Nitric oxide–endothelin-1 interaction in humans

GUNVOR AHLBORG AND JAN M. LUNDBERG
Department of Clinical Physiology, Huddinge University Hospital, S-141 86 Huddinge and Department of Physiology and Division of Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden

Ahlborg, Gunvor, and J an M. Lundberg. Nitric oxide–endothelin-1 interaction in humans. J. Appl. Physiol. 82(5): 1593–1600, 1997.—Healthy men received Ng-monomethyl-l-arginine (L-NMMA) intravenously to study cardiovascular and metabolic effects of nitric oxide synthase blockade and whether this alters the response to endothelin-1 (ET-1) infusion. Controls only received ET-1. L-NMMA effects were found with ET-1 infusion. Controls only received ET-1. L-NMMA like ET-1, induces prolonged cardiovascular effects and suppresses SGO. L-NMMA causes pulmonary ET-1 release and blocks responses to ET-1 infusion. The results indicate that nitric oxide inhibits ET-1 production and thereby interacts with ET-1 regarding increase in vascular tone and reduction of SGO in humans.

Ng-monomethyl-l-arginine and endothelin-1 infusion; cardiac output; splanchnic and renal blood flow; systemic and pulmonary vascular resistance; arterial levels and pulmonary release of endothelin-1; insulin and splanchnic glucose output.

EVER SINCE the endothelium-dependent vasodilatory effect of acetylcholine (14) was shown to be mediated by nitric oxide (NO) (23), there has been an increasing interest in the possible role of NO in health and disease. The systemic hypotension seen during endotoxic shock has been ascribed to an excess formation of NO (20). In patients with acute respiratory distress syndrome, NO administration causes reduction of the pulmonary hypertension (26). Other studies have shown that hypoxic vasoconstriction in healthy subjects is reversed by NO inhalation (12). However, there is no direct evidence that NO is involved in the regulation of pulmonary vascular resistance in healthy humans. NO is formed by two principally different types of cytosolic NO synthases (NOS). One type, which is found in neurons and endothelial cells, is constitutive, is Ca2+ calmodulin dependent, and releases picomoles of NO on receptor stimulation; the other type is induced, e.g., by immunological stimuli, is Ca2+ independent, and releases hundred- to thousand-fold higher amounts of NO. The substrate for NOS is l-arginine (22). The L-arginine analog Ng-monomethyl-l-arginine (L-NMMA), which occurs naturally in human blood (29), is a competitive inhibitor of NOS (24). Previous studies in healthy humans have shown that administration of L-NMMA into the brachial artery causes vasoconstriction in the forearm (28). Administration into a systemic vein causes increased systemic arterial blood pressure and reduces heart rate and cardiac index as estimated by noninvasive methods (15). The influence of NO synthesis in different systemic vascular beds in humans, with the exception of muscle, is incompletely known.

Apart from NO, vascular endothelial cells have been shown to synthesize a potent pressor substance called endothelin-1 (ET-1) (35). Previous studies of the effects of ET-1 infusion in healthy humans have shown that ET-1 causes vasoconstriction in the splanchic and renal vascular beds as well as reduction of cardiac output and splanchnic glucose production (1–3, 32, 33). Several substances stimulate the expression of preproendothelin mRNA (36). NO has been shown to suppress ET-1 release (8, 27, 38) by inhibition of preproendothelin synthesis (38). Furthermore, the ET-receptor antagonist bosentan has recently been shown to attenuate the pressor effects of NOS inhibition in rats (25). These observations suggest that NO might promote its vasodilatory effects by blocking the formation of ET-1. On the other hand, ET-1 stimulates NO formation via endothelial ET-receptor activation (11, 31). If NO and ET-1 interact at the smooth muscle contraction level, withdrawal of NO production would result in increased sensitivity to exogenous ET-1. In this study we have, therefore, examined the effects of intravenous administration of L-NMMA on cardiac output and vascular resistance in pulmonary, splanchnic, and renal vascular beds in healthy human subjects. We also studied the effects of the NOS blocker on ET-1 levels in plasma and splanchnic glucose output. Moreover, we examined whether L-NMMA changes the responses to infused exogenous ET-1.

METHODS

Subjects

Five healthy male subjects (age 26 ± 1.7 yr; height 183 ± 2.3 cm; weight 76 ± 1.9 kg) were studied in the resting 12- to 14-h postabsorptive basal state and received infusions of both L-NMMA and ET-1 (group 1). Another group of six subjects (age 28 ± 2.4 yr; height 181 ± 2.0 cm; weight 73 ± 3.2 kg) served as a control group and only received ET-1 infusion (group 2). All subjects were informed of the purpose and possible risks before giving their voluntary consent. Approval was obtained from the local Ethics Committee. The investigation conforms with the principles outlined in the Declaration of Helsinki (BMJ, 1964, xx: 177).
Procedure

All subjects were studied in the supine position after an overnight fast. All catheters were inserted percutaneously. One thin Teflon catheter was introduced into an antecubital vein for infusion of dye indicators for determination of blood flow. Another was inserted into the brachial artery for blood sampling and blood pressure measurements. A balloon-tipped catheter was introduced into an antecubital vein and advanced to a branch of the pulmonary artery under fluoroscopic control. Pulmonary capillary wedge pressure was monitored by pressure recording and fluoroscopy.

In group 1, another catheter (Cournand no. 7) was positioned in the hepatic vein, under fluoroscopic control. L-NMMA (Calbiochem-Novabiochem, Läufelfinger, Switzerland) dissolved in saline was administered intravenously during 5 min at a dose of 3 mg/kg as previously described (15). Pulmonary $O_2$ uptake ($V_{O_2}$) was determined, and blood samples were drawn simultaneously from the brachial and pulmonary arteries and the hepatic vein in the basal state and at 10, 20, and 30 min after the L-NMMA infusion for determinations of dye concentrations (in the brachial artery and hepatic vein), $O_2$ contents, and plasma ET-1 levels. Thirty minutes after L-NMMA, a 20-min ET-1 (Peninsula Laboratory, Belmont, CA) infusion was initiated. In two subjects, the infusion rate was 0.2 pmol·kg$^{-1}$·min$^{-1}$ for 20 min. In three subjects, the rate was 0.2 pmol·kg$^{-1}$·min$^{-1}$ for the first 10 min and 1 pmol·kg$^{-1}$·min$^{-1}$ for the last 10 min. Pulmonary $V_{O_2}$ was determined, and blood samples were drawn from all sites at 20 min of ET-1 infusion.

In group 2, ET-1, 0.2 pmol·kg$^{-1}$·min$^{-1}$, was infused for 60 min. Pulmonary $V_{O_2}$ was determined, and blood samples were drawn simultaneously from the catheters in the basal state repeatedly during and up to 90 min after the infusion (see Table 2).

In both groups, heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP) and mean pulmonary capillary wedge pressure (PCWP) were recorded continuously during the studies. Cardiac output (CO) was estimated according to the Fick principle on the basis of pulmonary $V_{O_2}$ and systemic arterial-pulmonary arterial $O_2$ difference [(a-pa)DO$_2$]. In group 1, splanchnic and renal blood flow were determined by constant infusions of cardiogreen and p-aminohippurate (PAH) as previously described (1). For calculation of renal blood flow, the fractional extraction of PAH (the arterial minus renal venous concentration divided by arterial concentration) was assumed to be 0.9. In group 1, arterial insulin, glucagon and glucose concentrations, as well as glucose concentration in the hepatic vein were determined before and after L-NMMA administration as well as during and after the ET-1 infusion.

Analyses

Plasma ET-1 concentrations were assessed by radioimmunoassay technique. Plasma aliquots (1 ml) were extracted with acid ethanol and dried under a nitrogen stream. For determination of ET-1, ET-1 antiserum (E1) and $^{125}$I-labeled ET-1 (Amersham) were used. The detection limit for the assay was 0.40 fmol/tube. Expressing the ET-1 value as 100%, the cross-reactivity of the used antiserum is 16% for ET-1 (16–21), 27% for ET-2, 8% for ET-3, and 0.045% for Big ET-1 (1–38). No cross-reactivity with Big ET-1 (22–38) was observed at concentrations up to 1 $\mu$M. The intra- and interassay variations were 6 and 14%, respectively (16). For characterization of the immunoreactivity in the extracted samples, extra samples were subjected to reverse phase high-performance liquid chromatography. $O_2$ saturation and hemoglobin concentration in blood were determined with an OSM 3 radiometer and blood gases with an ABL 520 radiometer (Radiometer, Copenhagen, Denmark). $O_2$ content in expired air was determined with a zirconium oxide cell (S-3A) $O_2$ analyzer (Ametek, Pittsburgh, PA) and CO$_2$ content by the infrared technique (Ametek CD-3A CO$_2$ analyzer). The radioimmunochemical techniques used to analyze insulin and glucagon as well as the glucose dehydrogenase method for glucose measurement have been described previously (1).

Calculations

Systemic vascular resistance (SVR) was calculated as MAP/CO and pulmonary vascular resistance (PVR) as (MAP – PCWP)/CO.

Data are means ± SE. Analysis of variance (ANOVA), according to the repeated-measures design, was used followed by the Fisher protected least-significant difference test (PLSD), i.e., the interpretation of a significant effect by computing Student’s $t$-test between all means in the effect; “protected” indicates that the test is preceded by a significant F-test. When the variances differed too much, giving F-values not allowing for ANOVA, logarithms were used to achieve homogenous variances (35).

RESULTS

Group 1

Effects of L-NMMA administration. HR, PULMONARY $V_{O_2}$, PULMONARY AND SPLANCHNIC ARTERIOVENOUS $O_2$ DIFFERENCES, CO, AND SPLANCHNIC AND RENAL BLOOD FLOW (Table 1). HR decreased from 58 ± 4 beats/min in the basal state to 48 ± 4 beats/min within 3 min after the start of the L-NMMA administration ($P < 0.001$). From 10 to 20 min after L-NMMA there was a small increase, but HR was still below basal values 30 min after L-NMMA. Pulmonary $V_{O_2}$ remained unchanged after L-NMMA administration (Table 1). (a-pa)DO$_2$ rose from 46 ± 2.0 to 56.0 ± 2.3 ml/min within 10 min after L-NMMA ($P < 0.001$), with no further change at 30 min after the administration. CO fell from 5.57 ± 0.25 to 4.64 ± 0.23 l/min at 10 min after L-NMMA ($P < 0.01$) and was still below basal value 30 min after the administration. Stroke volume (SV) did not change significantly during the study. The systemic arterial-hepatic venous $O_2$ difference had increased from 45 ± 4 to 64 ± 5 ml/l at 10 min after the L-NMMA administration ($P < 0.001$) and was still elevated 20 min later ($P < 0.05$). Splanchnic $V_{O_2}$ had fallen by an average of 33% at 10 min after L-NMMA ($P < 0.001$) and, although the values increased from 10 up to 30 min ($P < 0.01$) after L-NMMA, they were still 16–23% below basal values ($P < 0.01$). Splanchnic $V_{O_2}$ was unchanged during the study.

SYSTEMIC AND PULMONARY BLOOD PRESSURE AND VASCULAR RESISTANCES (Table 1). MAP increased from the basal value $91 ± 2$ mmHg within 3 min ($P < 0.05$) during the L-NMMA administration, was $96 ± 2$ mmHg at 10 min after the administration ($P < 0.01$), and remained unchanged 20 min later. MAPAP and mean PCWP did not change significantly after L-NMMA. SVR increased from $16.3 ± 0.9$ mmHg·min$^{-1}$·l$^{-1}$ in the basal state to $20.9 ± 1.1$ mmHg·min$^{-1}$·l$^{-1}$ ($P < 0.001$) at 10
min. PVR increased after L-NMMA from 0.72 ± 0.09 to 1.01 ± 0.11 mmHg·min·l−1 at 20 min after the L-NMMA administration (P < 0.05). Neither SVR or PVR showed any further change. Splanchnic (VRSpl) and renal (RVR) vascular resistances increased within 10 min after L-NMMA (P < 0.001), VR spl decreased from 10 to 20 min (P < 0.01), and RVR showed a progressive decrease from 10 to 30 min (P < 0.05) after L-NMMA. Both VRSpl and RVR were above basal levels 30 min after the infusion (range P < 0.01–0.001).

VENTILATION. There was no difference in respiratory rate or ventilation before (13.8 ± 1.8 breaths/min and 8.02 ± 0.59 l/min, respectively) compared with after L-NMMA (14.2 ± 1.7 breaths/min and 7.86 ± 0.42 l/min, respectively), as estimated from collection of expired air during mouth breathing with a nosedip.

ARTERIAL LEVELS AND PULMONARY AND SPLANCHNIC EXCHANGES OF PLASMA ET-1. Arterial ET-1 levels rose slightly (ANOVA, repeated measures, P < 0.05) after the L-NMMA administration from 7.57 ± 0.56 pmol/l in the basal state to maximally 9.56 ± 0.74 pmol/l at 20 min after L-NMMA administration (P < 0.05). Ten minutes later, arterial plasma ET-1 concentration had returned to basal value. Pulmonary arterial plasma ET-1 concentration was 7.42 ± 0.93 pmol/l in the basal state and did not show a significant change after L-NMMA. There was no pulmonary arterial-arterial plasma ET-1 difference except at 20 min after the administration of L-NMMA (−2.00 ± 0.38 pmol/l, P < 0.01), corresponding to a net ET-1 release of 5.30 ± 0.93 pmol/min (P < 0.005) in the pulmonary vascular bed. There was no arterial-hepatic venous ET-1 difference at any time, indicating that splanchnic release did not differ from local uptake of ET-1; i.e., there was no net production.

Table 1. HR, pulmonary VO2, (a-pa)DO2, (a-hv)DO2, BF, SV, MAP, MPAP, PCWP, SVR, PVR, VR spl, and RVR before and up to 30 min after infusion of L-NMMA followed by 20 min of ET-1 infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-L-NMMA</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>ET-1</th>
<th>ANOVA (Repeated Measures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>58 ± 4</td>
<td>48 ± 4*</td>
<td>54 ± 4*</td>
<td>52 ± 4*</td>
<td>54 ± 5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>VO2, ml/min</td>
<td>25 ± 10</td>
<td>25 ± 10</td>
<td>26 ± 8</td>
<td>26 ± 7</td>
<td>26 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>BF, l/min</td>
<td>45 ± 10</td>
<td>56 ± 2*</td>
<td>54 ± 4*</td>
<td>53 ± 2*</td>
<td>52 ± 2*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>BF spl, l/min</td>
<td>1.33 ± 0.033</td>
<td>0.889 ± 0.058*</td>
<td>1.105 ± 0.034*</td>
<td>1.114 ± 0.02*</td>
<td>1.098 ± 0.089*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>RBF, l/min</td>
<td>1.592 ± 0.128</td>
<td>1.081 ± 0.126*</td>
<td>1.174 ± 0.116*</td>
<td>1.219 ± 0.085*</td>
<td>1.108 ± 0.087*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.57 ± 0.25</td>
<td>4.64 ± 0.23*</td>
<td>4.86 ± 0.24*</td>
<td>4.95 ± 0.23*</td>
<td>4.95 ± 0.164</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>SV, ml</td>
<td>99 ± 9</td>
<td>99 ± 8</td>
<td>99 ± 10</td>
<td>97 ± 8</td>
<td>94 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 ± 2</td>
<td>96 ± 2*</td>
<td>97 ± 4*</td>
<td>95 ± 3*</td>
<td>98 ± 2*</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>13 ± 7</td>
<td>13 ± 3</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>9 ± 1</td>
<td>8 ± 0</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>SVR, mmHg·min·l−1</td>
<td>16.3 ± 0.9</td>
<td>20.9 ± 1.1*</td>
<td>19.8 ± 0.8*</td>
<td>20.0 ± 0.9*</td>
<td>19.8 ± 0.7*</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>PVRe, mmHg·min·l−1</td>
<td>0.72 ± 0.09</td>
<td>0.99 ± 0.04</td>
<td>1.01 ± 0.11</td>
<td>0.98 ± 0.07</td>
<td>0.97 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>VRspl, mmHg·min·l−1</td>
<td>67.6 ± 2.9</td>
<td>109.7 ± 6.0</td>
<td>86.4 ± 2.0</td>
<td>85.0 ± 2.2</td>
<td>94 ± 6.8*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>VR-RV, mmHg·min·l−1</td>
<td>57.7 ± 2.6</td>
<td>90.8 ± 10.3</td>
<td>81.8 ± 6.2</td>
<td>77.3 ± 3.4</td>
<td>89.3 ± 6.3*</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 subjects. HR, heart rate; VO2, O2 uptake; (a-pa)DO2, systemic arterial-pulmonary arterial O2 difference; (a-hv)DO2, arterial-hypoxic venous O2 difference; BF, splanchic blood flow; SV, stroke volume; MAP and MPAP, mean arterial and mean pulmonary arterial pressure, respectively; PCWP, pulmonary capillary wedge pressure; SVR, pulmonary vascular resistance; PVR, systemic pulmonary vascular resistance; VR spl and RVR, splanchnic and renal vascular pressure, respectively. L-NMMA, G-monomethyl-L-arginine; ET-1, endothelin-1; ANOVA, analysis of variance; NS, not significant. Significantly different from basal preinfusion values. *P < 0.01; †P < 0.05; ‡P < 0.001; §P < 0.01–0.001; °P < 0.01; †P < 0.05. ANOVA, repeated measures including all time points, followed by Fisher protected least-significant difference (PLSD) test. §Significantly different 20-min ET-1 infusion value vs. 30-min post-L-NMMA value, P < 0.05.
after L-NMMA (values at 20 min of ET-1 infusion corresponding to 4.18 ± 0.60 mmol/l, 7.5 ± 1.1 µU/ml, and 63 ± 5.9 pg/ml, respectively). Nor did splanchnic glucose production show a significant change (the value corresponding to 0.55 ± 0.07 mmol/min at 20 min of ET-1 infusion).

Group 2

Effects of ET-1 infusion. HR, PULMONARY VO₂, PULMONARY (a-pa)DO₂, and CO (Table 2). HR showed a transient decrease from 58 ± 3 beats/min in the basal state to 55 ± 4 beats/min at 20 min of the ET-1 infusion (P < 0.05). Pulmonary VO₂ did not change during the ET-1 infusion. (a-pa)DO₂ rose from 45 ± 2 to 49 ± 2 ml/l within 3 min of infusion (P < 0.001) and was still elevated 90 min after the infusion (P < 0.05). CO fell from 5.97 ± 0.18 to 5.28 ± 0.20 l/min at 10 min of the infusion (P < 0.001) and then remained unchanged during and up to 30 min after the infusion. SV decreased from 103 ± 5 to 95 ± 5 ml/min (P < 0.05) at 10 min of the infusion. CO and SV had returned to basal values 90 min after the infusion.

SYSTEMIC AND PULMONARY BLOOD PRESSURE AND VASCULAR RESISTANCES (Table 2). MAP increased during ET-1 infusion from 93 ± 3 mmHg within 10 min to 95 ± 4 mmHg (P < 0.05), with a further increase up to 60 min of infusion (P < 0.001). Ninety minutes after the infusion, MAP was still significantly increased (P < 0.001).

<table>
<thead>
<tr>
<th></th>
<th>Basal 0 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
<th>30 min</th>
<th>90 min</th>
<th>ANOVA (Repeated Measures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>58 ± 3</td>
<td>56 ± 4</td>
<td>55 ± 4*</td>
<td>59 ± 4</td>
<td>58 ± 4</td>
<td>57 ± 5</td>
<td>60 ± 3</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>258 ± 8.1</td>
<td>253 ± 19.4</td>
<td>257 ± 10</td>
<td>261 ± 10.2</td>
<td>263 ± 10.0</td>
<td>266 ± 11.8</td>
<td>273 ± 9.8†</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(a-pa)DO₂, ml/l</td>
<td>45 ± 2</td>
<td>50 ± 2*</td>
<td>50 ± 2*</td>
<td>49 ± 2*</td>
<td>50 ± 2*</td>
<td>49 ± 2*</td>
<td>47 ± 2*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.97 ± 0.18</td>
<td>5.28 ± 0.20†</td>
<td>5.40 ± 0.20†</td>
<td>5.51 ± 0.22†</td>
<td>5.40 ± 0.22†</td>
<td>5.47 ± 0.18†</td>
<td>5.97 ± 0.19†</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>SV, ml</td>
<td>103 ± 5</td>
<td>95 ± 5*</td>
<td>101 ± 5</td>
<td>96 ± 5*</td>
<td>96 ± 5*</td>
<td>97 ± 5*</td>
<td>98 ± 5*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>93 ± 3</td>
<td>95 ± 4*</td>
<td>95 ± 4†</td>
<td>97 ± 3†</td>
<td>100 ± 4‡</td>
<td>97 ± 5*</td>
<td>98 ± 5†</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>12 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1†</td>
<td>8 ± 1†</td>
<td>8 ± 1†</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>SVR, mmHg·min⁻¹</td>
<td>15.8 ± 0.7</td>
<td>18.1 ± 0.8†</td>
<td>17.8 ± 0.8‡</td>
<td>17.7 ± 0.7‡</td>
<td>18.7 ± 0.8‡</td>
<td>17.7 ± 0.8‡</td>
<td>16.3 ± 0.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PVR, mmHg·min⁻¹</td>
<td>0.79 ± 0.06</td>
<td>0.94 ± 0.12</td>
<td>0.86 ± 0.08</td>
<td>1.10 ± 0.06†</td>
<td>1.02 ± 0.06‡</td>
<td>1.14 ± 0.10†</td>
<td>0.98 ± 0.12</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. Significantly different from basal, *P < 0.05, †P < 0.01, ‡P < 0.001, respectively (ANOVA, repeated measures including all time points, followed by Fisher PLSD).
arterial levels of plasma ET-1. Arterial ET-1 was 4.87 ± 0.55 pmol/l in the basal state and rose during the ET-1 infusion (P < 0.001, ANOVA, repeated measures) to 5.72 ± 0.5 pmol/l at 2 min and 7.74 ± 0.88 pmol/l at 10 min (P < 0.05), with a further increase to 11.2 ± 2.13 pmol/l at 60 min of infusion. The value was still elevated at 60 min (6.78 ± 1.01 pmol/l, P < 0.01) but not 90 min after ET-1 infusion.

DISCUSSION

The present results demonstrate that intravenous administration of a NOS blocker, L-NMMA, to healthy humans induces marked cardiovascular effects as indicated by decreased HR, CO, and splanchnic and renal blood flow and splanchnic glucose output. In addition, MAP, SVR, and PVR increased. No effects were seen on pulmonary pressures. Furthermore, L-NMMA abolished the effects of subsequently infused ET-1 on these variables.

Cardiovascular Effects

The effects of L-NMMA on MAP agreed well with those reported in a previous study, in which a similar dose of L-NMMA was administered (15). These results indicate that NO synthesis is involved in the maintenance of basal vascular tone. The increase in MAP in this and the previous study (15) was associated with decreases in HR and CO. These were most likely baroreceptor reflex-mediated responses because neither SV nor left ventricular filling pressure, as represented by PCWP, changed in the present study. Thus it is unlikely that L-NMMA reduced the contractility of the heart. Furthermore, NO itself, if anything, reduces the contractility of cardiac myocytes in vitro (9).

The administration of L-NMMA has been shown to cause constriction of the human forearm vasculature (28). The present results show that both splanchnic and renal blood flow also fall in response to L-NMMA. Thus it is clear that effects in these vascular beds also contribute to the increased SVR. In fact, at the presently administered dose of L-NMMA, the reduction in splanchnic and renal blood flow totaled 0.9 l/min. The corresponding decrease in CO was ~1 l/min. Thus little of the reduction in CO could be due to flow reduction in other vascular beds such as muscle. In other words, the vasoconstriction in the splanchnic and renal vascular beds seems to be the most important determinant for the increased MAP after L-NMMA administration. To our knowledge, there are no previous reports, from in vivo human studies, that these vascular beds respond to NO or NO synthesis blockade, although indirect evidence from animal studies has suggested that this is the case (for a review, see Ref. 34). In addition, humans have been shown to synthesize more NO from intragastrointestinal vs. intravenous administration of arginine (10), suggesting that NO has a regulatory function in the splanchnic region.

The total mass of skeletal muscle is ~40–50% of body weight (21). However, the basal blood flow in skeletal muscle is only ~20–50 ml·kg⁻¹·min⁻¹ (5). The reduction in CO measured in the present study allows for a blood flow reduction of ~0.3 l/min in the musculature. This corresponds to a reduction by ~30%, i.e., the same proportional reduction in skeletal muscle as in the splanchnic and renal tissues, indicating that all these vascular beds are equally dependent on NO synthesis for maintenance of basal tone.

When L-NMMA was infused, we noticed a maximal increase in PVR (40%), which was of a magnitude similar to that for SVR (28%). We could not demonstrate any change in the pulmonary blood pressure. This could be due to the fact that basal pulmonary pressure is too low to allow a similar proportional increase in MPAP as that seen for MAP (5%) to be detected. Absence of a pulmonary pressure response after L-NMMA administration has also been noticed in isolated normoxic, but not hypoxic, rat lungs (6). Previous results also indicate that preconstriction of the pulmonary vessels is necessary to elicit NO release and pressure response (6). This could explain why inhalation of NO together with air has no effect on MPAP or CO in healthy humans (13) and suggests that endogenous NO has optimal vasodilatory effects under basal normoxic conditions.

Previous data indicate that NO inhibits synthesis of ET-1 (8, 27, 38) at the preproendothelin level in endothelial cells. Inhibition of NO synthesis would be expected to lead to increased ET-1 synthesis and possibly an overflow to the blood. This is consistent with our finding that the systemic arterial concentration of ET-1 was increased 20 min after L-NMMA infusion. A further support for this theory is the observation that ET-1 concentration was more greatly increased in systemic than in pulmonary arterial blood. Thus, although the para- and autocrine events elicited by ET-1 need not necessarily be accompanied by changes in circulating levels of ET-1, we noticed a net pulmonary release of ET-1 at 20 min after L-NMMA corresponding to 5.30 ± 0.93 pmol/min (P < 0.005).

In a previous study, we found that intravenous administration of ET-1 at doses that evoked a 37% elevation of systemic arterial ET-1 levels was accompanied by decreases in splanchnic and renal blood flow corresponding to 18 and 10%, respectively (3). In the present study, we observed only a 26% increase in systemic arterial ET-1 levels. However, because the present circulating ET-1 levels represent an "overflow" of ET-1 synthesized by the endothelium, the local concentration at the site of action can be assumed to be higher, especially because the main ET-1 release seems to occur abluminally (30). In experiments that used even higher ET-1 doses, leading to 10-fold increases
in systemic arterial concentration, the reduction in splanchnic and renal blood flow was 34 and 26%, respectively (32). Consequently, it seems possible that the reductions in splanchnic and renal blood flow (~33%) noted in the present study (Table 1) on infusion of L-NMMA could, at least partly, be ET-1 mediated and that a significant part of the dilating effect of NO on the vasculature might be due to inhibition of ET-1 synthesis. This hypothesis is supported by the findings that NO stimulates cGMP formation (18) and that a cGMP-dependent mechanism or NO formation inhibits ET-1 formation in cultured endothelial cells (8, 27, 38).

To further test our hypothesis that NO exerts a physiological effect in vivo by interfering with ET-1 formation, the NOS blockade was followed by intravenous infusion of ET-1 30 min after L-NMMA. The rate of ET-1 infusion was the same (3) as has previously been shown to cause significant, prolonged (>1- to 3-h) reductions in splanchnic and renal blood flow (3, 32) and, in the present control group (group 2), to cause prolonged increase in MAP and decrease in CO. No further fall in splanchnic blood flow was seen on ET-1 infusion after L-NMMA in the present study. At 20 min of ET-1 infusion, the fall in renal blood flow in the present study corresponded to 0.11 ± 0.04 l/min, which is significantly smaller (P < 0.05) than our previously noted reduction of 0.21 ± 0.04 l/min (3) with the same ET-1 dose. In fact, the small drop in renal blood flow at 20 min of ET-1 infusion after L-NMMA in the present study is in agreement with our previous results in resting control subjects, who did not receive L-NMMA or ET-1 but showed a slow progressive reduction in renal blood flow with time (3).

Our previous data have shown that infusion of ET-1 at 4 pmol·kg⁻¹·min⁻¹ causes reduction of CO (33). The present data show that infusion of ET-1 alone at 0.2 pmol·kg⁻¹·min⁻¹ (group 2) causes an 11% fall in CO within 10 min of infusion (P < 0.001), whereas the same dose of ET-1 infused after L-NMMA caused no further effects on CO or blood pressure. The prompt CO response (within 3 min) in group 2, which received only ET-1 infusion at a dose leading to an increase of arterial ET-1 (47%), comparable to the physiological increase previously seen during physical exercise (2) and the prolonged suppression of CO (Table 2), emphasizes the potency of ET-1 and also the potential importance of circulating ET-1. Both the observation that the vascular effect of ET-1 infusion was nearly abolished and the prolonged duration of blood flow reductions in lung, splanchnic, and renal tissues after L-NMMA infusion support interaction of NO with ET-1 by suppressing ET-1 synthesis. Had NO mainly exerted its vasodilatory effect by acting directly on smooth muscle contraction, the withdrawal of NO production would be expected to potentiate the vasoconstrictor response to ET-1 infusion. The explanation underlying the surprising observation that the vasoconstrictor effects of exogenous ET-1 were virtually abolished up to 50 min after L-NMMA may be related to long-term occupancy of ET₁ and ET₉ receptors by excess endogenously released peptide (or internalization of ET receptors), which prevents receptor access for the circulating exogenous ET counterpart. The notion that part of the vasodilatory action of nitrovasodilators (27) or NO (8, 38) might be due to suppression of ET-1 production has previously been suggested on the basis of studies in cultured human endothelial cells. Conversely, ET-1 has been shown to release NO from mesenteric arteries (11, 31). ET-1 stimulates NO formation (11, 31) presumably via ET₁-receptor stimulation and intracellular Ca²⁺ release (36). Support for this theory could be the unexplained increase in ET-1 levels found in patients with chronic heart failure, treated with the ET₁-receptor antagonist bosentan (19). Blockade of the ET₁ receptor would be expected to offset the stimulating effect of ET-1 on NO synthesis and, consequently, the inhibitory effect of NO on ET-1 synthesis and/or release, resulting in increased ET-1 levels. On the basis of these studies and the present data, we would like to suggest that there is a feedback mechanism between ET-1 and NO synthesis that acts reciprocally to regulate vascular tone in intact humans in vivo, at least in the basal state.

**Splanchnic Glucose Output**

Synthesis of NO does not only take place in the vascular endothelium. Consequently, for example, not only endothelial but also neuronal NOS have been identified in the human kidney (4), indicating the potential importance of NO in different signalling events. Interestingly, NOS blockade in the present study also caused a prolonged (>60-min) decrease in splanchnic glucose output, a phenomenon we have previously seen during and after ET-1 infusion in humans (1–3). That splanchnic glucose output is not dependent on the blood flow is shown by findings during exercise when glucose output may increase fivefold while splanchnic blood flow falls by 50% (2). Also, the hepatic venous-arterial glucose concentration difference falls with a higher rate of ET-1 infusion at rest (1). Furthermore, ET-1 infusion before exercise causes suppressed splanchnic glucose output during exercise compared with control exercise that is not directly related to the reductions in splanchnic blood flow. Thus exercise immediately after ET-1 infusion results in further depression of splanchnic blood flow but an increasing splanchnic glucose output (2). L-NMMA did not lead to any consistent change in arterial insulin or glucagon levels but to a persistent suppression of splanchnic glucose output, with no further change during the subsequent ET-1 infusion (Fig. 1). Similar changes in splanchnic glucose output with no or a minor change in arterial glucose concentrations indicating reduced peripheral utilization were seen in our previous ET-1 infusion studies (1–3). Therefore, the present results concerning the effect of NOS blockade on splanchnic glucose output and arterial glucose concentrations are also consistent with an ET₁-mediated effect. Lower insulin and glucagon plasma levels have been found in vivo in dogs after NOS blockade (7). In addition, suppression of arterial insulin and glucagon levels was also seen in humans
after infusion of ET-1 at rates higher than those used in the present study (1, 2). Our present results do not, however, allow an interpretation of the mechanism underlying the reduction in splanchnic glucose output. In freshly isolated islets, NO does not seem to mediate arginine- or glucose-stimulated insulin secretion because this occurs without changes in NO release or cGMP content and is not prevented by NOS blockade (18). In fact, our results show the same pattern of decreased splanchnic glucose output with NOS blockade as with previous ET-1 infusion without blockade of the NO synthesis (1–3). This observation favors a mediator other than NO, possibly ET-1, for the reduction in splanchnic glucose output.

In summary, the results show that NOS blockade was followed by increased arterial ET-1 levels, pulmonary ET-1 release, MAP, and SVR and PVR but reduced HR, CO, splanchnic and renal blood flow, and splanchnic glucose production. The pattern was similar to that previously reported for splanchnic and renal blood flow and splanchnic glucose output (3) and in the present control group for HR, MAP, CO, and SVR and PVR with increases in ET-1 levels 80 (3) to 130% above basal value. Although l-NMMA caused only a 25% increase in ET-1 levels, the intra- and paracellular concentrations are probably higher. This and the fact that the subsequent ET-1 infusion in the l-NMMA-treated subjects did not cause any further cardiovascular change or reduction in splanchnic glucose output support that NO suppresses ET-1 production and, thereby, interacts with ET-1 regarding vascular tone and splanchnic glucose output in humans. The results raise the question of whether pathologically increased ET-1 levels, which have been described for many pathological conditions in the cardio-pulmonary-vascular system, might, at least in part, be due to defective NO synthesis, which would be expected to disturb the normal control of vascular tone and splanchnic glucose output.

This study was supported by Swedish Medical Research Council Grants 14X-10374 and 14X-6554, the King Gustav V and Queen Victoria Foundation, and the Clas Groschinsky Foundation.

Address for reprint requests: G. Ahlborg, Dept. of Clinical Physiology, Huddinge Univ. Hospital, S-141 86 Huddinge, Sweden.

Received 10 April 1996; accepted in final form 24 Januay 1997.

REFERENCES