Nitric Oxide Release Accounts for Insulin's Vascular Effects in Humans

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Abstract

Insulin exerts effects on the vasculature that (*a*) may play a role in the regulation of blood pressure; and (*b*) by boosting its own delivery to target tissues, also have been proposed to play an integral part in its main action, the promotion of glucose disposal.

To study the role of nitric oxide (NO) in the mediation of insulin's effects on the peripheral vasculature, N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of the synthesis of endothelium-derived NO, was infused into the brachial arteries of healthy volunteers both before, and at the end of a 2-h hyperinsulinemic (6 pmol/kg per min) euglycemic clamp. L-NMMA (but not norepinephrine, an NO-independent vasoconstrictor) caused larger reductions in forearm blood flow during hyperinsulinemia than at baseline. Moreover, L-NMMA prevented insulin-induced vasodilation throughout the clamp. Prevention of vasodilation by L-NMMA led to significant increases in arterial pressure during insulin/glucose infusion but did not alter glucose uptake.

These findings indicate that insulin's vasodilatory effects are mediated by stimulation of NO release, and that they play a role in the regulation of arterial pressure during physiologic hyperinsulinemia. Abnormalities in insulin-induced NO release could contribute to altered vascular function and hypertension in insulin-resistant states. (*J. Clin. Invest.* 1994. 94:2511–2515.) Key words: hyperinsulinemic euglycemic clamp • muscle blood flow • blood pressure • acetylcholine infusion • norepinephrine infusion

Introduction

Insulin exerts effects on the cardiovascular system that may play a role in the regulation of blood pressure (1), and by boosting its own delivery to target tissues, also appears to play a role in its ability to promote glucose disposal in target tissues (2). For example, insulin's vasodilatory effects in skeletal muscle that were first reported more than half a century ago (3) have recently been suggested to represent an important determinant of insulin-mediated glucose uptake in skeletal muscle in humans (4). Using the euglycemic clamp technique, insulin-induced stimulation of muscle blood flow and glucose uptake were shown to be tightly coupled (4). While there is evidence that such stimulation of blood flow is directly related to insulin per se, rather than to insulin-induced stimulation of carbohydrate metabolism (5), the underlying mechanism by which insulin exerts its vasodilatory effects remains unknown.

Over the past few years, endothelium-derived relaxing factor, whose activity is accounted for by nitric oxide (NO),[1] has emerged as a major regulatory mechanism of peripheral vascular tone (6). This factor which is synthesized from the amino acid L-arginine by the enzyme NO synthase is a potent vasodilator. N^G-monomethyl-L-arginine (L-NMMA), an analogue of L-arginine, by its action as a competitive stereospecific inhibitor of NO-synthase, inhibits both basal and stimulated NO synthesis. For example, in the human forearm, intraarterial L-NMMA infusion causes vasoconstriction, indicating that basal NO release is an important determinant of resting blood flow (6). Furthermore, the forearm vasodilation evoked by local infusion of acetylcholine (6) and vasopressin (7) is attenuated after infusion of L-NMMA, suggesting that stimulation of NO synthesis contributes to the vasodilation evoked by these pharmacological agents. Finally, there is evidence in both experimental animals (8) and humans (9) that NO is a mediator of neural nonadrenergic, noncholinergic vasodilation.

In this study we examined the effects on forearm blood flow of local L-NMMA infusion performed before and at the end of a 2-h insulin/glucose infusion and compared these effects with those of norepinephrine infusion (an NO-independent vasoconstrictor). To assess effects of altered insulin-induced vasodilation by L-NMMA on glucose uptake, we also performed calorimetric determinations of carbohydrate metabolism.

Methods

Nine lean healthy men (wt 78±2 kg, height 183±1 cm, body mass index 23.2±0.6 kg/m^2, age 28±2 yr, mean±SE) participated in this study after providing informed written consent. All subjects were normotensive, were taking no medication, and had no evidence of metabolic or cardiovascular disease at the time of the study. Tests were all con-

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1. *Abbreviations used in this paper:* L-NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide.

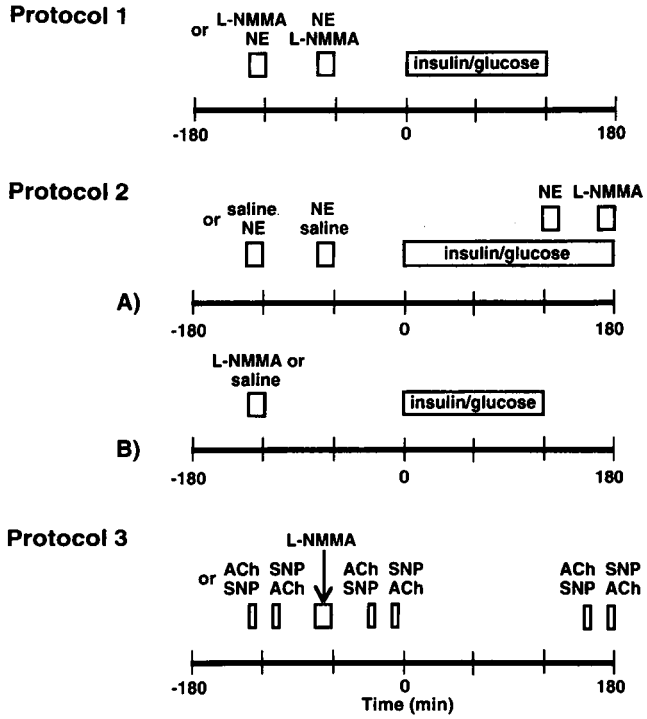

Figure 1. Study design. *NE*, norepinephrine; *ACh*, acetylcholine; and *SNP*, sodium nitroprusside. Vasoconstrictor and vasodilator agents were infused intraarterially (nondominant arm), whereas insulin-glucose was infused intravenously. During intraarterial drug infusions blood flow in both forearms was measured continuously throughout the entire infusion period. During insulin/glucose infusion forearm blood flow was recorded for 5 min out of every 30 min. Respiratory gas exchanges were constantly monitored from time −60 min to 120 min.

ducted in the morning after an overnight fast. Subjects had been on a weight-maintaining diet containing at least 40% energy as carbohydrates for 3 d before the tests. The experimental protocol was approved by the Institutional Review Board on Human Investigation.

General procedures

Subjects were studied in the supine position. Heart rate (electrocardiogram), respiratory excursions (pneumobelt), blood pressure (indwelling catheter in the brachial artery of the nondominant arm), and blood flow in both forearms (venous occlusion plethysmography) were recorded continuously on an electrostatic recorder. Drugs or physiological saline were infused continuously into the brachial artery of the nondominant arm through a 20-gauge needle introduced under local anesthesia. Intravenous catheters were inserted in a right and a left antecubital vein, one for substrate infusion (insulin/glucose), the other for blood sampling. Urine was collected before and at the end of the study for nitrogen determination.

Experimental protocols (Fig. 1)

Protocol 1: effects of L-NMMA and norepinephrine infusion on forearm blood flow at baseline and during insulin/glucose infusion (hyperinsulinemic euglycemic clamp). After resting control values of forearm blood flow had been measured, six subjects received intraarterial infusions (at a fixed rate of 2 ml/min) of four doses of norepinephrine (50, 100, 200, and 400 pmol/min) for 4 min each, and after an interval of 45 min (to allow blood flow to return to control) subjects received four doses of L-NMMA (1, 2, 4, and 8 μmol/min) for 4 min each. The order of L-NMMA and norepinephrine infusion was randomized. 60 min after completing the infusion of the second vasoconstrictor agent, a primed continuous infusion of crystalline insulin (Actrapid HM; Novo Industri S/A, Bagsvaerd, Denmark) was started at a rate of 1 mU/kg per min (6 pmol/kg per min) for 2 h. Euglycemia was maintained by determining plasma glucose concentration every 5 min and periodically adjusting a variable infusion of 20% dextrose (5). Hypokalemia was prevented by administration of KCl infused at a rate of 4 mmol/h. Hemodynamic measurements were recorded for 5 out of every 30 min throughout the insulin glucose/infusion. Blood samples were collected in the basal state and at timed intervals throughout the study for analysis of substrate and hormone concentrations.

Protocol 2: effects of norepinephrine and L-NMMA on forearm blood flow after 2 h of insulin/glucose infusion. Since we found that in protocol 1 insulin-induced forearm vasodilation was suppressed, the aim of this protocol was (*a*) to determine whether this suppression was caused by L-NMMA infusion; and (*b*) to examine vasoconstrictor effects of L-NMMA and norepinephrine during insulin-induced vasodilation. The same six subjects returned for this protocol (protocol 2A), in which they received (in randomized order) norepinephrine and saline infusion (but no L-NMMA) before the start of the clamp. The timing of the infusions (norepinephrine, saline, start of insulin/glucose) was identical to protocol 1. 2 h after the start of insulin/glucose infusion, subjects received intraarterial infusion of four doses of norepinephrine (50, 100, 200, and 400 pmol/min) for 4 min each. Insulin/glucose infusion was continued, and after an interval of 30 min (to allow blood flow to return to control), subjects received four doses of L-NMMA (1, 2, 4, and 8 μmol/min) for 4 min each.

To exclude the possibility that in protocol 1 a synergistic effect of combined L-NMMA and norepinephrine infusion was responsible for the suppression of insulin-induced vasodilation, three other subjects participated in an additional series of two experiments (protocol 2 B), in which they received either L-NMMA alone or normal saline alone (but no norepinephrine) before the start of the hyperinsulinemic clamp (timing of infusions as in protocol 1). Since we found that L-NMMA alone also suppressed the vasodilation evoked by subsequent insulin infusion (and that vasodilator responses evoked by insulin alone and insulin preceded by norepinephrine were comparable), for analysis, the results obtained in these three subjects were pooled with those obtained in the six initial subjects.

Protocol 3: effects of L-NMMA on forearm blood flow responses to acetylcholine and nitroprusside infusion. The aim of this protocol was to examine whether L-NMMA also had a prolonged inhibitory effect on vasodilator responses to a locally administered endothelium-dependent vasodilator. To this end, we examined in five subjects forearm vascular responses to acetylcholine (an endothelium-dependent dilator) and nitroprusside (an endothelium-independent dilator) infusion. Acetylcholine (80 nmol/min for 4 min) and nitroprusside (4 nmol/min for 4 min) were infused in randomized order before L-NMMA and 30 and 210 min after termination of L-NMMA infusion (performed as in protocol 1). The time interval between the infusion of the two vasodilator agents was 15 min.

Forearm blood flow

Blood flow in both forearms was measured with venous occlusion plethysmography, using mercury-in-silastic strain gauges (10). The forearms were elevated 10–15 cm above the level of the right atrium to collapse the veins. The circulation to the hand was arrested by inflating a cuff around the wrist during blood flow determinations, which were performed at 15-s intervals for 5 min out of every 30 min. During intraarterial drug infusions, blood flow was recorded continuously throughout the entire infusion period.

Indirect calorimetry

Substrate utilization was calculated from respiratory gas exchanges (determined by a computerized, flow-through canopy gas analyzer system [Deltatrac, Datex, Helsinki, Finland] and urinary nitrogen excretion, after correction for changes in the body urea nitrogen pool as described earlier (5). Total glucose uptake was assumed to be equal to exogenous glucose infusion. The rate of nonoxidative glucose disposal was calcu-

lated by subtracting the rate of glucose oxidation from the rate of steady-state glucose uptake.

Analytical methods

Plasma glucose was determined in duplicate by the glucose oxidase method on a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was measured by radioimmunoassay (5), blood urea nitrogen using a urea analyzer (Beckman Instruments), and urinary nitrogen by the Kjehldahl method.

Drugs

Drugs were dissolved in physiological saline immediately before use. L-NMMA was obtained from Clinalfa (Läufelfingen, Switzerland), norepinephrine from Hoechst (Frankfurt, Germany), acetylcholine from Dispersa (Hettlingen, Switzerland), and sodium nitroprusside from Roche (Basel, Switzerland).

Data analysis

Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Vascular resistance in the forearm was calculated as mean arterial pressure in millimeters of mercury divided by blood flow in milliliters per min per 100 ml tissue, and expressed in units. During intraarterial drug infusion, the ratio of blood flow in the infused arm compared with that in the control arm was calculated for each measurement period. The ratio of forearm blood flow measured in response to drugs was expressed as a percentage of the ratio measured during the control period (11).

During insulin/glucose infusion, the 5 min of data from forearm blood flow, blood pressure, and heart rate collected every 30 min were averaged to a single value. Blood pressure recordings were analyzed by an observer who was unaware of the study design. Whole body glucose uptake, energy expenditure, and substrate oxidation were averaged for 30-min periods. Statistical analysis was performed using analysis of variance for repeated measures, and paired t tests. A P value < 0.05 was considered statistically significant. Data are given as mean±SE.

Results

Effect of L-NMMA on forearm blood flow response to subsequent insulin/glucose infusion. L-NMMA suppressed both insulin-induced stimulation of forearm blood flow and decreases in forearm vascular resistance (Fig. 2). This inhibitory effect was observed in both the infused and the control (noninfused) arm (not shown). At the end of a 2-h insulin/glucose infusion that was not preceded by L-NMMA, blood flow increased ($P < 0.01$) by 1.04±0.20 ml/min per 100 ml in the infused arm and by 0.68±0.13 ml/min per 100 ml in the control arm ($P > 0.1$, infused vs. control arm), whereas during insulin infusion after L-NMMA, blood flow remained unchanged (changes in blood flow were 0.26±0.14 ml/min per 100 ml and 0.11±0.17 ml/min per 100 ml, respectively) in both arms ($P < 0.01$ vs. insulin infusion not preceded by L-NMMA).

L-NMMA infusion did not have any detectable effect on arterial pressure, heart rate, or contralateral forearm blood flow at baseline. At the time when insulin infusion was started, blood flow in the infused arm (2.70±0.27 ml/min per 100 ml) had returned to its preinfusion values (2.74±0.20 ml/min per ml). L-NMMA did not alter heart rate responses to insulin/glucose infusion; heart rate increased by 4±1 beats/min during insulin infusion not preceded by L-NMMA, and by 5±2 beats/min during insulin infusion after L-NMMA. In contrast, L-NMMA altered blood pressure responses to insulin infusion; after L-NMMA, mean arterial pressure increased slightly but significantly ($P < 0.03$) from 82±1 to 86±2 mmHg at 60 min of insulin infusion and remained above control until the end of the clamp (87±2 mmHg, $P < 0.0001$ vs. control), whereas it remained unchanged (83±2, 82±2, and 84±3 mmHg at 0, 60, and 120 min, respectively) during insulin/glucose infusion not preceded by L-NMMA. At the end of the clamp, arterial pressure tended to be higher ($P = 0.07$) after L-NMMA compared with insulin infusion not preceded by L-NMMA.

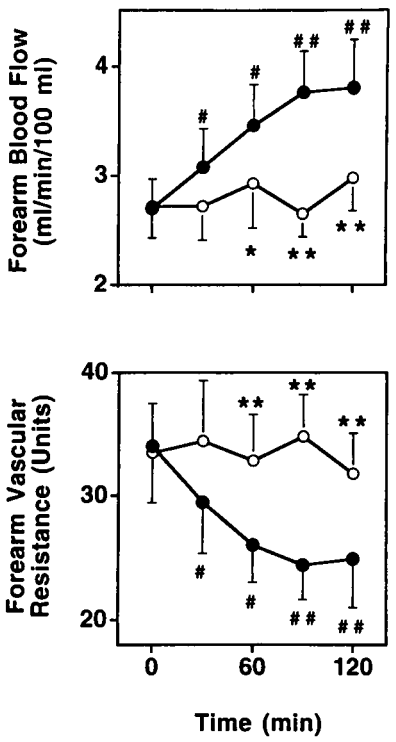

Figure 2. Effect of L-NMMA on forearm vascular responses (infused forearm) evoked by subsequent insulin/glucose infusion in nine subjects. L-NMMA infusion suppressed both insulin-induced stimulation of forearm blood flow and decreases in forearm vascular resistance. (●——●) Insulin infusion not preceded by L-NMMA, (○——○) insulin infusion after L-NMMA. #, $P < 0.05$; ##, $P < 0.005$ vs. corresponding time 0; *, $P < 0.05$; **, $P < 0.01$ vs. insulin infusion preceded by L-NMMA.

L-NMMA did not alter stimulation of glucose uptake, and glucose oxidation evoked by euglycemic hyperinsulinemia; during the last 30 min of the clamp, total glucose uptake was 44±3 μmol/kg per min during insulin infusion not preceded by L-NMMA, and 42±4 μmol/kg per min during insulin infusion performed after L-NMMA ($P > 0.1$); the values for glucose oxidation were 20±1 μmol/kg per min, and 19±2 μmol/kg per min, respectively ($P > 0.1$). Plasma insulin concentration also increased similarly under both conditions; it increased from 52±4 to 447±49 pmol/liter, and from 59±8 to 465±45 pmol/liter, respectively.

Effect of L-NMMA and norepinephrine on forearm blood flow at baseline and during insulin/glucose infusion. L-NMMA infusion caused decreases in forearm blood flow ratio (infused/control arm) that were significantly greater during euglycemic hyperinsulinemia than in the fasting state (Fig. 3). In contrast, norepinephrine evoked decreases in forearm blood flow ratio that were comparable during fasting and euglycemic hyperinsulinemia (Fig. 3). Moreover, vasoconstrictor responses to norepinephrine during fasting were reproducible on both study days; percent reductions in fasting forearm blood flow ratio evoked by graded norepinephrine infusion in protocol 1 (Fig. 3) were 10±4, 21±5, 29±4, and 34±6%, and those in protocol 2 (not shown) were 10±5, 24±1, 26±4, and 39±5%, respectively.

Effect of L-NMMA on forearm blood flow responses to intraarterial acetylcholine and nitroprusside infusion. Before L-NMMA, local acetylcholine and nitroprusside infusion increased forearm blood flow similarly by 152±27 and 144±28%, respectively. After L-NMMA, vasodilator responses to acetyl-

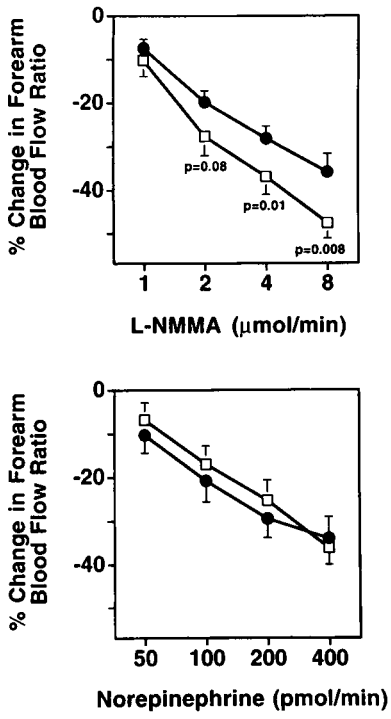

Figure 3. Percent changes in forearm blood flow ratio (infused/control arm) in response to increasing doses of intraarterial L-NMMA (*top*) and norepinephrine (*bottom*) infusion performed during fasting (*filled circles*) and performed during euglycemic hyperinsulinemia (*open squares*) in six subjects. L-NMMA infusion evoked significantly larger decreases in forearm blood flow ratio during hyperinsulinemia than during euinsulinemia. In contrast, vasoconstrictor effects evoked by norepinephrine (an NO-independent vasoconstrictor) were similar under both conditions.

choline were attenuated ($P < 0.05$ vs. control) both shortly (53±16%) and more than 3 h (75±22%) after termination of L-NMMA infusion, whereas the vasodilator responses to nitroprusside remained unchanged (170±24 and 168±36%, respectively).

Discussion

The major new findings are that (*a*) short term L-NMMA infusion prevented insulin-induced stimulation of muscle blood flow throughout a 2-h hyperinsulinemic euglycemic clamp; (*b*) L-NMMA infusion evoked larger reductions in forearm blood flow during hyperinsulinemia than at baseline; and (*c*) prevention of vasodilation by L-NMMA led to significant increases in arterial pressure during insulin/glucose infusion, but did not alter insulin-induced stimulation of whole body glucose uptake. These results indicate that NO-dependent mechanisms play a major role in the mediation of insulin-induced vasodilation in skeletal muscle tissue. Moreover, the present data suggest that during euglycemic hyperinsulinemia, insulin's vasodilatory effects play a role in the regulation of blood pressure, but may not be a primary determinant of glucose disposal.

There is abundant evidence that insulin is a potent vasodilatory stimulus in skeletal muscle tissue. Indeed, shortly after the introduction into clinical practice, insulin-induced stimulation of muscle blood flow was first reported (3). Since then numerous studies have confirmed this finding, and shown that insulin-induced stimulation of muscle blood flow is not dependent upon epinephrine release (12), nor mediated primarily by β-adrenergic (13, 14) or cholinergic (14) mechanisms. The present data demonstrate that NO-dependent mechanisms contribute to insulin-induced vasodilation in skeletal muscle. This interpretation is based on two lines of evidence. First, the decrease in forearm blood flow evoked by L-NMMA administered during insulin/glucose infusion was significantly larger than the one evoked in the absence of insulin infusion, indicating an augmented contribution of NO to overall forearm vascular tone (15) during hyperinsulinemia. In contrast, responses to norepinephrine, an NO-independent vasoconstrictor, were similar at basal and during insulin/glucose infusion. Second, in all our subjects, forearm vasodilation evoked by insulin/glucose infusion was prevented when insulin infusion was preceded by local, intraarterial L-NMMA infusion. This unexpected finding suggests that local intraarterial L-NMMA infusion that at the time when insulin glucose/infusion was started did not have any detectable vasoconstrictor effect (forearm blood flow had returned to its basal values), had a prolonged inhibitory effect on vasodilation (and presumably NO release) evoked by physiologic hyperinsulinemia, an interpretation that is further evidenced by the acetylcholine studies. Thus, these observations in humans are consistent with recent findings in vitro, showing prolonged inhibition of endothelium-dependent relaxation in vascular ring preparations pretreated with a competitive inhibitor of NO-synthase (16). Finally, local intraarterial L-NMMA infusion at the dose used in this study had been thought to have only local effects, because, as in the present study, vasoconstrictor effects were limited to the infused arm, and effects on blood pressure or heart rate were lacking. However, the present observation that local, intraarterial L-NMMA infusion abolished insulin-induced vasodilation not only in the infused, but also in the contralateral forearm, is consistent with the view that such infusion had systemic effects.

Insulin's vascular actions have been the focus of much interest, because recent findings suggest that an impairment of vascular responsiveness to insulin may contribute to insulin resistance. For example, there is evidence in both animals and humans that transcapillary transport of insulin from plasma to interstitium is rate limiting for glucose uptake (17, 18). Furthermore, in lean humans, insulin-induced stimulation of blood flow and glucose uptake in skeletal muscle are closely correlated (4). Finally, in obese insulin-resistant subjects, an impairment in insulin-induced stimulation of muscle blood flow has been proposed to contribute to impaired glucose uptake (4). In the present study, suppression of insulin-induced vasodilation by L-NMMA had no effect on insulin-induced stimulation of whole body glucose metabolism. These findings indicate that the inhibition of forearm blood flow responses to insulin by L-NMMA cannot be explained on the basis of an attenuated stimulation of glucose uptake. More important, this finding could suggest that insulin-induced stimulation of blood flow per se may not be a primary determinant of muscle glucose uptake.

Insulin infusion is associated with both sympathetic activation and vasodilation in skeletal muscle (5, 19, 20), and a balance between these two opposing pressor and depressor effects has been proposed to offer one potential explanation for the absence of an increase in blood pressure during insulin/glucose infusion in humans (19). Our findings provide direct experimental support for this hypothesis and show that prevention of insulin-induced vasodilation by L-NMMA leads to significant increases in blood pressure during euglycemic hyperinsulinemia.

While the present data demonstrate a dramatic impairment by L-NMMA of insulin-induced vasodilation, they do not allow us to determine the mechanism by which insulin stimulates NO release. Recent observations in insulin-resistant humans may suggest potential mechanisms. Insulin resistance is associated with an impairment in both insulin-induced vasodilation and

sympathetic activation in skeletal muscle tissue (21). There is evidence in both experimental animals (8) and humans (9) that neural nonadrenergic noncholinergic vasodilation is mediated by NO release. These observations could be consistent with the concept that insulin stimulates NO release by activation of neural noncholinergic nonadrenergic vasodilator pathways. Alternatively, the recent observation in patients with NIDDM of an impairment in stimulated NO release to local acetylcholine infusion (22) could be consistent with the hypothesis that insulin's actions on NO release may be locally mediated.

We do not know yet the exact mechanism by which insulin stimulates NO release, nor how it may be altered in insulin-resistant states. What this study shows, however, is that stimulation of NO release plays a major role in the mediation of insulin's vasodilatory effects in humans, and that suppression of such vasodilation leads to increases in arterial pressure during physiologic hyperinsulinemia. A defect in insulin-induced stimulation of NO release could be one of the factors that contributes to altered vascular function (9, 21, 23) and hypertension (1) in insulin-resistant states.

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