Circulating Levels of Endothelin-1 During Acute Hyperinsulinemia in Patients With Essential Hypertension Treated With Type 1 Angiotensin Receptor Antagonist or Placebo

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Insulin and angiotensin II (Ang II) are involved in the regulation of endothelin-1 (ET-1). This study investigates their possible influence on plasma levels of ET-1 in humans. Twenty patients with essential hypertension were included in a randomized, double-blind, placebo-controlled crossover study of 4 weeks' treatment with losartan, a selective type 1 angiotensin (AT_1) receptor antagonist. The effect was evaluated in the fasting state and during acute hyperinsulinemia physiologically induced by oral glucose ingestion (OGTT) and by euglycemic glucose clamp. Losartan lowered blood pressure significantly, but did not influence plasma levels of ET-1 in the fasting condition (5.2 ± 0.2 fmol/mL on placebo and 5.6 ± 0.3 fmol/mL after losartan treatment). During both models of acute hyperinsulinemia, there was a significant decrease in plasma ET-1. In the OGTT the mean values after placebo treatment decreased from 5.2 ± 0.2 fmol/mL at time 0 to 4.7 ± 0.4 ($P = .001$) and 4.0 ± 0.5 ($P = .001$) at 60 and 120 minutes, respectively. During the clamp the mean ET-1 values decreased from 5.7 ± 0.4 fmol/mL at time 0 to 4.6 ± 0.2 ($P < .001$) and 4.3 ± 0.3 ($P = .006$) at 60 and 120 minutes, respectively. No differences in these profiles occurred after losartan treatment. Significant inverse correlation between fasting levels of ET-1 and insulin sensitivity index was found, $r = -.51$, $P = .003$. In conclusion, losartan did not influence the circulating levels of ET-1 in basal condition or during acute hyperinsulinemia, whereas a significant decrease in plasma ET-1 occurred during acute hyperinsulinemia. A significant inverse correlation demonstrated between basal levels of plasma ET-1 and the insulin-stimulated glucose uptake could point to a possible regulatory influence of ET-1 production on glucose metabolism or vice versa.

INSULIN RESISTANCE and hyperinsulinemia are associated with hypertension, although the mechanisms of interaction still are not definitely clarified.[1,2] Furthermore, associations between hypertension and endothelin-1 (ET-1), the powerful endogenous vasoconstrictor produced mainly in the endothelium[3] but also in smooth muscle cells,[4] have also been suggested.[5,6] Elevated levels of ET-1 have been reported in patients with essential hypertension,[5,7] and similar associations have been found in experimental models of hypertension.[8] However, the pathophysiological role of ET-1 in hypertension is still controversial.[9]

The regulatory mechanisms of ET-1 production are not fully understood. It has been demonstrated that ET-1 synthesis and secretion are stimulated by vasoactive substances such as vasopressin and angiotensin II (Ang II)[10-12] and also by catecholamines.[3,12] In addition, insulin has been proposed as a mediator of ET-1 production, based on results from bovine and porcine endothelial cell culture studies, showing a stimulatory effect of insulin on ET-1 release.[13,14] This effect has been reproduced in some human endothelial cell culture studies,[15] but not all.[16] However, elevated levels of ET-1 have been found in insulin-treated diabetic patients,[17] as well as patients with non–insulin-dependent diabetes mellitus (NIDDM).[18] Furthermore, increased levels of ET-1 have been demonstrated during acute hyperinsulinemia in normotensive NIDDM patients and in obese hypertensive males, using a euglycemic glucose clamp model.[15,19]

Recent data have also demonstrated that increased blood pressure per se enhances the release of ET-1 from cultures of human endothelial cells.[20]

According to the above-mentioned associations, our hypothesis was that treatment with Ang II receptor antagonists may reduce circulating levels of ET-1 either directly or by decreasing blood pressure. Furthermore, acute hyperinsulinemia may increase circulating ET-1, which could be counteracted with Ang II receptor antagonism.

Therefore, in the present study, we investigated the effects of acute hyperinsulinemia on ET-1 levels in patients with essensial hypertension before and after treatment with the selective Ang II type 1 angiotensin (AT_1) receptor antagonist losartan or a placebo. Two different models of hyperinsulinemia were used: one euglycemic and one with physiological hyperglycemia.

SUBJECTS AND METHODS

Study Design

A randomized, double-blind, placebo-controlled crossover study of 4-week treatment periods with the Ang II AT_1 receptor antagonist losartan (Cozaar; Merck Sharp & Dohme Norge, Drammen, Norway) was performed. The dosage of losartan (or placebo) was increased from 50 to 100 mg (1 to 2 tablets) after 2 weeks if the diastolic blood pressure was still higher than 90 mm Hg.

Subjects

Twenty patients (seven women and 13 men; mean age, 48 years; range, 24 to 79) with essential hypertension (blood pressure < 140/95 mm Hg, and self-assessed home diastolic blood pressure > 90 mm Hg) were recruited from physicians in general practice. Seventeen patients were previously untreated for hypertension, and three had been without medication for the last 3 to 7 weeks. All patients were without other medication, except one patient treated with 2 mg estradiol and 1 mg thyroxine daily, kept constant under the study duration. After randomization, the patients began treatment with losartan or placebo. After 4 weeks' treatment, the regimens were changed without any washout period.

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The study was approved by the Regional Ethics Committee, and all participants provided written informed consent.

Procedures

All patients were examined with the oral glucose tolerance test (OGTT) 3 or 4 days before the glucose clamp examination at the end of each 4-week treatment period. Thus, all blood samples were collected after each 4-week period. All procedures began with the patients in a fasting state between 8 and 9 AM. For technial reasons, blood sampling for ET-1 determination was not successful in three patients on one occasion each during the clamp procedures.

For the OGTT, after 15 minutes' rest, the first blood sample was collected from an antecubital vein before the patients were administered 75 g glucose monohydrate in 400 mL water orally. Venous blood samples were drawn 1, 2, and 3 hours after glucose intake. The patients were in a quiet state during the study.

The glucose clamp procedure is described in detail elsewhere.[21] In brief, before starting, arterialization of the venous blood was performed. On the right arm, an antecubital vein was cannulated with a short teflon catheter (Venflon 17G; Viggo, Helsingborg, Sweden) and the arm was placed in a heating sleeve (Thermal Vascular Dilatator; Swetron, Veddesta, Sweden) with the temperature set to 52°C. This catheter was used for sampling arterialized venous blood during the procedure. On the left arm, an antecubital vein was cannulated for later infusion of insulin and glucose. The patients rested supine for 20 minutes before baseline blood collection.

The euglycemic-hyperinsulinemic glucose clamp was performed according to the method described by DeFronzo et al[22] as previously detailed.[21] Insulin was infused at a fixed rate of 1 mU $\cdot$ kg^{-1} $\cdot$ min^{-1}. Blood samples were taken every 5 minutes for determination of blood glucose using the Reflolux II (Boehringer, Mannheim, Germany). In addition, blood samples were collected at baseline and after 30, 60, 100, and 120 minutes for determination of insulin, catecholamines, and ET-1.

The glucose disposal rate ([GDR] milligrams per kilogram per minute) was calculated from the amount of glucose infused from 100 to 120 minutes, the mean serum insulin concentration was determined from the two samples obtained during the last 20 minutes, and the insulin sensitivity index (GDR/I) was calculated from the GDR in relation to the mean serum insulin concentration.

Blood Sampling Procedures and Laboratory Methods

Serum glucose was determined enzymatically with the glucose dehydrogenase method (Hoffmann-LaRoche, Basel, Switzerland), and insulin by radioimmunoassay using a specific antibody from Linco Research (St Louis, MO), with an intraassay coefficient of variation less than 9% at all levels. Plasma adrenaline and noradrenaline were determined by the radioenzymatic technique of Peuler and Johnson as previously detailed.[23] The interassay coefficient of variation was 10% for both.

Plasma for determination of ET-1 was prepared in ice-chilled Vacutainer (Becton Dickinson, Plymouth, UK) tubes containing 0.34 mol/L EDTA-K_3, kept on ice, and centrifuged within 15 minutes at 4°C and 4,000 $\times$ *g* for 15 minutes. All samples were kept at −70°C until analysis, and all samples from the same patient at different time points were analyzed at the same time. ET was extracted from 2 mL plasma acidified with 6 mL 4% acetic acid on Sep-Pak C18 cartridges (Millipore; Waters Chromatography Division, Milford, MA). The cartridges were pretreated with 10 mL 85% ethanol and 10 mL 4% acetic acid. After washing with 5 mL distilled water, ET was eluted with 4 mL 4% acetic acid in 86% ethanol, evaporated to dryness with nitrogen at 37°C, and reconstituted in 500 μL 0.02-mol/L borate buffer. Radioimmunoassay was performed using the Endothelin 1-21 specific [^{125}I] assay system (RPA 555; Amersham International, Cardiff, England). This Endothelin 1-21 specific assay system has no cross-reactivity with big ET, 144% cross-reactivity with ET-2, and 52% cross-reactivity with ET-3. Recovery of unlabeled ET-1 (synthetic) (Amersham International) was 61% ± 6% when the plasma was spiked with 0 and 4.9 fmol/mL. The ET-1 values presented are not corrected for loss during extraction. The interassay coefficient of variation was 8%, and intraassay variation was 5%.

Statistics

For differences between treatment regimens, the Mann-Whitney rank-sum test was used. For differences from baseline to various time points, Student's *t* test for paired data was used. For correlation analysis, the Pearson correlation coefficient was estimated. A two-tailed *P* value of .05 or less was considered statistically significant. Data are presented as the mean ± SEM.

RESULTS

Basal State

Supine blood pressure after 4 weeks of treatment was satisfactorily reduced with losartan (134 ± 4/83 ± 3 *v* 146 ± 3/90 ± 3 mm Hg for placebo, $P < .001$ for systolic and $P < .03$ for diastolic blood pressure).

In the basal fasting state, there were no differences between the two treatment regimens with regard to glucose, insulin, or catecholamines. Neither was there any significant difference in circulating ET-1 levels (5.6 ± 0.3 fmol/mL on losartan *v* 5.2 ± 0.2 fmol/mL on placebo). The results were similar in venous and arterialized blood (Table 1, venous blood samples taken before start of OGTT).

Response to Insulin

All patients were good responders to glucose intake in the OGTT. There were no significant differences in the measured values for insulin 1 hour after intake between the treatment regimens (910 ± 465 pmol/L on losartan and 833 ± 436 pmol/L on placebo, $P = .50$). The mean level of insulin during the last 20 minutes of the clamp procedure was 1,011 ± 59 pmol/L on losartan and 927 ± 52 pmol/L on placebo ($P = .09$). The GDR was 6.7 ± 0.6 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} on losartan and 6.2 ± 0.5 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} on placebo ($P = .41$), whereas the calculated GDR/I was 7.0 ± 0.8 arbitrary units on both regimens. There were no differences in the change from baseline between

Table 1. Blood Pressure and Glucose, Insulin, and Hormone Levels in the Basal State After 4 Weeks' Treatment With Losartan or Placebo

Parameter	Placebo	Losartan	*P*
Blood pressure (mm Hg)			
Diastolic	146 ± 3	134 ± 4	<.001
Systolic	90 ± 3	83 ± 3	<.03
Glucose (mmol/L)	5.3 ± 0.1	5.1 ± 0.1	NS
Insulin (pmol/L)	122 ± 7	137 ± 14	NS
Epinephrine (nmol/L)	0.43 ± 0.09	0.42 ± 0.08	NS
Norepinephrine (nmol/L)	1.89 ± 0.18	2.00 ± 0.18	NS
ET-1 (fmol/mL)	5.2 ± 0.2	5.6 ± 0.3	NS

NOTE. Results are the mean ± SEM from venous blood samples (n = 20). *P* values refer to differences between the treatment regimens.

Abbreviation: NS, nonsignificant.

treatment regimens with regard to catecholamines in either of the hyperinsulinemic models. Neither was there any significant increase in catecholamines from baseline to various time points during any of the procedures. Blood pressure was not altered during the clamp procedure in any of the treatment regimens (Fig 1).

ET-1 levels during both hyperinsulinemic states are shown in Fig 2. During the OGTT, there was a significant decrease in ET-1 after 1 hour with both losartan (from 5.6 ± 0.3 to 4.5 ± 0.2 fmol/mL, $P = .01$) and placebo (from 5.2 ± 0.2 to 4.7 ± 0.4 fmol/mL, $P = .001$), which was principally sustained throughout the 3-hour test period. The lowest values were obtained after 2 hours (4.0 ± 0.4 fmol/mL on both treatment regimens; Fig 2A). The same profiles were seen during the glucose clamp procedure, where the significant decrease in ET-1 had already occurred after 30 minutes (from 5.6 ± 0.2 to 4.2 ± 0.3 fmol/mL on losartan, $P = .002$, and from 5.7 ± 0.4 to 4.6 ± 0.2 fmol/mL on placebo, $P = .002$) and was thereafter sustained throughout the 2-hour test period (Fig 2B). There were no significant differences between treatment regimens with regard to changes in ET-1 values from baseline to different time points.

Correlations

The correlation coefficient (r) between basal levels of ET-1 and insulin was .50, which was not statistically significant ($P = .096$), whereas correlations between basal levels of ET-1 and the GDR and GDR/I were −.34 ($P = .054$) and −.51 ($P = .0026$), respectively, when all observations in the crossover model were used ($N = 37$). Using data with patients on placebo only (n = 18), the correlations still were statistically significant (ET-1 v GDR, $r = -.36$, $P = .042$; ET-1 v GDR/I, $r = -.52$, $P = .0050$). Figure 3 shows the correlation between basal arterialized plasma ET-1 and the GDR/I for the total number of observations.

DISCUSSION

In the present study, we investigated the effects of acute hyperinsulinemia on circulatory levels of ET-1 in patients with essential hypertension treated with the Ang II AT_1 receptor antagonist losartan or placebo in a crossover design. With this study design, the potential influence of interindividual differences is avoided. According to the pharmacokinetics of losartan, no carryover effect of the drug after 4 weeks could be expected.

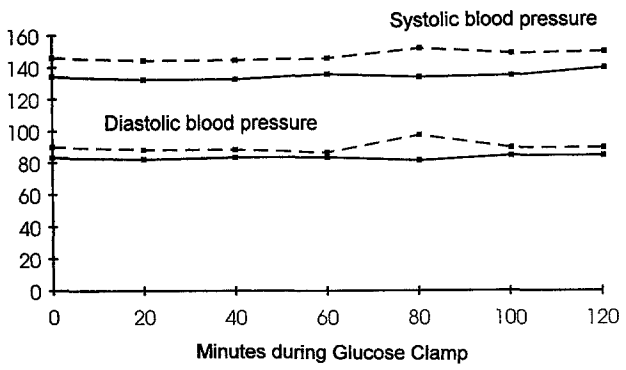

Fig 1. Mean values for systolic and diastolic blood pressure (mm Hg) during the glucose clamp. (- - -) Placebo; (—) losartan.

Blood pressure was satisfactorily reduced by losartan, indicating that AT_1 receptor antagonism was effectively achieved. We have previously reported on the neutral effects of losartan with regard to fibrinolysis, catecholamines, and insulin sensitivity.[24,25]

We could not demonstrate any effect of losartan on fasting levels of ET-1 despite the reduction in blood pressure in the present study. This finding contributes to the discussion of the possible association between circulating levels of ET-1 and hypertension and the pathophysiological role of ET-1 in hypertensive patients.[6,9] Since it has been reported that ET-1 secretion is stimulated in vitro by Ang II,[10-12] an effect of Ang II receptor blockade on ET-1 levels could be expected. This may have been produced not only by the reduction in blood pressure per se,[20] but also potentially by a reduction of ET-1 secretion induced by AT_1 receptor blockade with losartan. Chua et al[26] have demonstrated the involvement of this AT_1 receptor in ET-1 regulation in a study with losartan, although it was performed in vitro in rat heart endothelial cells. However, the in vivo experimental studies by Kohno et al[8] in rats did not show any effect of Ang II injection on the plasma level of ET-1. The lack of effect of losartan on circulating ET-1 levels in our study could be due to the selection of patients who were mildly hypertensive. However, there still could be an effect on ET-1 production that is not detectable in the plasma compartment, since ET-1 secretion from endothelial cells occurs mostly abluminally, toward the vascular wall.[27] Thus, the concentration found in the circulation may not reflect the vascular production.

As previously reported,[24] there was no effect of losartan on epinephrine or norepinephrine, both potentially regulators of ET-1 production.[3,12]

ET-1 production can be stimulated by insulin, as shown with in vitro cell culture studies[13,14] and with animal models in which rats implanted with a subcutaneous insulin pellet showed increased plasma ET-1.[14] Some studies in humans have demonstrated increased plasma levels of ET-1 in both insulin-dependent diabetic and NIDDM patients.[17,18]

However, we could not demonstrate any increase in circulating levels of ET-1 during acute hyperinsulinemia either with the model of physiologically increased insulin levels (OGTT) or with the glucose clamp technique. In contrast, a statistically significant decrease occurred after 1 hour in the OGTT and after 30 minutes in the glucose clamp model. To our knowledge, no reports have been published suggesting circadian variations of plasma ET-1, which might have explained our results. Blood pressure did not change during the glucose clamp, again pointing to the controversial association between blood pressure and ET-1.

The significant decrease in plasma ET-1 levels during both acute hyperinsulinemic models was unexpected, and is not in agreement with our hypothesis and results reported by others.[15,19,28] This could be due to differences in the patient populations. The patients in our study were mildly hypertensive and moderately insulin-resistant, with normal levels of fasting insulin.

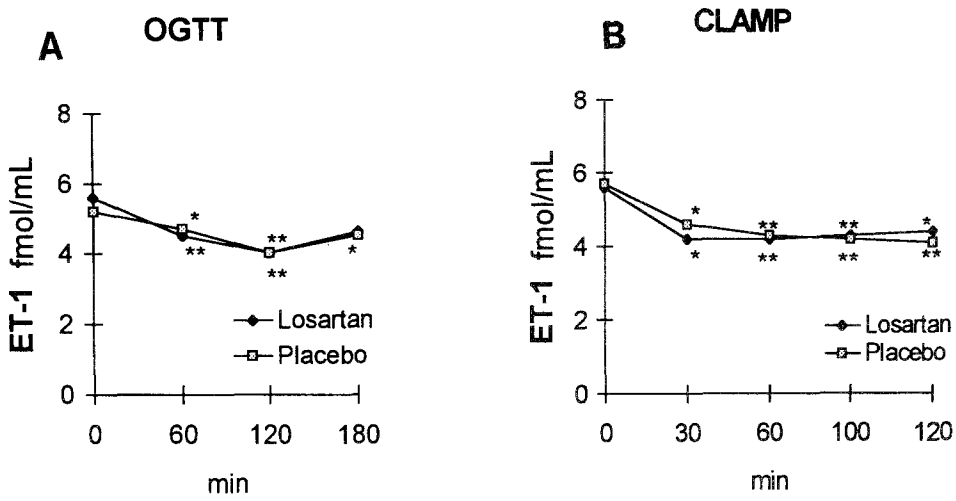

Fig 2. Mean circulating levels of ET-1 during the OGTT (A) and glucose clamp (B) after placebo or losartan treatment. *$P < .05$, **$P < .01$ (2-tailed tests, difference *v* baseline).

Glucose delivery to the peripheral tissues is important for determination of insulin-mediated glucose uptake.[29] The GDR/I provides the best estimate of glucose metabolism in peripheral tissues, as it takes into account both the amount of glucose taken up by the tissues and the prevailing insulin concentration during the clamp, ie, adjusting for differences in insulin clearance. Thus, the negative correlations demonstrated between ET-1 and the GDR and GDR/I, also found by Ferri et al,[19] could suggest that glucose uptake and metabolism rather than the direct influence of insulin per se are involved in ET-1 production. However, an inhibitory influence of ET-1 on insulin sensitivity in skeletal muscle would be more likely. Of course, caution should be taken when interpreting correlations less than .55, although they are statistically significant.

During the glucose clamp, peripheral glucose uptake and metabolism are maximally stimulated, and the observed decrease in ET-1 during the procedure could also indicate that glucose uptake is involved in the regulation of ET-1 production or vice versa.

Reduced ET-1 production in hyperglycemic conditions has been demontrated in cell culture studies.[13,15] However, since the same profiles of ET-1 occurred in the hyperinsulinemic models of both hyperglycemia and euglycemia in the present study, it should be due to insulin or insulin-related mechanisms and not to glucose per se. Apparently, the present results are in conflict with the findings of Zimmerman and Maymind,[30] who found that ET-1 infusion in rats was associated with a decrease in blood glucose. However, if hyperinsulinemia occurs as a consequence of glucose intake or infusion as in our models, the effect of ET-1 on glucose homeostasis observed by Zimmerman and Maymind[30] would probably not be needed, and a downregulation of this protein might be physiologically important.

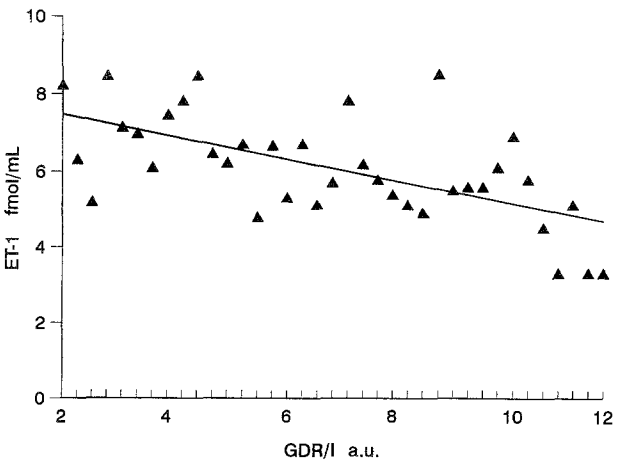

Fig 3. Correlation between the GDR/I and the fasting level of ET-1 ($r = -.51$, $P = .0026$) in patients after both treatment regimens.

It seems reasonable to suggest that the mild vasodilation that occurs during insulin infusion[31] might contribute to the decrease in ET-1 levels. In cell culture studies,[32] as well as human in vivo models of acute hyperinsulinemia,[33,34] it has been shown that vasodilation due to insulin is mediated through production of endothelium-derived nitric oxide. (NO). Furthermore, there is evidence for an inhibitory effect of endothelium-derived NO on the production of ET-1 from cell culture studies.[35,36] The discrepancy between the results of our study and results reported by Ferri et al[15,19] and Wolpert et al[28] might again be due to the patient population, as it has been shown that the increase in NO production during acute hyperinsulinemia differs in relation to the degree of insulin resistance.[34] It should also be emphasized that the effects of insulin on endothelial vasodilator pathways in acute hyperinsulinemia may be different from the effects in chronic hyperglycemia and insulin resistance.[32]

In summary, we have demonstrated a neutral effect of treatment with losartan, a selective Ang II AT_1 receptor antagonist, on plasma levels of ET-1 in the fasting condition and during acute hyperinsulinemia in patients with essential hypertension. We have also shown a significant decrease in circulating levels of ET-1 during both physiologically induced acute hyperinsulinemia (OGTT) and a euglycemic glucose clamp. Furthermore, we have demonstrated a significant inverse correlation between basal plasma ET-1 levels and glucose uptake and the insulin sensitivity index, pointing to a possible regulatory influence of ET-1 production on glucose metabolism or vice versa.

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