Physiological Hyperinsulinemia Is Not Associated With Alterations in Venous Plasma Levels of Endothelin-1 in Healthy Individuals

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Elevations in circulating levels of both endothelin-1 (ET-1) and insulin are found in coronary heart disease and chronic heart failure. Although several studies have shown that insulin can stimulate ET-1 release from endothelial cell cultures, in vivo studies have yielded equivocal results. We sought to determine whether endogenous insulin at physiological concentrations leads to alterations in venous plasma ET-1 levels in healthy subjects. In addition, we investigated the effects of physiological and supraphysiological doses of insulin on the release of ET-1 from human umbilical vein endothelial cells (HUVECs) in vitro. In the in vitro experiment, ET-1 and insulin levels were measured during an intravenous glucose tolerance test (IVGTT) in 10 healthy subjects. In the in vitro experiment, HUVECs were incubated in the absence of serum and with different concentrations of insulin (25 pmol/L to 1 μmol/L) for 4 hours before measurement of secreted ET-1. The in vivo study showed no significant alterations in venous plasma ET-1 levels during IVGTTs (maximum plasma insulin, 616.9 ± 147.0 pmol/L [mean ± SEM]). In the in vitro experiment, increases in ET-1 release were observed under serum-free conditions at 100 pmol/L (physiological) and 1 μmol/L (supraphysiological) insulin (ET-1, 22.4% and 46.4% higher than control cultures, respectively, both $P < .05$). Our results show that insulin at physiological concentrations does not alter plasma ET-1 levels in healthy individuals, but does stimulate its secretion from vascular endothelial cells in vitro. This may have implications for the study of elevated ET-1 in hyperinsulinemic states.

INCREASED levels of endothelin-1 (ET-1), the most potent natural vasoconstrictor, have been found in patients with coronary heart disease,[1-3] essential hypertension,[4,5] chronic heart failure,[6] and diabetes mellitus.[7,8] Hyperinsulinemia occurs in association with all of these conditions.[9,10] Despite considerable objections,[11] the concept of insulin as a pathogenetic factor[9] cannot be readily rejected. Plasma insulin levels have emerged as a predictor of coronary heart disease in five prospective studies,[12-16] independently of known confounders. Because ET-1 exerts a wide variety of effects on the vasculature,[17,18] demonstration of a positive association between insulin and ET-1 could provide a pathogenetic link between hyperinsulinemia and cardiovascular disorders.

Several studies have shown that insulin increases the release of ET-1 from endothelial cells from a variety of species,[19,20] including humans,[21] suggesting that insulin modulates ET-1 secretion. This association has also been investigated in vivo, albeit with conflicting results.[21,22] In this study, we sought to clarify whether physiological rather than supraphysiological levels of insulin significantly modulate venous plasma ET-1 levels in healthy individuals. In addition, we investigated the dose of insulin required to stimulate ET-1 production in normal human endothelial cells in vitro.

SUBJECTS AND METHODS

In Vivo Study

Ten healthy subjects (eight males and two females) were assessed in our metabolic day ward. Participants were asked to consume more than 200 g/d carbohydrate in their diet for 3 days before the visit, to have fasted for 12 hours, and to have refrained from smoking on the morning of the test. After resting for 15 minutes in a semirecumbent position, systolic and diastolic blood pressure was measured by a cuff method with a mercury sphygmomanometer. First- and fifth-phase Korotkoff sounds were recorded. A cannula was inserted into an antecubital vein in one arm for sampling, the arm having been previously rested on a heating pad to assist blood flow. Blood samples were taken to determine fasting plasma glucose, insulin, and plasma ET-1 concentrations. A further sample was taken for repeat measurement of fasting plasma glucose and insulin concentrations. The participant then underwent an intravenous glucose tolerance test ([IVGTT] 0.5 $g \cdot kg^{-1}$ body weight dextrose administered as a 50% solution) with sampling for plasma glucose, insulin, and ET-1 at 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes after injection of the glucose solution.

In Vitro Study

Cell cultures. Human umbilical cords were donated fresh from a local hospital after approval by the local Ethics Committee. Endothelial cells were extracted from the veins essentially according to a previously published protocol.[23] Cultures in passages 1 to 3 were used for experiments. Cultures were checked by phase-contrast microscopy for alterations in morphology both during routine culture and before and after experimentation.

Experimental design. Cells were plated onto 2% (wt/vol) gelatin-coated 24-well Falcon plates (Marathon Laboratory Supplies, London, UK) and grown to confluence (100,000 cells/cm^2). Supernatants were aspirated, and the cultures were washed in M199 before addition of media (M199 + 0.5% vol/vol BSA) plus 25 pmol/L bovine insulin (control) at 0.5 to 1 mL/10 cm^2. Insulin was added at concentrations up to 1 μmol/L. Cultures were incubated for 4 hours. Supernatants were harvested and stored at −20°C until assayed.

Laboratory Determinations

Plasma glucose was determined on the same day using glucose oxidase procedures with aminophenazone.[24] Plasma insulin concentrations were measured on samples stored at −20°C, using a radioimmunoassay procedure.[25] Within- and between-batch precision was monitored throughout the study using frozen plasma and serum pools and commercially available lyophilized sera, and by participation in national quality-assurance schemes.

ET-1 assays. For the in vitro study, ET-1 was determined using a two-site immunoenzymetric assay (Amersham Life Sciences, Amersham, UK) based on a highly specific well-characterized antiserum. The assay was performed according to the manufacturer's protocol. Intraas-

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say and interassay coefficients of variance were found to be 3.6% (320 pmol/L, n = 6) and 16.5% (160 pmol/L, n = 6), respectively. Given the low levels of ET-1 normally present in human plasma, a highly sensitive radioimmunoassay was used for measurement of ET-1 levels in the in vivo study, as previously described.[26]

RESULTS

In Vivo

Plasma insulin concentrations increased from 58.6 ± 11.7 pmol/L (mean ± SEM) at baseline to a maximum of 616.9 ± 147.0 pmol/L at 3 minutes. Plasma glucose increased from 5.03 ± 0.11 mmol/L at baseline to 23.95 ± 1.85 mmol/L at 3 minutes. Plasma ET-1 concentrations, which were 1.06 ± 0.20 pmol/L at baseline, did not change significantly in response to changes in the concentration of insulin or glucose throughout the course of the IVGTT (Fig 1).

In Vitro

Dose-dependent increases in ET-1 release were observed under serum-free conditions at 100 pmol/L (physiological) and 1 μmol/L (supraphysiological) insulin (ET-1, 22.4% and 46.4% higher than control cultures, respectively, both $P < .001$) (Fig 2).

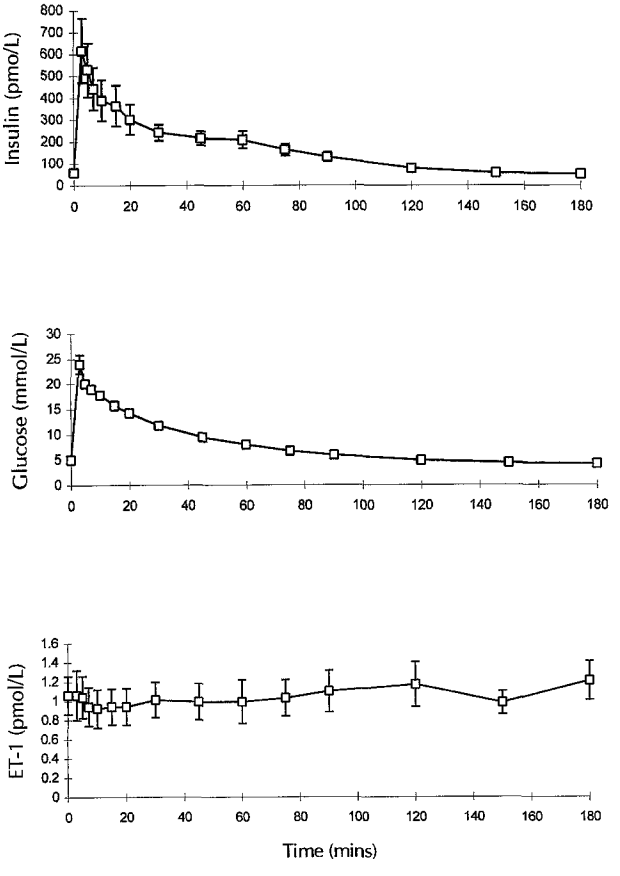

Fig 1. Plasma insulin, glucose, and ET-1 levels during IVGTTs in 10 healthy subjects. Data are the mean ± SEM.

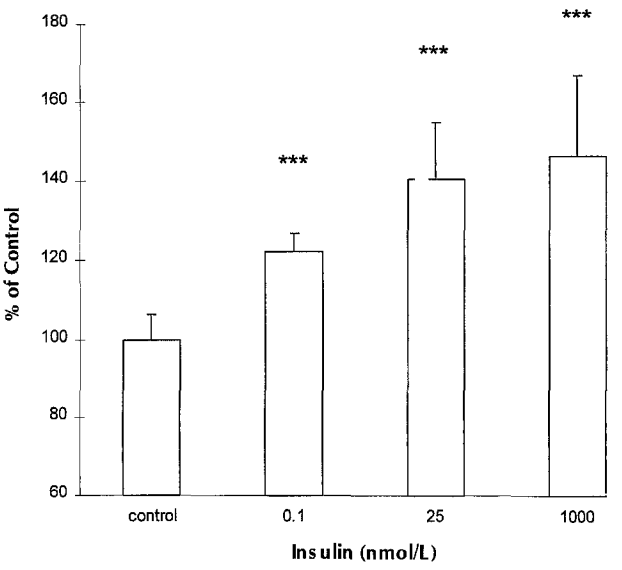

Fig 2. Effect of increasing insulin concentration on secretion of ET-1 by HUVECs in vitro. Data are presented as a % of control values. ***$P = .001$.

DISCUSSION

In this study, we have shown that insulin is capable of stimulating ET-1 secretion from human umbilical vein endothelial cells (HUVECs) in vitro. This accords with previous studies in which insulin has been shown to stimulate ET-1 gene expression[20,27] and release[19-21] in vascular endothelial cells. In the present study, significant elevations in ET-1 release from HUVECs occurred at all concentrations of insulin tested. In two previous studies,[19,21] high doses of insulin were required to stimulate release of ET-1 from endothelial cells. Our observation of a significant increase in ET-1 release at an ambient concentration of 100 pmol/L suggests that physiological concentrations should enhance ET-1 secretion in vivo.

However, we failed to detect an increase in venous plasma ET-1 in response to physiological hyperinsulinemia in healthy subjects. This coincides with the findings of Metsärinne et al,[21] who showed that 10-fold physiological levels of exogenously administered insulin failed to elicit an increase in venous plasma ET-1. In this study of healthy subjects, venous plasma ET-1 was only determined at 2 hours of the glucose and insulin infusions, and a transient increase in ET-1 therefore could not be excluded. This might be expected, considering that ET-1 mRNA can increase within minutes in response to insulin.[27] The findings of the present study using multiple sampling during IVGTTs indicate that such a transient effect does not occur. However, we cannot exclude the possibility that elevations in plasma ET-1 occur in response to prolonged hyperinsulinemia.

Our findings are in contrast to those of Ferri et al,[22] who found an increase in plasma ET-1 in response to exogenously administered insulin. However, the study involved euglycemic-hyperinsulinemic clamps, whereas the present study involves IVGTTs and healthy subjects. A possible explanation for our contrasting findings may relate to the different techniques used to achieve hyperinsulinemia. In contrast to the IVGTT, hyperinsulinemic clamps involve a state of constant hyperinsulinemia

in which glucose disappearance is progressively increasing. As pointed out by Prager et al,[28] the sustained hyperinsulinemia and the high rate of glucose disappearance achieved during hyperinsulinemic clamps seldom occur in normal physiology. As a further explanation for our findings, insulin may only modulate venous plasma ET-1 levels in disease states such as obesity, diabetes mellitus, or hypertension.

Failure to demonstrate elevations in circulating ET-1 in response to endogenous insulin may relate to the fact that up to 80% of ET-1 may be sequestered by the lungs.[29,30] In addition, most ET-1 secreted by endothelial cells is normally secreted toward the abluminal rather than the luminal side.[31] Thus, studies on putative links between insulin and ET-1 in vivo need to consider that plasma ET-1 levels are governed by the balance between abluminal and luminal secretion, and plasma clearance. We therefore conclude that insulin is a modulator of ET-1 secretion in vascular endothelial cells, but that this phenomenon is not reflected in venous plasma ET-1 levels in healthy individuals.

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