Endothelin-1 Decreases Glucose, Inhibits Glucagon, and Stimulates Insulin Release in the Rat

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The effects of endothelin-1 (ET-1) infusion at 0, 25, 50, and 75 ng/kg/min on blood glucose, insulin, and ET-1 levels were determined in anesthetized rats. In a separate group of rats, ET-1 was infused at 75 ng/kg/min and glucagon and glucose levels were determined. In another group of rats, the effect on blood glucose of glucagon infusion at 0.2 ng/kg/min with ET-1 infusion at 75 ng/kg/min for 30 minutes was determined. Glucose decreased 10 minutes after initiation of ET-1 infusion at 75 ng/kg/min and at 15 minutes during ET-1 infusion at 25 and 50 ng/kg/min. After 45 minutes, glucose decreased by 1.05 ± 0.1, 1.44 ± 0.11, and 1.39 ± 0.22 mmol/L and ET-1 increased by 4.4 ± 0.8, 5.2 ± 1.2, and 11.2 ± 0.8 pmol/L during ET-1 infusion at 25, 50, and 75 ng/kg/min, respectively. Insulin levels increased during ET-1 infusion of 50 ng/kg/min at 30 and 45 minutes by 300 ± 75 and 405 ± 120 pmol/L, respectively. During ET-1 infusion of 75 ng/kg/min, insulin increased at 45 minutes by 570 ± 180 pmol/L. Glucagon decreased during ET-1 infusion at 15 minutes associated with a decrease in glucose. Glucagon levels subsequently returned to baseline values despite a continued decline in glucose levels. Glucagon infusion at 0.2 μg/kg/min prevented the early ET-1–induced hypoglycemia. These findings demonstrate that ET-1 decreased blood glucose initially associated with a decrease in glucagon and subsequently associated with enhanced insulin release.

THE ENDOTHELIN (ET) FAMILY of peptides has recently been described.[1,2] ET-1 and ET-3 are stored in vascular endothelial cells,[1] and when infused in rats[3,4] or dogs,[5,6] cause hypertension, vasoconstriction, and decreased cardiac output. ET causes vasoconstriction by stimulating contraction of vascular smooth muscle[1] by increasing intracellular calcium through activation of phospholipase C.[7,8] Phospholipase C hydrolyzes inositol bisphosphate, resulting in inositol trisphosphate and diacylglycerol. Inositol trisphosphate mobilizes intracellular stores of calcium from the endoplasmic reticulum, and one effect of diacylglycerol is stimulation of protein kinase C, which activates membrane calcium channels.[9,10]

Recently, ET has been found to stimulate release of several hormones, including atrial natriuretic factor from the heart,[3] aldosterone[11] and epinephrine[12] from the adrenal gland, and luteinizing hormone and follicle-stimulating hormone[13] from the pituitary gland. ET-1 has also been shown to inhibit prolactin secretion from the pituitary gland.[14] Preliminary data indicate that ET levels are elevated in streptozotocin-induced diabetic rats[15] and in humans with insulin-dependent diabetes.[16,17] Further data indicate that ET receptors are increased by insulin in aortic vascular smooth muscle cells.[18] Furthermore, cholecystokinin has been shown to stimulate insulin release by activating phospholipase C[19] using the same signal transduction pathway used by ET-1 to induce vasoconstriction.

Based on these preliminary data, we hypothesized that ET will decrease blood glucose and enhance insulin release. The current studies were designed to determine the effect of ET infusion on blood glucose and insulin release, as well as the effects of ET on glucagon release.

MATERIALS AND METHODS

Four studies were performed using male Sprague-Dawley rats weighing 250 to 350 g that were anesthetized with inactin (100 mg/kg intraperitoneally) and were fasted before study. A 200-gauge polyethylene tube was inserted in the trachea to facilitate spontaneous respiration. A femoral artery catheter (PE-50) was inserted for blood withdrawal and to monitor blood pressure. An SV-31 catheter was inserted in the femoral vein for drug infusion. A second femoral vein SV-31 catheter was advanced into the inferior vena cava for measurement of central venous pressure. After a 30-minute surgical recovery period, studies were initiated.

In protocol 1, the effect of ET on circulating ET-1 and blood glucose levels in four groups of four rats was determined. ET-1 (ET-1; Peptides International, Louisville, KY) in saline was infused at 0, 25, 50, and 75 ng/kg/min at a rate of 0.6 mL/h for 45 minutes starting at 0 minutes. We have previously found that a 75-ng/kg/min infusion mimics circulating levels of ET elicited by stressing rats with hypotensive hemorrhage.[20] Three milliliters of blood was removed just before ET-1 infusion for baseline measurement of ET-1 and blood glucose levels. Three milliliters of heparinized blood obtained from a donor rat of the same strain was infused immediately after the initial blood withdrawal. Blood was removed (0.15 mL) every 5 minutes for 45 minutes for determination of blood glucose. At 45 minutes, 3 mL blood was removed for determination of circulating levels of ET by radioimmunoassay (RIA).

To determine the time course of insulin secretion after ET infusion, a second protocol was performed using four groups of rats that were studied after the identical surgical preparation. ET-1 in saline was infused at 0 (n = 6), 25 (n = 10), 50 (n = 10), and 75 (n = 6) ng/kg/min starting at 0 minutes. One milliliter of blood was removed at 0, 15, 30, and 45 minutes for determination of blood glucose and insulin levels. Immediately after blood withdrawal, 1 mL heparinized blood obtained from a donor rat of the same strain was infused.

To determine the effect of ET on glucagon secretion, a third protocol was performed. Following surgical preparation as described earlier, ET-1 was infused at 75 ng/kg/min at 0.6 mL/h for 45 minutes starting at time 0 in five rats, and 1 mL blood was removed every 15 minutes for 45 minutes and stored in EDTA tubes for measurement of glucagon and glucose levels. Immedi-

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ately following blood withdrawal, 1 mL heparinized blood obtained from a donor rat of the same strain was infused.

To determine if glucagon infusion can block ET-1–induced hypoglycemia, a fourth protocol was performed. Following inactin anesthesia and surgical preparation, ET was infused at 75 ng/kg/min and glucagon at 0.2 μg/kg/min in five additional rats for 30 minutes starting at time 0. One milliliter of blood was obtained every 15 minutes for 30 minutes and stored in EDTA tubes for determination of glucose and glucagon levels. Following each blood withdrawal, an equal volume of heparinized blood from a rat of the same strain was infused. In six additional rats, glucagon was infused alone at 0.2 μg/kg/min following inactin anesthesia for 30 minutes. One milliliter of blood was obtained every 15 minutes for 30 minutes for determination of glucose levels. Following each blood withdrawal, an equal volume of heparinized blood from a rat of the same strain was infused.

Circulating levels of insulin were determined by RIA using the Incstar Insulin ^{125}I RIA Kit (Incstar, Stillwater, MN) with rat standards. This assay has a 30% cross-reactivity with proinsulin. Before assay, 250 μL 25% polyethylene glycol was added to 250 μL serum and centrifuged at room temperature for 20 minutes at 760 × *g*. Two hundred microliters of the supernatant was placed into two separate tubes. This removes endogenous interfering substances. One hundred microliters of guinea pig antiinsulin serum was added to the standards and specimens. ^{125}I–porcine insulin in bovine serum albumin–borate buffer was added to insulin standards and the treated serum specimens and incubated at 2° to 8°C for 16 to 20 hours. Following incubation, 500 μL of the precipitating complex (normal guinea pig serum prepared with goat antiguinea serum and polyethylene glycol) was added to all tubes and incubated for 25 minutes at 20°C. Tubes were centrifuged for 20 minutes at 760 × *g* at 20°C. The supernatant was decanted, and the pellet was counted on an Auto Gamma 5000 series gamma counter (Packard Instruments, Downers Grove, Il). Insulin levels were determined from the standard curve. Interassay and intraassay coefficients of variation were 9.2% and 7.5%, respectively.

Immunoreactive ET was determined by RIA using the ET 1-21 specific RIA kit (Arlington Heights, IL) as previously described.[20] Cross-reactivity is 0.4% between ET-1 and big ET-1 in this kit, 144% for ET-2, and 52% for ET-3. The intraassay coefficient of variation was 8.8%, interassay coefficient of variation 13.8%, and sensitivity 3 pg/mL. Recovery of radioactive ET-1 is 76% ± 1%.

Glucagon levels were determined by RIA using a glucagon RIA kit (ICN, Costa Mesa, CA) specific for pancreatic glucagon, with a cross-reactivity for pancreatic glucagon of 100% and for gut glucagon of 0.0013%. Blood was collected in chilled EDTA tubes centrifuged at 4°C for 20 minutes and frozen at −70°C until assay. Plasma (0.2 mL) was incubated with rabbit antiporcine glucagon antibody for 6 hours. ^{125}I-glucagon (0.1 mL) was then added and incubated for 16 hours. Goat antirabbit γ-globulin (0.1 mL) and 0.1% polyethylene glycol in 0.01 mol/L phosphosaline buffer (0.1 mL) were subsequently added. Following centrifugation for 15 minutes at 1,000 × *g*, the supernatant was aspirated and the pellet was counted on an Auto Gamma 5000 series gamma counter. Glucagon levels were determined from the standard curve. The intraassay coefficient of variation was 3.8%, and interassay coefficient of variation was 5%.

Blood glucose was determined using a One Touch II glucose reflectance meter (Lifescan, Milpitas, CA). This method of blood glucose analysis was validated by comparing 36 glucose results obtained with the One Touch meter between 35 and 400 mg/dL from a separate group of rats with plasma glucose values obtained spectrophotometrically with the Sigma glucose kit (Sigma, St Louis, MO). Values for the One Touch meter were multiplied by 1.15 before comparison because the One Touch meter measures whole-blood glucose and the Sigma kit measures plasma glucose. A correlation coefficient of .994 and R^2 of .988 were obtained with P less than .0001.

Data were analyzed using the Statistica statistical program (Stat Soft, Tulsa, OK). Following ANOVA with repeated measures, post hoc comparisons between experimental and control means were performed using Tukey's honest significant difference analysis. Due to the nonlinear nature of glucagon results in protocol 3, ANOVA was analyzed in two groups. The first analysis compared 0- and 15-minute time points, and the second analysis compared 0- and 45-minute time points. Comparisons in which P was less than .05 were considered significant.

RESULTS

The effect of ET-1 infusion at 0, 25, 50, and 75 ng/kg/min is shown in Fig 1. Blood glucose was 4.33 ± 0.17 mmol/L before saline infusion began and did not change during saline infusion. Glucose decreased from 4.33 ± 0.11 mmol/L by 0.56 ± 0.11 mmol/L ($P < .05$ compared with baseline) at 15 minutes to 3.77 ± 0.11 mmol/L during ET-1 infusion at 25 ng/kg/min. After 45 minutes, the lowest ET-1 infusion rate resulted in a decrease of 1.05 ± 0.17 mmol/L ($P < .05$ compared with baseline) to 3.27 ± 0.11 mmol/L ($P < .05$). Similar effects of ET-1 are observed at the ET-1 infusion rate of 50 ng/kg/min. At the 75-ng/kg/min ET-1 infusion rate, blood glucose significantly decreased from a baseline of 4.77 ± 0.11 mmol/L by 0.72 ± 0.06 mmol/L ($P < .05$ compared with baseline) by 10 minutes of ET-1 infusion and ultimately decreased by 1.39 ± 0.22 mmol/L ($P < .05$ compared with baseline) to 3.39 ± 0.11 mmol/L at 45 minutes of ET-1 infusion.

The change in insulin concentration is illustrated in Fig 2. Insulin levels did not significantly change during saline infusion or ET-1 infusion at 25 ng/kg/min. During ET-1 infusion at 50 ng/kg/min, insulin increased from a baseline of 240 ± 30 pmol/L by 300 ± 75 pmol/L ($P < .05$ compared with baseline) and 405 ± 120 pmol/L ($P < .05$ compared with baseline) at 30 and 45 minutes, respectively.

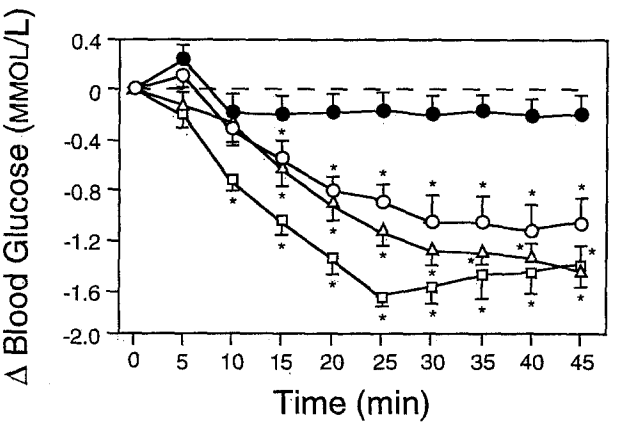

Fig 1. Effect of ET-1 infusion (starting at time 0) on change in blood glucose over 45 minutes in the rat. (●) Saline; (○) ET-1 at 25 ng/kg/min; (△) ET-1 at 50 ng/kg/min; (□) ET-1 at 75 ng/kg/min.

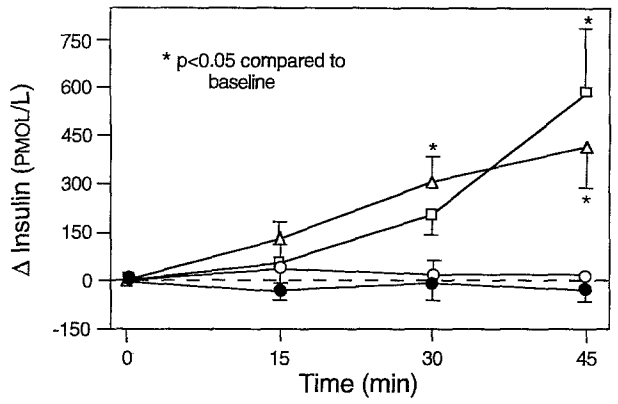

Fig 2. Effect of ET-1 infusion on change in insulin concentration over 45 minutes in the rat. (●) Saline; (○) ET-1 at 25 ng/kg/min; (△) ET-1 at 50 ng/kg/min; (□) ET-1 at 75 ng/kg/min.

At an ET-1 infusion rate of 75 ng/kg/min, insulin increased by 570 ± 180 pmol/L ($P < .05$ compared with baseline) from a baseline value of 180 ± 45 pmol/L after 45 minutes of infusion.

ET-1 levels did not significantly change from a baseline level of 16.8 ± 1.3 pmol/L to 17.2 ± 0.3 during saline infusion. ET-1 levels increased by 4.4 ± 0.8 pmol/L ($P < .05$ compared with baseline), 5.2 ± 1.2 pmol/L ($P < .05$ compared with baseline), and 11.2 ± 0.8 pmol/L ($P < .05$ compared with baseline) during ET-1 infusion rates of 25, 50, and 75 ng/kg/min, respectively (Fig 3).

The effects of ET-1 infusion at 75 ng/kg/min on changes in glucagon and glucose levels are illustrated in Fig 4. Similar to the previous study, glucose decreased starting at 15 minutes from 5.0 ± 0.17 to 4.44 ± 0.17 mmol/L. Glucose decreased further to 3.83 ± 0.22 mmol/L at 45 minutes. Glucagon levels decreased by 27 ± 6 ng/L at 15 minutes ($P = .0014$) and returned to baseline levels at 30 and 45 minutes. No significant change in glucose or glucagon was observed during saline infusion alone. In Fig 5, the effect of combined infusion of ET-1 and glucagon on glucagon and glucose over 30 minutes is illustrated. Glucagon was infused at a rate that produced no significant change in circulating

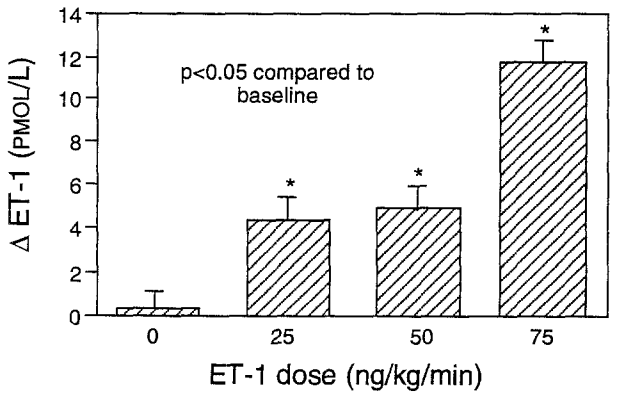

Fig 3. Effect of saline and ET-1 infusion at 25, 50, and 75 ng/kg/min for 45 minutes on circulating levels of ET in the rat.

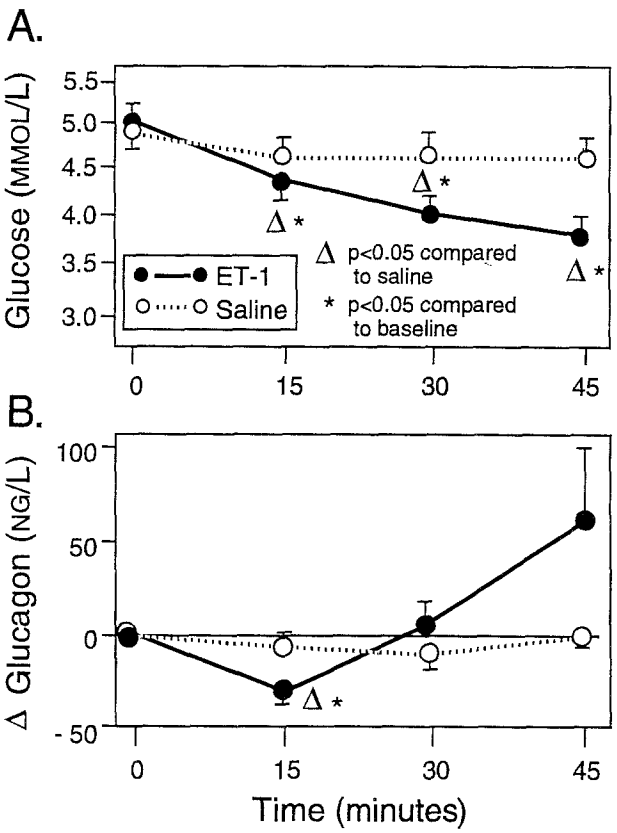

Fig 4. Effect of ET-1 infusion at 75 ng/kg/min (●) and saline at 0.6 mL/h (○) on (A) glucose (n = 5) and (B) glucagon in the rat.

glucagon levels at the 15-minute time point. Glucagon infusion at this rate prevented the early decreases in glucose observed during ET-1 infusion alone. In six additional rats, glucagon infusion alone increased glucose from 4.6 ± 0.11 to 7.17 ± 0.61 mmol/L at 15 minutes ($P < .05$) and to 9.17 ± 0.88 mmol/L at 30 minutes ($P < .05$).

DISCUSSION

The present study demonstrates that ET-1 infusion at 25, 50, and 75 ng/kg/min decreases blood glucose within 10 to 15 minutes. ET-1 also increases circulating levels of insulin, but only at the higher ET-1 infusion rates and after infusion of ET for a greater length of time than is required to decrease blood glucose. ET-1 infusion decreases circulating glucagon levels within 15 minutes of ET-1 infusion, corresponding to the initial decrement in blood glucose observed during ET-1 infusion. Furthermore, when glucagon was infused at a dose that prevented the ET-1–induced decrement in glucagon at 15 minutes, the early ET-1–induced hypoglycemia was prevented. These findings support the concept that ET-1 decreases blood glucose by initially decreasing glucagon and subsequently in part by increasing insulin release.

The finding that ET-1 decreased glucose in the present study can be caused by several mechanisms. First, ET-1 may increase circulating levels of insulin. Second, ET-1 may

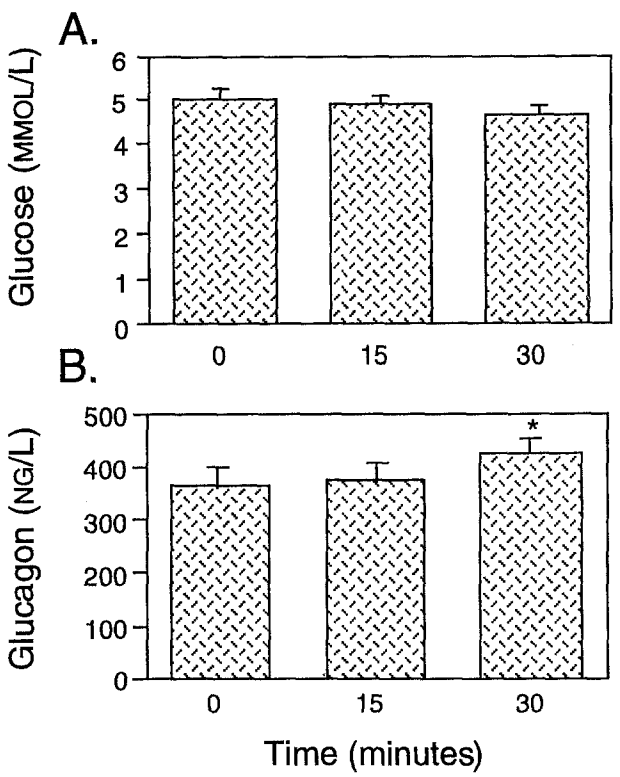

Fig 5. Effect of combined ET-1 infusion at 75 ng/kg/min and glucagon infusion at 0.2 ng/kg/min starting at time 0 for 30 minutes on (A) glucose (n t5) and (B) glucagon levels in the rat (n = 5). *$P <$ .05.

decrease counterregulatory hormones. ET-1 may independently decrease glucose production or independently enhance glucose disposal. The finding that insulin does not increase until after blood glucose decreases supports the concept that enhanced insulin release is not the only mechanism of ET-induced hypoglycemia. The finding that glucagon is decreased at a time when glucose is first decreased and that glucagon infusion prevents early ET-1–induced hypoglycemia supports the concept that early ET-1–induced hypoglycemia may be caused by ET-1 inhibition of glucagon. However, recent data reported by Roden et al[21] suggest that in the perfused liver, ET directly enhances glucose production. It is therefore possible that ET enhances hepatic glucose production, which inhibits glucagon production, but that glucose disposal is enhanced by ET more than glucose production, resulting in hypoglycemia.

The finding that insulin levels increase despite an initial decrease in glucose suggests that ET-1 also increases insulin release. The mechanism of ET-1–induced inhibition of glucagon and ET-1–induced insulin release was not determined in the present study. The mechanism of ET-induced insulin release may be similar to the mechanism by which ET-1 induces vasoconstriction, since ET-1 has been shown to increase intracellular calcium by activating phospholipase C, which causes phosphatidyl inositol hydrolysis and stimulates protein kinase C.[1,7-10] This mechanism both increases release of intracellular calcium from the endoplasmic reticulum and increases calcium entry into the cell through the cell membrane. A similar mechanism has been found for cholecystokinin-induced insulin release.[22,23] It is possible that this mechanism is involved in ET-1–induced insulin release by enhanced intracellular calcium stimulation of the exocytic apparatus. Alternatively, ET-1 may increase insulin release by increasing nitric oxide, since ET-1 has been shown to stimulate nitric oxide release[24] and nitric oxide has been shown to stimulate insulin release.[25] However, this is not likely since more recent studies do not support an insulin-secretory role for nitric oxide.[26,27]

It is possible that the changes in circulating hormones in the present study represent changes in clearance of the measured hormones. However, this is not likely, since the changes in insulin and glucagon levels went in opposite directions. If decreased clearance was the only cause of the change observed in the concentration of these hormones, one would expect both glucagon and insulin levels to increase. The finding in the present study that glucagon levels tended to increase 45 minutes after ET infusion most likely represents the effect of hypoglycemia overcoming the inhibitory effect of ET-1 on glucagon release.

The three effects of ET-1 in the present study of decreasing glucose, decreasing glucagon, and increasing insulin are similar to the effects of glucose itself and the insulin secretagogue, glucagon-like peptide-1.[28] Although not established in the present study, it is possible that ET may play a role in glucose homeostasis as an incretin-like, glucagon-like peptide-1.

Several recent preliminary studies support a role for ET in glucose homeostasis. First, Yamauchi et al[29] have found that glucose stimulates ET release in cultured bovine aortic endothelial cells. Second, Takeda et al[30] and Hegazy et al[15] have found that circulating levels of ET are increased in streptozotocin diabetic rats. Hegazy et al[17] have similarly found increased circulating ET-1 levels in humans with diabetes. Third, Frank et al[8] and Hu et al[31] have found that ET receptor density is increased by insulin and decreased by glucose and that insulin stimulates secretion of ET from bovine endothelial cells. Taken together with the current findings on ET, these studies support the concept that insulin is important for ET-1 action and that ET-1 has the ability to activate its receptors by stimulating insulin release. It is possible that ET-1 may be important in the postprandial state both as an insulin secretagogue and also as a vasoconstrictor perhaps in nongastrointestinal vascular beds.

The physiologic role of ET-1 on insulin release has yet to be defined. However, it is interesting that ET-1 has been found to affect hormone secretion from several endocrine glands, including atrial natriuretic factor from the heart,[3] aldosterone and epinephrine from the adrenal,[11,12] and luteinizing hormone, follicle-stimulating hormone,[13] and prolactin[14] from the anterior pituitary. Taken together, these studies suggest that ET-1 may act as a ubiquitous paracrine or endocrine regulator of hormone secretion

throughout the body. Clearly, factors that control ET-1 release will need to be elucidated to better understand physiologically how ET-1 affects hormone secretion.

The present study demonstrates for the first time that ET increases insulin, decreases glucagon, and decreases blood glucose. It demonstrates that the initial ET-1–induced hypoglycemia is associated with a decrease in glucagon and that the continued hypoglycemia is associated with enhanced insulin release.

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