Insulin-Induced Biphasic Responses in Rat Mesenteric Vascular Bed
Role of Endothelin

Derek A. Misurski, Sheng-Qian Wu, J. Robert McNeill, Thomas W. Wilson, Venkat Gopalakrishnan

Abstract—The vasodilatory capacity of insulin has been widely reported, yet some investigators have not noted this effect. Because insulin has been shown to enhance endothelin release, we speculated that endothelin could be attenuating insulin-evoked vasodilation. We examined the effect of ex vivo insulin perfusion on vascular resistance by using the Sprague-Dawley rat mesenteric vascular bed. In methoxamine-preconstricted preparations, insulin (3.0 pmol/L to 10 nmol/L) evoked a concentration-dependent decrease in perfusion pressure (PP) with a maximal response of 42.0±9.2%, whereas continuous exposure to 10 nmol/L insulin induced a 51.8±3.5% relaxation. Further exposure to 10 nmol/L insulin resulted in the generation of endothelin and a subsequent loss of the vasodilatory response. Indomethacin had no effect on vascular responses. The vasodilatory response was significantly inhibited by nitric oxide synthase inhibition (20.5±4.2%; P<0.01) and calcium-activated potassium channel blockade (28.5±3.7%; P<0.05). Endothelial denudation attenuated the vasodilatory component (20.3±7.1%; P<0.01) and altered the biphasic pattern of the response. The decline in insulin-evoked vasodilation was significantly prevented by an endothelin-A antagonist (BQ123), an endothelin-B antagonist (BQ788), and nonselective endothelin blockade with both BQ123 and BQ788. These results demonstrate that the endothelium is intimately involved in regulating the vascular response to insulin. Insulin promotes the release of nitric oxide and endothelium-derived hyperpolarizing factor. During sustained exposure to higher concentrations, this vasodilatory effect is countered by the pathological generation of endothelin. Endothelin receptor blockade facilitates the maintenance of vasodilation despite high insulin concentrations. (Hypertension. 2001;37:1298-1302.)

Key Words: insulin ■ nitric oxide ■ vasodilation ■ endothelin ■ endothelium ■ mesenteric arteries

The ability of insulin to induce vasodilation is integral to the regulation of skeletal muscle blood flow and glucose delivery.1–3 Insulin-mediated vasodilation is impaired in insulin-resistant patients, suggesting a close relation between insulin resistance and hypertension.4,5 However, despite the potential significance, the mechanism(s) underlying direct vascular effects of insulin remain controversial.1–16 Several studies in animals and humans attribute the vasodilatory effect of insulin to enhanced generation of endothelium-derived nitric oxide (EDNO).2,11–16 On the contrary, there have been reports supporting an endothelium-independent mechanism.5–10 Incubation with insulin has been shown to reduce cytosolic-free calcium levels6 and to increase the expression of the sodium pump gene, leading to hyperpolarization of vascular smooth muscle (VSM) cells.7 Insulin enhancement of vascular β-adrenergic responsiveness has also been reported in normotensive animals and humans.8,9 In the perfused rat mesenteric vascular bed (MVB), insulin-evoked vasodilation has been linked to the activation of calcitonin gene–related peptide receptors on the VSM cells.10 Interestingly, these investigators noted that endothelial denudation of the MVB enhanced vasodilator responses to insulin, implicating the release of a contracting factor from the endothelium.10 Moreover, a few reports have demonstrated the lack of a vasodilator response to insulin.17,18 Therefore, an explanation that insulin is simultaneously promoting the release of a contractile substance countering the release of vasodilator factor(s) is indeed possible, because several in vitro and in vivo studies have shown that insulin promotes endothelin-1 (ET-1) generation in the vascular endothelium.19–24 This activity may account for the paradoxical vascular effects of insulin. To assess this hypothesis, we investigated the endothelium-dependent and endothelium-independent effects of insulin on the vasculature. The perfused rat MVB was used because it is a well-established representation of vascular resistance function.25
Methods

MVX Perfusion

Experiments were performed with 80 male Sprague-Dawley rats (Charles River) weighing 410 to 515 g. Animals were killed under pentobarbital anesthesia (65 mg/kg IP). All procedures were conducted in accordance with the guidelines of the university animal care committee. The vascular bed was removed, cannulated, and perfused as previously described. An equilibrium period of 30 minutes was allowed to stabilize the baseline. This was followed by perfusion of a Krebs solution containing the α₁-agonist methoxamine (MTX, 70 μmol/L), either in the presence or absence of the following agents: (1) a combination of nitric oxide synthase (NOS) inhibitors Nω-nitro-L-arginine (L-NNA, 100 μmol/L) and Nω-nitro-L-arginine-methylester (L-NAME, 100 μmol/L) to maximally inhibit the enzyme; (2) a calcium-activated potassium channel blocker (BQ788), which serves as an endothelium-derived hyperpolarizing factor (EDHF) blocker, (tetrabutylammonium [TBA], 0.5 mmol/L); (3) a cyclooxygenase inhibitor (indomethacin, 10 μmol/L); (4) an ETα receptor blocker (BQ123, 100 nmol/L); (5) an ETβ receptor blocker (BQ788, 100 nmol/L); and (6) a combination of ETα and ETβ receptor blockers (BQ123 and BQ788, 100 nmol/L each). Denudation was accomplished as previously described. The MTX-evoked contraction. The potency of insulin-induced vasodilation was expressed as the negative logarithm of the half-maximal response (pD2 value). Both maximal responses and the pD2 were expressed as mean±SEM. Comparison of mean values among groups was performed by ANOVA methodology (Superanova program–SAS Institute). Simultaneous multiple comparisons were examined by Scheffe’s F test.

Assessment of Endothelin Generation

To assess whether the loss of insulin-induced vasodilation was attributable to ET, we collected the MVB perfusate for 10 minutes during both the baseline and MTX equilibration periods. After this, perfusate was collected every 10 minutes for 1 hour during exposure to fixed concentrations of insulin, IGF-1, or MTX as a control. The ET level in the perfusate was measured with a sensitive enzyme-linked immunosorbent assay kit as previously described.

Reagents

MTX, TBA, L-NNA, L-NAME, indomethacin, ACh, SNP, and insulin (human USP) were all purchased from Sigma. Alcohol was used to dissolve indomethacin at a final concentration of 1/2000 and was added to all solutions. BQ123 and BQ788 were obtained from American Peptide. Krebs solution salts were obtained from BDH. The enzyme-linked immunosorbent assay kit was obtained from Biomedica Gruppe. Amrep Octadecyl C18 columns were from Amersham.

Statistical Analysis

Cumulative concentration-response curves were analyzed individually. The results are expressed as the percentage of vasodilation of MTX-evoked contraction. The potency of insulin-induced vasodilation was expressed as the negative logarithm of the half-maximal response (pD2 value). Both maximal responses and the pD2 were expressed as mean±SEM. Comparison of mean values among various groups was performed by ANOVA methodology (Superanova program–SAS Institute). Simultaneous multiple comparisons were examined by Scheffe’s F test.

Results

Vascular Responses to Perfusion of Insulin

Perfusion of the MVB with MTX resulted in a vasoconstriction and an elevation in the PP (49.0±5.1 mm Hg). In MTX-preconstricted preparations, cumulative insulin perfusion resulted in a concentration-dependent decrease in PP. At concentrations of insulin >10 nmol/L, the decrease in PP gradually diminished such that at a maximal concentration of insulin (3 μmol/L), there was no decrease in PP. A representative tracing from a single experiment is shown in Figure 1. The pD2 value for the insulin-mediated vasodilation was 10.3±0.1 (−log mol/L), and the maximal relaxation of MTX-induced PP was 42.0±2.1% (Table). The continuous perfusion of a fixed concentration of insulin (10 nmol/L) induced vasodilation (31.0±2.2%) shortly after being exposed to the MVB, whereas perfusion of insulin (10 mmol/L) produced a larger relaxation of 51.8±3.5% (P<0.01). By comparison, the maximal relaxation response attained with ACh and SNP as vasodilatory agents were 93.0±7.8% and 98.4±4.4%, respectively. The basal ET level in the perfusate was 46.5±7.2 pg/50 mL. There was no significant change in ET levels when MTX alone was perfused for a period of 1 hour (Figure 2A). Although continuous perfusion of a fixed concentration of insulin (1 mmol/L) did not evoke an increase in ET production after 1 hour (data not shown), insulin (10 mmol/L) significantly increased (P<0.01) ET generation at a 20-minute time period and thereafter (Figure 2A). This was accompanied by a gradual loss of the vasodilatory response, as depicted in the representative tracing of a single experiment (see Figure 2B). The residual vasodilation after perfusion of 10 nmol/L insulin for 1 hour was 5.6±3.2%. Perfusion of IGF-1 for up to 1 hour did not affect the changes in PP to MTX (data not shown).

Effects of Treatment on Insulin Perfusion

Inclusion of inhibitors of endothelium-mediated vasodilation and contraction in the perfusion solution did not have any significant effect on the MTX-induced increase in PP. Endothelial denudation (P<0.01), NOS inhibition (P<0.05), and TBA (P<0.05) reduced the maximal vasodilator responses to ACh, whereas neither indomethacin nor ET antagonists had any effect. Endothelial denudation significantly attenuated (P<0.01) insulin-induced vasodilation (Figure 3A), resulting in a smaller response compared to insulin perfusion alone.
in both a decrease in the maximal vasodilation ($P<0.01$) and a right shift in the concentration-response curve ($P<0.01$; Table). NOS inhibition ($P<0.01$) and TBA ($P<0.05$; Figure 3B) both attenuated insulin-evoked maximal dilation (Table). ET$_{A}$ and nonselective ET-receptor blockade prevented the loss of vasodilation associated with insulin concentrations starting at 10 nmol/L (Figure 3C). Neither selective ET-receptor blockade nor nonselective ET blockade produced any significant change in the potency or maximal effect of insulin-evoked vasodilation (Table). Maximal SNP responses did not differ among the groups.

### Effects of Treatment on pD$_2$ and Percent Maximal Vasodilation of MTX-Induced (70 µmol/L) Tone Produced by Insulin in Perfused MVB of Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Concentrations</th>
<th>pD$_2$ (−log mol/L)</th>
<th>% Maximum Vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td></td>
<td>10.3±0.1</td>
<td>42.0±9.2</td>
<td></td>
</tr>
<tr>
<td>Indomethacin (8)</td>
<td></td>
<td>10 µmol/L</td>
<td>10.6±0.1</td>
<td>46.6±7.3</td>
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<tr>
<td>BQ123 (8)</td>
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<td>100 nmol/L</td>
<td>10.1±0.2</td>
<td>50.6±5.9</td>
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<tr>
<td>BQ788 (8)</td>
<td></td>
<td>100 nmol/L</td>
<td>10.0±0.3</td>
<td>48.0±8.8</td>
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<tr>
<td>BQ123+BQ788 (8)</td>
<td></td>
<td>100 nmol/L (each)</td>
<td>10.1±0.2</td>
<td>58.1±8.1</td>
</tr>
<tr>
<td>L-NAME+L-NNA (8)</td>
<td></td>
<td>100 µmol/L (each)</td>
<td>10.4±0.2</td>
<td>20.5±4.2†</td>
</tr>
<tr>
<td>TBA (8)</td>
<td></td>
<td>0.5 mmol/L</td>
<td>10.1±0.1</td>
<td>28.5±3.7*</td>
</tr>
<tr>
<td>Denudation (7)</td>
<td></td>
<td>Air perfusion</td>
<td>9.1±0.3†</td>
<td>20.3±7.1†</td>
</tr>
</tbody>
</table>

Values are represented as mean±SEM. Percentage of maximal vasodilation of MTX-induced constrictor tone. pD$_2$ is provided as negative logarithm of molar concentration required to provide half-maximal vasodilation of MTX-induced constrictor tone. $^*P<0.05$, $^{†}P<0.01$ vs control.
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Insulin Vasodilation Opposed by Endothelin 1301

Discussion

The results of this investigation demonstrate a concentration-dependent effect of insulin on vascular reactivity and that in the presence of higher concentrations, the duration of exposure is of consequence. The ability of NOS inhibition and K_\text{Ca} blockade to attenuate the initial vasodilatory response suggests the involvement of EDNO and EDHF. Although at higher concentrations, insulin initially produces a more profound vasodilation than at lower doses, sustained exposure stimulates the production and release of ET, which diminishes the preceding vasodilation. Subsequently, ET-receptor blockade maintains vasodilation. Because the MVB contributes significantly to the resistance function of the vasculature, the present study supports the notion that hyperinsulinemic-evoked imbalances in endothelium-derived factors may contribute to insulin-resistant hypertension. A recent investigation by Cardillo et al also demonstrated the ability of ET-receptor blockade to increase forearm blood flow during hyperinsulinemia.

Insulin has been shown to have profound effects on the ET system, promoting the release of ET-1 and upregulating ET receptors on VSM cells. Incubation of bovine aortic endothelial cells with insulin in vitro increases the production and release of ET-1 within 10 minutes, an effect that was maximal within 1 to 2 hours. Our results are consistent with these in vitro observations. Insulin also increases ET-1 production in cultured human VSM cells, supporting the finding of enhanced ET release in both healthy as well as obese non–insulin-dependent diabetes mellitus human subjects. The concentration of insulin (10 nmol/L) that evoked significant ET generation in the present study may be relevant to in vivo situations because similar concentrations of plasma insulin have been detected in hyperinsulinemic Zucker obese rats. Furthermore, an in vitro study with femoral artery isolated from Wistar rats used a much higher concentration of insulin (300 nmol/L) to demonstrate a significant increase in KCl-induced vascular contractility. This effect of insulin was attenuated by inclusion of either an ET antagonist or ET antiserum.

ET has an autocrine influence on endothelial-cell ET_\text{B} receptors, promoting a transient vasodilation through the release of NO. However, ET_\text{B}-selective antagonism failed to attenuate insulin-induced dilation. Because insulin-evoked NO generation has been shown to be dependent on tyrosine kinase/phosphatidylinositol 3-kinase, it is possible that although present, the transient ET_\text{B}-receptor–mediated NO release made a minimal contribution. It is also possible that ET is not being generated in substantial quantities in this vascular bed during the early vasodilatory phase of the response. The inability of ET_\text{B} receptor blockade to produce a significant shift in the vasodilatory pD_2 confirms that NO (and EDHF) activity is functionally independent of the ET_\text{B} receptor. Although the functional importance of ET_\text{B} receptors on VSM is thought to be minimal, the characterization of ET_\text{B} receptors on the rat mesenteric VSM cells helps to explain the ability of BQ788 to attenuate ET_\text{B}-mediated vasoconstriction.

Although the existence of a non-EDNO entity that induces vasodilation through TBA-sensitive potassium conductance has been established, this is the first report of two endothelium-derived autacoids mediating an insulin-evoked vasodilation. Little is conclusive about EDHF except that it produces vasodilation during NOS inhibition and that its release is probably linked to an increase in intracellular calcium. The vasodilator effect of insulin could not be attributed to its direct effects on VSM cells because no decrease in response to SNP after insulin infusion was noted. Because endothelial denudation prevented an increase in vascular tone, the involvement of an endothelium-derived vasoconstrictor is probable. Because indomethacin did not significantly alter the dynamics of the response, it is unlikely that a cyclooxygenase-dependent factor was responsible. However, because exogenous insulin may augment cardiovascular reactivity to norepinephrine (NE), it is possible that the loss of vasodilation was due to the effects of insulin on MTX-induced vasoconstriction. A recent study has indeed demonstrated that incubation with a high dose (715 nmol/L) but not a low dose (715 pmol/L) of insulin increases the vasoconstrictor effect of NE in resistance vessels of spontaneously hypertensive rats. This effect was attenuated by ET-receptor blockade, suggesting that enhanced NE responses were at least partially mediated by ET. Accordingly, ET-1 has been reported to enhance adrenergic vasoconstriction, suggesting that sympathetic activation in hyperinsulinemic states could be linked to ET.

This study demonstrates the importance of the endothelium in regulating the vascular effects of insulin. Interestingly, ET-receptor blockade maintains insulin-mediated vasodilation in the presence of hyperinsulinemia, suggesting a role for these agents in hyperinsulinemic hypertension. We have also demonstrated the involvement of both EDNO and EDHF; however, the recruitment of autacoids is dependent on the type of vascular tissue as well as on vascular pathology. Furthermore, comorbid factors such as hypertriglyceridemia and hypercholesterolemia may contribute to increased ET generation, which in turn could affect EDNO and/or EDHF. Because of this complexity of interaction between endothelial factors, more detailed studies of ET blockers in various vascular beds of insulin-resistant models are required.

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References


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