The impact of the endothelin type A receptor on regional endothelin-1 turnover, in particular renal endothelin-1 release, in humans

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Rullman E, Gustafsson T, Ahlborg G. The impact of the endothelin type A receptor on regional endothelin-1 turnover, in particular renal endothelin-1 release, in humans. J Appl Physiol 108: 1625–1630. 2010. First published February 11, 2010; doi:10.1152/japplphysiol.00881.2009.—The endothelin type A (ETA) receptor was studied in six healthy subjects on two occasions with or without an ETA receptor (BQ-123) blockade. At 40 min of either BQ-123 or NaCl infusion, a concomitant infusion of the endothelin-1 (ET-1) precursor, big ET-1, was initiated to augment ET-1 formation. Blood samples were taken from catheters in a peripheral artery, the renal and femoral veins, and the pulmonary artery. Forty minutes of infusion with BQ-123 alone increased heart rate (P < 0.001) and cardiac output (CO; P < 0.01) and depressed mean arterial blood pressure (P < 0.001) and systemic vascular resistance (SVR; P < 0.01). During infusion of big ET-1 alone, CO, stroke volume, and renal blood flow decreased (P < 0.01), whereas SVR and pulmonary and renal vascular resistance increased (P < 0.05). These responses to big ET-1 were abolished or diminished by ETA receptor-mediated vasoconstrictive effects of ET-1 in human subjects. The mechanism by which ETA receptors affect circulating levels of ET-1 in healthy subjects is increased by 35% to a level below that of patients with cardiovascular disease, a net vasoconstrictive effect and increased MAP are observed (5). This has mainly been attributed to ETA receptor-mediated vasoconstriction in splanchic and renal tissues (5), resulting in higher blood pressure and a decrease in cardiac output (CO) (2). Circulating ET-1 (21) and big ET-1 (4) are taken up by the skeletal muscle vascular bed. Despite the net whole body vasoconstrictive effect of ET-1 infusion, forearm skeletal muscle blood flow increases (4, 5, 21), indicating that endothelial ETB receptors in skeletal muscle have a greater response to circulating ET-1 than those in the splanchic and renal vascular beds.

Infusion of ET-1 induces insulin resistance (16), which suggests that it exerts effects other than only vascular effects. That this effect is reversed by an ETA receptor (BQ-123) blockade (1) is indicative of the presence of functional ETA receptors in skeletal muscle. Beyond their effects on the vascular wall, ETB receptors also function as clearing receptors, as shown in the rat (10); particularly the kidneys and lungs are rich in ETB receptors in humans (9). We have earlier demonstrated that ET-1 levels increase after ETB receptor blockade (7) but have also found evidence indicating that ETA receptors may play a role in regulating circulating ET-1 levels (1) in human subjects. The mechanism by which ETA receptors affect circulating levels of ET-1 may involve the release or uptake of ET-1 including possible effects on ECE-1 (17) in the pulmonary, renal, splanchic, and skeletal muscle vascular beds.

In this study, we investigated the effect of an ETA receptor blockade on the conversion of big ET-1 to ET-1, the subsequent ET-1 release, and blood flow responses in the pulmonary, renal, and leg vascular beds of healthy humans. Skeletal muscle biopsies were taken to elucidate the intracellular mechanisms involved in mediating ET-1 turnover after ETA receptor blockade. Big ET-1 was administered to stimulate the formation of ET-1 and to achieve optimal physiological ET-1 effects. In particular, we studied the role of the ETA receptor in 1) regulation of circulating ET-1 levels, 2) the conversion from its precursor big ET-1, release, and uptake of ET-1 in human pulmonary, renal, and skeletal muscle vascular beds, and 3) the gene expression of ET-1 and ECE-1 in human skeletal muscle.

MATERIALS AND METHODS

Subjects

Six healthy male subjects were included in the study. All subjects were exercising on a regular basis but not being extremely well

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trained as indicated by their basal heart rate (HR), see Table 1. Their age was 31 ± 1 yr; their height was 1.86 ± 0.03 m; and weight was 83 ± 3 kg. Subjects were studied in the basal resting state in the supine position after an overnight fast. The subjects were informed of the nature, purpose, and possible risk involved in the study before giving their written voluntary consent. The procedures used in this study were reviewed and approved by the Ethics Committee of Karolinska Institutet. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Procedure

The subjects were examined on two separate occasions with approximately 2 mo in between (range 1–4 mo). On both occasions thin catheters were inserted percutaneously in an antecubital vein, for infusion of big ET-1 [synthetic big ET-1 (Peninsula Laboratories Europe, St. Helens, UK), dissolved in 0.9% NaCl containing 0.5% albumin], saline, or the ETA receptor blocker BQ-123 and pararnohippurate (PAH) as well as in a peripheral artery (A) for intermittent sampling of blood and measurements of systemic arterial blood pressure. On one occasion 60 min of saline (control study) and on the other occasion 60 min of BQ-123 (blocker study) was infused. During the last 20 min an infusion of big ET-1 at a rate of 8 pmol·kg⁻¹·min⁻¹ was added.

On both occasions a balloon-tipped catheter was also inserted percutaneously in an antecubital vein and advanced under fluoroscopic control. A thin catheter was also inserted in one femoral vein (FV). Thereafter the continuous infusion of PAH (see Ref. 3) was started during inflation of the balloon. A Cournand catheter no. 7 was inserted percutaneously in an antecubital vein and advanced under fluoroscopic control. Pulmonary capillary wedge pressure (PCWP) was registered. Oxygen saturation and hemoglobin concentrations were determined in the basal state, after 40 min of saline or BQ-123, at 20 min after BQ-123 infusion in the blocker study. For analysis of ET-1 antiserum when the ET-1 (1–21) value is expressed as 100% say as previously described (4, 5, 13). The cross reactivity for the used ET-1 antiserum when the ET-1 (1–21) value is expressed as 100% was: ET-1 (16–21) 16%, ET-2 27%, ET-3 8%, and ET-1 0.03%. No cross reactivity with big ET-1 (22–38) was observed at concentrations up to 1 μM. The intra- and interassay variations were 5.6 and 12.7%, respectively (3).

Oxygen saturation and hemoglobin concentrations were determined with an OSM 3 radiometer and blood gases with an ABL 3 radiometer (ABL, Copenhagen, Denmark); the hematocrit was analyzed with a microcapillary hematocrit centrifuge and corrected for trapped plasma. Oxygen content in expired air was determined with a paramagnetic analyzer (Magnos 2T; Hartmann & Braun, Frankfurt, Germany) and with a zirconium oxide cell (oxygen analyzer S-3A/I; Ametek, Pittsburgh, PA).

RNA Measurements

Total RNA was prepared by the acid phenol method and quantified spectrophotometrically by absorbance at 260 nm. Integrity of total RNA was determined by 1% agarose gel electrophoresis. Two micrograms of RNA were reverse transcribed by Superscript reverse transcriptase (Life Technologies, Stockholm, Sweden) using random hexamer primers (Roche Diagnostics, Mannheim, Germany) in a total volume of 20 μl.

Detection of mRNA was performed on an ABI-PRISM 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster City, CA). Primers and TaqMan probes for ET-1 and ECE-1 were ordered as assay on demand (Perkin-Elmer Applied Biosystems). β-Actin mRNA were selected as endogenous controls to correct for potential variations in RNA loading (4310881E; Perkin-Elmer Applied Biosys-

Table 1. Measurements of subjects at various time points

<table>
<thead>
<tr>
<th>Time</th>
<th>Receptor or Control</th>
<th>Basal</th>
<th>40-Min BQ-123 or NaCl Infusion</th>
<th>20-Min Infusion of Big ET-1</th>
<th>10 Min Post BQ-123/NaCl + Big ET-1</th>
<th>ANOVA Within Group</th>
<th>ANOVA Between Groups, P and I</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>BQ-123</td>
<td>60 ± 3</td>
<td>68 ± 3‡</td>
<td>64 ± 3*</td>
<td>69 ± 4‡</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
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<tr>
<td>V̇O₂</td>
<td>C</td>
<td>58 ± 4</td>
<td>58 ± 3</td>
<td>56 ± 3</td>
<td>54 ± 2</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>CO</td>
<td>BQ-123</td>
<td>347 ± 9</td>
<td>344 ± 12</td>
<td>361 ± 15</td>
<td>364 ± 11</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>SV</td>
<td>C</td>
<td>335 ± 17</td>
<td>38 ± 13</td>
<td>380 ± 12</td>
<td>337 ± 10</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>RBF</td>
<td>BQ-123</td>
<td>45 ± 2</td>
<td>41 ± 2</td>
<td>43 ± 2</td>
<td>42 ± 1</td>
<td>n.s.</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(A-PA)₀₂</td>
<td>C</td>
<td>43 ± 1</td>
<td>43 ± 1</td>
<td>54 ± 2‡</td>
<td>57 ± 2‡</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>BQ-123</td>
<td>7.1 ± 0.3</td>
<td>8.76 ± 0.54†</td>
<td>8.45 ± 0.42</td>
<td>8.54 ± 0.31*</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>SV</td>
<td>C</td>
<td>7.71 ± 0.3</td>
<td>7.52 ± 0.22†</td>
<td>6.12 ± 0.17§</td>
<td>5.97 ± 0.21†</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>RBF</td>
<td>BQ-123</td>
<td>130 ± 7</td>
<td>127 ± 10</td>
<td>135 ± 11</td>
<td>124 ± 6</td>
<td>n.s.</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(A-FV)₀₂</td>
<td>C</td>
<td>135 ± 11</td>
<td>131 ± 8</td>
<td>111 ± 6</td>
<td>110 ± 7</td>
<td>n.s.</td>
<td>P &lt; 0.01</td>
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<tr>
<td>CO</td>
<td>BQ-123</td>
<td>146 ± 0.10</td>
<td>1.34 ± 0.12</td>
<td>1.25 ± 0.07</td>
<td>1.38 ± 0.13</td>
<td>P = 0.05</td>
<td>P &lt; 0.05</td>
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<tr>
<td>SV</td>
<td>C</td>
<td>1.55 ± 0.12</td>
<td>1.39 ± 0.08</td>
<td>0.97 ± 0.07‡</td>
<td>1.06 ± 0.07‡</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>RBF</td>
<td>BQ-123</td>
<td>62 ± 7</td>
<td>58 ± 4</td>
<td>48 ± 3†</td>
<td>58 ± 6</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(A-FV)₀₂</td>
<td>C</td>
<td>57 ± 5</td>
<td>59 ± 4</td>
<td>65 ± 6</td>
<td>71 ± 6*</td>
<td>P = 0.12</td>
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</tbody>
</table>

Heart rate (HR), cardiac output (CO), stroke volume (SV), and renal blood flow (RBF) in the basal state, at 40 min of BQ-123 or NaCl control, C followed by another 20 min of BQ-123 or NaCl + big endothelin-1 (ET-1) (8 pmol·kg⁻¹·min⁻¹) and at 10 min after the infusions. Applicable values are means ± SE. ANOVA within and between groups and interaction (l) are indicated. *P < 0.05, †P < 0.01, and ‡P < 0.001 compared with basal value. n.s., not significant; (A-PA)₀₂, systemic arterial-mixed venous oxygen difference; (A-FV)₀₂, arterial-femoral venous oxygen difference.

Table 1. Measurements of subjects at various time points
Effects of BQ-123 Per Se

Forty minutes of BQ-123 infusion per se before the big ET-1 infusion increased HR (P < 0.001) and CO (P < 0.01) and depressed MAP (P < 0.001) and SVR (P < 0.01, see Table 1). BQ-123 per se caused no significant change regarding arterial level or pulmonary, renal, or leg net exchange of ET-1.

Comparison of Big ET-1 Effects With or Without Concomitant BQ-123 Infusion

HR, VO$_2$, pulmonary, renal, and skeletal muscle arterial venous oxygen differences, CO, and renal blood flow. ANOVA showed a difference between trials regarding HR (P < 0.05, interaction group × time P = 0.01; see Table 1). Whereas there was no change in the control trial only receiving big ET-1, HR rose in the BQ-123 + big ET-1 trial (P < 0.001). ANOVA showed no difference in VO$_2$ between or within trials. (A-PA)$_O_2$ differed between trials (P < 0.01, group × time P < 0.001) with an increase after big ET-1 and no change after BQ-123 + big ET-1. CO also differed (P = 0.01, group × time P < 0.001) with a drop after big ET-1 alone (P < 0.001) but not after BQ-123 + big ET-1. Stroke volume differed between trials (P < 0.05) because of a drop in the big ET-1 trial, which was eliminated after BQ-123 + big ET-1 (group × time P < 0.05). Renal blood flow differed between trials (P < 0.05, group × time P < 0.001) with a drop after big ET-1 (P < 0.001), which disappeared following BQ-123. The arterial-renal venous oxygen difference, (A-RV)$_O_2$, increased from 14 ± 2 to 20 ± 2 ml/l after big ET-1 alone (P < 0.05) but remained unchanged after BQ-123 + big ET-1. As a consequence, resting renal oxygen uptake (19 ± 1 ml/min) remained unchanged at 20 min of big ET-1 with (17 ± 2 ml/min) or without (18 ± 1 ml/min) BQ-123. The arterial-femoral venous oxygen difference, (A-FV)$_O_2$, showed a difference between trials (group × time P = 0.05) with a transient 23% fall after BQ-123 + big ET-1 (P < 0.01). BQ-123 alone caused no change in (A-FV)$_O_2$.

Systemic and pulmonary blood pressures and resistances. MAP differed between trials (P < 0.01, group × time P < 0.001; see Table 2) because of a fall in the BQ-123 + big ET-1 trial (P < 0.001) and rise in the big ET-1 trial (P < 0.001). MAP showed no difference between or within trials. For PCWP there was interaction between trials (P < 0.05), and PCWP fell in the BQ-123 + big ET-1 trial but was unchanged after big ET-1 alone. SVR differed between trials (P < 0.01 group × time P < 0.001) and fell in the BQ-123 + big ET-1 (P < 0.01) group but rose after big ET-1 alone (P < 0.001). Regarding PVR, ANOVA showed a difference neither between trials (group × time P < 0.08) nor within trials although big ET-1 alone caused an increase (P < 0.05) compared with basal value, and PVR seemed to fall when BQ-123 was given together with big ET-1. Renal vascular resistance differed between trials (P = 0.001, group × time P < 0.001) and remained unchanged in the BQ-123 + big ET-1 trial but increased in the big ET-1 trial (P < 0.001).

Arterial levels of ET-1 and pulmonary, renal, and skeletal muscle exchanges of plasma ET-1. There was no difference in arterial ET-1 levels between groups (see Fig. 1). ANOVA within each group showed a similar twofold increase after big ET-1 infusion (P < 0.001) with no effect of prior BQ-123
administration (Fig. 1). Basal values were approached 10 min after cessation of the big ET-1 administration. The changes in arterial ET-1 concentration from 16 to 20 min of big ET-1 infusion were 0.2 ± 0.1 and 0.3 ± 0.1 pmol·l⁻¹·min⁻¹ in the BQ-123 and NaCl groups, respectively. Thus the different circulation times through the organs need not be corrected for when calculating the arterial venous oxygen differences, (A-V)O₂.

No significant pulmonary uptake was seen in the basal state or at 40 min of BQ-123. After big ET-1, however, a significant pulmonary uptake was seen in the BQ-123 group (P < 0.01) with an interaction between groups (group × time P < 0.05). ANOVA showed higher uptake in the BQ-123 group (P < 0.05). There was neither a net basal renal ET-1 exchange nor a release at 40 min of BQ-123 infusion. Renal net ET-1 release was seen in both trials (P < 0.001) only after big ET-1 infusion and differed between trials (P < 0.001, group × time P < 0.05) with approximately threefold higher ET-1 release in the BQ-123 group. There was no significant basal leg ET-1 exchange, nor did it change within or differ between groups during BQ-123 or big ET-1 infusions.

Muscle biopsy analyses. There was no change in mRNA for ET-1 or ECE, after BQ-123, BQ-123 + big ET-1, or big ET-1 alone compared with basal values (see Fig. 2).

DISCUSSION

Previous studies have shown that splanchnic tissues (3), the kidneys (3), and skeletal muscle (4), but not the brain (4), take up big ET-1 during systemic infusion. A concomitant big ET-1 and ET-1 release indicating big ET-1 conversion was only found in the kidneys (3, 4). Despite the abundance of ETB receptors in the kidneys, renal net ET-1 release after big ET-1 infusion was approximately threefold higher after ETA receptor blockade than after infusion of big ET-1 alone (Fig. 1). This indicates that ET-1 exerts negative feedback on renal ET-1 release to the blood by acting on the ETA receptor. The magnitude of the renal ET-1 release is substantial, considering the potent vasoconstrictive and hypertensive effect of ET-1 (2) and its other biological functions in the kidneys, e.g., the ETA receptor-mediated increase in urinary sodium retention (11). Several previous studies have proposed that the ETB receptor is a major regulatory factor associated with circulating ET-1 (9). Our results show that the ETA receptor is also a significant factor in this respect and emphasize the importance of the ET-1 system in the kidneys.

Pulmonary ET-1 uptake was increased after infusion of BQ-123 plus big ET-1 compared with infusion of big ET-1 alone (Fig. 1). Interestingly, the pulmonary fractional extraction of ET-1 was 34 ± 6% after the blockade, compared with our previous estimate of fractional extraction of 41 ± 4% during ET-1 infusion (21), indicating no pulmonary release. We have not previously been able to demonstrate any pulmonary big ET-1 uptake (13) or ET-1 release (4) during big ET-1

Fig. 1. A: arterial concentrations of endothelin-1 (ET-1) in the basal state, at 40 min of BQ-123 or NaCl infusion, at 60 min of BQ-123 or NaCl together with 20 min of big ET-1 infusion, and at 10 min after the infusions. Additional measurements were performed at 16 and 18 min of big ET-1 infusion as well as at 2 and 4 min after infusions. *** P < 0.001 compared with basal value within each trial. There was no difference between the trials. Values are presented as means ± SE. B: renal release, skeletal muscle release, and pulmonary uptake of ET-1 at 60 min of BQ-123 + 20 min of big ET-1 (hatched bars) vs. the control situation at 60 min of NaCl + 20 min of big ET-1 infusion (open bars). Total skeletal muscle release was estimated on the basis of the assumption that leg uptake mainly represents leg muscle uptake and that leg muscle corresponds to 50% of total skeletal muscle mass. Differences between trials are indicated by * P < 0.05. Values are presented as means ± SE. C: renal release, skeletal muscle release, and pulmonary uptake of ET-1 at 40 min of BQ-123 (hatched bars) or NaCl (open bars) are included. Differences between trials are indicated by * P < 0.05. Values are presented as means ± SE.
infusion. Taken together, these results support the fact that the lungs play a predominant role in ET-1 clearance in accordance with the high abundance of ETB receptors in the lungs (9).

The pulmonary uptake of ET-1 was greater than the renal release ($P < 0.05$; Fig. 1) after BQ-123 and big ET-1 infusion. Values are presented as median, second and third quartile, and range. 

The absence of a significant net leg muscle exchange of ET-1 may still reflect increased turnover of ET-1 associated with increases in ET-1 uptake and release of equal magnitude. Skeletal muscle has been shown to take up big ET-1 (3) and ET-1 (21) from the circulation, and the fractional extraction of ET-1 by skeletal muscle has been shown to be 30 ± 7% (21) of the arterial inflow. Neither in our previous studies nor in the present study have we been able to demonstrate any effect of big ET-1 or ET-1 on pulmonary or regional oxygen uptake (3, 4, 21, 22). Thus the 23% decrease in $(A-FV)O_2$ observed in the present study indicates a 30% increase in leg blood flow after the BQ-123 infusion. After administration of big ET-1 alone, the $(A-FV)O_2$, representing not only skeletal muscle but also skin, subcutaneous tissues, and bone, increased corresponding to a 15% decrease in leg blood flow. This is in contrast to the increase in skeletal muscle-specific blood flow (as determined by a catheter in a deep forearm vein only draining skeletal muscle) following ET-1 infusion (1). Consequently, at similar arterial ET-1 levels, the increase in leg blood flow after the blockade would be accompanied by ~40% higher ET-1 uptake and release compared with the control. A recent study reported that big ET-1 induces gene expression of ECE-1 in isolated bovine endothelial cells (18). A significant amount of the mRNA retrieved from a skeletal muscle biopsy is of endothelial and smooth muscle origin. Therefore, detectable effects of ETA blockade and big ET-1 infusion on endothelial and smooth muscle cells would be expected in skeletal muscle biopsies. Several enzymes are able to convert big ET-1 to ET-1, but ECE-1 is probably the most important. To determine whether gene expression of ECE-1 or big ET-1 is induced by Big ET-1 administration or ETA receptor blockade in humans, skeletal muscle biopsies from the vastus lateralis muscle were obtained after infusion with BQ-123, big ET-1 alone, or BQ-123 plus big ET-1. The mRNA level of ECE-1 or big ET-1 was not increased by any of these infusions. We cannot exclude the possibility that gene expression of ECE-1 is induced in the endothelial cells of the larger arteries because the endothelial cells contained in the skeletal muscle biopsy were mainly of capillary and small-resistance vessel origin. As the release of ET-1 was elevated after 60 min of BQ-123 infusion, posttranslational upregulation of endothelin-converting activity represents a more plausible explanation for our findings of increased ET-1 release than ECE-1 or big ET-1 gene expression. The rapid renal release of ET-1 also indicates that gene expression of ET-1 is not an important mechanism. Still, on the basis of the data in this study, we cannot exclude the possibility of an increased gene expression of ECE-1 in the kidneys because no tissue for such analysis was obtained.

Finally, an additional interesting finding in the present study was that 40 min of BQ-123 infusion before the big ET-1 infusion depressed MAP ($P < 0.001$) and SVR ($P < 0.01$) and increased CO ($P < 0.01$; Table 1). Analysis of basal vascular tone is somewhat outside the scope of the present paper but supports previous studies showing that basal vascular tone is at least partly mediated by the ETA receptor (12).

In summary, our data indicate that circulating ET-1 levels are regulated by negative feedback mediated by the ETA receptor. This mechanism is most potent in the kidneys and emphasizes that ETA receptors play an important physiological role in the kidneys by preventing excess ET-1 secretion and from the kidneys. The lung seems to act mainly as a clearing organ for ET-1 in healthy humans in agreement with the high abundance of ETB receptors in the lung. These results set the focus on treatment problems with selective ET-1 receptor blockers. Whole body effects of such treatments will, especially in patients with combined pulmonary/renal/vascular
disease, differ and may require blockade of the other receptor as well.

**REFERENCES**