Integrated Cardiac, Renal, and Endocrine Actions of Endothelin

Wayne L. Miller, Margaret M. Redfield, and John C. Burnett, Jr.
Cardiorenal Research Laboratory, Department of Internal Medicine and Physiology and Biochemistry, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Abstract

Endothelin, a newly discovered endothelial-derived peptide, has been demonstrated in vitro to have potent vasocontractile properties and has been speculated to play a role in vivo in arterial pressure-volume homeostasis. The present studies in anesthetized dogs were designed to determine the action of endothelin on cardiovascular-renal and endocrine function in vivo as in acute arterial pressure-volume regulation. Intravenous infusion of endothelin (50 ng/kg per min) increases arterial pressure by increasing peripheral vascular resistance but in association with an increase in coronary vascular resistance and decreases in cardiac output. Renal blood flow and glomerular filtration rate were markedly reduced in association with a sustained reduction in sodium excretion and an increase in plasma renin activity. Atrial natriuretic factor, vasopressin, and aldosterone were also elevated. These results indicate that endothelin is a potent vasoconstrictor that elevates systemic blood pressure in association with marked decreases in cardiovascular and renal function. This peptide may function as a counterregulatory hormone to the effects of endothelial-derived vasodilator agent(s).

Introduction

A new peptide of vascular endothelial origin has been recently isolated from the cultured supernatant of porcine aorta (1). This 21-amino acid residue peptide, endothelin, has been demonstrated to be a potent constrictor of vascular smooth muscle in vitro, which may be dependent upon extracellular calcium (1). In addition to the production of an endothelial-derived vasoconstriction, the vascular endothelium is well recognized to produce relaxation of vascular smooth muscle through the release of short-lived endothelium-derived relaxing factor(s) (EDRF)[1] (2). The production by the endothelium of these two substances with opposing vascular effects suggests a complex role of the endothelium in the regulation of vascular smooth muscle and of arterial pressure. Further, vascular tone may also be modulated indirectly by an endothelial effect on the kidney in which renin release is inhibited by EDRF in renal cortical slices (3).

To date, the effects of endothelin in integrated hemodynamic, cardiovascular, renal, and endocrine function in vivo in the regulation of cardiovascular volume homeostasis, however, have not been determined. Therefore, based on previous in vitro studies of endothelin, this study was designed to determine the integrated response to systemic administration of endothelin with a specific focus on cardiac function as well as renal action and renin activity in the overall cardiorenal regulation of body fluid homeostasis. In addition to plasma renin activity (PRA), the action of endothelin on other vasoactive sodium-regulating hormones was also studied.

Methods

Six male mongrel dogs weighing 18–26 kg were studied. Each dog was fasted beginning 18 h before the acute experiment. Water was available ad lib. Dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) with additional anesthetic given as needed to maintain a constant level of anesthesia. Pulmonary ventilation was accomplished after endotracheal intubation by a positive pressure respirator (model 6070; Harvard Apparatus Co., Inc., South Natick, MA) with room air supplemented with 100% oxygen. Respiratory rate and tidal volume were adjusted to body weight.

Polyethylene catheters were advanced centrally from a femoral artery and both femoral veins for measurement of arterial blood pressure and sampling of arterial blood, and intravenous infusion of inulin and endothelin. Inulin was infused in isotonic saline at 1 ml/min in a concentration sufficient to establish plasma levels of ~ 50 mg/dl. The left kidney was exposed retroperitoneally and the ureter cannulated for timed urine collections. A calibrated electromagnetic flow probe was placed on the left renal artery and connected to a flowmeter (model FM 5010; Carolina Medical Electronics, Inc., King, NC).

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 of surrounding tissue. A calibrated electromagnetic flow probe was positioned on the proximal circumflex coronary artery and connected to a similar flowmeter as described above. Blood flows and arterial pressure were recorded on a strip recorder (model 2200; Gould, Inc., Houston, TX).

A flow-directed, balloon-tipped thermodilution catheter (model 93-131A, 7 Fr, American Edwards Laboratories, Inc., Minneapolis,

Address reprint requests to Dr. John C. Burnett, Jr., Cardiorenal Research Laboratory, Mayo Clinic and Foundation, 200 First Street SW, Rochester, MN 55905.

Received for publication 22 August 1988 and in revised form 21 September 1988.

1. *Abbreviations used in this paper:* Aldo, aldosterone; ANF, atrial natriuretic factor; CBF, coronary blood flow; CO, cardiac output; CVR, coronary vascular resistance; EDRF, endothelium-derived relaxing factor(s); FE_{Na^+}, fractional excretion of sodium; (L)AP, left atrial pressure; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PRA, plasma renin activity; PVR, pulmonary vascular resistance; (R)AP, right atrial pressure; RBF, renal blood flow; RVR, renal vascular resistance; SVR, systemic vascular resistance; $U_{(Na^+)}V$, sodium excretion.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/89/01/0317/04 $2.00
Volume 83, January 1989, 317–320

MN) was advanced from the isolated right external jugular vein through the right cardiac chamber and positioned in a pulmonary artery. Right atrial, pulmonary artery, and pulmonary artery wedge pressures were measured. Cardiac output (CO) was determined in triplicate by the thermodilution technique with a cardiac output computer (model 9510-A, American Edwards Laboratories, Inc.).

Experimental protocol. After completion of surgical preparations and initiation of inulin and isotonic saline infusions at 1 ml/min the dogs were allowed to stabilize for 60 min without intervention. Subsequently, two consecutive 15-min baseline clearances were obtained. Each clearance, either baseline or subsequently with infusion of endothelin, consisted of a 15-min urine collection, measurement of hemodynamic parameters, and the withdrawal of 25 cm of arterial blood for hormone, electrolyte, and inulin determinations. Systemic intravenous infusion of endothelin (0.05 μg/kg per min; Peninsula Laboratories, Inc., Belmont, CA) was begun after the baseline clearances. The purity of this peptide is > 95% based upon HPLC analysis. After a 15-min lead-in infusion of endothelin, the first of three consecutive 15-min clearances was begun. The total time of endothelin infusion was 60 min. After the last endothelin clearance period the infusion was discontinued and a 60-min washout period was observed. A final 15-min clearance (recovery) was then obtained to complete the experimental protocol.

Analytical procedures. GFR was measured by the clearance of inulin. Plasma and urine inulin concentrations were measured by the anthrone method (4). Plasma and urinary sodium concentrations were quantified using ion-selective electrodes (system E2A, Beckman Instruments, Inc., Palo Alto, CA). PRA, aldosterone, and arginine vasopressin (AVP) levels were measured by RIA as previously reported (5). Extracted arterial plasma levels of atrial natriuretic factor (ANF) were measured by an RIA procedure previously described (6).

Data from the two baseline clearances were combined and the mean values calculated. Data from the endothelin infusion periods and the recovery periods are expressed separately with all data expressed as mean±SEM. Statistical analysis of data was completed by *t* tests for paired and unpaired observations. Statistical significance was accepted for $P < 0.05$.

Results

Table I summarizes the systemic, cardiac, and pulmonary hemodynamic data during baseline, intravenous infusion of endothelin, and 1-h postinfusion for six normal dogs. Mean arterial pressure (MAP) increased significantly above baseline with endothelin infusion, achieving the maximum increase in pressure after 45 min of infusion (99±7–134±6 mmHg, $P < 0.05$). Pressure then declined despite continued endothelin infusion and a further increase in systemic vascular resistance (SVR). This was also associated with a significant decrease in CO. At 1 h postinfusion MAP returned to baseline, but this was accomplished with persistent decreases in CO and increases in SVR.

Pulmonary artery wedge pressure as an index of left atrial pressure [(L)AP] was elevated with endothelin infusion. The most significant rise above baseline occurred after 45 min of continuous infusion (2.5±0.4–9.1±3.4 mmHg, $P < 0.05$). Wedge pressure decreased toward baseline at 1 h postinfusion, but nevertheless remained significantly elevated. In contrast, right atrial pressure [(R)AP] was not changed from baseline (3.1±1.3 mmHg) with endothelin infusion. Left circumflex coronary artery flow also tended to decrease with endothelin but the decrease was not statistically significant. Coronary vascular resistance (CVR), however, was significantly elevated in the second and third clearance periods of endothelin infusion but then returned to baseline in the recovery period. Mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR) were unchanged with endothelin infusion or with recovery.

Table II summarizes the renal hemodynamic and excretory function data during baseline, endothelin infusion, and 1-h postinfusion periods. With endothelin infusion glomerular filtration rate and renal blood flow (RBF) were significantly reduced beginning from the first clearance period of endothelin infusion. RBF was dramatically decreased by endothelin to ~ 23% of baseline flow. Renal vascular resistance (RVR) showed a correspondingly significant increase with endothelin infusion. At 1 h postinfusion neither blood flow nor RVR had recovered to baseline, with RBF remaining at ~ 65% of baseline. GFR, in contrast, was significantly elevated above baseline during recovery (38.5±3.6–47.7±2.1 ml/min, $P < 0.05$) in contrast to RBF, which remained depressed. Urine flow and sodium excretion [$U_{Na^+}V$] were immediately reduced in association with the decreased renal hemodynamics. While urine flow was restored to baseline at 1 h postinfusion, $U_{Na^+}V$ remained depressed compared with baseline (47.6±14.3–12.6±3.6 μeq/min). Fractional sodium excretion (FE_{Na^+}) was not significantly decreased from baseline during endothelin infusion, but did decline significantly despite termination of endothelin infusion. At 1 h postinfusion FE_{Na^+} was significantly depressed (0.95±0.71–0.18±0.05%, $P < 0.05$). FE_{Na^+} was the only parameter monitored, hemodynamic or renal function, that did not show some recovery toward baseline

Table I. Systemic and Cardio-Pulmonary Hemodynamic Effects of Endothelin

		Endothelin (*50 ng/kg per min*)			
	Control	1	2	3	Recovery (*1 h postinfusion*)
Heart rate (min^{-1})	136±9	122±11	126±7	129±8	135±8
MAP (*mmHg*)	98±7	127±10*	134±6*	123±7*	101±5
CO (*liters/min*)	3.34±0.25	2.30±0.14*	1.46±0.16*	0.99±0.10*	2.34±0.21*
SVR ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	0.029±0.002	0.054±0.005*	0.094±0.010*	0.129±0.017*	0.044±0.005*
CBF, LCx (*ml/min*)	60±15	50±4	46±11	41±13	55±15
CVR ($mmHg \cdot ml \cdot min^{-1}$)	2.156±0.501	3.154±0.663	3.554±0.779*	6.477±3.765*	2.484±0.655
(R)AP (*mmHg*)	3.1±1.26	3.5±1.57	2.9±1.72	3.5±1.69	3.2±1.50
(L)AP (*mmHg*)	2.5±0.35	3.9±1.02*	4.6±1.25*	9.1±3.45*	4.7±1.47*
MPAP (*mmHg*)	12.4±1.64	11.8±1.30	10.4±1.34	11.6±2.40	12.8±1.41

$\bar{x}$±SEM; $n = 6$. * $P < 0.05$.

Table II. Renal Hemodynamic and Excretory Effects of Endothelin

		Endothelin (*50 ng/kg per min*)			
	Control	1	2	3	Recovery (*1 h postinfusion*)
GFR (*ml/min*)	38.5±3.64	4.26±1.31*	1.43±0.39*	0.66±0.22*	47.7±2.10*
RBF (*ml/min*)	267±33	136±21*	87±14*	63±20*	173±28*
RVR ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	0.398±0.049	1.043±0.172*	1.702±0.212*	2.490±0.656*	0.667±0.115*
Urine volume (*ml/min*)	0.27±0.082	0.03±0.009*	0.012±0.001*	0.008±0.002*	0.30±0.085
$U_{Na^+}V$ (*μeq/min*)	47.6±14.3	4.56±0.48*	1.23±0.26*	—‡	12.6±3.61*
FE_{Na^+} (%)	0.95±0.31	0.88±0.17	0.66±0.14	—‡	0.18±0.05*

$\bar{x}$±SEM, $n = 6$. * $P < 0.05$. ‡ sodium detected; insufficient urine volume collected for determination.

when compared with the endothelin infusion periods. This persistent avid reabsorption of sodium occurred despite an overshoot recovery of GFR.

Table III summarizes the endocrine function data for baseline endothelin infusion and 1 h postinfusion periods. ANF was significantly and progressively elevated during endothelin infusion. This increase in ANF was associated with the progressive rise in (L)AP (Table I). ANF levels decreased at 1 h postendothelin infusion but remained elevated above baseline; this again corresponded to a persistent elevation in (L)AP. PRA was maximally increased with endothelin infusion beginning with the first clearance period (3.7±1.0–7.3±2.3 ng/ml) and returned to baseline level only during the 1-h postinfusion period. The plateaued elevations in renin occurred as ANF levels progressively increased. Aldosterone (Aldo) levels also were elevated progressively with endothelin infusion and remained significantly elevated at 1 h postinfusion. This maximum aldo elevation corresponded to the maximum suppression of FE_{Na^+} which also occurred in the postinfusion period (Table II). AVP level was significantly increased at 60 min of endothelin infusion. AVP then returned to baseline during the 60-min postinfusion recovery period as hemodynamic parameters improved.

Discussion

Intravenous infusion of endothelin produced a significant pressor response with systemic mean arterial blood pressure increasing maximally by 36 mmHg over a 45-min infusion period. This response was associated with a substantial decrease in CO, reflecting the effects of increased cardiac afterload as SVR increased 445% above baseline in association with coronary vasoconstriction. While left circumflex coronary blood flow (CBF) tended to decrease with endothelin infusion, this was not to a statistically significant extent. CVR, however, did increase 300% above baseline, which supports a coronary vasoconstrictive effect by endothelin. The progressive rise in mean (L)AP is also consistent with myocardial ischemia resulting in a depression in inotropic function. The increased afterload may have contributed as well to the progressive decline in left ventricular function but the maximum increase in MAP was not proportionate to the magnitude of left ventricle dysfunction observed; that is, a 37% increase in MAP was associated with a 70% reduction in CO. In contrast, Edwards et al. (5) demonstrated that angiotensin II infusion in the dog, which produced a 20% increase in MAP, resulted in only a 10% reduction in CO. The progressive increase in ANF may also have contributed to the further decline in CO observed during endothelin administration, perhaps by an effect to limit venous return. (R)AP, however, remained unchanged.

RBF was dramatically and preferentially reduced by endothelin when contrasted to the reduction in CBF. RBF was reduced to 24% of baseline flow with RVR increasing ~ sixfold above baseline. This increased resistance was sustained into the postinfusion period. Renal excretory function was also significantly affected by endothelin. Urine volume and $U_{Na^+}V$ were substantially decreased with endothelin infusion, with urine flow returning to baseline but with $U_{Na^+}V$ remaining significantly depressed in the recovery period. Neither $U_{Na^+}V$ nor FE_{Na^+} recovered with cessation of endothelin infusion. This continued avid sodium reabsorption effect may reflect a protracted direct effect of endothelin and/or an indirect effect through the activation of the renin-angiotensin-aldosterone system.

GFR was significantly reduced with endothelin infusion to ~ 2% of baseline. GFR, however, did recover to 24% above

Table III. Endocrine Effects of Endothelin

		Endothelin (*50 ng/kg per min*)			
	Control	1	2	3	Recovery (*1 h postinfusion*)
ANF (*pg/ml*)	39.4±5.90	64.9±12.81*	116.8±30.8*	180.5±52.9*	73.7±9.64*
PRA (*ng/ml*)	3.72±1.02	7.29±2.28*	7.08±2.49	6.94±1.74*	5.56±1.56
Aldo (*ng/ml*)	11.6±3.34	23.5±6.16*	28.9±8.11*	42.3±9.23*	46.8±10.0*
AVP (*pg/ml*)	8.45±2.76	5.05±2.60	9.43±2.97	16.43±3.52*	9.80±2.98

$\bar{x}$±SEM, $n = 6$. * $P < 0.05$.

baseline with cessation of endothelin infusion. This may reflect a waning effect of endothelin preferentially on afferent arteriolar resistance and/or the effect of a sustained elevation in ANF which has afferent arteriole vasodilatory properties and/or actions upon glomerular function to increase K_f, the ultrafiltration coefficient.

The endocrine data support the hypothesis that endothelin stimulates renin activity in vivo. This finding, in view of the recent report of Vidal et al. that EDRF inhibits renin production in canine renal cortical slices, suggests that endothelin may function physiologically to antagonize the effects of EDRF (3). Alternatively, endothelin may serve to activate renin by decreasing delivery of sodium to the macula densa, which is consistent with its action on GFR and proximal tubule reabsorption as GFR decreased in association with an increase in proximal tubule reabsorption. An interaction of renin with ANF is also suggested by these data as PRA was maximally stimulated in the initial period of endothelin infusion and thereafter declined as ANF levels progressively increased. This is based on the recognized action of ANF to inhibit renin activation. The elevated but plateaued levels of PRA may, therefore, reflect the influence of the progressive elevation of ANF. The progressive rise in ANF with endothelin infusion corresponds to the progressive rise in (L)AP also observed with endothelin infusion. This supports previous reports of the mechanism of ANF release through elevation in atrial pressure. The sustained elevation in ANF at 1 h postinfusion is also reflected in the sustained increase in (L)AP. In association with activation of renin, plasma aldo was also increased.

AVP was unchanged during the initial periods of endothelin infusion, but subsequently increased. This occurred despite elevated ANF as well as increased (L)AP, which are both inhibitors of vasopressin release.

In summary, these data demonstrate that endothelin is a potent vasoconstrictive peptide in vivo with a preferential effect on the renal circulation in association with a significant effect upon the peripheral circulation to increase overall systemic resistance. A sustained antinatriuretic effect was also produced by endothelin despite significant elevations in ANF. PRA is also stimulated by endothelin as is AVP. The potential for endothelin to contribute to pathophysiologic states such as hypertension, vasospasm, and congestive heart failure remains to be investigated as does the interaction of endothelin with other endothelium-derived vasoactive agents such as EDRF.

Acknowledgments

The authors wish to thank Larry Aarhus and Denise Heublein for their expert technical assistance, and June Hanke for preparing the manuscript.

This work was supported by National Institutes of Health grant HL-36634, American Heart Association grant 86-767, and the Mayo Foundation.

References

1. Yamagisawa, M., H. Kurihata, S. Kumura, Y. Tomabe, M. Kobayashi, Y. Mitsui, K. Goto, and T. Masaki. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond.).* 332:441–415.

2. Furchgott, R. F., and J. V. Zawadski. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.).* 288:372–376.

3. Vidal, M. J., J. C. Romero, and P. M. Vanhoutee. 1988. Endothelial-derived relaxing factor inhibits renin release. *Eur. J. Pharmacol.* 149:401–402.

4. Führ, J., J. Kaczmarczyk, and C. D. Krüttgen. 1955. Eine einfache colorimetrische Methode zur Inulinbestimmung für Nierenclearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* 33:729–730.

5. Edwards, B. S., T. R. Schwab, R. S. Zimmerman, D. M. Heublein, N. S. Jiang, and J. C. Burnett, Jr. 1986. Cardiovascular, renal, and endocrine response to atrial natriuretic peptide in angiotensin II mediated hypertension. *Circ. Res.* 59:663–667.

6. Burnett, J. C., Jr., P. C. Kao, D. C. Hu, D. W. Heser, D. Heublein, J. P. Granger, T. J. Opgenorth, and G. S. Reeder. 1986. Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science (Wash. DC).* 231:1145–1147.