Cardiovascular and Endocrine Effects of Endothelin-1 at Pathophysiological and Pharmacological Plasma Concentrations in Conscious Dogs

Julian E. Donckier, MD; Claude Hanet, MD; Adrien Berbinschi, MD; Laurence Galanti, MD; Annie Robert, MS, PhD; Henri Van Mechelen, MS; Hubert Pouleur, MD; and Jean-Marie Ketelslegers, MD

Background. Increased plasma concentrations of endothelin-1, a potent vasoconstrictor produced by the endothelium, have been reported in various pathological conditions. This study was conducted to evaluate effects of endothelin-1 at pathophysiological and pharmacological plasma concentrations.

Methods and Results. Endothelin-1 was infused at increasing doses (2.5, 5, 10, and 20 ng/kg/min for 1 hour each) in nine conscious dogs. During endothelin-1 infusion, plasma endothelin-1 rose from a basal value of 1.8±0.4 pmol/l to 5.8±1.1 (pathophysiological), 20.8±3.9 (pathophysiological), 85.4±18.9 (pharmacological), and 311.4±55.7 (pharmacological) pmol/l at each dose, respectively. Heart rate increased at 2.5 ng/kg/min (from 129±7 to 146±12 beats/min) but decreased at 20 ng/kg/min (97±7 beats/min) (p<0.001). Such a biphasic response was also observed for peak (+)dP/dt and (dP/dt)/DP0 (p<0.005). Left ventricular systolic pressures, mean aortic pressure, and left atrial pressure increased over time (p<0.05, p<0.005, and p<0.001, respectively). The time constant of early isovolumic relaxation rose progressively (p<0.001). The percent systolic shortening decreased at 10 and 20 ng/kg-min (p<0.005). Pressure–segment length loops showed a reduction in systolic shortening associated with an increase in left ventricular systolic pressure at 20 ng/kg-min. Atrial natriuretic factor rose after 5 ng/kg-min from 28.5±6.5 to 92.0±18.2 pmol/l (p<0.005). Angiotensin II and catecholamines did not change significantly. Serum urea and creatinine rose progressively (p<0.05), whereas glucose decreased (p<0.05). The above results differed significantly from measurements obtained in a time-control group of six dogs.

Conclusions. A fourfold increase of plasma endothelin-1 obtained after doubling the infusion rate suggests a reduction in endothelin-1 clearance or endothelin-1 endogenous production. The biphasic response of heart rate is consistent with baroreflex-mediated effects resulting from vasodilation at the pathophysiological level and vasoconstriction at the pharmacological level. Hemodynamic data suggest an increase followed by a decrease in contractility at both levels, respectively. Finally, endothelin-1 is a stimulator of atrial natriuretic factor. (Circulation 1991;84:2476–2484)

Endothelin-1, a newly discovered 21-amino-acid peptide,1 is a potent vasoconstrictor produced by the endothelium. Endothelin-1 may also play a role in the regulation of various endocrine and neuroendocrine systems.2–5

Very low circulating levels of endothelin-1 have been found in plasma from healthy volunteers and animals,6–8 but these levels are mildly increased in patients with uremia who are undergoing hemodialysis9 and in patients with acute renal failure,10 acute myocardial infarction,11 and essential hypertension.8

From the Divisions of Endocrinology and Internal Medicine (J.E.D., L.G.), University Hospital UCL of Mont-Godinne, Yvoir, Belgium; the Departments of Physiology and Cardiology (C.H., A.R., H.V.M., H.P.) and the Unité de Diabétologie et Nutrition, (A.B., J.-M.K.) University of Louvain, Brussels.

Supported by grant 3.4577.90 from the Fonds National de la Recherche Scientifique, Brussels, Belgium.

Address for correspondence: Julian Donckier, MD, Internal Medicine, University Hospital of Mont-Godinne, 5530 Yvoir, Belgium.

Received November 14, 1990; revision accepted July 16, 1991.
Cardiovascular and endocrine effects of such mild increases of circulating endothelin-1 are not yet extensively documented. Because endothelin-1 may affect reflex mechanisms, its actions should be evaluated in conscious animals. The cardiovascular and endocrine actions of endothelin-1 have been assessed in animals after intravenous bolus injection\(^1\,^2\) or infusion.\(^3\,^4\) Plasma concentrations of endothelin-1 were not determined in either of these studies, so the relevance to pathophysiological conditions is still unclear. In one recent study, endothelin-1 levels were measured during a low-dose infusion, but the effects were evaluated in anesthetized animals.\(^5\) In another recent study,\(^6\) plasma endothelin-1 levels were also determined in humans during low-dose infusion, but no detailed hemodynamic assessment was performed and no control group was included.

The present study was conducted to provide integrated information on the hemodynamic and endocrine effects of endothelin-1 in conscious, chronically instrumented dogs compared with a time-control group. Endothelin-1 was infused at different rates to create plasma levels within pathophysiological and pharmacological ranges with circulating levels constantly monitored.

**Methods**

Sixteen mongrel dogs weighing 15–32 kg were studied: 10 dogs received endothelin-1 (only nine were included in the analysis because one died during the study), and six dogs received the vehicle solution alone (control group). After general anesthesia (20 mg/kg sodium pentobarbital i.v.), intubation, and ventilation, the animals underwent thoracotomy under sterile conditions. Two Tygon catheters were implanted in the right atrial appendage; one was for measurement of right atrial pressure and the other was advanced into the inferior vena cava for infusion of endothelin-1. Another catheter was implanted in the left atrial appendage for measurement of left atrial pressure. A micromanometer (JSI 0400, Janssen Scientific Instruments) was inserted into the left ventricle through a stab incision of the apex. Electrodes for the monitoring of the electrocardiogram were sutured to the ventricular walls. A pair of piezoelectric crystals was implanted into the ventricular walls to record segment lengths (Sonomicrometer 120, Triton Technology). A last catheter was inserted into the descending aorta for blood pressure measurement and blood sampling. The catheters and wires were subcutaneously tunneled to the neck, and the animals were allowed 8–10 days to recover. At the time of the study, the dogs were afebrile and healthy.

**Experimental Protocol**

Experimental protocol is shown in Figure 1. Before starting the study, the animals were allowed to stabilize for 40 minutes. Endothelin-1 (Peninsula Laboratories, Belmont, Calif.), dissolved in acetic acid (1/10,000) was added to isotonic saline (50 ml) and infused into the inferior vena cava at a rate of 2.5, 5, 10, and 20 ng/kg/min (equivalent to 1.0, 2.0, 4.0, and 8.0 pmol/kg-min) for 1 hour each. The control group received the vehicle solution alone (1/10,000 acetic acid added to 50 ml isotonic saline). Hemodynamic parameters were recorded and blood was withdrawn at 40 and 60 minutes of each infusion period and 10 minutes after the end of the last infusion period. The experimental protocol is approved by the Commission for Animal Research of the Belgian Fonds National de la Recherche Scientifique Médicale.

**Data Analysis**

Hemodynamic data were recorded on analog magnetic tape (Honeywell 101) and on paper (Gould ES 1000). The JSI 0400 micromanometer has a linear gain up to 300 mm Hg and a flat frequency response of up to 15 kHz.\(^14\) Zero level was adjusted to match the systolic pressure recorded by means of the fluid-filled catheter connected to a Statham P23ID. Analog data were digitized every 2 msec and processed on-line by means of a Hewlett-Packard A900 computer as described previously.\(^15\) As indexes of inotropic state, we used the peak positive of the first derivative of left ventricular pressure (dP/dt) and the value of dP/dt at a developed pressure of 40 mm Hg and normalized this for developed pressure (i.e., [dP/dt]/DP\(_{40}\)).\(^16\) The time constant T\(_1\) of the exponential left ventricular pressure fall during the first 40 msec after peak (−)dP/dt was used as an index of left ventricular relaxation rate.\(^17\) For regional wall motion analysis, end-diastolic (ED) and end-systolic (ES) segment lengths (SL) were determined at the peak of the R wave and at the time of peak negative dP/dt, respectively. Percent systolic shortening was calculated as 100×(EDSL−ESSL)/EDSL. To further evaluate the effects on inotropic state and diastolic distensibility, the pressure–segment length loops were constructed.

The methods for endothelin-1 and atrial natriuretic factor (ANF) measurements have been previously described by our group.\(^7\,^18\) Angiotensin II was measured similarly. Briefly, these three peptides
were measured after plasma extraction on Sep-Pak C18 cartridges (Waters Associates, Milford, Mass.) by radioimmunoassays, using specific antibodies and synthetic peptides from Peninsula (Belmont, Calif.). Intra- and interassay coefficients of variation were, respectively: endothelin-1, 12% and 18%; ANF, 6.7% and 8.7%; and angiotensin II, 6.5% and 8.5%.

The sensitivities of the radioimmunoassays, defined as 10% tracer displacement, were 2 pg per tube for endothelin-1, 2.8 pg per tube for ANF, and 1.9 pg per tube for angiotensin II. The percent cross-reactivities of the antiserum for endothelin have been reported by Peninsula as 100% for endothelin-1, 7% for endothelin-2 and endothelin-3, 0% for angiotensin II, and 0% for ANF. Insulin was measured by a radioimmunoassay, using human insulin as standard. Urea, creatinine, and total protein were measured in a Dacos Analyzer (Coulter Electronics, Inc., Hialeah, Fla.). Concentrations of sodium, potassium, chloride, and glucose were determined by an Astra IV System (Beckman Instruments, Inc., Fullerton, Calif.). Plasma catecholamine activity was measured by high-pressure liquid chromatography with electrochemical detection and a cation exchange analytical column (Bio-Rad Clinical Division, Hercules, Calif.) as previously described.19 Intra- and interassay coefficients of variation were, respectively: epinephrine, 8.2% and 11%; norepinephrine, 6.6% and 12%; and dopamine, 4.5% and 17%. The sensitivities of the assays were 10 pg/ml for epinephrine, 25 pg/ml for norepinephrine, and 40 pg/ml for dopamine.

Statistical Analysis

Data were analyzed by two-way analysis of variance for repeated measurements with an orthogonal decomposition of the trial factor (time) because the levels were ordered and unequally spaced.20 Differences between treated and control groups (the grouping factor) were assessed using F tests and differences between the levels of the trial factor (the within factor) were assessed using conservative Greenhouse-Geisser tests. When the interaction between the two factors was significant, a one-way analysis of variance for repeated measurements was performed in each group. A detailed contrast analysis is also provided in the tables. A probability value of less than 0.05 was considered significant. Computations were performed using BMDPC 90 statistical software. Data are expressed as mean±SEM.

Results

Plasma Endothelin-1 Concentrations

Figure 2 and Tables 1 and 2 illustrate plasma endothelin concentrations in basal conditions and during infusion of either endothelin-1 or the vehicle solution. Basal plasma endothelin-1 was detectable in all dogs. A significant increase was observed at and after the dose of 2.5 ng/kg/min (p<0.001). It is noticeable that plasma endothelin-1 concentrations increased approximately four times after doubling the infusion rate.

In the control group, endothelin-1 levels displayed a mild increase (p<0.05). Endothelin-1 infusion obviously produced much higher levels (significant interaction time × group on endothelin-1 levels; p<0.001).

Side Effects

Two animals died during the study. The first one had ventricular fibrillation at the 5-ng/kg-min dose. This animal was excluded from the analysis. A second animal, which was included in the study, died after the experiment from acute left ventricular failure and shock. All animals were conscious during the endothelin infusion but slightly drowsy at the end of the large-dose infusion. None of them vomited.

Hemodynamic Effects

The infusion of endothelin-1 produced several cardiovascular changes (see Table 3). Table 4 shows the hemodynamic parameters in the control group. In the group receiving endothelin-1, changes of heart rate (p<0.001) were characterized by an initial increase at low-dose infusion (2.5 and 5 ng/kg-min) (p<0.001) followed by a decrease at larger doses (20 ng/kg-min) (p<0.02). Heart rate did not change in the control group. The evolution of heart rate was significantly different between the two groups (interaction time × group on heart rate; p<0.001).
Left ventricular systolic pressure and mean aortic pressure increased significantly (p<0.05 and p<0.005, respectively) during endothelin-1 infusion, whereas both parameters did not change in the control group (p>0.02, control versus endothelin-1 for left ventricular systolic pressure and p<0.005 for mean aortic pressure). No change and no difference between groups were evidenced for left ventricular diastolic pressure.

Changes of peak (+dP/dt) and (dP/dt)/DP40 (p<0.005) were also significantly different between groups (p<0.05 for peak [+dP/dt] and p<0.001 for [dP/dt]/DP40). In the group receiving endothelin-1, an initial increase of these variables at the dose of 2.5 and 5 ng/kg/min (p<0.05 for peak [+dP/dt] and p<0.005 for [dP/dt]/DP40) was followed by a decrease at the larger dose of 20 ng/kg/min (p<0.01 for both variables).

The time constant of early isovolumic relaxation (T1) rose progressively (p<0.001) when increasing the infusion dose of endothelin-1 but remained unchanged in the control group (p<0.005 versus control). In terms of regional function, it is noteworthy that end-systolic dimensions were significantly decreased at the dose of 2.5 ng/kg-min but at larger doses, end-diastolic and systolic lengths increased significantly (p<0.05 and p<0.005, respectively) and were unchanged in the control group (p<0.01 and p<0.002 versus control for both parameters, respectively). The percent systolic shortening deteriorated in the group receiving endothelin-1 (p<0.005) and was constant in the control group (p<0.005 versus control). Individual pressure–segment length loops were reconstructed at the end of the 5 ng/kg-min and 20 ng/kg-min infusion periods. Figure 3 illustrates typical loops in one animal. It is clear from these loops that endothelin-1 caused only a slight right and upward shift at the dose of 5 ng/kg-min but that at the dose of 20 ng/kg-min, a marked reduction in systolic shortening accompanied the increase in left ventricular systolic pressure.

At the largest doses, endothelin-1 produced a rise (p<0.001) of left atrial pressure that remained unchanged in the controls (p<0.05 versus control). Right atrial pressure did not change in either group. As shown in Table 3, effects of the drug on the cardiovascular variables were fading out after discontinuation of endothelin-1 infusion (significant con-

### Table 1. Effects of Four Cumulative Doses of Endothelin-1 in Nine Dogs on Plasma or Serum Values of Hormones, Glucose, Protein, Creatinine, Urea, and Electrolytes

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th></th>
<th>B</th>
<th></th>
<th>C</th>
<th></th>
<th>D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 ng/kg/min</td>
<td>5 ng/kg/min</td>
<td>10 ng/kg/min</td>
<td>20 ng/kg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1 (pmol/l)</td>
<td>1.8 ±0.4</td>
<td>4.4 ±0.8*</td>
<td>5.8 ±1.1†</td>
<td>19.1 ±3.5‡</td>
<td>20.8 ±3.9‡</td>
<td>69.5 ±16.0‡</td>
<td>85.4 ±18.9‡</td>
<td>307.3 ±58.6‡</td>
</tr>
<tr>
<td>ANF (pmol/l)</td>
<td>28.5 ±6.5</td>
<td>24.8 ±5.5</td>
<td>27.6 ±7.3</td>
<td>33.2 ±9.8</td>
<td>29.3 ±7.6</td>
<td>40.9 ±10.9</td>
<td>38.8 ±10.6</td>
<td>78 ±16.7†</td>
</tr>
<tr>
<td>ANG II (pmol/l)</td>
<td>116.0 ±43.9</td>
<td>217.1 ±100.5</td>
<td>250.7 ±127.7</td>
<td>221.8 ±104.9</td>
<td>198.0 ±93.3</td>
<td>131.7 ±62.9</td>
<td>120.8 ±56.4</td>
<td>123.9 ±48.8</td>
</tr>
<tr>
<td>NE (pmol/l)</td>
<td>6.862 ±2.752</td>
<td>9.723 ±3.235</td>
<td>8.669 ±2.843</td>
<td>5.531 ±2.206</td>
<td>5.144 ±1.843</td>
<td>6.017 ±2.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOPA (pmol/l)</td>
<td>1.116 ±6.2</td>
<td>2.126 ±929</td>
<td>2.341 ±1.027</td>
<td>1.645 ±876</td>
<td>1.199 ±739</td>
<td>1.434 ±849</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.9 ±0.7</td>
<td>7.5 ±1.1</td>
<td>7.2 ±1.2</td>
<td>6.1 ±1.1</td>
<td>5.6 ±1.1*</td>
<td>5.8 ±1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td>46 ±11</td>
<td>46 ±13</td>
<td>58 ±24</td>
<td>60 ±24</td>
<td>75 ±31</td>
<td>74 ±24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>65 ±2</td>
<td>67 ±3</td>
<td>66 ±3</td>
<td>67 ±3</td>
<td>68 ±4</td>
<td>67 ±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>80 ±9</td>
<td>87 ±11</td>
<td>87 ±13</td>
<td>91 ±14</td>
<td>102 ±15*</td>
<td>104 ±15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>6.2 ±1.4</td>
<td>6.4 ±1.4</td>
<td>6.6 ±1.5</td>
<td>7.2 ±1.6*</td>
<td>7.7 ±1.5‡</td>
<td>7.9 ±1.5‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na+ (mmol/l)</td>
<td>143.5 ±1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K+ (mmol/l)</td>
<td>3.4 ±0.2</td>
<td>3.2 ±0.2</td>
<td>3.4 ±0.2‡</td>
<td>4.0 ±0.2‡</td>
<td>4.5 ±0.2‡</td>
<td>4.2 ±0.2‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl- (mmol/l)</td>
<td>117 ±2</td>
<td>116 ±2</td>
<td>116 ±2</td>
<td>117 ±2</td>
<td>117 ±2</td>
<td>117 ±2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A, before infusion; B and C, at 40 and 60 minutes of each 1-hour infusion period, respectively; D, 10 minutes after discontinuation of the last dose; ET-1, endothelin-1; ANF, atrial natriuretic factor; ANG II, angiotensin II; NE, norepinephrine; E, epinephrine; DOPA, dopamine.

*p<0.05, †p<0.01, ‡p<0.005 vs. respective basal values.

§p<0.05, || p<0.01 vs. last measurement of last infusion dose.
Table 2. Values of Hormones, Glucose, Protein, Creatinine, Urea, and Electrolytes in a Time-Control Group of Six Dogs

<table>
<thead>
<tr>
<th></th>
<th>2.5 ng/kg-min</th>
<th>5 ng/kg-min</th>
<th>10 ng/kg-min</th>
<th>20 ng/kg-min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>ET-1 (pmol/l)</td>
<td>1.4 ±0.3</td>
<td>1.9 ±0.4</td>
<td>2.5 ±0.6</td>
<td>2.7 ±0.6</td>
</tr>
<tr>
<td>ANF (pmol/l)</td>
<td>25.3 ±4.6</td>
<td>21.6 ±4.0</td>
<td>22.2 ±4.3</td>
<td>22.6 ±3.5</td>
</tr>
<tr>
<td>ANG II (pmol/l)</td>
<td>50.0 ±20.1</td>
<td>79.0 ±32.8</td>
<td>50.7 ±34.0</td>
<td>102.6 ±37.7</td>
</tr>
<tr>
<td>NE (pmol/l)</td>
<td>4,091 ±2,919</td>
<td>...</td>
<td>2,672 ±1,221</td>
<td>...</td>
</tr>
<tr>
<td>E (pmol/l)</td>
<td>3,403 ±1,699</td>
<td>5,290 ±2,442</td>
<td>4,332 ±1,682</td>
<td>2,660 ±1,585</td>
</tr>
<tr>
<td>DOPA (pmol/l)</td>
<td>324 ±106</td>
<td>272 ±132</td>
<td>303 ±213</td>
<td>554 ±234</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.3 ±0.6</td>
<td>6.5 ±0.9</td>
<td>6.2 ±1.0</td>
<td>6.1 ±0.9</td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td>14 ±2</td>
<td>28 ±7</td>
<td>18 ±2</td>
<td>16 ±2</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>62 ±3</td>
<td>65 ±2</td>
<td>61 ±2</td>
<td>60 ±2</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>72 ±12</td>
<td>72 ±12</td>
<td>71 ±13</td>
<td>68 ±12</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>6.4 ±1.6</td>
<td>6.3 ±1.6</td>
<td>6.0 ±1.6</td>
<td>6.0 ±1.6</td>
</tr>
<tr>
<td>Na+ (mmol/l)</td>
<td>145.3 ±1.5</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>K+ (mmol/l)</td>
<td>3.6 ±0.2</td>
<td>3.2 ±0.2</td>
<td>3.3 ±0.2</td>
<td>3.8 ±0.2</td>
</tr>
<tr>
<td>Cl- (mmol/l)</td>
<td>116 ±2</td>
<td>116 ±2</td>
<td>116 ±2</td>
<td>116 ±2</td>
</tr>
</tbody>
</table>

A, before infusion; B and C, at 40 and 60 minutes of each 1-hour infusion period, respectively; D, 10 minutes after discontinuation of the last dose; ET-1, endothelin-1; ANF, atrial natriuretic factor; ANG II, angiotensin II; NE, norepinephrine; E, epinephrine; DOPA, dopamine.

*p<0.05, tp<0.01 vs. respective basal values.

Contrasts between last values during the dose of 20 ng/kg-min and values 10 minutes later. Interestingly, heart rate was higher (p<0.005) at the beginning of the 5 ng/kg-min dose than after discontinuation of the infusion despite similar endothelin-1 concentrations averaging 20 pmol/l.

Endocrine Effects

Atrial natriuretic factor did not change significantly at the dose of 2.5 ng/kg-min and 5 ng/kg-min but rose threefold thereafter (p<0.005) (Table 1 and Figure 2). In contrast, ANF remained stable in the control group (significant interaction on ANF; p<0.01).

There was a trend for angiotensin II, norepinephrine, epinephrine, and dopamine to display changes parallel to those in heart rate (increase at low dose and decrease at large dose) but these changes did not reach statistical significance because of important standard deviations of those variables and because changes did not occur at exactly the same dose.

Other Effects

Serum (or plasma) protein and chloride did not change significantly in either group (Tables 1 and 2). Although an increase of serum potassium (p<0.001) was observed with endothelin-1, no interaction time × group on potassium (p=0.07) could be established because of a slight rise in the control group. In contrast, in the presence of endothelin-1, there was a rise of serum urea (p<0.01) and creatinine (p<0.05). In the control group, serum creatinine did not change but urea decreased (p<0.001 versus endothelin-1). Endothelin-1 produced a decrease of serum glucose (p<0.05), whereas no change occurred in the controls (the interaction being at the limit of significance, p=0.06). Despite a tendency for insulin to increase in the group receiving endothelin-1, changes were not significant.

Sodium, which was measured only at the beginning and at the end of the experiment, increased (p<0.05) during endothelin-1 infusion but did not change significantly in the control group.

Discussion

Our group has previously established a range of plasma endothelin-1 concentration of 2–6 pg/ml (equivalent to 0.8–2.4 pmol/l) in eight normal subjects. Using the same radioimmunoassay, we found that end-stage renal failure before kidney transplan-
tation was associated with eightfold higher values ranging from 17 to 66 pg/ml (equivalent to 6.8–26.9 pmol/l) (unpublished data). In myocardial infarction and some cases of hypertension, plasma endothelin-1 concentrations have also been reported to increase approximately two to eight times above basal levels. At a dose of 2.5 and 5 ng/kg/min, the present study could thus create plasma endothelin-1 concentrations similar to those observed in renal failure or a proportional rise similar to that seen in myocardial infarction or hypertension. Plasma endothelin concentrations achieved with larger doses (10 and 20 ng/kg/min) were within the pharmacological range. The present study thus helps to clarify the respective endocrine and hemodynamic roles of pathophysiological and pharmacological endothelin-1 concentrations in vivo. Four cumulative doses were used in the present study by doubling the infusion rate. Interestingly, circulating plasma endothelin-1 concentrations increased approximately fourfold after each new dose. This pharmacokinetic effect is evident, although not discussed, in the results of a recent study. This could be explained by a reduction in endothelin clearance because endothelin is known to reduce glomerular filtration rate and some cases of hypertension. However, we cannot exclude the possibility that synthetic endothelin-1 induced a release of endogenous endothelin because endothelin-1-specific receptors have been demonstrated on endothelial cells secreting endothelin.

A major finding of this study is that the infusion of endothelin at low dose (2.5 and 5 ng/kg/min) increased heart rate without significant changes in mean aortic pressure, whereas administration of larger doses (20 ng/kg/min) resulted in a decrease of heart rate associated with a rise of mean aortic pressure. Transient tachycardia has also been reported after low-dose bolus injection. Previous infusion studies carried out in anesthetized or in conscious dogs did not demonstrate an increase in heart rate but only a decrease with a rise of blood pressure. However, the doses used were higher. In the recent low-dose infusion study in humans, the authors did not observe a change in heart rate at the plasma endothelin concentrations (4–20 pmol/l) that produced a rise of heart rate in our study. The difference may be caused by a lower duration of infusion in this study (15 minutes versus 1 hour in our study) and may reflect the latency of the effect of endothelin. Indeed, 15 minutes after starting the infusion, heart rate was still unchanged in our study, but the increase was apparent after 30 minutes. Thus, our study demonstrated for the first time an increase in heart

### Table 3. Hemodynamic Changes in Nine Dogs Receiving Four Cumulative Doses of Endothelin-1

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 ng/kg/min</td>
<td>5 ng/kg/min</td>
<td>10 ng/kg/min</td>
<td>20 ng/kg/min</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>129 ±7</td>
<td>140 ±10*</td>
<td>145 ±12*</td>
<td>127 ±7</td>
</tr>
<tr>
<td></td>
<td>146 ±12*</td>
<td>144 ±12*</td>
<td>117 ±11</td>
<td>101 ±9†</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>5.8 ±5.9</td>
<td>6.1 ±5.9</td>
<td>6.4 ±5.9</td>
<td>6.7 ±5.9</td>
</tr>
<tr>
<td></td>
<td>5.3 ±6.7</td>
<td>6.7 ±6.7</td>
<td>6.7 ±6.7</td>
<td>9.3 ±10.8</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>107 ±114</td>
<td>114 ±114</td>
<td>114 ±114</td>
<td>122 ±122</td>
</tr>
<tr>
<td></td>
<td>112 ±122</td>
<td>114 ±122</td>
<td>114 ±122</td>
<td>136 ±136</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>87 ±4</td>
<td>86 ±4</td>
<td>86 ±4</td>
<td>89 ±5*</td>
</tr>
<tr>
<td></td>
<td>86 ±5*</td>
<td>86 ±5*</td>
<td>99 ±5*</td>
<td>102 ±7†</td>
</tr>
<tr>
<td>Peak (+dp/dt) (mm Hg/sec)</td>
<td>2,854 ±236</td>
<td>3,448 ±392*</td>
<td>3,545 ±290*</td>
<td>2,854 ±236</td>
</tr>
<tr>
<td>(dp/dt)/ΔP (1/sec)</td>
<td>45.7 ±2.5*</td>
<td>52.0 ±2.8*</td>
<td>52.3 ±3.8*</td>
<td>46.3 ±3.6*</td>
</tr>
<tr>
<td>T1 (msec)</td>
<td>21.0 ±1.0</td>
<td>19.9 ±1.0</td>
<td>19.8 ±1.0</td>
<td>23.8 ±1.0</td>
</tr>
<tr>
<td></td>
<td>21.3 ±1.3</td>
<td>21.3 ±1.3</td>
<td>23.8 ±1.3</td>
<td>24.7 ±1.3</td>
</tr>
<tr>
<td>Dias (mm)</td>
<td>11.6 ±0.9</td>
<td>11.5 ±0.9</td>
<td>11.5 ±0.9</td>
<td>11.7 ±0.9</td>
</tr>
<tr>
<td></td>
<td>11.3 ±1.0</td>
<td>11.3 ±1.0</td>
<td>11.3 ±1.0</td>
<td>11.3 ±1.0</td>
</tr>
<tr>
<td>Syst (mm)</td>
<td>9.9 ±0.6</td>
<td>9.7 ±0.7</td>
<td>9.6 ±0.6</td>
<td>10.2 ±0.6</td>
</tr>
<tr>
<td></td>
<td>9.8 ±0.7</td>
<td>9.8 ±0.7</td>
<td>9.8 ±0.7</td>
<td>10.3 ±0.7</td>
</tr>
<tr>
<td>Shortening (%)</td>
<td>12.6 ±2.2</td>
<td>13.5 ±2.2</td>
<td>13.7 ±2.2</td>
<td>13.0 ±2.0</td>
</tr>
<tr>
<td></td>
<td>13.2 ±2.0</td>
<td>13.2 ±2.0</td>
<td>13.2 ±2.0</td>
<td>13.0 ±2.0</td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>2.7 ±0.7</td>
<td>2.2 ±0.6</td>
<td>2.0 ±0.8</td>
<td>2.1 ±0.8</td>
</tr>
<tr>
<td></td>
<td>2.7 ±0.8</td>
<td>2.7 ±0.8</td>
<td>2.7 ±0.8</td>
<td>2.7 ±0.8</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>0.3 ±0.3</td>
<td>0.3 ±0.3</td>
<td>0.7 ±0.3</td>
<td>0.3 ±0.3</td>
</tr>
<tr>
<td></td>
<td>0.3 ±1.0</td>
<td>0.3 ±1.0</td>
<td>0.3 ±1.0</td>
<td>0.3 ±1.0</td>
</tr>
</tbody>
</table>

A, before infusion; B and C, at 40 and 60 minutes of each 1-hour infusion period, respectively; D, 10 minutes after discontinuation of the last dose; LVEDP and LVSP, left ventricular end-diastolic and end-systolic pressure; Mean AoP, mean aortic pressure; T1, time constant of relaxation; Dias, left ventricular end-diastolic segmental length; Syst, left ventricular end-systolic segmental length; LAP, left atrial pressure; RAP, right atrial pressure.

* p<0.05, †p<0.01, ‡p<0.005 vs. respective basal values.

§p<0.05, ‡p<0.01 vs. last measurement of last infusion dose.
Table 4. Hemodynamic Data in a Time-Control Group of Six Dogs

<table>
<thead>
<tr>
<th></th>
<th>2.5 ng/kg-min</th>
<th>5 ng/kg-min</th>
<th>10 ng/kg-min</th>
<th>20 ng/kg-min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>130</td>
<td>129</td>
<td>127</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>±12</td>
<td>±13</td>
<td>±13</td>
<td>±12</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>3.8</td>
<td>±0.4</td>
<td>±5.2</td>
<td>±4.9</td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>±1.5</td>
<td>±5.2</td>
<td>±1.5</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>102</td>
<td>±3</td>
<td>110</td>
<td>±3</td>
</tr>
<tr>
<td></td>
<td>109</td>
<td>±2</td>
<td>111</td>
<td>±2</td>
</tr>
<tr>
<td>Mean AoP (mm Hg)</td>
<td>82</td>
<td>±6</td>
<td>92</td>
<td>±3</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>±2</td>
<td>92</td>
<td>±4</td>
</tr>
</tbody>
</table>

A, before infusion; B and C, at 40 and 60 minutes of each 1-hour infusion period, respectively; D, 10 minutes after discontinuation of the last dose; LVEDP and LVSP, left ventricular end-diastolic and end-systolic pressure; Mean AoP, mean aortic pressure; T1, time constant of relaxation; Dias, left ventricular end-diastolic segmental length; Syst, left ventricular end-systolic segmental length; LAP, left atrial pressure; RAP, right atrial pressure.

Rate at stable plasma endothelin concentrations corresponding to the levels observed in several pathological conditions (as described above).

To explain an increase in heart rate at low endothelin concentrations and a decrease at pharmacological levels, we would favor the hypothesis that these changes were mediated by a reflex from the arterial baroreceptors. At low concentrations, endothelin-1 has been shown to release endothelin-derived relaxing factor (EDRF) from isolated perfused arterial vessels.22 The increase in heart rate observed in our study might therefore reflect a sympathetic neural discharge to maintain blood pressure in the presence of a decrease in peripheral resistances induced by EDRF. Even if results of cardiac output or systemic vascular resistance are not directly available, our data clearly indicate in which direction these two variables were modified. Indeed, at a low dose of endothelin, there is evidence of an increase in stroke volume; end-diastolic dimensions and pressures were unchanged but end-systolic dimensions decreased significantly (Table 3). As heart rate was simultaneously increased, it is obvious that cardiac output was also increased by at least 13 to 15%. Because aortic pressure at that time tended to decrease by an average of 4 mm Hg, calculated systemic vascular resistance should be lower. At high endothelin concentrations, a decrease in sympathetic tone reducing heart rate would occur when the EDRF-mediated vasodilation was overwhelmed by endothelin-mediated vasoconstriction. The trends of circulating catecholamine changes (increase at low dose and decrease at high dose) could support this hypothesis of a change in baroreceptor activity. A role of an intermediate, transient factor mediating the effect of endothelin at low dose, such as EDRF, may also be suspected on the basis that heart rate was higher early at low dose than after discontinuation of the
infusion despite similar endothelin-1 concentrations. This phenomenon can also be explained by a rema-
nence of endothelin-1 effect on its receptor.

Peak (+)dp/dt and (dp/dt)/dp at also increased at low-dose infusion and declined thereafter. Although these indexes may be heart-rate dependent, studies in conscious dogs have demonstrated that increasing heart rate from 120 to 150 beats/min (as in our data) had no effect on peak (+)dp/dt. Likewise, the decrease of dp/dt at the dose of 10 ng/kg-min cannot be explained by heart rate only. Infusion of pharma-
cological doses of endothelin produced a rise of left ventricular end-systolic pressures and of the rate of isovolumic relaxation. End-diastolic and systolic di-

dmensions increased and the percent systolic shorten-
ing decreased by 50%. This decline in left ventricular function illustrated by the pressure-segment length loops as well as the increase of left atrial pressure can be the consequence of an increased ventricular after-
load. However, the depression of systolic function variables was proportionally more pronounced than the rise of blood pressure or the heart rate effects, conceivably reflecting a decrease in inotropic func-
tion. This effect might have been exerted by the peptide itself, by a coronary vasoconstrictive effect, or simply by a decrease in sympathetic tone and catecholamines.

At high doses, endothelin also stimulated the release of ANF. Among the mechanisms regulating the release of ANF in vivo, atrial distension appears to be the principal determinant as demonstrated in experiments of volume expansion,25 during exercise,26 or in congestive heart failure.27 Catechola-
mies,28,29 calcitonin gene-related peptide,30 and recently, endothelin31 have been shown to directly elicit the release of ANF. However, a direct effect of endothelin is difficult to establish because ANF and left atrial pressure increased concomitantly. A role of the sympathetic nervous system or of catecholamines can probably be ruled out because heart rate and catecholamines were at their lowest levels at that time.

A decrease of catecholamine release (mainly nor-

epinephrine) has been found by previous authors at the dose of 10 ng/kg-min in conscious dogs and attributed to a decrease in the reflex-mediated sympa-
thetic neural discharge to the heart.5 At doses larger than those used in our study (30 ng/kg-min), they observed a rise in plasma catecholamines that was attributed to a stimulation of adrenal medulla.5 In the present study, circulating catecholamines tended to increase at low dose and to decrease at larger doses. As explained above, these changes may be mediated by reflexes from the arterial baroreceptors. Angiotensin II, which has never been measured during endothelin infusion, tended to display changes similar to those of catecholamines.

An increase of urea and creatinine may reflect the alteration of renal function resulting from the vaso-
constrictive action of endothelin. An increase in potassium levels confirming previous reports13 was greater in magnitude in the group receiving endo-
thelin-1 than in the controls, although the interaction was not significant. A decrease in blood glucose was also observed, which does not seem to be due to the fasting state of the animals but possibly to a stimu-
lation of insulin secretion.

Conclusions

The present study has shown that circulating pathophysiological levels of endothelin-1 induced a rise of heart rate, possibly mediated by arterial baroreflexes. A direct or indirect positive inotropic effect can also be suspected at the same dose. In contrast, pharmacological levels of endothelin-1 pro-
duced a decrease in heart rate associated with a rise of blood pressure, a decline in contractility, and a release of ANF. Because endothelin-1 may act locally in a paracrine way rather than as a hormone, the relation between local levels and increased circulating levels still remains to be elucidated.

Acknowledgment

We thank Isabelle Mottard for her expert secre-
tarial help.

References


KEY WORDS: endothelium, vasoconstriction, atrial natriuretic factor, baroreflex, endothelin-1
Cardiovascular and endocrine effects of endothelin-1 at pathophysiological and pharmacological plasma concentrations in conscious dogs.

Circulation. 1991;84:2476-2484
doi: 10.1161/01.CIR.84.6.2476

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/84/6/2476