N^G-Methyl-L-Arginine and Somatostatin Decrease Glucose and Insulin and Block Endothelin-1 (ET-1)–Induced Insulin Release But Not ET-1–Induced Hypoglycemia

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We have previously demonstrated that endothelin-1 (ET-1) increases plasma insulin and decreases blood glucose. The present study was designed to determine if ET-1–induced hypoglycemia occurs in the presence of the insulin secretion inhibitor, somatostatin, and whether ET-1–induced insulin secretion is affected by the nitric oxide synthase I inhibitor, N^G-methyl-L-arginine (NMLA), in the anesthetized rat. ET-1 increased plasma insulin and decreased blood glucose in all protocols. Somatostatin alone decreased blood glucose and plasma insulin. Somatostatin blocked ET-1–induced plasma insulin release but did not completely block ET-1–induced hypoglycemia. NMLA alone decreased blood glucose and plasma insulin. NMLA also blocked ET-1–induced insulin release but not ET-1–induced hypoglycemia. The present study confirms our previous finding that ET-1 decreases blood glucose and increases plasma insulin. Because hypoglycemia occurs during insulin inhibition with somatostatin, the present study suggests that ET-1–induced hypoglycemia is partially caused by non–insulin-mediated mechanisms. Because insulin secretion is blocked by the nitric oxide synthase I inhibitor, NMLA, the present study suggests that ET-1– induced insulin release may be mediated by production of nitric oxide.

ENDOTHELIN-1 (ET-1) and ET-3 are stored in vascular endothelial cells.[1,2] Infusion of ET-1 or ET-3 in rats[3] or dogs[4,5] increases mean arterial pressure and decreases cardiac output by increasing systemic vascular resistance. The vasoconstriction is stimulated by contraction of vascular smooth muscle, which occurs due to ET-induced increases in intracellular calcium.[6] Increased intracellular calcium is produced by ET-1–induced activation of phospholipase C[7,8] and stimulation of membrane calcium channels.[9]

Recently, ET has been shown to stimulate release of several hormones, including atrial natriuretic factor from the heart,[3] aldosterone[10] and epinephrine[11] from the adrenal gland, and luteinizing hormone and follicle-stimulating hormone from the pituitary.[12] We have recently demonstrated that ET stimulates insulin release and decreases glucagon and glucose in the rat.[13] Preliminary studies in other laboratories have demonstrated that ET levels are increased in streptozotocin-induced diabetic rats[14] and in humans with insulin-dependent diabetes mellitus.[15,16] Further preliminary data indicate that insulin receptors are increased by ET in aortic vascular smooth muscle cells.[17] Taken together, these data suggest that ET may be a regulator of insulin secretion and glucose homeostasis and that insulin may regulate ET-1 action.

ET has also been shown to stimulate release of the vasodilator, nitric oxide, which acts in a negative-feedback loop for ET-1 action on vascular smooth muscle.[18-20] Nitric oxide has recently been shown to enhance insulin release from pancreatic β cells.[21] N^G-methyl-L-arginine (NMLA) has been shown to inhibit nitric oxide formation by competitively inhibiting the nitric oxide synthase I receptor.[22] Somatostatin has been shown to inhibit glucagon and insulin release.[23,24]

Based on these data, we hypothesized that (1) ET-1 decreases blood glucose by increasing insulin release, (2) blocking ET-1–induced insulin release with somatostatin blocks ET-1–induced hypoglycemia, (3) ET-1–induced insulin release is mediated by release of nitric oxide, and (4) blocking nitric oxide release with NMLA blocks ET-1–induced insulin release and ET-1–induced hypoglycemia. The current studies were performed to determine if ET-1–induced insulin release is mediated by nitric oxide, and whether increased insulin release is the only mechanism causing ET-1–induced hypoglycemia.

MATERIALS AND METHODS

Two protocols were performed using male Sprague-Dawley rats (Harlan-Sprague-Dawley, Indianapolis, IN) weighing 250 to 350 g that were anesthetized with Inactin (BYK Gulden, Konstanz, Germany) 100 mg/kg intraperitoneally. A 200-gauge polyethylene tube was inserted into the trachea to facilitate spontaneous respiration. A femoral artery catheter was inserted for blood withdrawal and to monitor blood pressure. A catheter was inserted in the femoral vein for drug infusion. After a 30-minute surgical recovery period, studies were initiated as outlined below.

In protocol 1, four groups of six rats were evaluated following surgical preparation. In group 1, saline was infused at 0.6 mL/h for 15 minutes and subsequently infused at 1.2 mL/h for 30 minutes. In group 2, the effect of ET-1 alone on insulin secretion was determined. Saline was infused for 45 minutes at 0.6 mL/h. ET-1 (Peptides International, Louisville, KY) in saline was infused at 75 ng/kg/min for 30 minutes starting 15 minutes after initiation of the saline infusion. In group 3, the effect of somatostatin (Peninsula Laboratories, Belmont, CA) infusion alone on glucose and insulin levels was evaluated. Somatostatin in saline was infused at 5 ng/kg/min at 0.6 mL/h for 45 minutes. Saline was infused for 30 minutes starting 15 minutes after initiation of somatostatin infusion at 0.6 mL/h. In group 4, the combined effect of somatostatin and ET-1 infusion on insulin and glucose levels was evaluated. Somatostatin was infused at 5 ng/kg/min at 0.6 mL/h for 45

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minutes. ET-1 in saline was infused for 30 minutes at 75 ng/kg/min at 0.6 mL/h starting 15 minutes after initiation of somatostatin infusion. One milliliter of blood for insulin and glucose measurement was obtained at 0, 15, 30, and 45 minutes. An equal volume of heparinized blood from a donor rat of the same strain was infused immediately after blood withdrawal in each group.

In protocol 2, four groups of six rats were evaluated following surgical preparation as above. In group 1, saline was infused for 45 minutes at 0.6 mL/h. In group 2, ET-1 in saline was infused for 30 minutes at 75 ng/kg/min. In group 3, the nitric oxide inhibitor, NMLA (Sigma, St Louis, MO), was administered as a 100-mg/kg intravenous bolus and saline was infused for 30 minutes, 15 minutes after NMLA was infused. In group 4, NMLA was administered as a 100-mg/kg intravenous bolus and ET-1 in saline was infused at 75 ng/kg/min, starting 15 minutes later for 30 minutes. One milliliter of blood was obtained at 0, 15, 30, and 45 minutes for determination of insulin and glucose. One milliliter of heparinized blood from a donor rat of the same strain was infused immediately after each blood withdrawal.

Circulating levels of insulin were determined by radioimmunoassay using the Incstar Insulin ^{125}I RIA Kit (Incstar, Stillwater, MN). 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 in two separate tubes to remove endogenous interfering substances. One hundred microliters of guinea pig antiinsulin serum was added to the standards and specimens. ^{125}I-labeled porcine insulin in bovine serum albumin–borate buffer was added to insulin standards and treated serum specimens and incubated at 1° to 8°C for 16 to 20 hours. Following incubation, 500 μL of the precipitating complex (normal guinea pig serum prepared with goat anti–guinea pig 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 gamma-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. Sensitivity of the assay is 0.01 ng/mL.

Blood glucose was determined using a One Touch II glucose reflectance meter (Lifescan, Milpitas, CA). The One Touch meter was validated as previously described[13] by comparing 36 glucose results obtained with the One Touch meter between 35 and 400 ng/dL from a separate group of rats with plasma glucose values obtained spectrophotometrically with the Sigma Glucose kit. One Touch values were multiplied by 1.15 before comparisons because the One Touch meter measures whole blood glucose levels and the Sigma kit measures plasma glucose levels. A correlation coefficient of .994 and R^2 of .988 were obtained with P less than .0001. The coefficient of variation for high glucose values is 1.75%, and for low glucose values, 3.04%.

Data were analyzed using the Statistica statistical program (Stat Soft, Tulsa, OK). Following ANOVA with repeated measures, post hoc comparisons between baseline and experimental means were performed with the Neuman-Keuls test. Comparisons in which P was less than .05 were considered significant.

RESULTS

The effects of saline alone, ET-1 alone, somatostatin alone, and combined ET-1 and somatostatin on blood glucose are illustrated in Fig 1A. Saline infusion alone did not significantly alter blood glucose levels from a baseline of 97 ± 2 mg/dL. ET-1 infusion at 75 ng/kg/min starting at 15 minutes caused a decrement in blood glucose of 13 ± 3 mg/dL ($P < .05$) and 22 ± 2 mg/dL at the 30- and 45-minutes time points, from a baseline of 91 ± 2 mg/dL. During somatostatin infusion at 5 ng/kg/min, glucose decreased by 10 ± 2 ($P < .05$), 16 ± 4 ($P < .05$), and 15 ± 3 mg/dL ($P < .05$) at 15, 30, and 45 minutes, respectively, from a baseline of 95 ± 3 mg/dL. During infusion of somatostatin starting at time 0 and ET-1 starting 15 minutes after initiation of somatostatin infusion, blood glucose decreased by 7 ± 1 ($P < .05$), 11 ± 1 ($P < .05$), and 12 ± 1 mg/dL ($P < .05$) at 15, 30, and 45 minutes, respectively, from a baseline blood glucose of 88 ± 3 mg/dL. Changes in plasma insulin levels are illustrated in Fig 1B. Plasma insulin levels were unchanged during saline infusion from a baseline of 3.3 ± 0.6 ng/mL. ET-1 infusion starting at 15 minutes at 75 ng/kg/min increased plasma insulin from a baseline level of 2.6 ± 0.5 ng/mL by 2.2 ± 0.3 ($P < .05$) and 3.8 ± 0.5 ng/mL ($P < .05$) at 30 and 45 minutes. Plasma insulin levels decreased during somatostatin infusion by 2.8 ± 0.5 ng/mL ($P < .05$) at 15 minutes and 2.9 ± 0.6 ng/mL ($P < .05$) at 30 and 45 minutes, from a baseline insulin level of 3.0 ± 0.6 ng/mL. During infusion of somatostatin starting at 0 minutes and ET-1 starting at 15 minutes, plasma insulin levels decreased from 3.3 ± 4 ng/mL by 2.8 ± 0.4 ($P < .05$), 3.1 ± 0.4 ($P < .05$), and 3.2 ± 0.4 ng/mL ($P < .05$) at 15, 30, and 45 minutes, respectively.

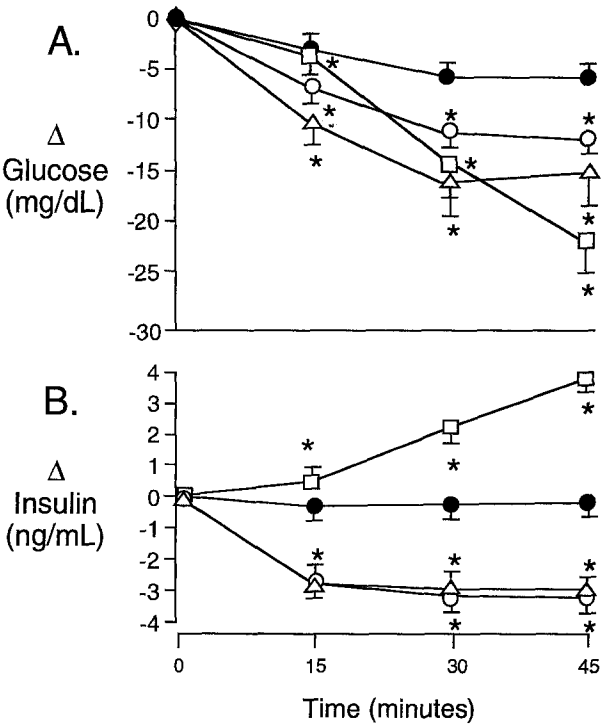

Fig 1. Effect of saline (●), saline plus ET-1 (□), saline plus somatostatin (△), and combined somatostatin and ET-1 (○) on the change in (A) glucose and (B) insulin in the anesthetized rat. *$P < .05$ *v* baseline.

The effect of NMLA on ET-1–induced hypoglycemia is illustrated in Fig 2A. As in the previous protocol, saline had no effect on blood glucose from a baseline value of 89 ± 6

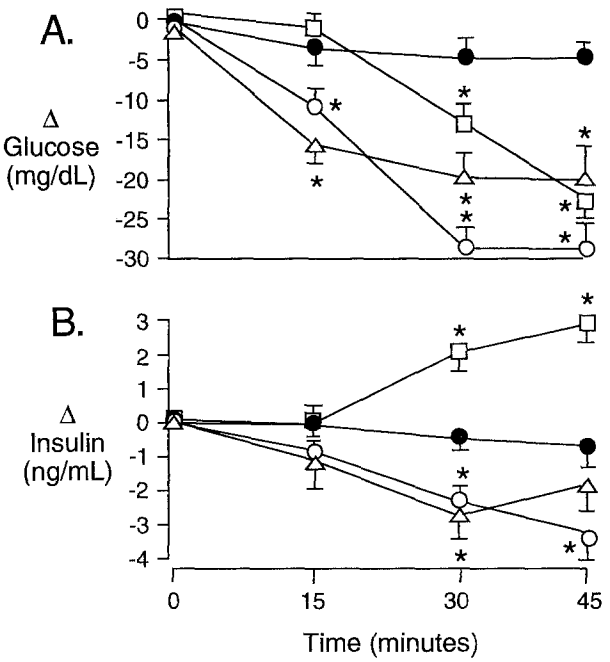

Fig 2. Effect of saline (●), ET-1 (□), NMLA (△), and combined ET-1 and NMLA (○) on the change in (A) glucose and (B) insulin in the anesthetized rat. *$P < .05$ v baseline.

mg/dL. ET-1 infusion alone starting at 15 minutes decreased blood glucose from 86 ± 4 mg/dL by 13 ± 3 mg/dL ($P < .05$) at 30 minutes and by 23 ± 2 mg/dL ($P < .05$) at 45 minutes. NMLA infusion alone starting at time 0 caused a decrease in blood glucose from 87 ± 7 mg/dL of 16 ± 3 ($P < .05$), 20 ± 5 ($P < .05$), and 22 ± 6 mg/dL ($P < .05$) at 15, 30, and 45 minutes, respectively. Combined infusion of NMLA at time 0 followed by ET-1 at 15 minutes similarly resulted in a decrease in blood glucose from 95 ± 2 mg/dL of 13 ± 3 ($P < .05$), 28 ± 3 ($P < .05$), and 29 ± 5 mg/dL ($P < .05$) at 15, 30, and 45 minutes, respectively. No significant difference between the group infused with NMLA alone and the group infused with NMLA plus ET-1 was observed.

The effect of NMLA on ET-1–induced insulin release is illustrated in Fig 2B. Saline did not alter plasma insulin levels from a baseline value of 5.8 ± 1.2 ng/mL. ET-1 starting at 15 minutes increased plasma insulin from 3.6 ± 0.6 ng/mL by 2.1 ± 0.5 ng/mL at 30 minutes and 2.4 ± 0.5 ng/mL ($P < .05$) at 45 minutes. NMLA decreased plasma insulin from 5.7 ± 1 ng/mL by 2.8 ± 0.5 ng/mL at 30 minutes ($P < .05$) and by 1.8 ± 0.9 ng/mL at 45 minutes. NMLA infusion at time 0 followed by ET-1 infusion at 15 minutes resulted in a decrease in plasma insulin from 5.6 ± 0.1 ng/mL of 2.6 ± 0.9 ng/mL ($P < .05$) at 30 minutes and 3.3 ± 0.8 ng/mL ($P < .05$) at 45 minutes. No significant difference between the group infused with NMLA alone and the group infused with NMLA plus ET-1 was observed.

DISCUSSION

The present study demonstrates that ET-1 infusion decreases blood glucose and increases plasma insulin release. Furthermore, inhibition of insulin release with somatostatin did not completely prevent ET-1–induced hypoglycemia, suggesting that ET-1 decreases blood glucose partially by enhancement of insulin release and partially by a non–insulin-mediated mechanism. Finally, the observation that NMLA inhibits ET-1–induced insulin release supports the hypothesis that ET-1–induced insulin release is mediated by nitric oxide.

In both protocols presented, ET-1 decreased blood glucose and increased plasma insulin. This is in agreement with previous studies in our laboratory.[13] However, we had suspected that enhanced insulin release was not the only mechanism of ET-1–induced hypoglycemia because our previous studies demonstrated that ET-1 causes hypoglycemia before circulating insulin levels are increased. The finding in the current study that ET-1 causes hypoglycemia during inhibition of insulin secretion with somatostatin lends further support to the concept that ET-1 decreases glucose in part by a non–insulin-mediated mechanism. Indeed, we have previously demonstrated that ET-1 decreases glucagon early after ET-1 infusion and that glucagon infusion can block early ET-1–induced hypoglycemia.[13] Taken together, these studies suggest that early ET-1–induced hypoglycemia is mediated by inhibition of glucagon. Although early ET-1–induced hypoglycemia appears to be mediated by glucagon inhibition,[13] our previous observation that plasma insulin increases later, despite a reduction in blood glucose, supports the concept that insulin release partially causes or maintains ET-1–induced hypoglycemia. The concept that insulin partially mediates the hypoglycemic response is also supported by the observation in the present study that ET-1–induced hypoglycemia is attenuated by prior administration of the insulin-secretory inhibitor, somatostatin.

The observation in the present study that somatostatin alone decreased blood glucose is most likely due to the fact that somatostatin decreases glucagon and growth hormone.[23-26] The observation that combined infusion of ET-1 and somatostatin did not decrease blood glucose to a greater extent than infusion of somatostatin alone suggests that somatostatin is as potent or more potent than ET-1 in inhibiting counterregulatory hormones. Many previous studies assessing the effect of somatostatin on insulin release replaced glucagon, which was not the case in the current study.

Several mechanisms of ET-1–induced insulin release are possible. ET-1 causes vasoconstriction by activating phospholipase C, which enhances release of intracellular calcium.[7,8] Cholecystokinin-induced insulin release has been shown to be mediated by this same mechanism, since increased intracellular calcium stimulates insulin exocytosis.[27] It is possible that ET-1 stimulates insulin release by this mechanism. Alternatively, ET-1 has been shown to stimulate release of nitric oxide,[18-20] and nitric oxide has been shown to stimulate insulin release.[21] Furthermore, NMLA has been shown to inhibit nitric oxide production by inhibiting the type I nitric oxide synthase receptor.[22] The observation in the current study that nitric oxide inhibition with NMLA blocks ET-1–induced insulin release strongly supports the concept that ET-1 stimulates insulin release by stimulating nitric oxide production.

It is perplexing that nitric oxide inhibition with NMLA decreased glucose and insulin. The current studies suggest that NMLA decreases glucose by a mechanism independent of insulin release. The mechanism of NMLA-induced hypoglycemia is not clear, but may be due to a decrease in one of the counterregulatory hormones such as glucagon or to direct stimulation of glucose transporters. It may decrease glucose production or increase glucose metabolism. It is possible that NMLA decreases circulating insulin levels by inhibiting arginine-induced insulin release or by inhibiting nitric oxide–stimulated insulin release.

The findings in the present study suggest that ET-1 increases insulin and decreases glucose. Furthermore, because ET-1–induced hypoglycemia persists despite inhibition of insulin release with somatostatin, this study suggests that ET-1–induced hypoglycemia is at least partially regulated by non–insulin-mediated mechanisms. The finding that the nitric oxide synthase I receptor blocker, NMLA, blocks ET-1–induced insulin release suggests that ET-1 may enhance insulin release by stimulation of nitric oxide production. Finally, the observation that NMLA alone decreases both insulin and glucose suggests a possible effect of nitric oxide on glucose metabolism independent of insulin release.

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