Vasodilator Response to Systemic But Not to Local Hyperinsulinemia in the Human Forearm

Carmine Cardillo, Crescence M. Kilcoyne, Sridhar S. Nambi, Richard O. Cannon III, Michael J. Quon, Julio A. Panza

Abstract—Insulin-mediated vasodilation has been proposed as an important determinant of whole-body insulin-stimulated glucose disposal. However, it is not clear whether the vasodilator effect of insulin results from a direct action of the hormone or whether alternative mechanisms are involved. To better characterize the mechanism of insulin-mediated vasorelaxation, we compared forearm blood flow (FBF) responses to local (intra-arterial) and systemic (intravenous, euglycemic clamp) hyperinsulinemia in 10 healthy lean subjects using venous occlusion plethysmography. In addition, we assessed the effect of nitric oxide (NO) synthase inhibition by \( \text{N}^\text{G}\)-monomethyl-L-arginine (L-NMMA) on the vasodilator and metabolic responses to hyperinsulinemia. Similar forearm concentrations of insulin were achieved during local and systemic infusion (231±39 versus 265±22 \( \mu \text{U/mL} \); \( P=0.54 \)). Of note, FBF did not change significantly in response to local hyperinsulinemia (from 2.6±0.3 to 2.4±0.3 mL \( \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \); \( P=0.50 \)). In contrast, systemic hyperinsulinemia caused a 52% increase in FBF (from 2.5±0.2 to 3.8±0.5 mL \( \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \); \( P<0.004 \)), which was reversed by L-NMMA (FBF decreased from 3.8±0.5 to 2.3±0.2 mL \( \cdot \text{min}^{-1} \cdot \text{dL}^{-1} \); \( P=0.004 \)). We conclude that systemic, but not local, hyperinsulinemia induces vasodilation in the forearm. Our findings suggest that insulin-mediated vasodilation is not due solely to a direct stimulatory effect of insulin but involves additional mechanisms activated only during systemic hyperinsulinemia. (Hypertension. 1998;32:740-745.)

Key Words: insulin \( \Box \) vasodilation \( \Box \) glucose \( \Box \) nitric oxide \( \Box \) L-NMMA

The vasoactive properties of insulin have recently received growing attention. It has been reported that physiological hyperinsulinemia during euglycemic clamp increases skeletal muscle perfusion.\(^1,2\) This effect correlates with the ability of insulin to stimulate glucose uptake in muscle, thus suggesting a potential role for perfusion in determining insulin-mediated glucose disposal.\(^1\) Moreover, insulin-mediated vasodilation is defective in insulin-resistant states such as obesity, non– insulin-dependent diabetes, and essential hypertension.\(^1,3\) Thus, an impaired ability of the vasculature to dilate in response to insulin may contribute to insulin resistance.

The release of nitric oxide (NO) from vascular endothelium appears to participate in determining the effect of insulin on skeletal muscle perfusion, as indicated by recent studies showing that the vasodilator response to systemic hyperinsulinemia can be blunted by infusion of the NO synthase inhibitor \( \text{N}^\text{G}\)-monomethyl-L-arginine (L-NMMA).\(^4,5\) Also, NO-mediated increase in skeletal muscle perfusion may contribute to glucose uptake, since L-NMMA infusion significantly reduces muscle glucose disposal in response to hyperinsulinemia.\(^6\) Moreover, it has been reported that obese insulin-resistant patients have endothelial dysfunction and impaired NO-dependent vasodilator response to insulin.\(^7\)

It must be noted, however, that all studies reporting an involvement of NO in determining hemodynamic and metabolic effects of insulin have used systemic hyperinsulinemia during euglycemic clamp. Therefore, it is not clear whether the vasodilator effect of systemic hyperinsulinemia stems solely from a direct action of the hormone on endothelial NO production or whether other mechanisms are also involved. To clarify this issue, in the present study we compared the vasodilator response to local and systemic hyperinsulinemia in normal subjects and assessed the effect of NO synthase inhibition by L-NMMA on insulin-mediated vasodilation.

**Methods**

**Study Population**

Ten healthy normal volunteers with no family history of diabetes or hypertension, whose clinical and metabolic characteristics are reported in Table 1, were selected for this study. Before inclusion, subjects were screened by clinical history, physical examination, routine chemical analyses, ECG, and chest radiography. Exclusion criteria were a history or evidence of present or past arterial hypertension, hypercholesterolemia, diabetes mellitus, cardiac disease, peripheral vascular disease, coagulopathy or any other disease predisposing them to vasculitis, or Raynaud’s phenomenon. All subjects were on an isocaloric diet with an approximate caloric distribution of 50% carbohydrate, 30% fat, and 20% protein. None of the volunteers was taking any medication or vitamin supplement. The study protocol was approved by the National Heart, Lung, and...
Blood Institute Investigational Review Board, and all participants gave written informed consent.

Study Protocol
After subjects had fasted overnight, all studies were performed the next morning in a quiet room with a temperature of approximately 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before studies. While the participants were supine, a 20-gauge Teflon catheter (Arrow Inc) was inserted into the brachial artery of the left arm for drug infusion and blood sampling. Another 20-gauge catheter (Abbott Laboratories) was inserted into a deep antecubital vein of the same arm for blood sampling. Forearm blood flow (FFB) was measured by venous occlusion strain-gauge plethysmography, blood pressure was recorded directly from the intra-arterial catheter immediately after each flow measurement, and heart rate was continuously recorded by ECG.

Study 1: Local Hyperinsulinemia
All subjects, after the forearm was instrumented, received intra-arterial infusion of saline for 15 minutes at 1 mL/min; subsequently, baseline blood flow was measured, and arterial and venous blood samples were obtained. Then, infusion of regular insulin (Humulin; Eli Lilly) was superimposed at 0.2 mU per kilogram of body weight per minute (1 mL/min infusion rate) for 2 hours. During this infusion period, FFB was measured, and arterial and venous blood samples were collected.

Study 2: Systemic Hyperinsulinemia
On a different occasion, after the forearm was instrumented, subjects underwent euglycemic hyperinsulinemic clamp with infusion of insulin in a deep vein of the contralateral arm at a dose of 120 mU/m² per minute for 3 hours. Euglycemia was maintained by determining blood glucose concentration every 5 to 10 minutes and periodically adjusting an infusion of 20% dextrose. Hypokalemia was prevented by administration of KCl at 0.23 mEq/kg per hour. Hemodynamic measurements were recorded at baseline and every 30 minutes throughout the insulin/glucose infusion. Blood samples were collected at baseline and throughout the study for analysis of hormone concentrations. Blood glucose concentration every 5 to 10 minutes and periodically adjusting an infusion of 20% dextrose. Hypokalemia was prevented by administration of KCl at 0.23 mEq/kg per hour. Hemodynamic measurements were recorded at baseline and every 30 minutes throughout the insulin/glucose infusion. Blood samples were collected at baseline and throughout the study for analysis of hormone concentrations.

RESULTS

Clinical and metabolic data of our study population are reported in Table 1. The age of our study subjects ranged from 46 to 64 years, their body mass index ranged from 20.7 to 26.7 kg/m², and the glucose infusion rate during the euglycemic clamp ranged from 1.8 to 4.4 mmol/min. As expected, the glucose infusion rate per insulin unit was inversely correlated with body mass index (r = −0.72, P = 0.027).

Table 1. Individual Demographic and Metabolic Characteristics

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<th>Subject</th>
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<th>BMI, kg/m²</th>
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<th>Local FGU, μmol/min</th>
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Mean ± SEM: 52 ± 2 64/4 74 ± 4 24.6 ± 0.7 169 ± 8 3.2 ± 0.3 3.68 ± 0.48 6.65 ± 0.85

GIR indicates glucose infusion rate during steady state of euglycemic hyperinsulinemia; Local FGU, forearm glucose uptake during local hyperinsulinemia; and Systemic FGU, forearm glucose uptake during systemic hyperinsulinemia.
Vasodilator Response to Insulin

In the basal state (during saline infusion), plasma insulin concentration was 6.2 ± 1.5 μU/mL; after 2 hours of intra-arterial infusion of insulin (0.2 mU · kg⁻¹ · min⁻¹), local venous insulin concentration increased to 231 ± 39 μU/mL (P < 0.001 versus baseline). Local administration of insulin for 2 hours did not produce any significant change in either blood flow (P = 0.50 versus baseline) or vascular resistance (P = 0.63) (Figure 1). Similarly, insulin administration did not significantly affect mean arterial pressure (85 ± 2 mm Hg at baseline versus 86 ± 2 mm Hg during hyperinsulinemia; P = 0.84) or heart rate (59 ± 3 bpm at baseline versus 58 ± 3 bpm during hyperinsulinemia; P = 0.72).

In the basal state, forearm fractional glucose extraction was 6.8 ± 0.7%, and forearm glucose uptake was 0.92 ± 0.17 μmol · min⁻¹ · dL⁻¹. After 2 hours of intra-arterial infusion of insulin, fractional glucose extraction increased to 38 ± 6% (P < 0.001 versus baseline), and glucose uptake increased to 3.7 ± 0.5 μmol · min⁻¹ · dL⁻¹ (P < 0.001 versus baseline).

Study 2: Systemic Hyperinsulinemia
Plasma insulin concentrations were 8 ± 2 μU/mL in the basal state and 265 ± 22 μU/mL (P < 0.001 versus baseline) during systemic infusion of insulin. During steady-state euglycemic hyperinsulinemic clamp, plasma insulin concentrations were not significantly different from effluent venous plasma levels achieved during local hyperinsulinemia (P = 0.54). Arterial glucose levels were clamped at 5.1 ± 0.3 mmol/L, a level similar to baseline values (5.0 ± 0.2 mmol/L; P = 0.83). During euglycemic clamp, plasma insulin levels were not significantly different before (265 ± 22 μU/mL) or after L-NMMA administration (257 ± 22 μU/mL; P = 0.07).

In contrast with the results obtained with local infusion of insulin, a significant increase in FBF from baseline was observed during systemic hyperinsulinemia. The increase in FBF during euglycemic clamp was 12% (P = 0.13) after 30 minutes and 21% (P = 0.03) after 60 minutes of systemic hyperinsulinemia (before steady-state conditions were achieved). Between 2 and 3 hours after insulin infusion was begun, when steady-state euglycemic hyperinsulinemia was achieved, there was a 52% increase in FBF (P < 0.004) (Table 2) and a 31% decrease in vascular resistance (from 36 ± 5 to

**TABLE 2.** Individual Hemodynamic Data at Baseline, After 2 Hours of Intra-Arterial Infusion of Insulin (0.2 mU · kg⁻¹ · min⁻¹), and During Steady State of Euglycemic Hyperinsulinemic Clamp (120 mU/m² per min) Before and During L-NMMA (4 μmol/min) Administration

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<td>FBE, mL · min⁻¹ · dL⁻¹</td>
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Mean ± SEM

MAP indicates mean arterial pressure; HR, heart rate.
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L-NMMA. There was no significant difference in forearm glucose uptake during euglycemic clamp before and after L-NMMA. There was no significant difference in forearm glucose uptake during euglycemic clamp before and after L-NMMA. (Figure 2). Insulin-stimulated forearm glucose uptake was significantly higher during systemic than during local hyperinsulinemia (r=0.42, P=0.27). Systemic insulin infusion did not significantly affect mean arterial pressure (82±2 mm Hg at baseline versus 83±3 mm Hg during hyperinsulinemia; P=0.85) but induced a significant increase in heart rate. The increase in heart rate was already observed after 30 minutes of hyperinsulinemia (9%; P=0.01 versus baseline) and reached 18% (from 57±2 bpm to 67±2 bpm; P=0.003) after 3 hours of insulin infusion. A significant correlation was observed between the increase in heart rate and the vasodilator response to systemic hyperinsulinemia (r=0.80, P=0.005).

In the basal state, forearm fractional glucose extraction was 7.6±1.1%, and forearm glucose uptake was 0.92±0.17 µmol·min⁻¹·dL⁻¹ (Figure 2). During hyperinsulinemic clamp, fractional glucose extraction rose to 42.8±4.8% (P<0.001 versus baseline) and forearm glucose uptake to 6.65±0.85 µmol·min⁻¹·dL⁻¹ (P<0.001 versus baseline) (Figure 2). Insulin-stimulated forearm glucose uptake was significantly higher during systemic than during local hyperinsulinemia (P<0.001). No significant correlation was observed between changes in FBF from baseline induced by systemic hyperinsulinemia and forearm glucose uptake during euglycemic clamp (r=0.44, P=0.24).

During steady-state hyperinsulinemia, intra-arterial infusion of L-NMMA abolished the vasodilator effect of insulin. L-NMMA administration decreased FBF from 3.8±0.5 to 2.3±0.2 mL·min⁻¹·dL⁻¹ (P=0.004) and increased vascular resistance (from 25±3 to 39±4 mm Hg per mL·min⁻¹·dL⁻¹; P<0.004). The infusion of L-NMMA during euglycemic hyperinsulinemia resulted in a 23% increase in forearm glucose extraction (from 42.8±4.8% to 52.1±5.5%; P=0.008) and a 15% decrease in forearm glucose uptake (from 6.65±0.85 to 5.68±0.66 µmol·min⁻¹·dL⁻¹; P=0.35) (Figure 2).

Discussion

The results of the present study show that systemic, but not local, hyperinsulinemia results in vasodilation of the forearm circulation in a group of normal lean subjects. This vasodilator response to systemic hyperinsulinemia was reversed after administration of L-NMMA, a competitive inhibitor of NO synthesis, thereby suggesting that endothelial release of NO is involved at least partially in this circulatory effect of insulin, in agreement with the results of previous studies. In contrast, local hyperinsulinemia did not produce vasodilation in the same subjects, even though local intravascular concentrations of the hormone were similar to those achieved during systemic hyperinsulinemia. Thus, the findings of our study extend previous observations by suggesting that insulin-mediated vasodilation may be related to mechanisms activated by systemic hyperinsulinemia that are not stimulated during local insulin infusion. In this regard, it is interesting to note that in our study a significant vasodilator response to systemic hyperinsulinemia occurred only after 60 minutes, and the maximal vasodilator effect was seen after 2 hours of insulin infusion. This finding is consistent with the results of previous investigations reporting a relatively slow-onset vasodilator effect of hyperinsulinemia (see Reference 12 for review).

Among the mechanisms activated by systemic hyperinsulinemia that could potentially contribute to vasodilation, one candidate is the sympathetic nervous system. Increased sympathetic activity during systemic hyperinsulinemia has been reported by several investigators, who have found enhanced muscle sympathetic traffic at microneurography in response to insulin infusion. The concept of a forearm vasodilator response to enhanced sympathetic activity is supported by the findings of Anderson et al, who reported both sympathetic stimulation and forearm vasodilation during systemic hyperinsulinemia. This observation is strengthened by the findings of Vollenweider et al, who reported that the absence of sympathetic activation is associated with defective vasodilator response to insulin in obese insulin-resistant patients, and those of Hausberg et al, who recently observed a lower degree of sympathetic activation and lack of vasodilator response to insulin in elderly subjects. The significant correlation between changes in heart rate and FBF observed in our study during systemic hyperinsulinemia also supports this concept.

In our study, in agreement with a previous report, insulin-stimulated forearm glucose uptake was considerably higher during systemic than during regional hyperinsulinemia, even in the presence of similar intravascular concentrations of the hormone. Thus, a higher rate of glucose metabolism during systemic hyperinsulinemia could have been a stronger stimulus toward vasodilation, leading to activation of the NO system through some undiscovered...
pathway. This hypothesis, however, seems unlikely because in our study local hyperinsulinemia, albeit to a lesser extent than systemic insulin infusion, did indeed induce an approximately 4-fold increase in forearm glucose uptake over baseline. Hence, if insulin-stimulated glucose metabolism were a primary determinant of the hemodynamic action of the hormone, one would expect some degree of vasodilation even in response to local hyperinsulinemia, whereas, in fact, after 2 hours of regional insulin infusion, FBF remained unchanged from baseline. Moreover, Vollenweider et al. have previously investigated the relationship between carbohydrate metabolism and insulin-mediated sympathetic activation and vasodilation. They observed that for equivalent rates of carbohydrate oxidation achieved with infusion of insulin/glucose, insulin/glucose, and fructose, the degree of sympathetic activation and skeletal muscle vasodilation was highest with insulin/glucose (high insulin circulating levels), intermediate with glucose (intermediate insulin levels), and lowest with fructose (low insulin levels). These findings indicate that insulin per se rather than insulin-stimulated carbohydrate metabolism is responsible for sympathetic stimulation and skeletal muscle vasodilation. The same groups of investigators have also reported that attenuation by 40% of insulin-mediated glucose uptake by free fatty acid infusion does not result in any detectable effect on insulin-induced sympathetic discharge and vasodilation. Finally, a recent report from Baron et al. has shown that prevention of insulin-induced NO-dependent vasodilation by pretreatment with L-NMMA markedly reduces leg glucose uptake; importantly, the inhibitory action of L-NMMA on insulin-mediated leg blood flow increase had a faster time course than that on leg glucose uptake, thereby suggesting that changes in blood flow in response to insulin are primary and instrumental in modulating the rate of glucose metabolism rather than vice versa.

It must be acknowledged that in our study, the lack of a proper experimental control does not provide conclusive evidence about the specificity of the effect of NO inhibition on the vasodilator response to systemic hyperinsulinemia. Our findings, however, are in line with those of previous studies in which the effects of L-NMMA on skeletal muscle vasodilation observed during euglycemic hyperinsulinemic clamp have been compared with those produced by L-NMMA on baseline flow or with those induced by a different vasoconstrictor, norepinephrine. The results of those studies have indicated that the vasodilator response to systemic hyperinsulinemia is indeed related to increased NO activity.

Our observation that local hyperinsulinemia does not result in vasodilation is in agreement with the results of previous studies showing no significant vasmotor response after intra-arterial infusion of insulin, but it contrasts with other reports of vasodilator effect of local hyperinsulinemia (see References 12 and 21 for review). The reasons for these discrepancies are not entirely clear, but it is possible that differences in insulin doses, infusion times, muscle forearm content, or methodology used to measure blood flow could have contributed to the different results. It is important to notice, however, that even in studies reporting a vasodilator effect of local hyperinsulinemia, the degree of the observed vasodilation was generally mild (≈25%) and much lower than that observed in this and other studies during systemic hyperinsulinemia, further supporting the concept that additional mechanisms activated only by systemic hyperinsulinemia contribute importantly to the vasodilator effect of the hormone.

Another finding of our study is related to the effect of NO synthase inhibition on the metabolic response to insulin. During euglycemic clamp, we did not observe a significant decrease in the disposal of plasma glucose by forearm skeletal muscle tissue in response to hyperinsulinemia after NO synthase inhibition by L-NMMA. This finding is explained by the fact that the decrease in FBF induced by L-NMMA was counteracted by an increase in the forearm glucose extraction, so that forearm glucose uptake was not affected. Our results are in keeping with those of a recent study showing that L-NMMA infusion into human forearm abolishes the NO-dependent increase in blood flow in response to local administration of insulin growth factor-I (IGF-I) without affecting the insulin-like metabolic response of the skeletal muscle tissue to IGF-I. These observations, however, are in contrast with the findings of Baron and coworkers, who have reported that when NO-mediated insulin-induced vasodilation in skeletal muscle is reversed or prevented by L-NMMA, there is a significant decrease in leg glucose uptake. Although the reasons for these discrepancies are unclear, a different regulation of the vasoactive and metabolic response of the microcirculatory bed of the forearm and the leg might be hypothesized as a potential explanation.

In conclusion, the present study demonstrates that systemic, but not local, hyperinsulinemia induces a vasodilator effect in the forearm circulation that is likely mediated by NO release. These findings suggest that insulin-dependent vasodilation is not due solely to a direct stimulatory effect of insulin but involves additional mechanisms activated only during systemic hyperinsulinemia.

References


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