ET_A receptors mediate vasoconstriction, whereas ET_B receptors clear endothelin-1 in the splanchnic and renal circulation of healthy men

Felix BÖHM*, John PERNOW*, Jonas LINDSTRÖM† and Gunvor AHLBORG†
*Department of Cardiology, Karolinska Hospital, S-171 76 Stockholm, Sweden, and †Department of Clinical Physiology, Huddinge Hospital, S-141 86 Huddinge, Sweden

ABSTRACT
The contribution of the endothelin (ET) receptors ET_A and ET_B to basal vascular tone and ET-1-induced vasoconstriction in the renal and splanchnic vasculature was investigated in six healthy humans. ET-1 was infused alone and in combination with the selective ET_A receptor antagonist BQ123 or the selective ET_B receptor antagonist BQ788 on three different occasions. BQ123 did not affect basal arterial blood pressure, splanchnic vascular resistance (SplVR) or renal vascular resistance (RVR), but inhibited the increase in vascular resistance induced by ET-1 [64±18 versus 1±7% in SplVR (P<0.05); 36±6 versus 12±3% in RVR (P<0.0001)]. BQ788 increased basal SplVR and RVR [38±16% (P=0.01) and 21±5% (P<0.0001) respectively], and potentiated the ET-1-induced vasoconstriction. Plasma ET-1 increased more after ETB blockade than under control conditions or after ETA blockade. These findings suggest that the ETA receptor mediates the splanchnic and renal vasoconstriction induced by ET-1 in healthy humans. The ET_B receptor seems to function as a clearance receptor and may modulate vascular tone by altering the plasma concentration of ET-1.

INTRODUCTION
The endothelins (ETs) are a family of peptides with potent and long-lasting vasoconstrictor actions [1]. ET-1, which is produced by endothelial cells [2] is the most important isoform in the cardiovascular system. Two ET receptors have been identified in humans: ET_A and ET_B [3,4]. ET_A is present on vascular smooth muscle cells where it mediates vasoconstriction. ET_B is present both on endothelial cells, where it mediates vasodilatation through the release of nitric oxide and prostacyclin [5], and on smooth-muscle cells, where it mediates vasoconstriction. Administration of ET-1 to healthy humans results in vasoconstriction in several vascular beds, such as the heart [6], renal and splanchnic tissues [7], and skeletal muscle [8]. The selective ET_A receptor antagonist BQ123 evokes increase in forearm blood flow [9], suggesting that endogenous ET-1 contributes to basal vascular tone mainly via this receptor subtype in the forearm vascular bed.

ET-1 may contribute to the progression of several cardiovascular disorders like congestive heart failure, hypertension, ischaemic heart disease and atherosclerosis [10,11]. It has also been speculated that ET-1 is important in patients with renal failure [12–14], in portal hypertension [15,16], as well as in several lung diseases.

Key words: endothelin, regional blood flow, vascular resistance.
Abbreviations: ET, endothelin; ET_A and ET_B, ET receptors A and B; MAP, mean arterial blood pressure; PAH, para-amohippuric acid; RBF, renal blood flow; RVR, renal vascular resistance; SBF, splanchnic blood flow; SplVR, splanchnic vascular resistance; SVR, systemic vascular resistance.
Correspondence: Dr Felix Böhm (e-mail felix.bohm@meds.ki.se).
including primary pulmonary hypertension [17,18]. Sustained afferent and efferent arteriolar vasoconstriction induced by ET-1 may contribute to ischaemia in acute renal failure [19,20]. Besides evoking vasoconstriction, ET-1 stimulates the development of glomerulosclerosis and interstitial fibrosis as demonstrated in ET-1 transgenic mice [21]. Therefore, ET-1 is an interesting target for therapy aimed at preserving renal and splanchnic function. This is further supported by the fact that sensitivity to ET-1 is preserved in renal insufficiency [22] and is even enhanced in atherosclerosis [23]. In a recent study, selective ET$_A$ receptor blockade was found to attenuate renal effects of ET-1 in humans [24]. Apart from that, the effects of ET-1 on the different receptors in the renal and splanchnic vasculature in healthy humans are not known. It is of importance to characterize the effects mediated via the ET$_A$ and ET$_B$ receptors in order to understand the changes of the ET system that occur under pathological conditions. In addition, the splanchnic and renal circulation account for a considerable part of the cardiac output, and thus, the haemodynamic effects evoked by the ET receptors in these vascular beds are of great importance.

The present study was undertaken to characterize the effect of ET-1 on renal blood flow (RBF) and splanchnic blood flow (SBF) mediated via the ET$_A$ and ET$_B$ receptors in humans in vivo. We evaluated the response to exogenous ET-1, both alone and in combination with selective blockade of ET$_A$ or ET$_B$ receptors, in the renal and splanchnic vasculature of healthy humans.

### MATERIALS AND METHODS

#### Subjects

Six healthy, male, non-smoking volunteers (age, 25.2 ± 1.2 years; height, 182 ± 3 cm; mass, 77 ± 2 kg) were included in the study. All subjects were informed of the nature, purpose and possible risks involved in the study before giving their voluntary consent. The investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the regional ethical committee of the Karolinska Institute.

#### Catheterization

The subjects were studied in the supine position after an overnight fast in a quiet laboratory. A thin catheter was inserted percutaneously in an antecubital vein for infusion of cardiogreen, para-amino hippuric acid (PAH), ET-1 and BQ123 (a selective ET$_A$ receptor antagonist; Clinalfa AG, Läufelfingen, Switzerland) or BQ788 (a selective ET$_B$ receptor antagonist; Clinalfa AG, Läufelfingen, Switzerland). Another catheter was inserted in the brachial artery for blood sampling and measurement of blood pressure. A balloon-tipped catheter was inserted percutaneously in an antecubital vein and advanced under fluoroscopic guidance to a branch of the pulmonary artery for blood sampling and measurement of cardiac output. In a subgroup of the subjects (n = 5), a Cournand catheter no. 7 was inserted into a femoral vein and positioned in either the right renal vein or a central hepatic vein under fluoroscopic guidance. This was done to ascertain that the fractional extraction of cardiogreen and PAH was uninfluenced by the ET receptor blockers or ET-1. We found that neither ET-1, BQ123 nor BQ788 induced any change in the fractional extraction of cardiogreen or PAH. Moreover, we have never observed any effect on the fractional extraction of cardiogreen or PAH by ET-1 in any of our previous studies [7,25].

#### Study protocols

Each subject participated in three different study protocols on 3 separate days with at least 1 week in between each protocol (Figure 1). In all three protocols, there was an initial resting period of 1 h following the catheterization, whereafter the infusions began (time 0). In protocol 1, 0.9% NaCl was infused intravenously for 15 min starting at time 0; in protocol 2, BQ788 (4 nmol kg$^{-1}$ min$^{-1}$) was infused for 15 min; in protocol 3, the BQ123 (2.5 nmol kg$^{-1}$ min$^{-1}$; n = 2; or 5 nmol kg$^{-1}$ min$^{-1}$; n = 4) was infused intravenously for 50 min starting at time 0. In the present study, we did not observe complete blockade of renal vasoconstriction in the first two subjects receiving the low dose of BQ123. Therefore, in the remainder of the study we doubled the dose of BQ123 in order to obtain a greater antagonism of the ET$_A$ receptor. In these four subjects, the vasoconstrictor response was similar in magnitude to that observed in the first two subjects. Therefore, the results from all six subjects are pooled. ET-1 (4 pmol kg$^{-1}$ min$^{-1}$) was infused intravenously in all

![Figure 1 Study protocols](image-url)
three protocols for 20 min. This started 30 min after the start of infusions of 0.9% NaCl, BQ788 or BQ123. The doses and the duration of the infusions of the ET receptor antagonists were selected on the basis of previous reports [26,27]. Strachan et al. [27] showed that the maximum effect on total peripheral vascular resistance in response to a 15-min infusion of BQ788 (300 nmol · min⁻¹) was obtained between 30 and 50 min after the start of the BQ788 infusion. In the study by Cowburn et al. [26], BQ123 was administered for 60 min with significant effects on mean arterial blood pressure (MAP) and systemic vascular resistance (SVR) from 30 min onwards. Based on this, we chose to infuse ET-1 between 30 and 50 min after the start of the ET receptor antagonist infusions. Heart rate and MAP were recorded continuously. Cardiogreen and PAH were infused intravenously at constant rates for determination of SBF and RBF as previously described [7]. Pulmonary oxygen uptake was determined in the basal state and at 20 min of ET-1 infusion in each protocol. Cardiac output was determined by Fick’s principle, based on the pulmonary oxygen uptake divided by the systemic arterial-pulmonary arterial oxygen difference. Blood samples, for determination of plasma ET-1, oxygen content, PAH and cardiogreen, were collected in the basal state, at 15 and 30 min of each study protocol, at 20 min of the ET-1 infusion, as well as 10 and 60 min after the ET-1 infusion was completed. To determine the elimination of ET-1 from plasma, additional blood samples were collected at 20 and 40 s, as well as 1, 2, 3, 4, 5, 10, 30 and 60 min after the end of the ET-1 infusion.

### Analysis

Blood was sampled into test tubes containing EDTA (10 mmol · l⁻¹ final concentration) on ice. After centrifugation (15 min, 4 °C, 2000 g), plasma (1 ml) was stored at −80 °C until analysis. After ethanol extraction, ET-1-like immunoreactivity was analysed by radioimmunoassay using commercially available antisera (rabbit anti-ET-1 6901; Peninsula Laboratories, Merseyside, U.K.) as described previously [28]. The intra-assay and inter-assay variations were 3% and 6% respectively. The haemoglobin concentration and oxygen saturation were determined with an ABL 520 Radiometer (Radiometer, Copenhagen, Denmark). The haematocrit was measured with a microcapillary haematocrit centrifuge and corrected for trapped plasma. Oxygen content in expired air was determined with a mass flow sensor (Vmax229, Sensor Medics, Bilthoven, The Netherlands).

### Drugs

BQ123 and BQ788 were dissolved in sterile 0.9% NaCl and stored frozen at −80 °C. The affinity of BQ123 is > 100000 times greater for the ET₁ receptor than the ET₃ receptor [29] and the affinity of BQ788 is > 1000

---

**Table 1**  Haemodynamic changes during the infusions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>NaCl</th>
<th>NaCl/antagonist</th>
<th>ET-1</th>
<th>ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>57 ± 4</td>
<td>61 ± 4</td>
<td>56 ± 5</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>BQ123 (n = 6)</td>
<td>56 ± 2</td>
<td>62 ± 3</td>
<td>63 ± 5 †</td>
<td>60 ± 2 *</td>
</tr>
<tr>
<td>BQ788 (n = 6)</td>
<td>57 ± 2</td>
<td>59 ± 1</td>
<td>51 ± 3</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>HR (beats · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>7.0 ± 0.6</td>
<td>7.5 ± 1.0</td>
<td>6.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>BQ123 (n = 5)</td>
<td>7.7 ± 0.4</td>
<td>8.2 ± 0.4</td>
<td>9.1 ± 0.6 †</td>
<td></td>
</tr>
<tr>
<td>BQ788 (n = 4)</td>
<td>7.4 ± 0.8</td>
<td>7.7 ± 1.5</td>
<td>6.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>91 ± 4</td>
<td>93 ± 6</td>
<td>96 ± 5</td>
<td>97 ± 6</td>
</tr>
<tr>
<td>BQ123 (n = 6)</td>
<td>88 ± 4</td>
<td>88 ± 5</td>
<td>88 ± 5 *</td>
<td>89 ± 5 †</td>
</tr>
<tr>
<td>BQ788 (n = 6)</td>
<td>91 ± 5</td>
<td>94 ± 4</td>
<td>99 ± 5</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>13.2 ± 0.8</td>
<td>13.0 ± 1.6</td>
<td>16.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>BQ123 (n = 5)</td>
<td>11.7 ± 0.9</td>
<td>11.0 ± 0.5 *</td>
<td>10.1 ± 1.1 †</td>
<td></td>
</tr>
<tr>
<td>BQ788 (n = 4)</td>
<td>12.8 ± 1.5</td>
<td>13.0 ± 2.6</td>
<td>16.0 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>CO (l · min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>7.0 ± 0.6</td>
<td>7.5 ± 1.0</td>
<td>6.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>BQ123 (n = 5)</td>
<td>7.7 ± 0.4</td>
<td>8.2 ± 0.4</td>
<td>9.1 ± 0.6 †</td>
<td></td>
</tr>
<tr>
<td>BQ788 (n = 4)</td>
<td>7.4 ± 0.8</td>
<td>7.7 ± 1.5</td>
<td>6.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>SVR (mmHg · min · l⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>13.2 ± 0.8</td>
<td>13.0 ± 1.6</td>
<td>16.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>BQ123 (n = 5)</td>
<td>11.7 ± 0.9</td>
<td>11.0 ± 0.5 *</td>
<td>10.1 ± 1.1 †</td>
<td></td>
</tr>
<tr>
<td>BQ788 (n = 4)</td>
<td>12.8 ± 1.5</td>
<td>13.0 ± 2.6</td>
<td>16.0 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

P-values are presented as means ± S.E.M. Bold values represent values during the ET-1 infusion.

*P < 0.05; †P < 0.01; ‡P < 0.001 represent significant differences compared to the control protocol at each time point.
times greater for the ET\textsubscript{B} receptor than the ET\textsubscript{A} receptor [30]. ET-1 (Peninsula Laboratories) was dissolved in 0.9\% NaCl/0.5\% albumin, and thereafter passed through a Millipore sterile filter [8]. The peptide was then stored at –80 °C until use. On the day of the experiments all substances were diluted to the appropriate concentrations in sterile 0.9\% NaCl.

Calculations and definitions
Renal vascular resistance (RVR) was calculated as MAP\times RBF\superscript{–1} and splanchnic vascular resistance (SplVR) as MAP\times SBF\superscript{–1}. All results are given as mean values and S.E.M.s. Statistical differences between the groups were calculated using repeated measures ANOVA, followed by Fischer’s protected least-significant difference test. P < 0.05 was regarded as significant.

RESULTS

Haemodynamic changes
There were no significant differences in basal haemodynamic parameters between the three study protocols (Table 1). Administration of ET-1 increased MAP in the control protocol and in the BQ788 protocol, but not in the BQ123 protocol. There were significant differences in SBF, SplVR, RBF and RVR between the three protocols as measured by repeated measures ANOVA (P < 0.0001; Figures 2 and 3). In the control protocol, administration of ET-1 reduced SBF by 31 ± 5\% (P < 0.01) and increased SplVR by 64 ± 18\% (P < 0.0001; Figure 2). SBF was significantly decreased by BQ788 alone (24 ± 5%; P < 0.01; Figure 2A). Moreover, the increase in SplVR in response to ET-1 was enhanced following administration of BQ788 (140 ± 37 versus 64 ± 18\% in the control protocol; P < 0.05; Figure 2B). Although BQ123 alone did not affect SBF or SplVR, it completely blocked the effect of ET-1. In the control protocol, ET-1 induced a significant reduction in RBF and increase in RVR (Figure 3). RBF was reduced significantly and RVR was increased by BQ788 alone (24 ± 5\%; P < 0.01; Figure 2B). The increase in RVR induced by ET-1 was significantly greater in the BQ788 protocol than in the control protocol (67 ± 14 versus 36 ± 6\%; P < 0.01). ET-1 evoked a slight increase in RVR also in the presence of BQ123 (12 ± 3\%; P < 0.05), but this increase was significantly smaller than that obtained in the control protocol (P < 0.01, Figure 3B). The differences in RVR between the three protocols were maintained even 60 min after the ET-1 infusion. ET-1 induced a significant increase in SVR in the control protocol (P < 0.01; Table 1). There was an increase in cardiac output and a decrease in SVR in the BQ123 protocol (P < 0.05; Table 1). The changes in cardiac output and SVR in the BQ788 protocol did not differ from the changes in the control protocol.

ET-1-like immunoreactivity
During intravenous infusion of ET-1, the ET-1 plasma levels in the pulmonary artery rose significantly in all three protocols (P < 0.0001; Figure 4A). The pulmonary artery ET-1 levels rose significantly more in the BQ788 protocol than in the control protocol (54 ± 11 versus 42 ± 6 pmol \cdot l\textsuperscript{–1} respectively; P < 0.01; Figure 4A). The
increase in systemic arterial ET-1 levels was smaller than the corresponding increase in pulmonary artery ET-1 levels during the ET-1 infusion. The increase in arterial ET-1 levels was significantly greater in the BQ788 protocol than in the control protocol (29 ± 5 versus 12 ± 2 pmol l⁻¹ respectively; P < 0.001; Figure 4B). The half-life of plasma ET-1 was similar in all three protocols (Figure 4C).

**Fractional extraction of ET-1**

Although we did not achieve absolute steady-state levels of ET-1 in the systemic or pulmonary artery, the plasma levels of ET-1 at 20 min of ET-1 infusion can still be used for calculating regional uptake owing to the short circulation times. Based on previously determined frac-
The main findings in the present study are that selective ET<sub>A</sub> receptor blockade completely blocks the vasoconstrictor effect of ET-1 in the splanchic vascular bed and that it greatly attenuates the vasoconstrictor effect of ET-1 in the human kidney in vivo. In addition, selective ET<sub>B</sub> receptor blockade causes splanchic and renal vasoconstriction, enhances vasoconstriction induced by ET-1 and raises plasma levels of ET-1. These findings suggest that ET<sub>A</sub> plays a more important role than ET<sub>B</sub> in mediating vasoconstriction in the splanchic and renal vasculature in healthy humans. Furthermore, the results support the notion that ET<sub>B</sub> has significant clearance properties.

BQ123 had no effects on basal splanchic or renal haemodynamics. This indicates that activation of ET<sub>A</sub> by endogenous ET-1 does not contribute significantly to basal splanchic or renal vascular tone in healthy humans. The vasoconstrictor effect induced by the ET-1 infusion in the splanchic vascular bed was completely blocked by BQ123. In addition, BQ123 significantly attenuated the vasoconstriction induced by ET-1 in the renal vasculature, in accordance with a recent study by Honing et al. [24]. The observation that BQ123 did not completely block the vasoconstrictor effect of ET-1 in the renal circulation could indicate incomplete ET<sub>A</sub> receptor blockade, or that vasoconstrictive ET<sub>B</sub> receptors mediate a minor part of the effect. Incomplete ET<sub>A</sub> receptor blockade, however, seems unlikely, since there was no further reduction of the response when the dose of BQ123 was doubled. The remaining reduction in RBF may also be due to the spontaneous reduction in RBF, as we have shown previously by using the same technique with PAH clearance. Accordingly, in a previous study using the same technique, RBF was reduced by 0.12±0.06 l·min<sup>-1</sup> after 60 min in subjects receiving only saline [25]. For comparison, in the present study, the decrease in RBF during administration of ET-1 in the presence of BQ123 was 0.12±0.04 l·min<sup>-1</sup>. This suggests that also the renal vasoconstrictor effect of ET-1 was abolished by BQ123 in the present study. The possibility that ET<sub>B</sub> receptors mediate the dilating and constricting of vascular tone equally cannot be excluded. This would be in agreement with the finding that little or no vascular effect remains in the presence of ET<sub>A</sub> receptor blockade. Taken together, these findings suggest that the constrictor effects of ET-1 in the splanchic and renal vascular beds of healthy humans are mainly ET<sub>A</sub>-mediated. The situation may be different in patients with renal failure [32] and in vessels from rats with portal hypertension [15], where the ET<sub>B</sub> seem to be up-regulated and thereby may contribute to a greater extent to the vascular tone induced by ET-1.

Administration of the ET<sub>B</sub> antagonist BQ788 reduced SBF and RBF<sub>1</sub> with corresponding increases in vascular resistance. This may be due to a basal vasodilator tone that is mediated via endothelial ET<sub>B</sub> receptors. Alternatively, as ET<sub>B</sub> acts as a clearance receptor [33], binding of
BQ788 to ET\textsubscript{B} leads to elevated plasma concentration of ET-1, which may induce vasoconstriction via ET\textsubscript{A}. In line with this hypothesis, the plasma levels of ET-1 were increased by BQ788. The finding that the effects of ET-1 on SBF and RBF was completely blocked or greatly reduced by the selective ET\textsubscript{A} receptor antagonist BQ123 indicate that ET\textsubscript{A} accounts for all or nearly all haemodynamic effects of ET-1 in the splanchnic and renal circulation. Therefore, a plausible explanation for the vasoconstriction in response to ET\textsubscript{B} receptor blockade is increased ET\textsubscript{A} receptor activation as a result of decreased clearance of ET-1. The same explanation applies for the enhanced splanchnic and renal vasoconstriction induced by the ET-1 infusion after BQ788. The relative distribution of ET\textsubscript{B} is larger than that of ET\textsubscript{A} in the human kidney [34]. Thus, although the ET\textsubscript{A} receptor is more generally distributed with concentration in the collecting system, the ET\textsubscript{A} receptor seems to be the predominant vasoconstrictive receptor in accordance with its localization mainly on the vascular smooth muscle [34]. In normal human liver tissue, ET\textsubscript{B} is predominantly expressed on hepatic sinusoidal endothelial cells and hepatic stellate cells, whereas ET\textsubscript{A} is only expressed in small amounts [35]. However, ET-1 has been shown to constrict the portal vein predominantly via ET\textsubscript{A} in isolated rabbit livers [36]. These findings are in accordance with the present results in humans that ET-1-mediated vasoconstriction in the splanchnic and renal circulation was effectively inhibited by ET\textsubscript{A} receptor blockade. Taken together, these observations suggest that ET\textsubscript{B} is important for clearance of circulating ET-1, whereas ET\textsubscript{A} is the most important receptor for mediating vasoconstriction in the human renal and splanchnic vascular beds.

There was an increase in MAP and SVR in response to ET-1 in both the control and the BQ788 protocols, but not in the BQ123 protocol, which suggests that these effects of ET-1 are mainly ET\textsubscript{A}-mediated. During the BQ123 infusion, the response to ET-1 was an increase in cardiac output and a decrease in SVR. The reason for this is unclear; one explanation might be loss of ET\textsubscript{A}-mediated basal vasoconstriction, which is not located to the splanchnic and renal vascular beds. Another possibility is that the effects of endothelial vasodilator ET\textsubscript{B} receptors outweigh those of vasoconstrictor ET\textsubscript{A} receptors located on smooth-muscle cells. Other studies have demonstrated increased forearm and systemic vascular resistance after administration of BQ788 in healthy men [27,37]. Previous studies have also reported that BQ123 decreased peripheral vascular resistance in healthy men [38] and that BQ123, but not BQ788, induced vasodilatation in the human forearm [37]. Collectively, available results indicate that the reduction in peripheral vascular resistance induced by ET\textsubscript{A} receptor blockade is due to vasodilatation in skeletal muscle, rather than in the renal and splanchnic vascular beds.

The plasma ET levels obtained in the pulmonary artery during the infusion of ET-1 were higher than those in the systemic artery. These findings are in agreement with clearance of circulating ET-1 in the pulmonary vascular bed [31]. Furthermore, the plasma levels during the ET-1 infusion were consistently higher following administration of BQ788 than under control conditions or in the presence of BQ123. This emphasizes the importance of ET\textsubscript{B} for clearance of circulating ET-1 [31,33]. On the other hand, the elimination of circulating ET-1 was very similar in all three protocols. The finding that the half-life of ET-1 in plasma was not significantly increased following administration of BQ788 suggests that clearance of circulating ET-1, via binding to ET\textsubscript{B} receptors, only contributes to a small proportion of the total ET-1 clearance in humans in accordance with observations in the rat [39]. Even though we did not see any significant changes in ET-1 plasma clearance in the present study during acute administration of selective ET receptor blockers it cannot be excluded that long-term treatment with ET receptor blockers may affect the clearance of ET-1 from the circulation as previously described in rats using a combined ET\textsubscript{A}/ET\textsubscript{B} receptor antagonist [39].

The ET-1 uptake was higher in all three vascular beds when BQ788 was present and in the pulmonary vascular bed in the presence of BQ123, compared with the control protocol (Table 2). This occurred in the BQ123 protocol, with similar arterial ET-1 levels, and in the BQ788 protocol, with higher systemic and pulmonary arterial levels of ET-1, in comparison with the control protocol. The results indicate increased ET-1 outflow following infusion of the antagonists. The mechanism behind this remains to be established. The higher ET-1 outflow in the BQ788 protocol may at least partly be due to blockade of ET\textsubscript{B}-mediated clearance.

Our results show that the effects of the BQ788 infusion were sustained after cessation of the infusion in agreement with previous observations [27]. Thus, the study protocols enabled us to demonstrate significant differences in the responses to ET-1 between the BQ788 protocol and the BQ123 protocol, as well as when compared with ET-1 alone, although the infusion times of the antagonists were not identical. Therefore, it seems unlikely that the differences in experimental protocols can account for the differences in haemodynamic responses to ET-1 between the protocols.

In conclusion, this study demonstrates that ET-1-mediated splanchnic and renal vasoconstriction is blocked by selective ET\textsubscript{A} receptor antagonism in healthy humans. To our knowledge, no results on ET\textsubscript{A}-mediated responses in the splanchnic circulation in humans have been published previously. For the first time, we also demonstrated that ET\textsubscript{B} receptor blockade alone reduces SBF and RBF and enhances ET-1-mediated vasoconstriction in humans in vivo. The findings demonstrate that ET\textsubscript{A} plays a dominant role in mediating vaso-
constriction in the splanchnic and renal vasculature. The results also suggest that ET$_B$ plays an important role in regulating plasma ET-1 levels as indicated by the increase after ET$_B$ receptor blockade. Disturbed endothelial ET$_B$ function in cardiovascular diseases such as atherosclerosis and hypertension might thus contribute to the increased ET-1 levels and thereby mediate vasoconstriction and increase MAP. Our results show that ET$_A$ and ET$_B$ receptor blockers exert clear haemodynamic effects at physiological and supraphysiological levels of ET-1. These findings in humans in vivo are important for understanding the role of the ET receptor subtypes and their potential role in pathogenesis.

ACKNOWLEDGMENTS

The study was supported by grants from the Swedish Medical Research Council (10374 and 10857), the Swedish Heart and Lung Foundation and the Karolinska Medical Research Council (10374 and 10857), the Swedish Heart and Lung Foundation and the Karolinska Medical Research Council (10374 and 10857), the Swedish Medical Research Council (10374 and 10857).

REFERENCES

18 Rubens, C., Ewert, R., Halank, M. et al. (2001) Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. Chest 120, 1562–1569


Received 12 July 2002/15 October 2002; accepted 21 November 2002