Insulin sensitivity and big ET-1 conversion to ET-1 after ET_{A}- or ET_{B}-receptor blockade in humans

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Ahlborg, Gunvor, and Jonas Lindström. Insulin sensitivity and big ET-1 conversion to ET-1 after ET_{A} or ET_{B} receptor blockade in humans. J Appl Physiol 93: 2112–2121, 2002. First published September 6, 2002; 10.1152/japplphysiol.00477.2002.—Cardiovascular diseases are characterized by insulin resistance and elevated endothelin (ET)-1 levels. Furthermore, ET-1 induces insulin resistance. To elucidate this mechanism, six healthy subjects were studied during a hyperinsulinemic euglycemic clamp procedure during infusion of the (ET-1 precursor) big ET-1 alone or after ET_{A}- or ET_{B}-receptor blockade. Insulin levels rose after big ET-1 with or without the ET_{A} antagonist BQ-788 (P < 0.05) but were unchanged after the ET_{B} antagonist BQ-123 + big ET-1. Infused glucose divided by insulin fell after big ET-1 with or without BQ-788 (P < 0.05). Insulin and infused glucose divided by insulin values were normalized by ETA blockade. Mean arterial blood pressure rose during big ET-1 with or without BQ-788 (P < 0.001) but was unchanged after BQ-123. Skeletal muscle, splanchnic, and renal blood flow responses to big ET-1 were abolished by BQ-123. ET-1 levels rose after big ET-1 (P < 0.01) in a similar way after BQ-123 or BQ-788, despite higher elimination capacity after ETA blockade. In conclusion, ET-1-induced reduction in insulin sensitivity and clearance as well as splanchnic and renal vasoconstriction are ETA mediated. ETA-receptor stimulation seems to inhibit the conversion of big ET-1 to ET-1.

skeletal muscle vascular beds (4, 21, 39, 40), and ET-1 is eliminated in the pulmonary circulation as well (40). At a given arterial ET-1 level, the vascular responses to infused big ET-1 are more pronounced than to ET-1 infusion (3, 4), indicating that locally synthesized ET-1 reaches more vascular smooth muscle ET-1 receptors than ET-1 taken up from the blood.

The vascular responses can be explained by the presence of two different ET-receptor subtypes: ET_{A} and ET_{B} (8, 34). ET_{A} and ET_{B} are found on vascular smooth muscle cells where both mediate contraction (12, 15), whereas only ET_{B} receptors are located on the endothelial cells and stimulate the production of the vasodilators prostacyclin (16) and nitric oxide (NO) (16, 28). ET_{B} receptors also take part in the elimination of ET-1 (20). When given intravenously to humans, ET-1 (39) and big ET-1 (21) are rapidly eliminated from the circulation, with shorter half-lives for ET-1.

Elevated plasma ET-1 levels have been demonstrated in virtually all diseases involving the cardiovascular or pulmonary systems, such as in patients with, e.g., myocardial infarction (30), atherosclerosis (26), systemic (33) and pulmonary (36) hypertension, and diabetes (38).

A metabolic disturbance characterized by elevated insulin levels and increased insulin resistance, i.e., reduced glucose uptake in response to insulin, is a common denominator for many cardiovascular diseases. ET-1 has previously been shown to reduce total body glucose turnover at rest (5, 7). That ET-1 may take part in the development of the metabolic syndrome finds support in a previous study in which ET-1 caused increased insulin resistance (31). The exact mechanism behind this finding is not known.

We hypothesize that ET-1 is one common factor for the development of insulin resistance and atherosclerosis. Therefore, we wanted to investigate the influence of the different ET-1 receptors on insulin resistance and ET-1 turnover. Accordingly, the responses to either ETA or ETB-receptor blockade during a hyperinsulinemic euglycemic clamp procedure were studied in healthy humans. To reinforce the endogenous big ET-1/ET-1 system, big ET-1 was also infused. Big ET-1
was chosen to mimic the actions of endogenous, locally synthesized ET-1 to achieve more physiological ET-receptor stimulation than infused ET-1 might have had.

METHODS

Subjects. Six healthy, nonsmoking male subjects without a family history of diabetes Type 1 or 2 (age: 28 ± 0.9 yr; height: 187 ± 2 cm; weight: 85 ± 3 kg; body mass index: 24.3 ± 0.8 kg/m²) 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 voluntary consent. The procedures used in this study were reviewed and approved by the Ethics Committee at Karolinska Institutet. The investigation conforms with the principles outlined in the Declaration of Helsinki (BMJ 1964, ii:177).

Procedure. All six subjects took part in the blocker study, which consisted of four different hyperinsulinemic euglycemic clamp protocols: a control clamp, a clamp with only big ET-1 infusion, and two clamp studies with big ET-1 infusion preceded by the infusion of either an ETB-receptor (BQ-788) or ETA-receptor (BQ-123) blocker with at least 1 wk in between the protocols. Average time for all four protocols was 4 mo. Care was taken that weight, diet, and physical activity were kept constant. The subjects did not experience any discomfort. There were no dropouts. The blocker doses and rate of infusions were based on previous investigations (13, 37). With these procedures, optimal effects on peripheral vascular resistance were seen from 30 min after onset of the blocker infusions, and near-optimal effects seemed to persist for at least another 30 min (13, 37). The affinity of BQ-123 is &gt;100,000 times greater for the ET_{B} than for the ET_{A} receptor (22), and the affinity of BQ-788 is &gt;1,000 times greater for the ET_{B} than for the ET_{A} receptor (23). For each subject, the big ET-1 infusion was initiated at the same time point in the protocols. The blocker investigations were performed in random order. Saline replaced the blockers in the protocols without blockers and big ET-1 in the control clamp. In addition, three of the subjects took part in an extra clamp study using a higher dose of big ET-1.

Thin catheters were inserted percutaneously into antecubital veins in one arm for infusion of big ET-1 [synthetic big ET-1 (Bachem, Bubendorf, Switzerland) dissolved in 0.9% NaCl containing 0.5% albumin], the receptor blocker BQ-123 or BQ-788 (both from Clinalfa, Läufelfingen, Switzerland), cardiorgog (CG), and p-aminohippurate (PAH) and in the other arm for infusion of glucose and insulin. Another thin catheter was introduced in the brachial artery (A) for sampling of blood and measurement of systemic arterial blood pressure. Heart rate (HR) and mean arterial blood pressure (MAP) were recorded continuously throughout the studies.

In the protocols with the ET-receptor blockers, a Courmand catheter no. 7 was inserted in the femoral vein and advanced to a right-sided central hepatic vein (HV) under fluoroscopic control (n = 5 in the BQ-788 protocol and n = 6 in the BQ-123 protocol, see below). Splanchnic (SBF) and renal blood flows (RBF) were determined by constant infusions of CG and PAH, as previously described (5). The HV catheter was introduced to ascertain that fractional uptake (F; equal to the A-HV difference divided by the arterial concentration) of CG was not influenced by the infusion of the ET-1 blockers or big ET-1. As there was no change in the F values for CG over time or between the blocker protocols, the mean F value 0.8 [0.799 ± 0.022 (SE)] for all subjects was used when CG concentrations in HV were not available. For PAH, a fractional extraction of 0.9 was used. In addition, another thin catheter was introduced into a deep forearm vein (DV) in five subjects in the big ET-1 clamp, four subjects in the BQ-788, and five subjects in the BQ-123 protocol, for blood sampling from the skeletal muscle vasculature.

After a 60-min resting period, a 120-min euglycemic hyperinsulinemic clamp, as previously described (for a review, see Ref. 18), was initiated. The clamp procedure in all protocols was as follows. During the first 10 min, insulin (Actrapid, 100 IE/ml; Novo Nordisk A/S, Bagsverd, Denmark) dissolved in 0.9% saline and blood were infused stepwise, corresponding to 804 mU/m² body surface area followed by 40 mU·m⁻²·min⁻¹ for 110 min. Arterial blood samples were taken every 5 min for determination of blood glucose. Fasting blood glucose level was maintained by adjusting the infusion rate of a 20% glucose solution. During euglycemia and relative steady-state glucose levels, the amount of glucose infused represents the total body glucose uptake (M; mg·kg⁻¹·min⁻¹). M was calculated during three 20-min periods from 60 to 120 min during the clamp. The M values were corrected for insulin by dividing the M values by the mean plasma insulin concentration (I; mU/ml) during the same period and multiplied by 100, according to established practice. The M/I value represents a measure of insulin sensitivity (IS).

Studies to investigate the effect of big ET-1 on M/I values. To determine an adequate dose of big ET-1, the first two subjects followed the control and the big ET-1 protocols (see below), the latter with big ET-1 infusion rates of both 4 and 8 pmol·kg⁻¹·min⁻¹, on three different occasions. The results indicated that 4 pmol·kg⁻¹·min⁻¹ was sufficient to cause reduced IS, and, therefore, this dose was used in these two subjects as well as in the next two subjects (subjects 3 and 4) in the blocker study protocols (see below). As it turned out, however, subjects 3 and 4 did not show a drop in M/I at this dose, and, therefore, we doubled the big ET-1 dose in subjects 5 and 6 in the blocker study protocols. To confirm that big ET-1 infusion per se causes insulin resistance, subject 4, who received the lower big ET-1 dose in the four protocols below, was also studied with the higher dose of big ET-1. Thus the effect of 8 pmol·kg⁻¹·min⁻¹ on M/I values was studied in five subjects.

The blocker study protocols. See Fig. 1. In the control clamp, in addition to glucose and insulin, nothing was infused except saline (see above). In the big ET-1 clamp, big ET-1 was infused at a rate of 4 (n = 4) or 8 (n = 2) pmol·kg⁻¹·min⁻¹ for 20 min via an antecubital vein, initiated at 60 min (n = 2), both receiving the lower dose of big ET-1) or 80 min (n = 4) into the clamp procedure. In the BQ-788 clamp, the big ET-1 infusion, starting at the same time as in the big ET-1 clamp, was preceded by infusion of an ET_{B}-receptor blocker, BQ-788, at a rate of 4 nmol·kg⁻¹·min⁻¹ for 15 min in all six subjects, starting 30 min before the big ET-1 infusion. In the BQ-123 clamp, the big ET-1 infusion, starting at the same time as in the big ET-1 clamp, was preceded by infusion of an ET_{B}-receptor blocker, BQ-123, at a rate of 2.5 (n = 4) or 5 (n = 2) nmol·kg⁻¹·min⁻¹ for 50 min, starting 30 min before the big ET-1 infusion. The higher dose was given to the two subjects who received the higher big ET-1 dose. The results of the two subjects who received double amounts of big ET-1 and BQ-123 were pooled with the results of the other four subjects in the BQ-123 clamp.

Blood sampling. Blood glucose was determined in arterial samples every 5 min during the clamp procedures and in HV samples in the blocker protocols. Arterial samples for deter-
ministration of plasma insulin were taken in the basal state, i.e., immediately before the clamp was initiated, as well as at 60, 80, 100, and 120 min into the clamp procedure. In addition, blood samples for analysis of CG and PAH concentrations from the catheters in A and HV (when available for CG concentrations) were taken in the basal state and at time points corresponding to immediately before and at 20 min of big ET-1 infusion. Oxygen content was determined in A and DV samples in the two ET-1-receptor blocker protocols in the basal state, after the blockers as well as at 20 min of big ET-1 infusion. In the big ET-1 clamp, the A-DV oxygen difference was determined before and at 20 min of big ET-1 infusion. In addition, the A-HV oxygen difference was determined in the basal state and after administration of the blockers. Arterial blood samples for determination of plasma ET-1 concentrations were taken at time points corresponding to those in the basal state, immediately before, at 20 min of big ET-1 infusion, and at 20 min after the clamp procedures in all protocols. HV concentrations of ET-1 were determined at 20 min of big ET-1 infusion in the blocker protocols.

Analyses. Glucose was analyzed in whole blood according to the glucose dehydrogenase method by using a HemoCue B-glucose photometer (HemoCue, Angelholm, Sweden) with a precision corresponding to a SD ≤ 0.3 mmol/l. Plasma insulin (5) and ET-1 (21) immunoreactivity were analyzed by radioimmunoassay, as previously described. The cross-reactivity for the E1 antiserum when the ET-1-(1–21) value is expressed as 100% was 16% ET-1-(16–21), 27% ET-2, 8% ET-3, and 0.03% big ET-1. 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.

Oxygen saturation and hemoglobin concentration were determined with an OSM 3 Radiometer, and blood gases with an ABL 3 Radiometer (Copenhagen, Denmark). The hematocrit was analyzed with a microcapillary hematocrit centrifuge and corrected for trapped plasma.

Calculations and statistics. Splanchnic (SplVR) and renal vascular resistances (RVR) were calculated as MAP divided by SBF or RBF, respectively. F represents the local arteriovenous difference for a substance divided by its arterial concentration.

A two-way ANOVA with repeated measures on two factors was used to analyze the data. The factors were clamp (4 levels) and time (3 or 4 levels). The interaction in the ANOVA refers to the statistical test of whether the effect of one factor, as measured by differences in the response averages, is different for different levels of the other factor. In case of significant interaction, post hoc interaction tests were performed between each pair of the clamp conditions across the time intervals. Simple effects tests were also performed, i.e., effects of one factor holding the other factor fixed. For these tests, Fisher’s protected least significant difference was performed. Data are presented as means ± SE.

RESULTS

HR, MAP, arterial blood glucose, arterial-hepatic venous and arterial-deep venous glucose differences, plasma insulin, M, and M/I values. ANOVA showed a difference between the four clamps regarding HR (interaction clamp × time, P < 0.05). HR increased in the control and the BQ-123 clamps (P < 0.05, Table 1). In contrast, there was no change in HR in the big ET-1 or BQ-788 clamps. For MAP, a significant difference was found between the four clamps (clamp × time, P < 0.01). Whereas MAP did not change in the control or BQ-123 clamp, it increased both in the big ET-1 and BQ-788 clamps (P < 0.001, Table 1).

ANOVA did not show any significant difference in arterial glucose concentration (Table 1) over time within or between the clamps. The basal hepatic glucose output determined in the two blocker clamp studies (0.81 ± 0.14 mmol/l in the BQ-788 and 0.49 ± 0.06 mmol/l in the BQ-123 clamp) did not differ. During the clamps, the A-HV differences as determined from 60 min onward did not differ from zero at any time except for at 80 min in the BQ-123 study when a splanchnic glucose uptake was seen (0.22 ± 0.08 mmol/min; P < 0.05), corresponding to 7% of total glucose infusion rate at that time.

Basal plasma insulin, I, did not differ in the four clamp protocols. Nor did arterial insulin at 60 min in the control clamp (66 ± 3 μU/l) differ from the 60-min value in the big ET-1 clamp (73 ± 5 μU/l) before the big ET-1 infusion. ANOVA comparing the I values from the four protocols during the clamps (Fig. 2) showed a significant difference between clamp protocols (P < 0.05). ANOVA with pairwise comparisons showed higher values in the big ET-1 and the BQ-788 clamps compared with the control clamp (P < 0.05) or the BQ-123 clamp (P < 0.01). There was no difference between the two latter clamp protocols or the big ET-1 vs. the BQ-788 protocols.

ANOVA comparing the M values in the four clamp protocols (Fig. 3) showed no significant difference nor did ANOVA comparing the M values in the control clamp with each of the other three protocols separately.

The M/I values from the four protocols (Fig. 4) showed a significant difference (clamp × time, P <
differences at different time points during the control clamp and the clamps with either big ET-1, BQ-788, or BQ-123 + big ET-1 infusions.

Table 1. HR, MAP, arterial glucose in the basal state and at different time points, as well as the A-HV glucose differences at different time points during the control clamp and the clamps with either big ET-1, BQ-788 + big ET-1, or BQ-123 + big ET-1 infusions

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>Basal State</th>
<th>60 Min</th>
<th>80 Min</th>
<th>100 Min</th>
<th>120 Min</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53 ± 2</td>
<td>53 ± 2</td>
<td>57 ± 3</td>
<td>62 ± 5*</td>
<td>61 ± 3*</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Big ET-1</td>
<td>53 ± 2</td>
<td>57 ± 4</td>
<td>53 ± 3</td>
<td>49 ± 3</td>
<td>51 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>BQ-788 + big ET-1</td>
<td>49 ± 2</td>
<td>52 ± 3</td>
<td>51 ± 3</td>
<td>48 ± 4</td>
<td>53 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>54 ± 3</td>
<td>60 ± 2</td>
<td>64 ± 3*</td>
<td>61 ± 1*</td>
<td>62 ± 3*</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>89 ± 2</td>
<td>90 ± 4</td>
<td>90 ± 4</td>
<td>92 ± 4</td>
<td>92 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Big ET-1</td>
<td>89 ± 5</td>
<td>90 ± 6</td>
<td>96 ± 7†</td>
<td>101 ± 6*</td>
<td>101 ± 6*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>BQ-788 + big ET-1</td>
<td>90 ± 4</td>
<td>91 ± 4</td>
<td>96 ± 5*</td>
<td>102 ± 5†</td>
<td>105 ± 5‡</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>90 ± 2</td>
<td>87 ± 4</td>
<td>89 ± 4</td>
<td>91 ± 4</td>
<td>92 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose arterial concentration, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.06 ± 0.11</td>
<td>4.94 ± 0.17</td>
<td>4.89 ± 0.11</td>
<td>4.83 ± 0.17</td>
<td>4.94 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Big ET-1</td>
<td>5.00 ± 0.11</td>
<td>5.33 ± 0.17</td>
<td>5.06 ± 0.28</td>
<td>4.94 ± 0.33</td>
<td>5.00 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>BQ-788 + big ET-1</td>
<td>4.94 ± 0.17</td>
<td>4.78 ± 0.17</td>
<td>4.89 ± 0.22</td>
<td>4.72 ± 0.17</td>
<td>4.83 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>5.06 ± 0.17</td>
<td>5.39 ± 0.11</td>
<td>5.00 ± 0.22</td>
<td>5.06 ± 0.17</td>
<td>4.94 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (A-HV), mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-788 + big ET-1 (n = 5)</td>
<td>-0.07 ± 0.11</td>
<td>0.06 ± 0.08</td>
<td>-0.11 ± 0.06</td>
<td>-0.01 ± 0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>0.21 ± 0.09</td>
<td>0.22 ± 0.08</td>
<td>0.14 ± 0.08</td>
<td>0 ± 0.07</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 unless otherwise stated. ET, endothelin; HR, heart rate; MAP, mean arterial pressure; glucose (A-HV), glucose brachial artery-hepatic vein difference; NS, not significant. ANOVA values are for all time points within each group. Significantly different from basal value: *P < 0.05, †P < 0.01, ‡P < 0.001.

0.01). ANOVA comparing the M/I values in the control clamp and big ET-1 clamp did not show any difference in trend. The difference between the M/I values in the control clamp and the BQ-788 clamp, however, was highly significant (clamp × time, P < 0.001), and the M/I values became lower in the BQ-788 clamp (P < 0.05). The M/I values after BQ-123 did not differ from those in the control clamp but differed from both the big ET-1 clamp (clamp × time, P < 0.05) and the BQ-788 clamp (clamp × time, P < 0.001), with lower values in the big ET-1 and BQ-788 clamps (both P < 0.05). There was no difference between the big ET-1 and BQ-788 protocols.

ANOVA comparing the M/I values for the five subjects receiving the higher big ET-1 dose (8 pmol·kg⁻¹·min⁻¹ for 20 min, see Studies to investigate the effect of big ET-1 on M/I values in methods) showed a significant difference compared with the control clamp (P < 0.05). There was no interaction, which was not unexpected due to the fact that two of the five subjects had already gotten the big ET-1 dose in the 60- to 80-min period. The M/I values in the 100- to 120-min period were lower in the big ET-1 clamp (P < 0.05).
SplVR and RVR. As can be seen from the basal A-DV oxygen differences in the two blocker protocols, differences between the I values in the clamp with the higher dose control clamp. ANOVA showed a significant difference compared with before the blocker. Nor did big ET-1 after BQ-123 cause any change in A-DV oxygen differences.

In the control clamp, there was no change in SBF (Table 2). ANOVA showed a significant difference (clamp × time, \( P < 0.01 \)) for the four clamp SBF values as determined in the basal state and at the time points corresponding to immediately before and at 20 min of big ET-1 infusion. Significant differences were found for the control clamp vs. the big ET-1 protocol as well as the BQ-788 protocol (interactions, clamp × time, \( P < 0.05 \)), with lower values in the two latter at 20 min of big ET-1 infusion compared with the corresponding control value (\( P < 0.01 \)). There was no difference between the two latter protocols nor a difference between the control and the BQ-123 protocol. Accordingly, the BQ-123 protocol also showed significant difference (clamp × time, \( P < 0.05 \)) and higher values compared with the big ET-1 (\( P < 0.05 \)) and the BQ-788 (\( P < 0.01 \)) clamp protocols at 20 min of big ET-1 infusion. In addition, ANOVA for the BQ-788 protocol (\( P < 0.001 \)) showed lower SBF values after BQ-788 per se (immediately before onset of the big ET-1 infusion, \( P < 0.05 \)). ANOVA for the BQ-123 protocol demonstrated that BQ-123 per se increases SBF (\( P < 0.05 \)).

RBF (Table 2) showed a small gradual decline in the control clamp corresponding to 0.14 ± 0.03 l/min (8 ± 2%, \( P < 0.01 \)), in accordance with what our laboratory

period were 8.14 ± 0.80 mg·kg\(^{-1}\)·min\(^{-1}\)·mU\(^{-1}\)·l·100 after the higher dose of big ET-1 compared with 12.18 ± 1.43 mg·kg\(^{-1}\)·min\(^{-1}\)·mU\(^{-1}\)·l·100 in the control clamp. ANOVA showed a significant difference between the I values in the clamp with the higher dose of big ET-1 compared with those in the control clamp (\( P < 0.05 \)), and the I values in the 100- to 120-min period increased to 104 ± 7.4 mU/l after big ET-1 vs. 68.5 ± 3.8 mU/l in the control clamp.

Arteriovenous oxygen differences, SBF and RBF, and SplVR and RVR. As can be seen from the basal A-DV oxygen differences in the two blocker protocols, different DV were drained by the catheter. Therefore, each clamp was tested separately with a one-way repeated-measures ANOVA. During the big ET-1 infusion in the big ET-1 clamp, the A-DV oxygen differences (Table 2) fell by 33% (\( n = 5 \), \( P < 0.02 \)). ANOVA showed differences between the A-DV oxygen values in the BQ-788 clamp (\( n = 4 \), \( P < 0.05 \)), with increasing values from the basal to after BQ-788 alone (\( P < 0.05 \)), and falling values during the subsequent big ET-1 infusion (\( P < 0.05 \)), when the values were 17% lower compared with basal value. After BQ-123 (\( n = 5 \)), there was no significant difference compared with before the blocker. Nor did big ET-1 after BQ-123 cause any change in A-DV oxygen differences.

Table 2. (A-DVO\(_{2}\), SBF and RBF at time points corresponding to the basal state, immediately before (30 min after onset of blocker infusion) and at 20 min of big ET-1 infusion in the four different clamp protocols.

<table>
<thead>
<tr>
<th>(A-DVO(_{2}), ml/l)</th>
<th>Basal</th>
<th>Before Big ET-1</th>
<th>After Big ET-1</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84 ± 8</td>
<td>56 ± 7*</td>
<td></td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Big ET-1 (( n = 5 ))</td>
<td>67 ± 12</td>
<td>79 ± 13*</td>
<td>55 ± 7</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BQ-788 + big ET-1 (( n = 4 ))</td>
<td>85 ± 5</td>
<td>95 ± 9</td>
<td>86 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>SBF, l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.19 ± 0.07</td>
<td>1.18 ± 0.10</td>
<td>1.13 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Big ET-1</td>
<td>1.24 ± 0.08</td>
<td>1.21 ± 0.08</td>
<td>0.96 ± 0.09†</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>BQ-788 + big ET-1</td>
<td>1.22 ± 0.07</td>
<td>1.07 ± 0.08*</td>
<td>0.86 ± 0.08‡</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>1.20 ± 0.07</td>
<td>1.37 ± 0.09*</td>
<td>1.23 ± 0.10</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>RBF, l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.70 ± 0.16</td>
<td>1.55 ± 0.15†</td>
<td>1.55 ± 0.14†</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Big ET-1</td>
<td>1.56 ± 0.12</td>
<td>1.46 ± 0.11</td>
<td>1.06 ± 0.09‡</td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>BQ-788 + big ET-1</td>
<td>1.56 ± 0.10</td>
<td>1.33 ± 0.08‡</td>
<td>0.99 ± 0.08‡</td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>BQ-123 + big ET-1</td>
<td>1.74 ± 0.13</td>
<td>1.65 ± 0.14*</td>
<td>1.50 ± 0.16‡</td>
<td>( P &lt; 0.0001 )</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) unless otherwise stated. Data from the control clamp and the clamps with big ET-1, BQ-788 + big ET-1, and BQ-123 + big ET-1 infusions are presented. (A-DVO\(_{2}\), arterial-deep venous oxygen difference; SBF, splanchnic blood flow; RBF, renal blood flow. ANOVA values are for all time points within each group. Significantly different from basal value: *\( P < 0.05 \); †\( P < 0.01 \); ‡\( P < 0.001 \).
ET receptors, insulin sensitivity, and ET-1 formation

big ET-1 infusion, \( P < 0.001 \). ANOVA for the BQ-123 protocol could not demonstrate that BQ-123 per se increased RBF, although there was a tendency toward a smaller decrease in RBF (5 ± 2%) compared with the corresponding control value (see above).

SplVR and RVR (not shown) did not change in the control clamp or in the other clamps before big ET-1 infusion. The latter was followed by increased (\( P < 0.001 \)) SplVR and RVR without, as well as after, BQ-788, whereas the values after BQ-123 did not differ from control values.

ANOVA for splanchnic oxygen uptake in the basal state and at 30 min of blocker infusion did not show any difference between the BQ-788 or BQ-123 protocols. In the BQ-123 protocol, these values were 48 ± 2 and 44 ± 3 ml/min (\( n = 6 \); not significant), and, in the BQ-788 protocol, these values were 47 ± 3 and 41 ± 1 ml/min (\( n = 5 \); not significant).

Arterial ET-1 values and splanchnic and renal uptakes of ET-1. ANOVA comparing the ET-1 values for the time points according to Fig. 5 showed a difference between groups (\( P < 0.01 \)) as well as a significant interaction (clamp \( \times \) time, \( P < 0.01 \)). No significant change in ET-1 was found in the control clamp protocol. There was a significant difference between the control and big ET-1 protocols (clamp \( \times \) time, \( P < 0.05 \)), and the value at 20 min postclamp (Fig. 5) became higher in the big ET-1 protocol (\( P < 0.01 \)). The ET-1 values at 20 min of big ET-1 infusion were higher in the BQ-788 and BQ-123 protocols compared with those at the corresponding time point in the control (\( P < 0.001 \)) or the big ET-1 (\( P < 0.01 \)) protocols. There was no difference in arterial ET-1 values between the BQ-788 and BQ-123 clamps. The blockers per se did not cause any significant change in ET-1 levels. Thus any cross-reactivity of the blockers with ET-1 in the ET-1 assay can be excluded as an explanation of the increase in ET-1 levels during the big ET-1 infusion after the blockers. By using our laboratory’s previously estimated values for F in the splanchnic and renal circulations (4), the ET-1 uptakes can be estimated (see Table 3). The calculations show significant ET-1 uptakes in all four clamp protocols at the time point corresponding to 20 min of big ET-1 infusion. The splanchnic and renal ET-1 uptakes were highest dur-

Table 3. Calculation of splanchnic and renal uptakes of ET-1 at time points corresponding to 20 min of big ET-1 infusion during the control clamp and the clamps with either big ET-1, BQ-788 + big ET-1, or BQ-123 + big ET-1 infusions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Big ET-1</th>
<th>BQ-788 + Big ET-1</th>
<th>BQ-123 + Big ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1 arterial concentration, pmol/l</td>
<td>4.68 ± 0.25</td>
<td>5.59 ± 0.68</td>
<td>7.72 ± 0.80*</td>
<td>7.86 ± 0.46*</td>
</tr>
<tr>
<td>SPF, l/min</td>
<td>0.69 ± 0.04</td>
<td>0.57 ± 0.05†</td>
<td>0.50 ± 0.05*</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Splanchnic uptake, pmol/min</td>
<td>2.43 ± 0.23</td>
<td>2.40 ± 0.25</td>
<td>2.31 ± 0.20</td>
<td>4.27 ± 0.38*</td>
</tr>
<tr>
<td>RPF, l/min</td>
<td>0.89 ± 0.08</td>
<td>0.63 ± 0.06*</td>
<td>0.57 ± 0.04*</td>
<td>0.86 ± 0.08</td>
</tr>
<tr>
<td>Renal uptake, pmol/min</td>
<td>2.57 ± 0.10</td>
<td>2.07 ± 0.30</td>
<td>2.58 ± 0.29</td>
<td>4.05 ± 0.44*</td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n = 6 \). SPF, splanchnic plasma flow; RPF, renal plasma flow. Splanchnic uptake was calculated as splanchnic fractional uptake (FSPL) \( \times \) ET-1 arterial concentration \( \times \) SPF. FSPL (0.75) was taken from Ref. 3. Renal uptake was calculated as renal fractional uptake (FR) \( \times \) ET-1 arterial concentration \( \times \) RPF. FR (0.60) was taken from Ref. 3. *\( P < 0.001 \) and †\( P < 0.05 \) compared with control.

Fig. 5. Arterial plasma ET-1 levels in the clamps: control, big ET-1, BQ-788 + big ET-1, and BQ-123 + big ET-1 at the time points corresponding to the basal state, 30 min after the start of the infusion of blocker, at 20 min of the big ET-1 infusion, and 140 min after the onset of the clamp procedure. Values are means ± SE.
ing the big ET-1 infusion after BQ-123 ($P < 0.001$ compared with the other protocols).

**DISCUSSION**

Hyperinsulinemic euglycemic clamp studies were performed with or without ETA-receptor (BQ-123) or ETB-receptor (BQ-788) blocker administration to study the mechanism behind ET-1-induced reduction of IS. It has previously been demonstrated, by using the same clamp model, that ET-1 reduces IS and that the hepatic glucose production is effectively turned off, both in the control situation as well as during ET-1 infusion (31). The present results demonstrate that cessation of hepatic glucose output still prevails after administration of the blockers, with or without big ET-1 infusion. Administration of big ET-1 was followed by reduced clearance of I, which was not reversed by ETB-, but was reversed by ETA-receptor blockade. As the I values changed and differed between the clamp protocols, the M values, representing total body glucose disposal, are not directly comparable as a measure of IS. The relatively minor changes in I values in the present study (Fig. 2) allow for correction of the M values (18). Therefore, the M/I values in the present study were used to represent IS. The divergent M/I values (Fig. 4) and significantly different values with time elucidate the mechanism by which the big ET-1/ET-1 system causes insulin resistance. In the present study, the M/I values after big ET-1 with or without prior ETB-receptor blockade were lower or unchanged with higher I values (Table 2) compared with the control clamp, demonstrating reduced IS. In contrast, the M/I values after ETA-receptor blockade + big ET-1 infusion were higher, with lower I values compared with after big ET-1 alone or in combination with ETB blockade, in accordance with higher IS after ETA blockade. In addition, neither the M/I nor I values differed from those in the control clamp when big ET-1 infusion was preceded by ETB blockade, indicating reversal of big ET-1-induced reduction of IS and I clearance. Consequently, the clamp protocols show that the big ET-1/ET-1 system reduces IS and I clearance by an ETA-receptor-mediated mechanism, whereas ETB receptors protect against ET-1-induced reduction of IS.

Although there was a tendency toward lower M/I values in the big ET-1 clamp in the present study, the average big ET-1 dose was too low to cause a statistically significant drop (Fig. 4), despite the vascular responses (Table 2), indicating conversion of big ET-1 to the vasoactive ET-1. With 8 pmol·kg$^{-1}$·min$^{-1}$ for 20 min in five subjects, it could be shown that big ET-1 reduces the M/I values (see RESULTS), further supporting previous results (22) that the big ET-1/ET-1 system affects IS.

In contrast to that in the big ET-1 clamp, the M/I values became significantly lower in the protocol when big ET-1 infusion was preceded by ETB-receptor blockade. The lower M/I values after ETB blockade + big ET-1, but not after big ET-1 infusion alone, are in accordance with previous results demonstrating that ETB receptors take part in ET-1 elimination (20) and, consequently, that blockade of the receptor would increase the local concentration of ET-1. The reduced degradation of ET-1 and ET-1 dislocated from the ETB receptors may have caused increased ETA-receptor stimulation. As NO is known to inhibit ET-1 synthesis (10), increased endogenous ET-1 formation from prepro-ET might also have occurred in a feedback manner, due to less ETB-receptor-mediated NO formation.

The results underscore the importance of intact ETB receptors to maintain IS. Interestingly, intact ETB receptors seem to be of importance in cardiovascular disease in general. Thus increased ET-1 levels are seen in patients with, e.g., atherosclerosis (26). ET-1 is presumed to play a part in atherosclerosis (24), and enhanced expression of ET-1 has been described in vascular smooth muscle (43) and atherosclerotic plaques (25, 43). Enhanced expression and activity of ET-1 converting enzyme (ECE-1) has also been described in atherosclerosis (29), further supporting increased local ET-1 synthesis. The vasoconstrictor response to ET-1 is increased in atherosclerotic vessels, e.g., coronary arteries, due to upregulation of functionally vasoconstrictive ETB receptors (14). ET-1 may thus serve as one common factor for insulin resistance and the development of atherosclerosis. During the development of atherosclerosis, it seems reasonable to assume that an early disturbance is in the function of endothelial cells, including reduced endothelial ETB-receptor numbers and/or function. If so, the result would be diminished clearance and elevated ET-1 levels. The known disturbance of endothelial NO synthesis in atherosclerosis (17, 27) and other cardiovascular disorders would be expected to increase ET-1 synthesis (10). Disturbed endothelial ETB-receptor function, including disturbed production of its second messenger NO, might initiate uncontrolled ET-1 synthesis and further increase the ET-1 levels.

After big ET-1 infusion, the arterial I level increased. This indicates reduced clearance, and not an increased release of I, as ET-1 per se has been shown only to cause a transient decrease in I (5–7). The effect was not reversed by ETB-receptor blockade, but by ETA-receptor blockade. Thus the latter not only reduces the I levels but also increases the efficiency of I and thus normalizes the M/I values in ET-1-induced insulin resistance. The change in clearance may well be related to the changes in SBF and RBF as I is extracted by these vascular beds to 40 and 25%, respectively, within a very wide range during a hyperinsulinemic euglycemic clamp (19). ETA-receptor blockers would seem to be of benefit to prevent the negative effects of ET-1 in the development of insulin resistance and atherosclerosis. The validity for patients is further supported by findings indicating that chronic ETA-receptor antagonism reduced glucose and I responses, following a meal challenge, in Zucker fatty rats, suggesting improved IS (9).

Much interest has been focused on the correlation between insulin resistance and skeletal muscle blood flow. The A-DV oxygen differences were used to determine skeletal muscle blood flow in the present study.
The rationale for this is that neither big ET-1 nor ET-1 changes pulmonary (4, 40), myocardial (32), splanchnic, or renal (3, 39) oxygen uptake. Neither splanchnic nor renal oxygen uptake is changed by ET-1 infusion during a hyperinsulinemic euglycemic clamp (31), nor did the E\(\text{T}\)_A- or E\(\text{T}\)_B-receptor blocker change the splanchnic oxygen uptake in the present study. Therefore, the skeletal muscle blood flow could be considered as inversely correlated to the A-DV oxygen differences. As opposed to the widespread use of plethysmography, which includes determination of skeletal muscle as well as skin blood flow and thus cannot differ between these, deep venous forearm blood mainly represents skeletal muscle. Our data show that big ET-1 infusion at the present dose decreased the A-DV oxygen difference by 33% (Table 2), indicating increased blood flow in skeletal muscle. This is in accordance with our laboratory’s previous studies during ET-1 (40) or big ET-1 (4) infusion, as well as in a clamp study during ET-1 infusion demonstrating increased skeletal muscle blood flow at the same time as IS decreases (31). ET-1-induced vasodilation is caused by NO and prostacyclin (16), also shown to be operating in vivo in humans (1, 2). Interestingly, the NO synthase inhibitor N-monomethyl-L-arginine caused increased IS in healthy young men during a hyperinsulinemic euglycemic clamp (11). The A-DV oxygen difference increased after E\(\text{T}\)_B blockade, indicating reduction of blood flow with a simultaneous small increase in MAP from 90 \(\pm\) 4 mmHg immediately before E\(\text{T}\)_B blockade to 93 \(\pm\) 5 mmHg after the blockade, immediately before onset of big ET-1 infusion (\(P < 0.05\)). Consequently, this vasoconstrictor response was not baroreceptor mediated but rather speaks in favor of blockade of a basal E\(\text{T}\)_B-mediated vasodilatory tonus. The subsequent big ET-1 infusion seemed to cause a smaller reduction in A-DV oxygen difference, indicating a smaller blood flow increase compared with big ET-1 infusion without blockade. Thus the net skeletal muscle basal vascular ET-1 receptor response to abluminally released and circulating ET-1, as demonstrated by up to 10-fold increases in ET-1 during ET-1 infusion (40), is vasodilatation. This is in contrast to the splanchnic and renal (7) as well as pulmonary (3) vasoconstrictive responses to less than doubled arterial ET-1 levels. The same is true for the myocardial vascular smooth muscle cells, which respond to ET-1 as well as big ET-1 with vasoconstriction with the same systemic infusion rates (32). The only vascular bed that counteracts the vasoconstrictive and blood pressure increasing effect of ET-1 is the skeletal muscle vascular bed.

The A-DV oxygen difference was unchanged by E\(\text{T}\)_A blockade, suggesting that, if there is a basal E\(\text{T}\)_A-receptor tonus, it is small. Nor did the subsequent big ET-1 infusion cause skeletal muscle vasodilatation. A tempting explanation would be that E\(\text{T}\)_A-receptor blockers also block the conversion of big ET-1 to ET-1. Alternatively, the endothelial E\(\text{T}\)_B-receptor activation may have been counteracted by some persisting E\(\text{T}\)_A-receptor activation or by an equal smooth muscle vasoconstrictive E\(\text{T}\)_B-receptor activation. This could possibly be due to increased local ET-1 concentration that is, at least partly, due to displacement from E\(\text{T}\)_A receptors. Another explanation could be that ET\(\text{T}\)_A-receptor activation inhibits the conversion of big ET-1 to ET-1, and that blockade of the receptor causes increased local ET-1 formation. In fact, results from studies on vascular smooth muscle cells showing that ET\(\text{T}\)_A-receptor stimulation induces expression of ECE-1 (41) suggest that this may be the case. Further support for this theory is the present observation that arterial ET-1 values increased during big ET-1 infusion alone but increased even more during big ET-1 infusion after E\(\text{T}\)_A-receptor blockade on a level with what is found after E\(\text{T}\)_B-receptor blockade (Fig. 5). Infused ET-1 is not only eliminated in the splanchnic and renal vascular beds (see Table 3) but also by the skeletal muscle and pulmonary vascular beds (\(F\) values corresponding to 30 and 40%, respectively; see Ref. 40). After E\(\text{T}\)_B blockade, the more elevated ET-1 levels during the big ET-1 infusion might to some degree reflect reduced skeletal muscle, splanchnic, and renal (and indirectly reduced pulmonary) clearance of ET-1 secondary to the lower blood flows compared with after big ET-1 alone (Table 2), as well as increased release of ET-1 secondary to less E\(\text{T}\)_B-receptor-mediated degradation and increased formation of ET-1, as mentioned above. After E\(\text{T}\)_A blockade, the arterial ET-1 increase was similar during big ET-1 infusion, despite intact E\(\text{T}\)_B receptors and higher SBF and RBF (Table 2), which, together with uninfluenced skeletal muscle blood flow, are in accordance with increased pulmonary blood flow too, indicating increased ET-1 elimination capacity. The 50–60% higher splanchnic and renal ET-1 uptakes (Table 3) after E\(\text{T}\)_A blockade (\(P < 0.001\)) compared with after big ET-1 alone suggest a similar increase in big ET-1 conversion to ET-1 and ET-1 spillover. As big ET-1 infusion alone caused a 7% increase in arterial ET-1 levels, the expected increase after E\(\text{T}\)_A blockade would be 11%. The increase was 35% (\(P < 0.01\)), indicating a bigger ET-1 release and indicating that other factors than the big ET-1 inflow per se had stimulated the ET-1 output. In addition, we found similar increases in ET-1 levels during ET-1 infusion with or without BQ-123 administration (unpublished observations), demonstrating that E\(\text{T}\)_A blockade per se does not alter the ET-1 transport from tissue to blood. The results are in conformity with increased conversion of big ET-1 to ET-1, and thus ECE-1 stimulation, suggesting that ET\(\text{T}\)_A-receptor activation suppresses the conversion of big ET-1 to ET-1 in a feedback manner. In addition, the results indicate that the clearance of ET-1 by E\(\text{T}\)_B receptors cannot compensate for the effect of E\(\text{T}\)_A blockade on ET-1 synthesis. Nor can E\(\text{T}\)_A receptors compensate for lack of E\(\text{T}\)_B-receptor clearance. As shown by the small increase in ET-1 levels after big ET-1 infusion alone (Table 3), intact E\(\text{T}\)_A and E\(\text{T}\)_B receptors seem to be perfectly balanced to maintain the arterial ET-1 level in healthy individuals.

Big ET-1 infusion after the E\(\text{T}\)_A blockade caused no change in SBF or RBF compared with the control clamp values. The slight drops in RBF in the present
control and ET_A blockade protocols (Table 2) are in agreement with those previously found for RBF in a control group without any intervention (7). ET_A blockade thus abolished the splanchic and renal vasoconstrictive responses to big ET-1 in conformity with the effect being ET_A-receptor mediated. These splanchic and renal responses agree with those found during ET-1 infusion with or without ET_A blockade (unpublished observations). The sensitivity of the splanchic and renal vascular beds to circulating ET-1 has previously been demonstrated by significant splanchic and renal vasoconstriction in healthy individuals with <37% increase in circulating ET-1 levels (7). No change occurred in blood flow, despite 35% higher levels of ET-1 and the indication of increased synthesis of ET-1 from big ET-1 after ET_A blockade, again suggesting that endothelial vasodilating and smooth muscle vasoconstrictive ET_B receptors balance each other in healthy subjects. The ET_B blockade per se resulted in a net vasoconstriction. This was followed by a smaller response to big ET-1, and the total reduction in SBF and RBF did not differ from the responses after big ET-1 alone, confirming that the vasoconstriction was ET_A mediated. Big ET-1 infusion caused an increase in MAP as previously reported (3, 4). The increase in MAP after big ET-1 was abolished after ET_A but uninfluenced by ET_B-receptor blockade and is, therefore, ET_A mediated. It would seem that the main purpose of ET_B receptors in these vascular beds is clearance of ET-1.

The increase in circulating ET-1 seen in the present study is smaller than the transient ET-1 increases found during moderately heavy physical exercise in humans (6). This, and the rapid elimination of big ET-1 (21) and ET-1 (39) in normal conditions, and the preserved responsiveness to ET-1 in atherosclerosis (14) demonstrate that the persistence of even slightly elevated basal ET-1 levels should be taken as an indicator of major local disturbance of ET-1 metabolism. Thus even small elevations in basal ET-1 levels should be considered for treatment. Neither the ET_A nor the ET_B receptor seems to be able to compensate for the other in counteracting elevated ET-1 levels. The adequate treatment in these conditions seems to be ET_A-receptor blockade and/or ECE-1 inhibition.

Summary and conclusion. Our data are the first to show that big ET-1/ET-1-induced reduction in IS and I clearance as well as splanchic and renal vasoconstriction can be counteracted by ET_A-receptor blockade in intact men. ET_A receptors also seem to inhibit the conversion of big ET-1 to ET-1, another new receptor mechanism not previously shown to be operating in vivo, by which ET-1 may regulate its own synthesis. The main role of ET_B receptors in healthy individuals is to keep ET-1 concentrations low, and, therefore, intact ET_B receptors are of importance for maintaining IS and I clearance.

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