Insulin Reduces Reflex Forearm Sympathetic Vasoconstriction in Healthy Humans

Giuseppe Lembo, Virgilio Rendina, Guido Iaccarino, Fausto Lamenza, Massimo Volpe, and Bruno Trimarco

Previous in vitro studies indicate that insulin modifies vascular reactivity to different agents. We have previously demonstrated that in normotensive humans physiological hyperinsulinemia is associated with an increase of forearm norepinephrine release but does not modify vascular resistance. To explore whether insulin modulates peripheral vasoconstriction induced by reflex sympathetic activation, we studied its effects on forearm hemodynamics (strain-gauge plethysmography) during graded levels of lower body negative pressure (−5, −10, −15, and −20 mm Hg, each for 5 minutes) in normotensive subjects. For this purpose, eight subjects received an intrabrachial artery infusion of regular insulin at a systemically ineffective rate (0.05 milliunits/kg per minute) so that deep-venous insulin levels increased in the experimental forearm from 16.5±2.9 to 379.6±30 pmol/L (p<0.01), whereas arterial insulin levels remained unchanged (from 40.9±8.6 to 43.1±7.9 pmol/L, NS). In the control arm, forearm vascular resistance (units) increased from 52.3±3 to a peak of 78.4±5 (p<0.001) during lower body negative pressure. In the insulin-exposed forearm, vascular resistance (46.4±2 at baseline) remained unchanged during insulin infusion (45.8±3, NS) and rose to a peak of 54.8±6 (p<0.05) during lower body negative pressure. The response of forearm vascular resistance to lower body negative pressure was different in the two forearms (F=4.506, p<0.01, repeated-measures analysis of variance with grouping factor). Our results demonstrate that in normotensive subjects local physiological hyperinsulinemia reduces the forearm vasoconstrictive response to reflex sympathetic activation. (Hypertension 1993;21:1015-1019)

KEY WORDS • insulin • vascular resistance • sympathetic nervous system • norepinephrine • lower body negative pressure

A lthough in vitro studies have clearly demonstrated that insulin induces an attenuation of the vascular reactivity,1,2 only indirect evidence supports such an effect of insulin on cardiovascular reactivity in humans. In particular, Anderson et al,3 recording muscle sympathetic nerve activity, forearm blood flow, and blood pressure in normotensive subjects during euglycemic hyperinsulinemia, found an increase in sympathetic discharge accompanied by an increase in forearm blood flow and a decrease in forearm vascular resistance. Thus, these authors concluded that with acute increases in plasma insulin within the physiologically postprandial range, the pressor actions mediated by the sympathoexcitatory effects of insulin are offset by the vasodilator actions. More recently, we reported4 that in both normotensive subjects and hypertensive patients during euglycemic clamp a physiological increase in plasma insulin levels is associated with a marked increase in forearm norepinephrine release with no increase in blood pressure and forearm vascular resistance. This observation further supports the hypothesis that insulin blunts vascular reactivity.

However, in both those studies, insulin itself represents the stimulus for sympathetic overactivity, and therefore the direct effect of insulin on vascular reactivity cannot be adequately quantified. The present study was planned to assess in normotensive subjects the effect of a local hyperinsulinemia on the forearm vascular response to neurogenic vasoconstriction induced by lower body negative pressure (LBNP).

Methods

Subjects

The study group consisted of eight normotensive volunteers (mean age, 28±3 years). A medical history and physical examination were performed to exclude any illness, hypertension, or use of medication. Renal, liver, and endocrine functions were normal. No subject had recent changes in body weight or dietary habits. Normal body weight (<20% above or below desirable body weight, according to life insurance tables5) and normal tolerance to a 75-g oral glucose load (according to the criteria of the National Diabetes Data Group6) were additional criteria for inclusion in the study. No subject was engaged in competitive sports or did intense physical activity during the 2 days preceding the study. Written informed consent was obtained from all participants. The experimental protocol was approved by the Ethics Committee of the University of Naples School of Medicine.

Procedures

The studies began at 8 AM in a quiet room with a constant temperature of 22–24°C. All subjects were

From the Institute of Internal Medicine, School of Medicine, Federico II University, Napoli, Italy.

Address for correspondence: Bruno Trimarco, MD, Medicina Interna, Via Pansini 5, 80131 Napoli, Italy.
studied in the postabsorptive state while they were in the supine position after a 12-15-hour overnight fast. No premedication was administered. On a subject's arrival at the laboratory, forearm volume was measured by water displacement. The forearm perfusion technique was performed as previously described. A plastic cannula was introduced in a retrograde manner into a large antecubital vein. In the same arm, a second double-lumen catheter with the distal hole separated by approximately 3 cm from the proximal one (Arrow International Inc., Reading, Pa.) was introduced into the brachial artery. The distal lumen was used for the infusion of insulin and other test substances, and the proximal lumen was used to sample arterial blood entering the forearm, uncontaminated by solutions infused downstream, and to measure arterial blood pressure, by means of a Statham P23Db pressure transducer (Cleveland, Ohio). Systolic and diastolic blood pressures were simultaneously recorded on a multichannel polygraph (Gould Instruments, Oxford, Calif.). Heart rate was determined from a simultaneously obtained electrocardiographic signal and calculated from the RR interval. Bilateral forearm blood flow (expressed in milliliters per minute per 100 milliliters) was measured by strain-gauge plethysmography using a Digimatic DM2000 (Medimatic, Copenaghen, Denmark) with calibrated mercury-in-Silastic strain gauges applied around the arms. The strain gauge was placed approximately 5 cm below the antecubital crease. Each arm was supported above heart level. Forearm blood flow was measured simultaneously in both arms from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating a cuff around the upper arm. Forearm vascular resistance was calculated as the ratio of mean arterial pressure (diastolic pressure plus one third of the pulse pressure) to the forearm blood flow and expressed as units reflecting millimeters of mercury per milliliter per 100 milliliters of tissue per minute.

LBNP was applied to induce unloading of cardiopulmonary baroreceptors and to evoke reflex vasoconstriction. A LBNP chamber, similar to that described by Mark and Kerber, was placed over the subject's body below the iliac crest and was sealed. Negative pressure was applied incrementally at -5, -10, -15, and -20 mm Hg for 5 minutes each. Forearm blood flow was measured in both arms during the last 90 seconds of each level of LBNP.

After complete instrumentation, a minimum of 20 minutes of quiet rest preceded the measurements of basal hemodynamics and venous and arterial blood samplings.

Protocol

Infusions were made only into the experimental arm. In each subject, we assessed the effect of vehicle infusion (0.9% saline) on the LBNP-induced forearm vascular response by comparing the hemodynamic response in the two arms. In particular, during graded LBNP, we measured arterial blood pressure, heart rate, and forearm blood flow in the two arms. In addition, arterial blood samples were obtained for the assessment of plasma norepinephrine levels. Subsequently, to evaluate the effects of insulin on the forearm vasoconstrictor response to LBNP, we administered human regular insulin, diluted in 0.9% saline, by constant infusion into one brachial artery at a rate of 0.05 milliunits/kg per minute to produce an increment in arterial plasma insulin levels of approximately 600 pmol/L in the experimental arm. After 30 minutes, hemodynamic and neurohumoral responses to LBNP were assessed again during insulin infusion. To demonstrate that the infused insulin had only local effects, we also measured glucose and potassium levels in forearm arterial and venous blood samples.

Analytical Methods

Plasma glucose was determined on a glucose analyzer (Beckman Instruments Inc., Fullerton, Calif.). Serum potassium concentrations were measured by a Beckman electrolyte analyzer. Plasma insulin was measured by radioimmunoassay. Plasma catecholamines were partially purified by batch alumina extraction, separated using ion-pairing reversed-phase high performance liquid chromatography (μBondapak C18 column, Powerline 600A chromatography system, and WISP 700 autoinjector, Waters Chromatography Division, Milford, Mass.), and quantified by a current produced on exposure of the column effluent to oxidizing and then reducing potentials connected in series (Coulochem II, ESA, Inc., Bedford, Mass.). Recovery through the alumina extraction step, calculated using dihydroxybenzylamine as an internal standard, ranged 60-70%; each sample was corrected for its recovery, and detection limits were 3 pg. Intra-assay and interassay variation coefficients were for norepinephrine 4.1% and 9.8%, respectively.

Statistical Design and Analysis

The main purpose was to compare the responses to LBNP in the experimental and control arms during vehicle and insulin infusions. In particular, the assessment of forearm vascular resistance in the control arm during graded LBNP was used to test the specificity of the effect of insulin on the vasoconstrictive response to LBNP in the experimental arm and to test the repeatability of the stimulus applied (i.e., LBNP), thus supporting the hypothesis that possible changes in the vasoconstrictive response in the experimental arm should be ascribed to local infusion of insulin rather than to a change in the magnitude of the stimulus.

Results were presented as mean±SEM. Data were evaluated, as appropriate, by Student's t test, one-way repeated-measures analysis of variance, with post hoc contrast across dependent variables for within-subjects effects, and repeated-measures analysis of variance with grouping factor to evaluate in each infusion period (vehicle or insulin) the interaction between LBNP effects and treatments.

Results

Resting Values

As shown in Tables 1 and 2, there were no significant changes in systolic and diastolic blood pressures, heart rate, and arterial plasma norepinephrine levels during intrabrachial infusion of insulin or vehicle. In addition, blood flow and vascular resistance in the experimental and control forearms were not affected by the intrabrachial infusions (Tables 1 and 2). During intrabrachial...
insulin administration, levels of arterial insulin (from 40.9 ± 8.6 to 43.1 ± 7.9 pmol/L, NS), glucose (from 4.3 ± 0.02 to 4.3 ± 0.03 mmol/L, NS), and potassium (from 4.18 ± 0.14 to 4.07 ± 0.13 mmol/L, NS) remained unmodified. However, in the experimental arm, the deep-venous insulin levels increased (from 16.5 ± 2.9 to 37.6 ± 30 pmol/L, p < 0.01), and concentrations of glucose (from 4.1 ± 0.03 to 3.7 ± 0.02 mmol/L, p < 0.05) and potassium (from 4.3 ± 0.13 to 3.92 ± 0.17 mmol/L, p < 0.05) fell, thus suggesting a significant rise in glucose and potassium uptake.

**Vasoconstrictive Responses**

Systolic and diastolic blood pressures and heart rate remained unchanged during graded LBNP during both intrabrachial infusion of vehicle and insulin administration. LBNP raised arterial norepinephrine concentration to a comparable extent during vehicle infusion and during local hyperinsulinemia (F = 0.246, NS) (Tables 1 and 2). During intrabrachial insulin infusion, LBNP induced in the experimental arm a significant increase of forearm vascular resistance only at the levels of -10 and -15 mm Hg, and the whole vascular response was statistically different from that observed when LBNP was applied during vehicle infusion (F = 11.518, p < 0.001). In contrast, in the control arm, the forearm vascular response to LBNP during insulin infusion was similar to that observed during vehicle infusion (F = 0.435, NS) (Tables 1 and 2). Therefore, LBNP raised forearm vascular resistance to a comparable extent in the two arms during vehicle infusion, whereas the vasoconstrictive response in the experimental arm was blunted and significantly different compared with that of the control arm during intrabrachial insulin administration (F = 4.506, p < 0.01) (Figure 1).

**Discussion**

Although the effect of insulin on vascular reactivity has been intensively investigated in in vitro studies, it still remains unclear whether in vivo insulin exerts a direct action on target tissues that can modulate physiological vasoconstriction. To address this issue, we compared the neurogenic vasoconstriction induced by the application of graded LBNP in one arm, which was simultaneously exposed to an intra-arterial infusion of insulin, and in a control arm, in which no perturbation in hormones and substrates was induced. The difference between the responses measured in the experimental and control arms reflects the net effect of insulin on the vascular bed. It is not possible to measure the increase in insulin concentration at the forearm arterial level.

**Table 1. Hemodynamic and Neurohumoral Effects of Graded Lower Body Negative Pressure During Intrabrachial Saline Infusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base</th>
<th>Base infusion</th>
<th>Lower body negative pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mm Hg)</td>
<td>139±3</td>
<td>138±3</td>
<td>142±3</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>77±2</td>
<td>78±2</td>
<td>79±2</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>98±2</td>
<td>98±2</td>
<td>100±2</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>76±4</td>
<td>74±5</td>
<td>75±5</td>
</tr>
<tr>
<td>FBF experimental arm (mL/min per 100 mL)</td>
<td>1.98±0.10</td>
<td>2.06±0.11</td>
<td>1.56±0.06*</td>
</tr>
<tr>
<td>FBF control arm (mL/min per 100 mL)</td>
<td>1.87±0.14</td>
<td>1.79±0.14</td>
<td>1.57±0.12†</td>
</tr>
<tr>
<td>FVR experimental arm (units)</td>
<td>50±1.92</td>
<td>48±1.85</td>
<td>65.2±3.1*</td>
</tr>
<tr>
<td>FVR control arm (units)</td>
<td>51.4±3.6</td>
<td>56.4±3.8</td>
<td>65.2±3.6*</td>
</tr>
<tr>
<td>NE (nmol/L)</td>
<td>1.41±0.24</td>
<td>1.37±0.25</td>
<td>1.49±0.24†</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; FVR, forearm vascular resistance; NE, arterial plasma norepinephrine concentration. Data are mean±SEM.

*p < 0.05, †p < 0.01 compared with base during saline infusion (base infusion).

**Table 2. Hemodynamic and Neurohumoral Effects of Graded Lower Body Negative Pressure During Intrabrachial Insulin Infusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base</th>
<th>Base infusion</th>
<th>Lower body negative pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mm Hg)</td>
<td>140±3</td>
<td>140±4</td>
<td>141±4</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>78±2</td>
<td>78±2</td>
<td>78±3</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>99±2</td>
<td>98±3</td>
<td>99±3</td>
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<tr>
<td>HR (bpm)</td>
<td>75±5</td>
<td>75±5</td>
<td>76±5</td>
</tr>
<tr>
<td>FBF experimental arm (mL/min per 100 mL)</td>
<td>2.15±0.11</td>
<td>2.26±0.23</td>
<td>2.14±0.27</td>
</tr>
<tr>
<td>FBF control arm (mL/min per 100 mL)</td>
<td>2.04±0.15</td>
<td>1.96±0.16</td>
<td>1.63±0.13*</td>
</tr>
<tr>
<td>FVR experimental arm (units)</td>
<td>46.4±1.8</td>
<td>45.8±3.13</td>
<td>49.8±4.1</td>
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<tr>
<td>FVR control arm (units)</td>
<td>49.9±3.4</td>
<td>52.3±3.5</td>
<td>62.9±4.2*</td>
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<tr>
<td>NE (nmol/L)</td>
<td>1.43±0.24</td>
<td>1.52±0.25</td>
<td>1.62±0.26†</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; FVR, forearm vascular resistance; NE, arterial plasma norepinephrine concentration. Data are mean±SEM.

*p < 0.05, †p < 0.01 compared with base during insulin infusion (base infusion).
during intrabrachial infusion of the hormone. However, because we can exclude an incomplete mixing of infused substance in the arterial blood resulting in ununiform exposure of forearm tissues to the infused substance, the observation that the increase in the forearm deep-venous plasma insulin level was similar to that observed after oral glucose load suggests that the arteriolar levels of insulin achieved in our study are in the physiological range. To simplify the experimental protocol, we decided not to measure the changes in intracardiac pressure induced by LBNP, which represent the stimulus for sympathetic reflex activation. Thus, we used the simultaneous assessment of forearm blood flow in the control arm to rule out the possibility that the changes in the vascular response in the experimental arm could result from a different fall in cardiac filling pressure during LBNP. The observation that no difference could be detected between the vascular response observed in the control arm during vehicle or insulin infusion demonstrates that the changes in neurogenic vasoconstriction in the experimental arm are exclusively due to insulin effect. As expected, the application of levels of LBNP up to 20 mm Hg, which unload prevalently cardiopulmonary receptors, induced a significant increase in sympathetic discharge to skeletal muscle and no change in heart rate. Thus, our finding that insulin attenuates the increase in forearm vascular resistance induced by LBNP provides the first evidence that an increase in insulin plasma levels, comparable to those commonly observed in the postprandial state, reduces the neurogenic vasoconstriction in humans.

Our results do not allow any definite conclusion about the mechanisms underlying this effect of insulin in normotensive subjects. Creager et al. found a dose-dependent forearm vasodilation during insulin infusion in the brachial artery, thus suggesting a nonspecific antagonistic effect of insulin on vasoconstrictor tone. However, in this study, as well as in previous studies from our laboratory and others, no changes in baseline forearm vascular resistance were detected during intra-arterial insulin infusion. This finding rules out the possibility that the blunted vascular response to LBNP may be due to a direct vasodilating effect of insulin. Similarly, our finding that baseline forearm vascular resistance was unmodified during insulin infusion seems to exclude the possibility that insulin might promote vasodilation by activating sympathetic neural vasodilator pathways to skeletal muscle.

In vitro studies suggest several alternative mechanisms by which insulin can attenuate neurogenic vasoconstriction: a direct effect of insulin on many transmembrane cation exchange systems, such as the stimulation of the Na⁺,K⁺-ATPase pump, which hyperpolarizes the vascular smooth muscle cell, making it less responsive to stimuli; or an attenuation of smooth muscle contractile responses by agonist-mediated rises in intracellular Ca²⁺ or a downregulation of α₁-adrenergic receptors. These latter mechanisms could also account for the observation that local insulin infusion does not modify baseline forearm vascular resistance but blunts the vascular response to sympathetic activation.

Our previous observation that hypertensive patients show an abnormal sympathetic overactivity during euglycemic hyperinsulinemia may suggest that a primary abnormality of sympathetic responsiveness may in turn induce insulin resistance. The results of the present study, indicating that in normotensive subjects insulin attenuates the sympathetic vasoconstriction, may suggest that insulin resistance also can reduce this property of insulin. From this perspective, loss of attenuation of sympathetic vasoconstrictive responses may act synergistically with sympathetic overresponsiveness to lead to increased peripheral resistance, resulting in hypertension.

References


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