Intra-arterial Infusion of Insulin Attenuates Vasoreactivity in Human Forearm

Kikuo Sakai, Tsutomu Imaizumi, Hiroyuki Masaki, Akira Takeshita

Hyperinsulinemia may contribute to the development of hypertension. The aim of the present study was to determine whether hyperinsulinemia modulates vascular reactivity to phenylephrine or angiotensin II. In 10 young, healthy volunteers, the left brachial artery was cannulated for drug infusion and direct measurements of arterial pressure. We measured forearm blood flow by a strain-gauge plethysmograph while infusing phenylephrine (0.2, 0.8, and 2.4 μg/min) and angiotensin II (5, 10, and 20 ng/min) locally into the brachial artery before and during simultaneous intra-arterial infusion of insulin (0.15 mU/kg per minute). Forearm vascular resistance was calculated from directly measured arterial pressure and forearm blood flow. Intra-arterial infusion of insulin raised the local plasma insulin level from 10.3 ±1.4 to 133.3±21.1 μU/mL (P<.01) and did not change blood glucose level in the venous effluents of the forearm. Insulin infusion slightly but not significantly increased basal forearm blood flow (4.6 ±1.5 to 5.5±0.9 mL/min per 100 milliliters, NS) and decreased forearm vascular resistance (22.1±2.1 to 20.3±2.8 U, NS). Phenylephrine and angiotensin II increased forearm vascular resistance dose dependently before and during simultaneous insulin infusion (P<.01 for both). Intra-arterial infusion of insulin attenuated vascular reactivity to phenylephrine (P<.01) and angiotensin II (P<.01). None of these drugs changed blood pressure or heart rate. Our results suggest that hyperinsulinemia attenuates vascular reactivity in the forearm resistance vessels in healthy humans. (Hypertension 1993;22:67-73)

KEY WORDS • hyperinsulinism • phenylephrine • angiotensin II • human studies • forearm • plethysmography

The possibility that hyperinsulinemia may contribute to the development of hypertension has been suggested by several investigators.1-3 The potential mechanisms by which hyperinsulinemia may cause hypertension are (1) sodium retention resulting from renal tubular sodium reabsorption,4 (2) stimulation of sympathetic nerve activity,5 (3) increased intracellular sodium and calcium accumulation in the vascular smooth muscle cell by transmembranous cation transport,6,7 and (4) proliferation of vascular cells.8 The potential mechanism that insulin may increase intracellular sodium and calcium accumulation in the vascular smooth muscle is intriguing, because the increase in calcium would cause vasoconstriction and augment reactivity to vasoconstricting stimuli.9,10 However, in animals, insulin attenuated vascular reactivity to norepinephrine and angiotensin II (Ang II) of the isolated rabbit femoral artery11 and isolated rat tail artery.12 In humans, intravenous insulin infusion with the euglycemic clamp method has been used to examine vascular reactivity to various vasoconstricting stimuli. Most of these studies demonstrated that insulin decreased or did not alter vascular reactivity to vasoconstricting stimuli.13,14 However, Gans et al15 recently reported that intravenous insulin augmented blood pressure responses to norepinephrine but not to Ang II. Thus, reported results are conflicting. More importantly, these studies were performed with intravenous insulin. It is well known that insulin stimulates sympathetic nerve activity by acting on the central nervous system.15,16 Therefore, the results of intravenous insulin should be interpreted with great caution.

The purpose of the present study was to examine whether hyperinsulinemia enhanced vascular reactivity to phenylephrine or Ang II in isolated peripheral resistance vessels in humans. Accordingly, we examined vasoconstrictor responses to phenylephrine infused intra-arterially and Ang II before and during simultaneous intra-arterial infusion of insulin. With this method, we could evaluate effects of insulin on vascular reactivity without systemic effects of insulin.

Methods

Subjects

Studies were performed in 10 young, healthy subjects. Their ages ranged from 19 to 23 years (mean, 20.4 years). Their body weights ranged from 50 to 76 kg (mean, 61.4 kg) and were within 20% of the ideal body weight. All were normotensive, with a mean systolic blood pressure of 131±2 mm Hg and diastolic blood pressure of 74±2 mm Hg. Their family history was negative for hypertension and diabetes mellitus. The study protocol was approved by the Human Study Committee of our institution, and informed consent was obtained from each subject.
General Procedures

The study was performed with the subjects in the supine position after overnight fast. Room temperature was maintained at 25°C. With subjects under local anesthesia with 2% (wt/vol) procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle PFTE (Teflon) cannula (Quick-Cath, Travenol Laboratories Inc), which was used for drug infusion as well as for recording of arterial pressure. Arterial pressure was recorded by connecting the arterial line to a pressure transducer (Viggo-Spectramed, Oxford, Calif) with the use of a three-way stopcock. The arterial line was kept open by infusing heparinized saline (0.1 mL/min) when no drug was being infused. In all subjects, obtain blood samples for measurement of blood glucose and plasma insulin concentrations, we cannulated an antecubital vein of the ipsilateral arm with the same cannula as that used for the heart. Heart rate was obtained by counting the pulse rate for a few minutes on arterial pressure recordings.

Measurements of Forearm Blood Flow

Forearm blood flow was measured with a mercury-in-Silastic strain-gauge plethysmograph by a venous occlusion technique.17,18 The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 milliliters) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff at the upper arm. The pressure in the venous occlusion of the congesting cuff at the upper arm was 40 mm Hg. Circulation to the hand was arrested by inflating a cuff around the wrist to a suprasystolic pressure (approximately 200 mm Hg). The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean blood pressure (diastolic blood pressure plus one third pulse pressure in millimeters of mercury) by forearm blood flow. These values are expressed as arbitrary units throughout this report. An average of four flow measurements made at 15-second intervals calculated by two authors independently was used for analysis.

Forearm Vascular Responses to Drugs

After placement of cannulas and a strain-gauge plethysmograph, at least 15 minutes was allowed for subjects to become accustomed to the study conditions before the experiments were begun. We examined responses to intra-arterial infusion of phenylephrine (n=10) and Ang II (n=10) at graded doses before and during intra-arterial infusion of insulin. First, we examined forearm responses to intra-arterial infusions of phenylephrine (0.2, 0.8, and 2.4 µg/min) and Ang II (5, 10, and 20 ng/min) for 2 minutes at each dose with simultaneous infusion of saline at 0.1 mL/min. Forearm blood flow reached the steady state by 1 minute after infusion of drugs was started. Infusion of these two drugs was alternated, and infusion of the second drug was begun at least 15 minutes after the termination of the first drug, when forearm blood flow had returned to the baseline value. Fifteen minutes after the second drug infusion, intra-arterial insulin infusion (0.15 mU/kg per minute) was started and continued. After insulin infusion for 20 minutes, baseline parameters were obtained. While insulin was infused, intra-arterial infusion of phenylephrine or Ang II was performed in the same way as before insulin infusion. Infusions of phenylephrine and Ang II were alternated. All drug infusions were finished within 1 hour after an insulin administration was started. Forearm blood flow was recorded continuously during drug infusion. The concentration of all drugs was adjusted so that the infusion volume did not exceed 0.6 mL/min. We had confirmed that the volume of infusion by itself did not alter forearm blood flow. The last 1-minute measurements of forearm blood flow during infusion of each dose of drugs were used for later analysis.

Measurements of Plasma Insulin and Blood Glucose Levels

Five milliliters blood was drawn for measurement of plasma insulin and blood glucose levels from the venous cannula of the ipsilateral arm at control and during intra-arterial infusion of insulin before simultaneous infusion of phenylephrine or Ang II. Five milliliters blood was obtained during insulin infusion from the contralateral arm for measurement of systemic concentration of insulin. Blood was centrifuged immediately and stored in a freezer. Plasma insulin level was measured by radioimmunoassay at a commercially available laboratory (SRL, Fukuoka, Japan). Blood glucose level was measured by the glucose oxidase method.

Preparation of Drugs

Insulin (Insulin Actrapid Human, Novo Nordisk A/S, Bagsvaerd, Denmark) was diluted in saline containing 1% serum albumin, and a 10-mL sample of stock solution at a concentration of 0.1 U/mL of insulin was made by the pharmaceutical department of our hospital. Ang II (Hypertensin CIBA, CIBA-GEIGY, Basel, Switzerland) was dissolved in saline at a concentration of 100 ng/mL, and 10 mL stock solution was made as well. Phenylephrine (Neo synthel Kowa, Kowa, Nagoya, Japan) was diluted in saline at a concentration of 2 µg/mL immediately before use.

Statistical Analysis

One-way analysis of variance was used to compare resting hemodynamic values and plasma insulin and blood glucose levels. Because insulin infusion altered resting forearm blood flow and forearm vascular resistance, changes in forearm blood flow and forearm vascular resistance in response to phenylephrine and Ang II were normalized and examined statistically by one-way analysis of variance. The percent increases in forearm vascular resistance in response to phenylephrine or Ang II infusion during insulin infusion were compared with those during saline infusion (control) by two-way analysis of variance. All values are expressed as mean±SEM; a value of P<.05 was considered statistically significant.

Results

Responses to Intra-arterial Infusion of Insulin

Insulin infusion at 0.15 mU/kg per minute raised the insulin level of the venous effluents of the ipsilateral
arm significantly ($P<.01$) and did not alter either arterial pressure or glucose level (Fig 1). The insulin level at the contralateral arm was 11.0±0.8 μU/mL during insulin infusion. Heart rate was not altered by insulin infusion (data not shown). All raw values of the baseline data are summarized in Table 1. The baseline forearm blood flow before phenylephrine infusion was 4.4±0.5 mL/min per 100 milliliters at control and 5.6±1.0 mL/min per 100 milliliters during insulin infusion; before Ang II infusion, it was 4.9±0.5 mL/min per 100 milliliters at control and 5.4±0.7 mL/min per 100 milliliters during insulin infusion (Table 2). Thus, insulin infusion increased forearm blood flow slightly but not significantly. The baseline forearm vascular resistance before phenylephrine infusion was 23.4±2.2 U at control and 21.0±3.2 U during insulin infusion (NS); before Ang II infusion, it was 20.8±2.0 U at control and 19.5±2.4 U during insulin infusion (NS) (Table 2).

Responses to Intra-arterial Infusions of Phenylephrine and Angiotensin II

Fig 2 shows representative plethysmographic recordings of forearm blood flow in a subject in response to intra-arterial infusion of phenylephrine at graded doses before and during simultaneous infusion of insulin at a rate of 0.15 mU/kg per minute. Phenylephrine infusion decreased forearm blood flow dose dependently. Insulin infusion decreased forearm blood flow slightly at control. The decreases in forearm blood flow in response to phenylephrine were attenuated during insulin infusion. In Table 2, pooled data of forearm hemodynamics in response to phenylephrine and Angiotensin II are shown. The values are presented as mean±SEM. The baseline forearm blood flow before phenylephrine infusion was 4.4±0.5 mL/min per 100 milliliters at control and 5.6±1.0 mL/min per 100 milliliters during insulin infusion; before Ang II infusion, it was 4.9±0.5 mL/min per 100 milliliters at control and 5.4±0.7 mL/min per 100 milliliters during insulin infusion (Table 2).

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**TABLE 1. Clinical Profiles and Baseline Forearm Hemodynamics Before and After Insulin Infusion**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>B.P. (mmHg)</th>
<th>FBF (mL/min/100 mL)</th>
<th>FVR (U)</th>
<th>Insulin level (μU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
<td>168</td>
<td>58</td>
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<td>22.0</td>
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<tr>
<td>2</td>
<td>178</td>
<td>55</td>
<td>116/66</td>
<td>2.78</td>
<td>2.66</td>
<td>29.0</td>
</tr>
<tr>
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<td>68</td>
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<td>8.27</td>
<td>24.5</td>
</tr>
<tr>
<td>4</td>
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<td>3.68</td>
<td>27.0</td>
</tr>
<tr>
<td>5</td>
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<td>66</td>
<td>134/66</td>
<td>7.12</td>
<td>8.33</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>70</td>
<td>132/72</td>
<td>6.39</td>
<td>5.24</td>
<td>15.1</td>
</tr>
<tr>
<td>7</td>
<td>175</td>
<td>55</td>
<td>120/68</td>
<td>5.48</td>
<td>11.70</td>
<td>15.6</td>
</tr>
<tr>
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<td>2.68</td>
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<td>5.80</td>
<td>27.1</td>
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<tr>
<td>10</td>
<td>170</td>
<td>60</td>
<td>126/68</td>
<td>3.20</td>
<td>2.78</td>
<td>26.7</td>
</tr>
</tbody>
</table>

B.P., blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance.
TABLE 2. Forearm Hemodynamics Before and After Insulin Infusion

<table>
<thead>
<tr>
<th>Phenylephrine (µg/min)</th>
<th>Angiotensin II (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2</td>
</tr>
<tr>
<td>MBP (mm Hg) Before</td>
<td>92.7±2.3</td>
</tr>
<tr>
<td>After</td>
<td>92.3±2.1</td>
</tr>
<tr>
<td>FBF ([mL/min]/100 mL) Before</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td>After</td>
<td>5.6±1.0</td>
</tr>
<tr>
<td>FVR (U) Before</td>
<td>23.4±2.2</td>
</tr>
<tr>
<td>After</td>
<td>21.0±3.2</td>
</tr>
</tbody>
</table>

MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance. Data are mean±SEM.

and Ang II are presented. Fig 3 shows pooled data of changes in forearm vascular resistance evoked by phenylephrine and Ang II. The increases in forearm vascular resistance in response to phenylephrine and Ang II were attenuated (P<.01) during insulin infusion. Because the baseline forearm blood flow and vascular resistance were slightly different before and during insulin infusion, these were normalized, regarding the control value as 100%. Fig 4 shows pooled data of the percent change in forearm blood flow and the percent change in forearm vascular resistance evoked by Ang II at graded doses before and during simultaneous infusion of insulin. Intra-arterial infusion of Ang II decreased forearm blood flow (P<.01) and increased forearm vascular resistance (P<.01) dose dependently before and during insulin infusion. The percent decreases in forearm blood flow and the percent increases in forearm vascular resistance were smaller during simultaneous insulin infusion than during saline infusion (P<.01 for both). Similar results were obtained for phenylephrine (Fig 5).

Discussion

The major finding of the present study was that local hyperinsulinemia at physiological levels attenuated the vascular reactivity to phenylephrine and Ang II in the forearm resistance vessels of healthy humans.

Effects of Insulin on Resting Forearm Vascular Tone

Previous reports in humans described increased forearm blood flow19 and leg blood flow with intravenous insulin20 using the euglycemic clamp method. The results of intravenous insulin should be interpreted with caution, because insulin alters sympathetic tone by acting on the central nervous system.16 Therefore, we infused a small amount of insulin (0.15 mU/kg per minute) locally into the brachial artery of the human forearm to examine the direct effects of insulin on resistance vessels. The major advantage of the perfused-forearm technique was that the systemic effects of insulin on regional hemodynamics were minimized. In the present study, intra-arterial infusion did not elevate systemic insulin concentration, and therefore, the systemic effects of insulin were eliminated.

Reports on the effects of intra-arterial infusion of insulin on resting forearm vascular tone are few and the results diverse. Creager et al21 reported that intra-arterial insulin at 0.1 mU/kg per minute slightly decreased forearm vascular resistance but not signifi-

FIG 2. Representative plethysmographic recordings show changes in forearm blood flow (FBF) during intra-arterial infusion of phenylephrine (PE) at graded doses before (a) and during (b) simultaneous intra-arterial infusion of insulin. Phenylephrine caused progressive decreases in forearm blood flow. Note that decreases in forearm blood flow in response to phenylephrine were smaller during insulin infusion than before insulin infusion.
significantly. They did not measure plasma insulin level. Gelfand and Barret\textsuperscript{22} reported a small but significant increase (25\%) in forearm blood flow with forearm hyperinsulinemia (124±11 μU/mL). Natali et al\textsuperscript{23} and Ferrannini et al\textsuperscript{24} demonstrated that intra-arterial infusion of insulin with forearm hyperinsulinemia at 125±11 μU/mL had no detectable effect on resting forearm vascular resistance.

We infused insulin intra-arterially at a dose of 0.15 mU/kg per minute, which resulted in forearm hyperinsulinemia at 133±21 μU/mL. This level of hyperinsulinemia was physiological. The local hyperinsulinemia slightly increased forearm blood flow and decreased forearm vascular resistance, but these changes were not statistically significant. As shown in Table 1, the responses of baseline hemodynamics are variable. In three subjects (No. 1, 3, and 7), insulin caused a large increase in baseline forearm blood flow. However, in these subjects, baseline hemodynamics before another vasoconstrictor were not altered by insulin infusion. In the other seven subjects, insulin caused no significant change in baseline forearm hemodynamics. It is not clear why baseline hemodynamics fluctuated after insulin infusion in these three particular subjects; they were not insulin resistant, hypertensive, or obese. Our impression was that local hyperinsulinemia at a physiological level did not alter forearm hemodynamics.

**Effect of Insulin on Vascular Reactivity to Pressor Agents**

Insulin stimulates Na\(^+\)-H\(^+\) exchange and increases intracellular sodium and calcium concentrations of the vascular smooth muscle.\textsuperscript{16} Increases in intracellular calcium concentration would enhance vascular reactivity to pressor agents.\textsuperscript{9} Thus, hyperinsulinemia may enhance vascular reactivity to pressor agents such as phenylephrine or Ang II. However, opposite results were reported for the acute effect of insulin on cardiovascular reactivity. Insulin attenuated the vasoconstriction induced by norepinephrine and angiotensin of

**FIG 3.** Line graphs show changes in forearm vascular resistance (FVR) evoked by intra-arterial infusion of graded doses of phenylephrine (PE) (left panel) and of angiotensin II (AgII) (right panel). Note that vasoconstrictor responses to drugs were attenuated during insulin infusion. Cont, control.

**FIG 4.** Line graphs show percent changes in forearm blood flow (FBF) and forearm vascular resistance (FVR) evoked by intra-arterial infusion of graded doses of angiotensin II (AgII) before (○) and during (●) simultaneous infusion of insulin. Angiotensin II caused progressive vasoconstriction. Note that vasoconstrictor responses to angiotensin II were attenuated during insulin infusion. Control value was 100\%.
which may have stimulated sympathetic nerve activity.

Most of them assessed vascular reactivity by measuring forearm blood flow (FBF) and forearm vascular resistance (FVR) evoked by intra-arterial infusions of graded doses of phenylephrine (PE) before (○) and during (●) simultaneous infusion of insulin. Phenylephrine caused progressive vasoconstriction. Note that vasoconstrictor responses to phenylephrine were attenuated during insulin infusion. Control value was 100%.

Possible Mechanisms for Attenuated Vascular Reactivity by Insulin

There are some possible mechanisms for attenuated vascular reactivity to pressor agents by insulin. Several in vitro studies suggest that insulin may reduce the vascular reactivity to norepinephrine by enhancing the ability of tissue to take up norepinephrine. Because insulin attenuated vasoreactivity not only to phenylephrine but also to Ang II in the present study, this possibility may not be likely. In the skeletal muscle, insulin is known to stimulate sodium-potassium ATPase, which will hyperpolarize the cell membrane, making the cell less responsive to stimuli. It has been suggested that this may occur in the vascular smooth muscle and thereby alter reactivity to vasoconstrictor agents. It is not clear from our study whether the attenuated vasoreactivity is due to the effects of insulin on receptors of phenylephrine and Ang II or is related to some intracellular mechanisms.

Limitations

Because we examined only short-term effects of insulin, our results are not necessarily applicable to long-term effects. It has been shown in dogs that hyperinsulinemia for several days may alter vascular reactivity to pressor agents. Moreover, we examined the effects of insulin in healthy young subjects; it is possible that hypertensive, diabetic, obese, or aged individuals may have different responses to insulin.

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