of these baseline clearances, ET-1 (Peninsula Laboratories, Inc., Belmont, Calif.) was infused intravenously at 1 ml/min in dogs in the experimental group 1 (n=6) at 2.5 ng/kg/min. This dose was chosen to double circulating ET based on pilot studies. Group 2 (n=8) received saline vehicle at 1 ml/min. After a lead-in period of 30 minutes, three 20-minute clearances were obtained, with measurements and collections as described above for the control periods. The total time of ET infusion was 90 minutes.

When the ET infusion was completed, two 60-minute recovery periods were observed during which no peptide was infused. A 20-minute recovery clearance was obtained at the end of each recovery period; these values were averaged.

The biological activity of the ET used in these experiments was tested using a bioassay in Wistar-Kyoto rats. Each lot number of ET supplied was verified as biologically active if a bolus of 1 μg into the anesthetized rat produced a rapid hypertensive response.

Collection of samples. Blood for plasma chemistries was placed in heparinized tubes, packed in ice, centrifuged at 2,500 rpm, and refrigerated along with urine chemistry samples pending analysis. Blood for hormone assays was placed in EDTA tubes on ice, and after centrifugation at 2,500 rpm, plasma was decanted and stored at −20°C until analysis. Plasma ANF was measured by radioimmunoassay to human ANF-(99–126) as previously described. Plasma renin activity was determined by radioimmunoassay using the method of Haber et al. Plasma renin activity was measured after dichloromethane extraction by radioimmunoassay (immuno-aldocolesone-iodine-125 kit D18, Pantex, Santa Monica, Calif.).

Plasma ET was determined by the ET-1,2[125I] assay system (Amersham International, Amersham, UK). Blood was drawn from dogs into tubes containing chilled potassium EDTA and immediately placed on ice until being centrifuged at 4°C. Plasma was separated and frozen at −20°C until assay. Before the radioimmunoassay, plasma was acidified with 0.5% trifluoroacetic acid. C8 Bond Elut cartridges (Analyticchem International, Harbor City, Calif.) were washed with 4 ml methanol and 4 ml water to
extract the plasma. After the plasma was applied, cartridges were washed with 2 ml normal saline and 6 ml water. ET was eluted from the cartridges with 2 ml of 90% methanol in 1% trifluoroacetic acid, then dried, and reconstituted for the radioimmunoassay. The recovery of the extraction procedure was 81%, as determined by addition of synthetic ET to plasma, and interassay and intraassay variations were 9% and 5%, respectively.

Serum and urine sodium concentrations were quantified by use of ion-selective electrodes (Beckman E2A analyzer, Beckman Instruments, Inc., Brea, Calif.). Glomerular filtration rate was determined by the clearance of inulin, and plasma inulin concentrations were determined by the anthrone method. Whole-kidney proximal reabsorption of sodium was estimated by the lithium-clearance technique. This technique has been shown to be a reliable method for estimating whole-kidney delivery of sodium from the proximal tubule, since lithium is reabsorbed exclusively by the proximal tubule in euvolemic animals.

**Statistics**

For each experimental group, data from the two baseline and two recovery clearances were combined, and the means were calculated. Data from all clearance periods were measured and expressed as mean±SEM. All data were analyzed by analysis of variance for repeated measurements and by paired and unpaired t tests. Significance was accepted at p<0.05.

**Results**

Tables 1, 2, and 3 summarize the cardiovascular, renal, and endocrine data for group 1. No statistical differences were observed between the two experimental groups at baseline.

In group 1, in which ET was administered to double circulating concentrations (Figure 1), an immediate and significant increase in systemic vascular resistance and a decrease in both heart rate and cardiac output were observed; these responses persisted through the recovery period. These responses were also significant when compared with the saline time control group (Figure 2). No effect on either mean arterial pressure, coronary blood flow, coronary vascular resistance, right atrial pressure, or pulmonary wedge pressure was noted.

During the first clearance period after ET administration, renal blood flow decreased and renal vascular resistance increased. A partial reversal to values not different from baseline was observed during the recovery period. An initial decrease in glomerular filtration rate was noted during the first clearance; however, this effect was transient, and glomerular

<table>
<thead>
<tr>
<th>TABLE 1. Systemic and Cardiovascular Effects of Endothelin Infusion in Dogs</th>
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<tr>
<td>HR (beats/min)</td>
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<td>MAP (mm Hg)</td>
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<td>CO (l/min)</td>
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<td>SVR (mm Hg/ml/min)</td>
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<td>CBF (ml/min)</td>
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<tr>
<td>CVR (mm Hg/ml/min)</td>
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<tr>
<td>RAP (mm Hg)</td>
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<td>PWP (mm Hg)</td>
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</table>

Values are mean±SEM; n=6 dogs. HR, heart rate; MAP, mean arterial blood pressure; CO, cardiac output; SVR, systemic vascular resistance; CBF, coronary blood flow; CVR, coronary vascular resistance; RAP, right atrial pressure; PWP, pulmonary wedge pressure.

*p<0.05 compared with baseline.

<table>
<thead>
<tr>
<th>TABLE 2. Renal Hemodynamic and Excretory Effects of Endothelin Infusion in Dogs</th>
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<td>GFR (ml/min)</td>
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<td>RBF (ml/min)</td>
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<tr>
<td>RVR (mm Hg/ml/min)</td>
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<tr>
<td>UV (ml/min)</td>
</tr>
<tr>
<td>UNaV (μeq/min)</td>
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<tr>
<td>FENa (%)</td>
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<td>FE Li (%)</td>
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</tbody>
</table>

Values are mean±SEM; n=6 dogs. GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance; UV, urine flow; UNaV, urinary sodium excretion; FE Na, fractional excretion of sodium; FE Li, fractional excretion of lithium.

*p<0.05 compared with baseline.
filtration rate returned to baseline during the second clearance period (Figure 3). Urine flow, urinary sodium excretion, fractional excretion of sodium, and fractional excretion of lithium were unchanged during the infusion. However, when compared with the vehicle group (group 2), the changes in urinary and fractional sodium excretion were significantly lower (Table 2).

No significant alteration in plasma ANF, aldosterone, or PRA was observed throughout the protocol (Table 3).

In group 2 (time control), systemic hemodynamic, renal, and endocrine parameters did not change during infusion of saline vehicle. Urinary and fractional sodium excretion increased modestly and not significantly from 66.5±13.7 to 95.1±29.4 μeq/min and from 1.1±0.4% to 2.1±0.6%, respectively. Plasma ET increased modestly during the first clearance period but returned to baseline values subsequently without further change.

**Discussion**

The present studies demonstrate for the first time that systemic administration of ET to produce a twofold increase in its circulating concentrations results in significant cardiovascular and renal biological activity. Several investigators have reviewed the pharmacological activities, regulation, and possible roles for endothelin in pathophysiological states. These reports have emphasized that, although increased plasma ET concentrations were detected in various diseases characterized by vascular injury, no evidence exists to indicate that a twofold increase in plasma ET concentrations is associated with biological activity. The current studies demonstrate clearly that such circulating concentrations do have actions that include systemic and renal vasoconstriction in association with a decrease in heart rate and cardiac output in the absence of an increase in arterial pressure. These studies are consistent with an important pathophysiological role for ET in cardiorenal homeostasis.

Pathophysiological concentrations of ET affected systemic hemodynamics, producing a significant increase in systemic vascular resistance and a decrease in cardiac output (Figure 2). Such actions have been observed in previous studies, using pharmacological and pressor doses of ET.34 In such previous investigations, the pressor response to exogenous ET was associated with a progressive bradycardic response.4,16,19 In the present study, much lower concentrations of plasma ET were also associated with progressive bradycardia. Since this decrease in heart rate was not accompanied by changes in mean arterial pressure, ET may have a direct effect on the cardiac conduction system, as suggested by Yamasaki et al.20 or it may serve as a neuromodulator21,22 to alter chronotropic activity. However, we cannot rule out the possibility that the bradycardia observed in this study was a baroreflex-mediated mechanism to maintain constant mean arterial pressure. Despite the negative chronotropic action of ET in the current study, coronary blood flow and

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**Table 3. Endocrine Effects of Endothelin Infusion in Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET (pg/ml)</td>
<td>8.8±0.9</td>
<td>17.2±1.3*</td>
<td>21.3±2.7*</td>
<td>19.5±2.4*</td>
<td>10.9±1.1</td>
</tr>
<tr>
<td>ANF (pg/ml)</td>
<td>32.8±6.3</td>
<td>32.6±7.0</td>
<td>35.5±8.0</td>
<td>53.6±20.0</td>
<td>42.0±10.0</td>
</tr>
<tr>
<td>Aldo (ng/ml)</td>
<td>10.2±4.2</td>
<td>12.5±5</td>
<td>13.5±5.1</td>
<td>15.7±5.8</td>
<td>17.2±5.7</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>3.1±1.7</td>
<td>3.5±2.4</td>
<td>2.4±1.4</td>
<td>2.3±1.2</td>
<td>3.7±1.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=6 dogs. ET, endothelin; ANF, atrial natriuretic factor; Aldo, aldosterone; PRA, plasma renin activity.

*p<0.05 compared with baseline.

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**Figure 1.** Graph showing plasma endothelin concentrations during vehicle and exogenous endothelin infusion in dogs. C1, C2, and C3, clearances 1–3, respectively (consecutive 20-minute clearances after 30-minute lead-in of endothelin); REC, recovery; ■, group infused with 2.5 ng/kg/min endothelin; ▲, vehicle-infused group. Collection and hemodynamic measurements were made at midpoint of each clearance. *p<0.05 compared with baseline; †p<0.05 compared with vehicle-infused group.
coronary vascular resistance were unchanged. This suggests that at pathophysiological concentrations ET may not have a potent coronary vasoconstrictor action as compared with the other vascular beds.

Consistent with the preferential renal vasoconstrictor action of ET at pharmacological concentrations, exogenous ET caused significant reductions in renal blood flow and increases in renal vascular resistance at the doses used in the present study (Figure 3). The effect on glomerular filtration rate was more complex, characterized by a reversible decrease in glomerular filtration rate. At pathophysiological concentrations, the glomerular filtration rate decreased only during the first clearance. The effect of these plasma concentrations on glomerular filtration rate can only be speculated. Because infusions of higher pharmacological concentrations of ET typically result in a progressive decrease in glomerular filtration rate,23,24 the observations of the present studies suggest that ET may have varying threshold actions on both the afferent and efferent arterioles.25 Its interaction with endogenous vasodilators, such as ANF and endothelium-derived relaxing factor, at these sites may also regulate local vascular tone.

Despite the renal vasoconstrictor action, no significant changes in sodium excretion were observed in the group that received exogenous ET. However, the twofold increase in plasma ET concentrations did prevent the modest natriuresis that was observed in the vehicle group, suggesting that pathophysiological concentrations of ET may evoke the onset of a decrease in sodium excretion as demonstrated with more pharmacological concentrations. Since whole-kidney delivery of sodium from the proximal tubule did not change significantly, as determined by the fractional excretion of lithium, this antinatriuretic effect may be secondary to enhanced tubular reabsorption of sodium beyond the proximal tubule. Such a distal tubular action of ET has been suggested in preliminary studies that demonstrate possible uptake or production of ET by distal tubular cells.26

Baseline plasma ET concentrations in the anesthetized dogs in the current study were higher than concentrations reported in previous studies. This may

![Bar graphs comparing percent change of heart rate (HR), cardiac output (CO), and systemic vascular resistance (SVR) between vehicle-infused dogs (solid bars) and dogs infused with 2.5 ng/kg/min endothelin (hatched bars). C1, C2, and C3, clearances 1–3, respectively (consecutive 20-minute clearances after 30-minute lead-in of endothelin); REC, recovery. Collection and hemodynamic measurements were made at midpoint of each clearance. *p<0.05 compared with vehicle-infused group.](http://circ.ahajournals.org/content/83/5/1812/F2.large.jpg)
reflect the effect of pentobarbital anesthesia or the surgical manipulation. With regard to plasma renin activity, studies using pharmacological doses of ET in vivo have demonstrated a significant increase in both plasma renin activity and plasma aldosterone,\(^3,4\) in contrast to in vitro studies that report an inhibitory action of ET on renin release from juxtaglomerular cells\(^27,28\) and a stimulation of aldosterone biosynthesis from adrenal cells.\(^29,30\) In the present study, at pathophysiological plasma concentrations a trend of the inhibitory action on plasma renin activity was observed. However, these values were not statistically significant compared with baseline values or with vehicle group values. Circulating ANF concentration did not change significantly during ET infusion in the absence of any change in atrial pressures. This is in contrast to in vitro studies that suggest that ET may stimulate release of ANF from atrial myocytes.\(^31,32\)

The present study, together with previous investigations, supports the conclusion that ET exerts different biological actions at different plasma concentrations. At pathophysiological concentrations, which double the plasma concentration and approximate levels observed in disorders of cardiovascular and renal function, the principal actions appear to be a decrease in cardiac output and heart rate in association with systemic and renal vasoconstriction. Previous studies have demonstrated that higher circulating concentrations produced by pharmacological doses of ET may increase mean arterial pressure and decrease coronary blood flow\(^33,34\); these responses are not observed in the current studies. These differential responses may suggest receptor variability between cardiovascular and renal tissues.\(^33,34\) The hemodynamic effects of ET were not reversible in the recovery period, even though plasma ET levels returned toward control values. This observation is consistent with previous in vitro and in vivo studies establishing ET as a long-acting vasoconstrictor\(^1,3,11\) and may reflect an increase in tissue concentrations, as was suggested by Shibouta et al.\(^35\)

Since the discovery of ET by Yanagisawa et al.,\(^1\) several studies have established its vasoconstrictor action in vivo at pharmacological concentrations. Increasing evidence suggests that ET is normally present in the circulation and that its plasma concen-

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**FIGURE 3.** Bar graphs comparing percent change of glomerular filtration rate (GFR), renal blood flow (RBF), and renal vascular resistance (RVR) between vehicle-infused dogs (solid bars) and dogs infused with 2.5 ng/kg/min endothelin (hatched bars). C1, C2, and C3, clearances 1–3, respectively (consecutive 20-minute clearances after 30-minute lead-in of endothelin); REC, recovery. Collection and hemodynamic measurements were made at midpoint of each clearance. *p < 0.05 compared with vehicle-infused group.
trations are increased in various pathological states in humans.5-12,36 Nevertheless, the biological role for ET at these pathophysiological concentrations has remained unclear. The present studies demonstrate that exogenous ET, administered at doses that approximate pathophysiological concentrations, has biological action, although the significance of physiological concentrations of ET remains to be resolved. These studies, for the first time, support a functional role for ET as a pathophysiological vasoconstrictor peptide hormone in the regulation of renal, cardiovascular, and endocrine function.

References

KEY WORDS • endothelin • vasoconstrictor agents • renal blood flow
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