Specific Endothelin ETA Receptor Antagonism Does Not Modulate Insulin-Induced Hemodynamic Effects in the Human Kidney, Eye, or Forearm

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ABSTRACT

There is evidence that hyperinsulinemia may stimulate endothelin-1 (ET-1) generation or release, which may affect diabetic vascular complications. BQ-123, a specific ETα receptor antagonist, was used to investigate if insulin-induced vascular effects are influenced by an acute ET-1 release. Two randomized, placebo-controlled, double-blind, cross-over studies were performed. In protocol 1, 12 healthy subjects received, on separate study days, infusions of BQ-123 (60 μg/min for 30 min) during placebo clamp conditions, BQ-123 during euglycemic hyperinsulinemia (3 mU/kg/min for 390 min), or placebo during euglycemic hyperinsulinemia. Fundus pulsation amplitude (FPA) was measured to assess pulsatile choroidal blood flow, and mean flow velocity (MFV) of the ophthalmic artery was measured by color Doppler imaging. In protocol 2, eight healthy subjects received, on separate study days, intra-arterial infusions of BQ-123 (32 μg/min for 120 min) during placebo or insulin clamp. Forearm blood flow was measured with bilateral plethysmography, expressing the ratio of responses in the intervention arm and in the control arm. Insulin alone increased FPA (10%, p < 0.001) and forearm blood flow (19%). BQ-123 increased FPA, MFV, and forearm blood flow ratio in the absence and presence of exogenous insulin, but this effect was not different between normo- and hyperinsulinemic conditions. ET-1 plasma concentrations were not affected by insulin. In conclusion, these data do not support the concept that hyperinsulinemia increases ET-1 generation in healthy subjects. Our results, however, cannot necessarily be extrapolated to diabetic and obese subjects.

It has been reported that plasma endothelin-1 (ET-1) concentrations are significantly increased in some patients with type I (Takahashi et al., 1990) and type II diabetes mellitus (Laurenti et al., 1997), which may contribute to the vascular complications in diabetes. Several studies demonstrated that insulin can stimulate ET-1 release in endothelial cell cultures (Metsärinne et al., 1994; Ferri et al., 1995). In addition, hyperinsulinemia elevates plasma ET-1 levels in rats (Hokanson et al., 1975), suggesting increased production and/or secretion of ET-1. This effect of insulin on circulating ET-1 levels was confirmed in obese patients (Wolpert et al., 1993) and type II diabetic patients (Ferri and de Mattia, 1995; Ferri et al., 1995) but was not seen in healthy subjects during a standard glucose tolerance test (Leyva et al., 1997) or during mild hyperinsulinemia (Metsärinne et al., 1994).

We decided to rechallenge the question of whether insulin may stimulate ET-1 release in healthy subjects for several reasons. On the one hand, studies of the effect of hyperinsulinemia on circulating ET-1 levels may be of limited value since ET-1 is predominantly a paracrine modulator of vascular tone (Webb, 1997). Hence, ET-1 plasma concentrations do not necessarily predict the level of vascular ET-1 activity. Furthermore, ET-1 concentrations are subject to considerable within-subject variability (Jilma et al., 1997). Thus, determination of ET-1 plasma levels cannot be considered an adequate approach to investigate the effect of hyperinsulinemia on local ET-1 effects. On the other hand, a previous study on the effect of insulin on ET-1 plasma levels in healthy subjects used an insulin infusion period of only 90 min (Metsärinne et al., 1994). Because ET-1 concentrations in isolated endothelial cells increase linearly with time, we decided to use a 390-min insulin infusion period to increase the likelihood to detect a possible effect of insulin on ET-1.

In an effort to answer the question of whether hyperinsulinemia affects ET-1 generation or release, we therefore measured systemic hemodynamics and ocular blood flow or forearm blood flow during normoglycemic hyperinsulinemia in

ABBREVIATIONS. ET-1, endothelin-1; FPA, fundus pulsation amplitude; MFV, mean flow velocity.
the presence or absence of blockade of vasoconstrictive ET<sub>Α</sub> receptors using the specific antagonist BQ-123 under control conditions in healthy subjects. Previous in vivo experiments suggest that this antagonist is appropriate to block vasoconstrictor effects of endogenous, i.e., paracrine (Haynes and Webb, 1994; Berrazueta et al., 1997) and exogenous ET-1 (Haynes and Webb, 1994; Schmetterer et al., 1998). The study was performed in healthy subjects to investigate whether potential ET-1 release occurs under physiological conditions.

**Materials and Methods**

**Subjects**

After approval of the study protocols by the Ethics Committee of the Vienna University School of Medicine and written informed consent, 20 healthy male subjects were studied (27.4 ± 3.0 years old, mean SD). The body mass index of all subjects was between the 20th and 80th percentiles. All volunteers passed a pre-study screening during the 4 weeks before the 1st study day, which included a physical examination and medical history, 12-lead electrocardiogram, complete blood cell count, clinical chemistry, urine drug screen, and a standardized oral glucose tolerance test. Those subjects who participated in protocol 1 also had an ophthalmic examination. Subjects with normal findings in the screening examinations and who participated in protocol 1 also had an ophthalmic examination. Subjects with normal findings in the screening examinations and ametropia of less than 3 diopters (protocol 1) were included in the trial.

**Study Design**

**Protocol 1.** The study design was a randomized, placebo-controlled, double-blind, balanced, three-way cross-over with a washout period of at least 5 days. Twelve healthy subjects received, on different study days, infusions of BQ-123 during placebo (saline) clamp conditions, BQ-123 during euglycemic hyperinsulinemia, or placebo during euglycemic hyperinsulinemia. Subjects were asked to refrain from alcohol and caffeine for at least 12 h before trial days and were studied after an overnight fast.

After an initial resting period of 45 min, the euglycemic clamps were performed according to DeFronzo et al. (1979). Each clamp was started with a primed infusion of insulin for 6 min followed by a constant infusion rate of insulin at 3 mU/kg/min over 6.5 h with a concomitant infusion BQ-123 (dose: 60 μg/min) or placebo over 30 min, commencing 6 h after starting insulin administration. Hemodynamic measurements were performed at frequent intervals in a predetermined sequence during drug administrations.

KCl was infused at a rate necessary to prevent hypokalemia. Glucose was infused at a rate necessary to maintain a constant blood glucose level of approximately 100 mg/dl. Arterialized venous blood samples were drawn for measurement of glucose concentration every 5 min from the contralateral arm placed in a heating blanket. All infusions were administered using an automatic device. Venous blood samples for analysis of endothelin-1 plasma levels were drawn at frequent intervals.

**Protocol 2.** The study was a balanced, randomized, placebo-controlled two-way cross-over with a washout period of at least 5 study days. Eight healthy subjects received on the 2 study days infusions of intra-arterial BQ-123 (dose: 32 μg/min) during placebo (saline) clamp conditions or intraretal BQ-123 during euglycemic hyperinsulinemia. Subjects were asked to refrain from alcohol and caffeine for at least 12 h before trial days and were studied after an overnight fast.

After a 20-min resting period, baseline measurements were done. Thereafter, the euglycemic insulin clamp at a dose of 3 mU/kg/min (or placebo) was started as described above. The clamping conditions lasted for 180 min, and after 60 min, the intra-arterial administration of BQ-123 was started. Measurements of forearm blood flow and of systemic hemodynamics were done at frequent intervals during drug administrations.

**Rationale for Doses.** Our own previous experiments in healthy subjects have shown that 60 μg/min BQ-123 caused no adverse clinical events but prevented the renal pressor effect of exogenous ET-1 (Schmetterer et al., 1998). The dosage of intra-arterial BQ-123 was based on a previous trial investigating the effect of this ET<sub>Α</sub> receptor antagonist on forearm blood flow (Berrazueta et al., 1997). A euglycemic insulin clamp of 1.5 mU/kg/min increased insulin levels approximately 10-fold and caused a 10% increase in ocular blood flow (Schmetterer et al., 1997b). In the present study, a dose of 3 mU/kg/min insulin was selected to cause clear-cut insulin-mediated vasodilation over a prolonged period to maximize paracrine ET-1 release.

**Methods**

**Systemic Hemodynamics.** Systolic and diastolic blood pressures were measured on the upper arm by an automated oscillometric device. Pulse rate was automatically recorded from a finger pulse-oxymetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA) (Wolzt et al., 1995).

**Ocular Hemodynamics.** In all subjects, the right eye was studied. Ocular fundus pulsation was assessed by laser interferometry as described by Schmetterer et al. (1995). In brief, the eye is illuminated by the beam of a single mode laser diode (∆ = 783 nm) along the optical axis. The light is reflected at both the front side of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. These distance changes are caused by the pulsatile inflow of blood through the arteries and by the non-pulsatile outflow through the veins. The maximum change in cornea-retinal distance is called FPA. The method has been shown to estimate the pulsatile blood flow in the choroidal vasculature (Schmetterer and Wolzt, 1998).

MFV in the ophthalmic artery was measured by color Doppler imaging. This noninvasive method is based on the backscattering of ultrasound by the formed elements in the blood vessels. Measurement of the frequency shift due to the Doppler effect yields information about the blood velocity. Peak systolic flow velocity and end diastolic flow velocity in the ophthalmic artery were assessed with a 3.25-MHz probe with pulsed Doppler device and simultaneous ECG recording (Lieb et al., 1991) (CFM 750; Vingmed Sound, Horten, Norway). From these parameters, the mean flow velocity (∫ integral of the Doppler curve/duration of the cardiac cycle) was calculated.

**Flowmetry of Forearm Blood Flow.** Subjects were in supine position with both arms on a support above the level of the right atrium. Pulse rate was measured continuously. A fine needle (27G-needle Sterican; B. Braun, Melsungen, Germany) was inserted into the brachial artery of the nondominant arm and physiologic saline infused at 1 ml/min. Subjects were allowed to acclimatize to the needle for at least 15 min before drug infusion.

Forearm blood flow was measured as described previously (Hokanson et al., 1975). In brief, mercury-in-rubber silastic strain gauges were placed on the greatest circumference of the forearms. The strain gauges were connected to plethysmographs (EC-6; D.E. Hokanson, Bellevue, WA), and traces were analyzed using the NIVP2 software (version 5.25; Hokanson). Bilateral plethysmography was used, expressing the ratio of responses in the intervention arm and in the control arm (Petrie et al., 1998). Cuffs were placed around both upper arms and inflated to 45 mmHg by a rapid cuff inflator (AG 101; Hokanson) during the measurements to occlude venous outflow. Wrist cuffs were inflated to suprasystolic pressures during each measurement to exclude circulation of the hands. Flow measurements were recorded for 9 s at 30-s intervals during drug infusion.

**Laboratory Analysis.** Glucose concentrations were measured using a glucose analyzer (Beckman Glucose Analyzer 2; Beckman Coulter, Fullerton, CA). ET-1 plasma levels were determined with a
radioimmunoassay (ET-1 RIA Kit; Peninsula Laboratories, Belmont, Belmont, CA). Plasma endothelin levels were measured after solid-phase extraction in duplicates. Intra-assay variation was less than 10%, and interassay variation was less than 20%. Insulin plasma levels were determined by routine laboratory procedures.

**Drugs Used.** BQ-123 was obtained from Clinalfa (Läufelfingen, Switzerland), and Huminsulin normal 40 IE was obtained from Eli Lilly (Fegersheim, France). Potassium chloride and 20% glucose were obtained from Leopold (Vienna, Austria).

**Data Analysis.** Data are presented as means ± S.E.M. The effect of insulin alone versus placebo conditions was analyzed with analysis of variance. The maximum effect of BQ-123 was calculated as a percentage of change of predose values during hyperinsulinemia or placebo. Differences between groups were analyzed using analysis of variance. Post hoc analysis was done with paired Student’s t tests using Bonferroni correction for multiple comparisons. A p value of <0.05 was considered significant.

**Results**

**Protocol 1.** There were no significant differences between the baseline values on the 3 trial days (Table 1). Placebo had no consistent effect on systemic or ocular hemodynamics. Insulin alone did not exert significant changes in systolic and diastolic blood pressure or pulse rate but increased FPA by 10% ± 2% (p < 0.001 versus predose and placebo) and tended to increase MFV (+13% ± 6%, p = 0.039 versus predose, Fig. 1). During hyperinsulinemia BQ-123 caused an additional effect on FPA (+8% ± 2% from insulin alone, p < 0.001 versus insulin, Fig. 1) and on MFV (+17% ± 4%, p < 0.01, Fig. 1). The effect of BQ-123 on FPA and MFV was comparable in the absence and presence of exogenous insulin (Fig. 2).

Insulin and glucose plasma levels are presented in Table 2. As expected, insulin plasma levels increased to approximately 200 μU/ml, whereas glucose plasma levels did not change. Plasma ET-1 levels had a high within-subject variability resulting in a coefficient of variation during placebo of 61%. Insulin, BQ-123, and the coinfusion of insulin with BQ-123 had no detectable effect on ET-1 concentrations (Table 3).

**Protocol 2.** There were no significant differences between the baseline values on the 2 trial days (Table 1). Intravenous BQ-123 alone did not affect systemic hemodynamics but increased forearm blood flow by 26% ± 5% (maximum effect; p < 0.001 versus baseline, Fig. 3) and increased forearm blood flow ratio by 28% ± 6%, (maximum effect; p < 0.001 versus baseline).

During hyperinsulinemia forearm blood flow increased by 19% ± 4% (p < 0.001 versus baseline), whereas forearm blood flow ratio remained unchanged (−2% ± 6%). Coadministration of BQ-123 caused an additional effect on forearm blood flow (+29% ± 7%, p < 0.001 versus predose level) and forearm blood flow ratio (+24% ± 6%, p < 0.001 versus predose level). The effect of BQ-123 on forearm blood flow and forearm blood flow ratio was comparable in the absence

![Fig. 1. Time course of ocular hemodynamic parameters (FPA and MFV) during hyperinsulinemia or placebo. The effect of coinfusion of insulin with placebo (•), insulin with BQ-123 (○), and placebo (saline) with BQ-123 (▼) are shown as means ± S.E.M. (n = 12). Drug administration is indicated by boxes.](image)

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Hemodynamic outcome variables at baseline (protocol 1: n = 12; protocol 2: n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>Insulin + Placebo</td>
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<tr>
<td>Protocol 1</td>
<td>Mean arterial blood pressure (mmHg)</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (beats/min)</td>
</tr>
<tr>
<td></td>
<td>Fundus pulsation amplitude (μm)</td>
</tr>
<tr>
<td></td>
<td>Mean flow velocity (cm/s)</td>
</tr>
<tr>
<td>Protocol 2</td>
<td>Mean arterial blood pressure (mmHg)</td>
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<tr>
<td></td>
<td>Pulse rate (beats/min)</td>
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<td></td>
<td>Forearm blood flow</td>
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<td></td>
<td>Forearm blood flow ratio</td>
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and presence of exogenous insulin (Fig. 3). Insulin and glucose plasma levels were in the same range as those seen in protocol 1 (Table 2).

**Discussion**

The main finding of our study is that the specific endothelin ET\(_A\) receptor antagonist BQ-123 caused comparable hemodynamic effects under prolonged hyperinsulinemia as compared with control conditions in healthy subjects, suggesting that high circulating levels of insulin do not stimulate systemic or local vasoconstrictor effects of ET-1 to a relevant degree. This is further supported by the lack of changes in ET-1 plasma levels in response to insulin although in a study of this sample size the sensitivity to detect small changes in plasma ET-1 levels is limited.

We have shown previously that exogenous ET-1 causes vasoconstriction in the human eye in vivo (Schmetterer et al., 1997a), which can be antagonized by administration of BQ-123 (Polak et al., 2001). Hence, it is conceivable that a relevant insulin-induced local increase of ET-1 should exert local vasoconstriction and that the specific ET\(_A\) receptor antagonist should counteract this effect. Therefore, the present investigation does not support the concept that insulin influences local or systemic ET-1 generation, release, or vasoconstrictor action. Given that hyperinsulinemia was present in the subjects under study at systemic doses over 6 h in protocol 1 and that BQ-123 alone was equally effective under placebo, an acute counterregulatory potential of ET-1 on vascular tone, which could have been missed in previous studies due to the short infusion period used (Wolpert et al., 1993; Metsä-Kärne et al., 1994; Katsumori et al., 1996), is very unlikely. Therefore, our results suggest that data from animal experiments showing markedly elevated plasma ET-1 levels in hyperinsulinemic rats (Hu et al., 1993) cannot be extrapolated to humans. This is also supported from the results of protocol 2, where vasodilator effects of BQ-123 were not altered during systemic hyperinsulinemia. Moreover, in this protocol, a different vascular bed was studied, and a different time schedule was used.

It is apparent that the dose of BQ-123 was adequate to block endogenous ET-1 and altered basal vascular tone as indicated by the significant increase in FPA and MFV in the ophthalmic artery and forearm blood flow ratio. Conceivably, effects of ET\(_A\) receptor blockade were detectable in the choroid, in the ophthalmic artery supplying the choroids, and in the forearm. This is in agreement with previous

### Table 2

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Insulin or Placebo</th>
<th>BQ-123 or Placebo</th>
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</thead>
<tbody>
<tr>
<td>0 min (baseline)</td>
<td>390 min</td>
<td>345 min</td>
</tr>
<tr>
<td>Insulin plasma levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin + placebo</td>
<td>6.5 ± 0.9</td>
<td>217.0 ± 31.4</td>
</tr>
<tr>
<td>Insulin + BQ-123</td>
<td>6.3 ± 0.8</td>
<td>202.5 ± 27.3</td>
</tr>
<tr>
<td>Placebo + BQ-123</td>
<td>6.1 ± 0.7</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>Glucose plasma levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin + placebo</td>
<td>84.4 ± 2.3</td>
<td>89.3 ± 3.0</td>
</tr>
<tr>
<td>Insulin + BQ-123</td>
<td>82.3 ± 2.8</td>
<td>90.0 ± 3.3</td>
</tr>
<tr>
<td>Placebo + BQ-123</td>
<td>84.3 ± 1.9</td>
<td>82.7 ± 2.4</td>
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### Table 3

<table>
<thead>
<tr>
<th>Insulin or Placebo</th>
<th>BQ-123 or Placebo</th>
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<tbody>
<tr>
<td>0 min (baseline)</td>
<td>345 min</td>
</tr>
<tr>
<td>Insulin + placebo</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Insulin + BQ-123</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Placebo + BQ-123</td>
<td>4.3 ± 1.9</td>
</tr>
</tbody>
</table>
reports indicating that endogenous generation of ET-1 maintains vascular tone of normal subjects in some vascular beds (Haynes and Webb, 1994; Haynes, 1995; Berrazueta et al., 1997; Schmetterer et al., 1998).

A limitation of the present study is that we cannot rule out possible effects of exogenous insulin on ET receptors. However, we have shown previously that in humans ET-1 exerts its vasoconstrictor effect in the kidney and the eye predominantly by its action on the ETA receptor (Schmetterer et al., 1998; Polak et al., 2001). In addition, there is evidence that in humans BQ-788, a specific ETB receptor antagonist induces possible effects of exogenous insulin on ET B receptors. However, we have shown previously that in humans ET-1 exerts a specific ETB receptor antagonist induces peripheral vasoconstriction, indicating that endogenous ET-1 rather favors vasodilation at this receptor subtype in the peripheral vasculature (Haynes, 1995).

In conclusion, our experiments do not support the concept that hyperinsulinemia increases ET-1 generation or functional activity in healthy subjects. This was shown from a study using intravenous BQ-123 assessing blood flow in the eye and a study using intra-arterial BQ-123 assessing blood flow in the forearm. Although this holds true during physiological conditions, our results cannot necessarily be extrapolated to diabetic or obese subjects.

References

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