Effect of Endothelin-1 in Man

H. Vierhapper, MD, O. Wagner, MD, P. Nowotny, and W. Waldhäusl, MD

The effect of an intravenous infusion of human endothelin-1 on blood pressure and plasma concentrations of endothelin-1, potassium, sodium, renin, aldosterone, and atrial natriuretic factor was investigated in six healthy, sodium-loaded men. During the peptide's exogenous application (1.0, 2.5, and 5.0 ng/kg·min), its plasma concentrations rose from a basal value of 1.2±0.3 to 3.2±1.9, 9.9±7.6, and 56.5±50.3 pmol/l (p<0.01), respectively, and mean blood pressure rose from a basal value of 87.1±7.3 to 92.6±8.2 mm Hg (p<0.01). A rise in serum concentrations of potassium (from 4.0±0.3 to 4.6±0.2 mmol/l; p<0.005) and a concomitant fall in serum concentrations of sodium (from 142.7±1.0 to 139.5±2.3 mmol/l; p<0.05) was seen in each subject. Plasma concentrations of renin, aldosterone, and atrial natriuretic factor did not change during the infusion of endothelin-1. Thus, in the doses used, endothelin-1 induces a rise in blood pressure and serum potassium concentrations. (Circulation 1990;81:1415–1418)

Endothelin-1, a 21-amino acid endothelium-derived peptide first described by Yanagisawa et al., induces vasoconstriction in a variety of vascular beds, possibly by directly or indirectly modulating vascular smooth muscle dihydropyridine-sensitive calcium channels or by activating other pathways of transmembrane signaling. In the isolated perfused rat kidney and porcine kidneys perfused in vivo, endothelin-1 (concentrations of 100–800 pmol/l) increases renal vascular resistance and decreases glomerular filtration rate. In conscious dogs, the peptide increases blood pressure as well as the plasma concentrations of vasopressin, renin, aldosterone, norepinephrine, epinephrine, and atrial natriuretic peptide. In this model, the pressor effect of endothelin-1 was similar to that of angiotensin II and vasopressin. Because no information is available on the effect of exogenous endothelin-1 in humans, we have in the present study investigated the effect of an intravenous infusion of endothelin-1 in healthy men.

Methods
Six healthy, nonobese male volunteers (aged 22–32 years) were informed of the purpose and the potential risks of the study protocol, which was approved by the ethical committee of the hospital. Written, voluntary consent was obtained before their participation. To achieve a positive state of salt balance, all subjects were told to consume their regular diet with 3 g of added salt (sodium chloride) per day for 3 days on an outpatient basis. No medication was permitted for at least 4 weeks before the study. All subjects were fasting overnight, and alcoholic beverages, coffee, and cigarette smoking were discontinued for at least 12 hours. On the day of the study, the subjects were in the supine position for at least 2 hours. Indwelling catheters were inserted into an antecubital vein of each arm—one for infusion and the other for blood sampling. Human endothelin-1 (Peptides International, Louisville, Kentucky) that had been shown to be chromato graphically pure and nonpyrogenic was dissolved in Haemac/cel (Behring Werke, Marburg/Lahn, Mar burg, FRG) and infused at a rate of 1.0, 2.5, and 5.0 ng/kg·min (equivalent to 0.4, 1.0, and 2.0 pmol/kg·min) for 15 minutes each.

Arterial blood pressure was measured by the same person with a cuff sphygmomanometer every 5 minutes for 30 minutes before and until 60 minutes after the infusion of endothelin-1. Mean blood pressure was calculated as diastolic blood pressure plus one third of the pulse height. Basal blood pressure was defined as the mean of all blood pressure readings obtained in the 30-minute preinfusion period. Blood samples were drawn for the determination of potassium and sodium before and 60 minutes after the termination of the infusion of endothelin-1. Samples for the determination of the plasma concentrations of renin, aldosterone, and atrial natriuretic factor (ANF) were obtained at −15, 0, 15, 30, 45, 60, 75, 90, and 105 minutes. To define more closely the time course in plasma, endothelin-1 concentration samples for the determination of peptide's plasma concentrations were obtained at −15, −5, 0, 14, 15, 29, 30, 44, 45, 50, 55, 60, 75, 90, and 105 minutes.

To determine the plasma concentrations of endothelin-1, 5 ml blood was collected into tubes
containing 100 μl 0.33 mol/l Na₂ EDTA · 5 ml blood. The samples were chilled immediately and centrifuged at 4,000 rpm for 15 minutes at 4°C, and the plasma was stored at −80°C until further processing. After thawing, 3,000 dpm 125 J-Endothelin-1 (Peninsula, Belmont, California) was added to each sample for the estimation of recovery. The samples were subsequently diluted with 10 ml trifluoroacetic acid (TFA) and, after 15 minutes, with another 5 ml of water and applied to C8-columns (Analytichem International, Harbor City, California) pretreated with successive application of 5 ml methanol, 20 ml water, 5 ml 0.5% trifluoroacetic acid (TFA) (solvent A); 5 ml 40% methanol–0.5% TFA (solvent B); 5 ml 90% methanol–0.5% TFA (solvent C); and, finally, 2×2 ml 0.5% TFA. After sample application, solvents B and C were reapplied. Endothelin-1 was eluted in solvent C. After concentration by evaporation under a stream of nitrogen at 37°C and subsequent lyophilization, the eluted samples were reconstituted in 500-μl assay buffer. Radioimmunoassay of endothelin-1 was performed in triplicate using standard material (Peninsula) in the range of 0.5–100 pg/tube, anti–endothelin-1 (Peninsula), and 100 μl plasma extract. Separation was achieved using goat anti-rabbit serum. The sensitivity of this radioimmunoassay was 0.5 pg at 90% binding, and the intra-assay and interassay coefficients of variation were less than 8%. (O. Wagner, P. Nowotny, H. Vierhapper, W. Waldhäusl; submitted for publication). The results were corrected for individual recovery. Mean individual recovery of endothelin-1 (calculated for 60 plasma samples) was 55.1±4.6%.

Renin was determined radioimmunologically as reported previously and expressed in Goldblatt units (GU) per milliliter. Plasma aldosterone was determined radioimmunologically after extraction by dichloromethane and thin-layer chromatography (cylohexane-ethylacetate 20:80). Concentrations of ANF were determined by radioimmunoassay after prepurification on Sep-Pak C-18 cartridges. Serum concentrations of serum and potassium were determined by American Monitor Parallel (Indianapolis, Indiana).

Data in the text and the figures are given as mean±SD. Analysis of variance for sequential data and Duncan’s multiple-range test were used for statistical evaluation.

Results

As shown in Figure 1, the infusion of 1.0, 2.5, and 5.0 endothelin-1 ng/kg · min induced a rise in plasma concentrations of endothelin-1 from a basal value of 1.2±0.3 to 3.2±1.9, 9.9±7.6, and 56.5±50.3 pmol/l (p<0.01), respectively. Despite a comparatively small range in the basal concentrations of endothelin-1, the maximum plasma concentrations induced by the peptide’s exogenous administration were heterogeneous and ranged from 450% to 10,000% above basal levels in individuals. A prompt decrease to 4.3±2.0 pmol/l was seen within 5 minutes after the termination of the infusion. The half-life of human endothelin-1 calculated from this initial rapid phase of the disappearance curve was 3.6±2.2 minutes. Thereafter, plasma concentrations continued to decrease but failed to reach basal concentrations during the next 55 minutes (Figure 1).

During the infusion of endothelin-1, a moderate rise in mean blood pressure and a concomitant fall in pulse rate were observed (Table 1). Specifically, mean blood pressure rose by 5–10 mm Hg in four individuals and remained unchanged in two subjects, including the volunteer with the smallest rise in estimated plasma concentrations of endothelin-1.

A rise in serum concentrations of potassium to (4.6±0.2 mmol/l, basal: 4.0±0.3 mmol/l; p<0.005) was seen 60 minutes after the end of the infusion of endothelin-1 in each single subject; this was paralleled by a fall in serum sodium concentrations from a basal value of 142.7±1.0 to 139.5±2.3 mmol/l (p<0.05). Plasma concentrations of aldosterone, renin, and ANF did not show major changes during either the infusion of endothelin-1 or the postinfusion period (Table 1).

A transient, local erythema in the area of the cubital vein was seen in one volunteer during the highest infused dose of endothelin-1. No other side effects were observed.

Discussion

Experiments recently carried out in conscious dogs have demonstrated that endothelin-1, a 21-residue vasoconstrictor peptide, given as an intravenous infusion of 10 ng/kg · min for 1 hour induces a rise in arterial blood pressure and decreases in heart
rate and cardiac output. Larger doses resulted in gastrointestinal side effects. In regard to the lack of information on the effects of exogenous endothelin-1 in man, the doses used for the purpose of the present pilot study were well below those used in experimental animals. This may explain the only moderate pressor action seen in our healthy volunteers. Plasma concentrations of endothelin-1 seen during the infusion of the smaller doses were comparable to those observed in a number of pathological conditions such as uremia, surgery, and subarachnoid hemorrhage. Because endothelin-1 may act locally on underlying smooth muscle cells rather than as a circulating hormone, the pathophysiological importance of these observations remains to be established. In regard to the potential pharmacological use of endothelin-1, it should be pointed out that plasma concentrations of endothelin-1 achieved in single individuals during the largest infused dose (5 ng/kg min) were above 100 pmol/l, and this concentration of endothelin-1 has been demonstrated to affect renal blood flow and glomerular filtration rate in vitro. Thus, it appears mandatory not to use higher doses of endothelin-1 in man before having studied in greater detail the hemodynamic effect of those hitherto used.

Despite the narrow range in basal plasma concentrations of endothelin-1, which was comparable to results recently published by others, the rise in the peptide’s plasma concentrations during its infusion was quite heterogenous. The reason for this observation is as yet unclear, particularly because the rapid postinfusion fall in endothelin-1 plasma concentrations in each of the six volunteers argues against marked interindividual differences in metabolic clearance rates. In analogy to observations recently made in the rat, this rapid postinfusion decrease in plasma endothelin-1 concentrations occurs in the presence of a sustained elevation of blood pressure. Thus, the clearance of endothelin-1 from the circulation is not congruent with its biological activity.

Plasma concentrations of renin, aldosterone, and ANF remained unchanged during the infusion of endothelin-1. The peptide was infused to men in the salt-repleted state, and this may have blunted possible effects of endothelin-1 on the renin-angiotensin-aldosterone axis. Furthermore, in regard to possible species differences and the smaller doses of infused endothelin-1 used in the present study, our results are not necessarily in contrast to the rise in ANF, aldosterone, and renin seen in dogs. The latter has been interpreted as a consequence of vasoconstriction of the renal vessels proximal to the juxtaglomerular cells, of a reduction in the amount of sodium reaching the macula densa, or of an increase in circulating catecholamines. On the other hand, endothelin-1, in concentrations comparable to those achieved in our study, has been shown to inhibit the release of renin in dispersed rat juxtaglomerular cells, possibly by a calcium-dependent mechanism. While we hesitate to suggest that the tendency of plasma renin concentration to decrease toward the end of our experimental protocol may be due to this action, the discrepancies in published experimental data indicate that the question of the potential influence of endothelin-1 on the release of renin is not as yet settled.

Plasma concentrations of aldosterone did not change in the healthy subjects investigated in this study. Whether the potential consequence of decreasing plasma renin concentrations was counterbalanced by the simultaneous rise in serum potassium concentrations cannot be deduced from our data, but it appears safe to say that the rise in serum potassium is not a consequence of a decrease in aldosterone secretion. At present, there is no ready explanation for the observed changes in serum electrolyte concentrations. The possibility of mild hemoysis as the cause of the observed rise in serum potassium concentrations cannot be ruled out and may indeed be suggested by the structural homology between endothelin-1 and the snake venom saraf-

### Table 1. Mean Blood Pressure, Pulse Rate, and Serum (Plasma) Concentrations of Potassium, Sodium, Renin, Aldosterone, and Atrial Natriuretic Factor in Six Healthy Men Before, During, and After the Intravenous Administration of Endothelin-1 (1.0, 2.5, and 5.0 ng/kg min)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Pulse rate (× min E-1)</th>
<th>K⁺ (mmol/l)</th>
<th>Na⁺ (mmol/l)</th>
<th>Renin (GU · 10E−4/ml)</th>
<th>Aldosterone (pmol/l)</th>
<th>ANF (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>87.1±7.3</td>
<td>63.3±1.6</td>
<td>4.0±0.3</td>
<td>142.7±1.0</td>
<td>0.60±0.20</td>
<td>213.6±27.7</td>
<td>7.9±1.7</td>
</tr>
<tr>
<td>+15</td>
<td>88.7±8.0</td>
<td>61.3±4.8</td>
<td></td>
<td></td>
<td>0.59±0.21</td>
<td>199.7±38.8</td>
<td>7.1±1.6</td>
</tr>
<tr>
<td>+30</td>
<td>91.2±7.5‡</td>
<td>62.6±3.3</td>
<td></td>
<td></td>
<td>0.61±0.23</td>
<td>197.0±80.4</td>
<td>6.0±2.7</td>
</tr>
<tr>
<td>+45</td>
<td>92.6±8.2‡</td>
<td>58.0±4.2†</td>
<td></td>
<td></td>
<td>0.51±0.22</td>
<td>213.6±80.4</td>
<td>6.8±2.5</td>
</tr>
<tr>
<td>+60</td>
<td>91.2±6.3‡</td>
<td>58.0±4.2†</td>
<td></td>
<td></td>
<td>0.48±0.17</td>
<td>246.9±97.1</td>
<td>6.4±3.2</td>
</tr>
<tr>
<td>+75</td>
<td>88.3±5.8</td>
<td>61.3±3.3</td>
<td></td>
<td></td>
<td>0.56±0.24</td>
<td>235.8±108.2</td>
<td>6.3±2.2</td>
</tr>
<tr>
<td>+90</td>
<td>87.8±5.6</td>
<td>58.7±3.3</td>
<td></td>
<td></td>
<td>0.48±0.19</td>
<td>216.4±88.8</td>
<td>5.0±2.0*</td>
</tr>
<tr>
<td>+105</td>
<td>87.0±6.4</td>
<td>62.0±4.9</td>
<td>4.6±0.2‡</td>
<td>139.5±2.3*</td>
<td>0.46±0.18*</td>
<td>235.8±99.9</td>
<td>6.0±2.1</td>
</tr>
</tbody>
</table>

ANF, atrial natriuretic factor.
Values for mean blood pressure and pulse rate represent the mean of measurements taken in 5-minute intervals during the respective period of time.

* p<0.05, † p<0.01, ‡ p<0.005 vs. respective basal values.
toxin. On the other hand, it is tempting to speculate that endothelin-1 might influence and/or impair transmembrane potassium-sodium transport, but there is no experimental evidence to support this assumption at this point. Even so, the effect of endothelin-1 on serum potassium concentrations deserves close attention in future studies concerning the effect of this peptide in man.

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References


Key Words • endothelin-1 • serum potassium • blood pressure • clinical trials
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