Endothelin Antagonism Uncovers Insulin-Mediated Vasorelaxation In Vitro and In Vivo

Subodh Verma, Linfu Yao, Duncan J. Stewart, Aaron S. Dumont, Todd J. Anderson, John H. McNeill

Abstract—The endothelial actions of insulin remain an area of intense research because they relate to both insulin sensitivity and vascular tone. Physiological doses of insulin evoke endothelium-dependent vasorelaxation in humans; however, this remains a pharmacological phenomenon in rat aortas. Because insulin may stimulate the divergent production of both nitric oxide and endothelin-1, we hypothesized that the lack of insulin-induced vasorelaxation at low/subthreshold concentrations may be due to the concurrent production of endothelin-1, which in turn serves to inhibit nitric oxide-dependent, insulin-mediated dilation. To investigate this, we studied the effects of subthreshold concentrations of insulin (100 mU/L) on norepinephrine-induced contraction in rat aortas following short-term and long-term endothelin blockade. In addition, the effects of tetrahydrobiopterin inhibition (with diaminohydroxyprimidine) on norepinephrine-induced contraction in the presence of insulin and endothelin receptor blockade were investigated. Subthreshold concentrations of insulin failed to evoke vasorelaxation in rat aortas. Strikingly, short-term endothelin A/B receptor blockade with bosentan (10^{-2} mmol/L) uncovered insulin-mediated dilation; the percent maximum contraction and sensitivity of aortas to norepinephrine were attenuated (% maximum relaxation: bosentan + insulin 74±4%* versus bosentan 92±3%, insulin 107±5% P<0.002; pD_{2} values: bosentan + insulin 6.87±0.14* versus bosentan 7.40±0.15, insulin 7.63±0.11, *P<0.002). This effect was mediated through endothelin A receptors because bosentan and BQ-123 (10^{-2} mmol/L) attenuated norepinephrine-induced contraction to a similar degree. In addition, insulin evoked vasorelaxation in aortas isolated from rats after long-term bosentan treatment (100 mg·kg^{-1}·d^{-1}, 3 weeks). The component of insulin-mediated vasorelaxation uncovered by endothelin receptor blockade was tetrahydrobiopterin-dependent because it was reversed by diaminohydroxyprimidine. These data demonstrate, for the first time, the functional interaction between endothelin-1, and tetrahydrobiopterin in modulating vascular tone in rat aortas in vitro and in vivo. (Hypertension. 2001;37:328-333.)

Key Words: insulin ■ endothelin ■ vasodilation ■ insulin resistance ■ hypertension ■ rat aorta ■ tetrahydrobiopterin ■ bosentan

The vascular actions of insulin represent an area of much current interest as they relate to both whole-body glucose metabolism/insulin sensitivity and vascular tone/hypertension.1,2 The balance of published information suggests that the vascular actions of insulin are mediated chiefly through the regulation of endothelium-derived factors.2 In this regard, insulin can stimulate the production of tetrahydrobiopterin (BH4)-dependent nitric oxide (NO) formation while concurrently augmenting the production of the potent endothelium-derived vasoconstrictor endothelin-1 (ET-1) (Figure 1).3,4 Although insulin-induced vasorelaxation occurs at physiological concentrations in humans, this effect appears to be mainly a pharmacological one in rat aortas. The mechanism underlying the lack of insulin-mediated dilation at low/subthreshold concentrations (in vitro) remains unknown but could relate to the simultaneous production of ET-1 by insulin. To further characterize the functional interaction between insulin, ET-1, and BH4/NO, the present study examined the effects of short-term and long-term endothelin (ET) receptor blockade on insulin-mediated vasorelaxation in isolated segments of rat aortas. We herein report, for the first time, that in the presence of an ET receptor blockade, subthreshold concentrations of insulin evoke vasorelaxation in a BH4-dependent fashion.

Methods

Animals and Experimental Design

All protocols described in this paper were approved by the institutional review committee at the University of British Columbia, Vancouver, Canada.

Received June 22, 2000; first decision July 17, 2000; revision accepted August 22, 2000.

From the Division of Pharmacology and Toxicology (L.Y., J.H.M.), Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, Canada; the Division of Cardiology (S.V., A.S.D., T.J.A.), Faculty of Medicine, The University of Calgary, Calgary, Canada; and the Division of Cardiology (S.V., D.J.S.), St Michael’s Hospital, The University of Toronto, Toronto, Canada.

Correspondence to Subodh Verma, Division of Cardiology, Foothills Hospital, 8th Floor, 1403-29th Street N.W., Calgary, AB, Canada, T2N 2T9.

E-mail subodhverma@home.com

© 2001 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org
INSULIN

Endothelium

PreProET

ET-1

BH4

NO

ET-3

ET-2

NO

Contraction

Relaxation

VSM

Figure 1. Schematic representation of insulin regulation of endothelium-derived vasoactive factors. Insulin exerts divergent actions on endothelial function. It stimulates the release of NO (by interacting with BH4) while concurrently stimulating the production of the potent vasoconstrictor ET-1. Alterations in the balance between these pathways may predispose to increased vascular tone/reactivity and changes in whole-body glucose homeostasis.

Study 1: Effects of Short-Term ET<sub>A</sub> Receptor Blockade With Bosentan on Vascular Responses to Subthreshold Concentrations of Insulin

Twenty-five male Sprague-Dawley rats were procured at 8 weeks of age from the University of British Columbia Animal Care Facility, Vancouver, British Columbia, Canada. At week 9, the rats were killed with an overdose of pentobarbital, and the thoracic aortas were cut into rings. Four rings from each rat (~3 to 6 mm in length) were obtained for the study. The tissues were suspended in an isolated tissue bath (volume 20 mL) containing modified Krebs-Ringer bicarbonate solution with the composition (in mmol/L): NaCl (118), KCl (4.7), CaCl<sub>2</sub> (2.5), KH<sub>2</sub>PO<sub>4</sub> (1.2), MgSO<sub>4</sub> (1.2), NaHCO<sub>3</sub> (25), dextrose (11.1), and disodium calcium EDTA (0.026), maintained at 37°C and oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Each ring was placed under a resting tension of 2.5 g determined in preliminary experiments to provide for maximum length-tension relationship. The tissues were then allowed to equilibrate for 90 to 120 minutes before the experiments were conducted. Isometric responses were recorded on a Grass polygraph (Grass Instruments). The tissues were stimulated according to the following protocol: (a) cumulative dose response curve (DRC) to norepinephrine (NE) (control); (b) cumulative DRC to NE in the presence of 100 mU/L insulin (subthreshold concentration) (3 hours) (control+insulin); (c) cumulative DRC to NE in the presence of bosentan (ET<sub>A</sub> receptor blocker, 10<sup>-2</sup> mmol/L for 1 hour) (control+bosentan), and (d) cumulative DRC to NE in the presence of bosentan and insulin (control+bosentan+insulin).

For each concentration, a plateau was obtained before the subsequent dose was added. After each DRC, the buffer was replaced several times to wash the tissues until the resting tension of each tissue was reached. The presence of the endothelium was confirmed by vasorelaxation to acetylcholine (ACh, 10<sup>-5</sup> mol/L). A time-control was run with all experiments, and individual recordings were corrected for time-related changes in contractile force (if applicable).

Study 2: Effects of Short-Term ET<sub>A</sub> Receptor Blockade With BQ-123 on Vascular Responses to Subthreshold Concentrations of Insulin

To determine the contribution of ET<sub>A</sub> receptor toward insulin-mediated vasorelaxation, we studied the effects of BQ-123 on NE-induced contraction in the presence of subthreshold concentrations of insulin. Thoracic aortas from male Sprague-Dawley rats (n=4) were studied with the organ bath procedure as outlined above. The tissues were stimulated according to the following protocol: (a) cumulative DRC to NE (control); (b) cumulative DRC to NE in the presence of 100 mU/L insulin (subthreshold concentration) (3 hours); (c) cumulative DRC to NE in the presence of BQ-123 (ET<sub>A</sub> receptor blocker, 10<sup>-2</sup> mmol/L for 1 hour) (control+BQ-123); and (d) cumulative DRC to NE in the presence of BQ-123 and insulin (control+BQ-123+insulin).

Study 3: Effects of Long-Term Bosentan Treatment on Vascular Responses to Subthreshold Concentrations of Insulin

To examine the effects of ET receptor blockade toward insulin-induced vasorelaxation in vivo, we studied the vascular responses to NE (in the presence of subthreshold insulin concentrations) after long-term bosentan treatment. Male Sprague-Dawley rats were divided into 2 groups: control (n=6) and control bosentan-treated (n=6). The treated group received bosentan (100 mg/kg per day via oral gavage) for a period of 3 weeks as described previously. After long-term treatment, thoracic aortas were removed from both groups, and isometric dose-response curves were constructed according to the protocol outlined in Study 1. Systolic blood pressure was measured using the tail-cuff method (without external preheating) as described previously.

Study 4: Effects of Short-Term Bosentan Treatment on Vascular Responses to Pharmacological Concentrations of Insulin

The experiments outlined in Studies 1 through 3 were designed to evaluate the effects of ET blockade on vascular responses to subthreshold insulin concentrations. To determine whether ET receptor blockade augments insulin-mediated vasodilation evoked by pharmacological concentrations, we studied, in a separate experiment, the effects of short-term bosentan incubation on NE responses in the presence and absence of 10<sup>-4</sup> mU/L insulin (for 3 hours).

Study 5: Effects of DAHP on the Vascular Responses to Insulin and Bosentan

Because insulin evoked vasorelaxation in the presence of ET receptor blockade (see Results), we examined whether these effects were mediated via insulin stimulation of NO production. To this aim, we studied the effects of NO synthesis inhibition (using 2,4-diamino-6-hydroxypyrimidine [DAHP], a specific inhibitor of tetrahydrobiopterin synthesis, [2×10<sup>-4</sup> mmol/L for 2 hours]) on NE-induced contraction in the presence of insulin and bosentan. This concentration of DAHP was chosen based on control experiments that demonstrated effective inhibition of ACh (and hence NO) mediated vasodilation (see Results). Our reasons to use DAHP to inhibit endothelium-dependent relaxation were based on studies in rat arteries that indicated that insulin-mediated vasodilation is dependent on BH<sub>4</sub> synthesis.

Calculations and Statistics

Percent maximum contraction (%ET<sub>A</sub>) and agonist sensitivity (pD<sub>2</sub> values calculated by non-linear regression) were compared between groups. All experiments were conducted in the presence of indomethacin (10<sup>-6</sup> mol/L) to prevent the synthesis of vascular prostaglandins. 0.05% albumin was added to the buffer to prevent the adsorption of insulin by glassware. Pilot studies were conducted that indicated that the order of addition of the agonists and antagonists did not affect the net NE contractile response reported in this paper. All chemicals were obtained from Sigma Chemical Co. Bosentan was a gift from Actelion Ltd, Allschwil, Switzerland.

Results are expressed as mean±SE. N indicates the number of rats. Multiple tracings from each rat were averaged for each intervention. The DRCs were compared using repeated measures ANOVA followed by a Newman Keuls’ test for post-hoc comparisons. A P value of less than 0.05 was considered to be statistically significant.
Results

Effects of Short-Term Endothelin Receptor Blockade on Insulin-Induced Vasorelaxation

The effects of 3-hour insulin incubation (100 mU/L) on the reactivity of aortic rings (with intact endothelium) in the presence and absence of bosentan (10\(^{-7}\) mmol/L for 1 hour) are depicted in Figure 2. Neither insulin nor bosentan alone affected NE-induced contraction; both the \(\%E_{\text{max}}\) and \(pD_2\) values were similar in the control+insulin and control+bosentan groups (Table). The inability of insulin to evoke in vitro relaxation in the \(\mu\)g/mL (versus \(\mu\)g/mL) range is well documented.1,7-9 Strikingly, after 1-hour incubation with bosentan, subthreshold concentrations of insulin (100 mU/L) attenuated the contractile responses to NE (\(\%E_{\text{max}}\) values [control 100%]: control+bosentan+insulin 74±4%* versus control+bosentan 92±3%, control+insulin 107±5%, \(P<0.002\); \(pD_2\) values: control+bosentan+insulin 6.87±0.14* versus control 7.52±0.04, control+insulin 7.63±0.11, control+bosentan 7.40±0.15, *\(P<0.0002\) different from the other 3 groups).

The effects of insulin (100 mU/L) and BQ-123 on NE-induced contraction in aortas are depicted in Figure 3. As with bosentan, in the presence of BQ-123, subthreshold concentrations of insulin attenuated the contractile responses to NE (Figure 3, Table). BQ-123 augmented insulin-mediated vasodilation to a similar degree to that achieved with mixed ET\(_{A/B}\) blockade with bosentan (\(\%E_{\text{max}}\) BQ-123+insulin 79±5% versus bosentan+insulin 74±4%, \(P>0.05\), Table).

Effects of Long-Term Endothelin Receptor Blockade on Insulin-Induced Vasorelaxation

To examine the in vivo contribution of ET-1 toward the vascular actions of insulin, we studied the effects of subthreshold insulin concentrations on NE responses in aortas isolated from rats that received long-term treatment with bosentan. After 3 weeks of bosentan treatment, the rats remained normotensive versus the untreated group (systolic BP: 122±8 versus untreated 135±4 mm Hg, \(P>0.05\)), which is consistent with previous reports.5,6 Figure 4 depicts the vascular responses to NE in the control and control bosentan-treated animals in the presence and absence of insulin (100 mU/L). The key observation from this study is that long-term bosentan treatment uncovered insulin-mediated vasodilation; in the presence of insulin, a downward and rightward shift of the DRC in the bosentan-treated rats became apparent (Figure 4, Table). Long-term bosentan treatment per se did not affect the contractile responses to NE (Figure 4, Table).

Pharmacological Insulin Concentrations and Endothelin Receptor Blockade

Because pharmacological insulin concentrations (10\(^5\) mU/L) are known to cause vasorelaxation in rat aortas, we examined whether this response can be further augmented by short-term ET receptor blockade. Figure 5 depicts the effects of 10\(^5\) mU/L insulin and bosentan on NE-induced contraction. In the presence of insulin alone, NE-induced contractile responses were significantly attenuated (Table) consistent with a vasodilatory action of insulin at these concentrations. In the presence of bosentan, insulin-mediated vasodilation was further augmented (\(\%E_{\text{max}}\) control+bosentan+insulin [10\(^5\) mU/L]: 51±6 versus control+insulin 69±4, \(P<0.05\), Figure 5, Table).

NO Synthesis Inhibition With DAHP and Vascular Responses to Insulin and Bosentan

To examine whether the vasorelaxant effects of subthreshold insulin concentrations (in the presence of bosentan) were mediated by BH\(_4\) (and hence NO production), we studied the effects of NO synthesis inhibition during concurrent ET receptor blockade in the presence and absence of insulin. Figure 6 depicts the effects of DAHP (inhibitor of BH\(_4\) synthesis) on insulin-induced attenuation of NE responses during simultaneous ET blockade. As observed earlier, insulin attenuated the contractile responses to NE in the presence of bosentan (Figure 6, C+B+I). The vasodepressor effects of
The primary purpose of the present study was to examine the interaction between insulin, ET-1, and NO in isolated rat aortas. We and others have demonstrated that pharmacological (versus physiological) concentrations of insulin attenuate the contractile responses to a variety of vasoconstrictors in vitro.1,7–9 Because insulin can stimulate the production of NO and ET-1 simultaneously,3 we hypothesized that the lack of insulin-mediated vasorelaxation at low subthreshold concentrations may be due, in part, to the production and subsequent inhibition of BH4/NO-dependent, insulin-mediated vasorelaxation by ET-1. To examine the validity of this hypothesis, we studied the effects of 100 mU/L insulin on NE-induced contraction in the presence of either BQ-123 or bosentan. Third, these dynamics were operative in vivo; long-term blockade of ET receptors from this study. First, insulin failed to evoke vasorelaxation at subthreshold concentrations, Figure 2 to 4). Insulin-induced vasorelaxation was further augmented by bosentan. *P < 0.05 different from control, control + bosentan, and control + bosentan + insulin.

**Discussion**

The ability of DAHP to inhibit endothelium-mediated dilation is consistent with other reports.10 insulin noted after ET blockade (C+B+I group) were reversed by simultaneous incubation with DAHP (Figure 6, C+B+I+D), suggesting that the component of insulin-induced vasodilation uncovered by bosentan was BH4/NO dependent. Control experiments that demonstrated that DAHP attenuated endothelium-dependent vasodilation to ACh in a fashion similar to L-NMMA (Figure 7). The ability of DAHP to inhibit endothelium-mediated dilation is consistent with other reports.10

**Functional Characteristics of the Norepinephrine Responses**

<table>
<thead>
<tr>
<th>Study/Groups</th>
<th>%E&lt;sub&gt;max&lt;/sub&gt;</th>
<th>pD&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>7.52 ± 0.04</td>
</tr>
<tr>
<td>Control + insulin (100 mU/L)</td>
<td>107 ± 5</td>
<td>7.63 ± 0.11</td>
</tr>
<tr>
<td>Control + bosentan</td>
<td>92 ± 3</td>
<td>7.40 ± 0.15</td>
</tr>
<tr>
<td>Control + bosentan + insulin (100 mU/L)</td>
<td>74 ± 4*</td>
<td>6.87 ± 0.14*</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>7.52 ± 0.04</td>
</tr>
<tr>
<td>Control + insulin (100 mU/L)</td>
<td>107 ± 5</td>
<td>7.63 ± 0.11</td>
</tr>
<tr>
<td>Control + BQ-123</td>
<td>98 ± 5</td>
<td>7.29 ± 0.22</td>
</tr>
<tr>
<td>Control + BQ-123 + insulin</td>
<td>79 ± 5*</td>
<td>6.77 ± 0.10*</td>
</tr>
<tr>
<td>Study 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>7.52 ± 0.04</td>
</tr>
<tr>
<td>Control + insulin (10^7 mU/L)</td>
<td>69 ± 5*</td>
<td>6.58 ± 0.18*</td>
</tr>
<tr>
<td>Control + bosentan</td>
<td>93 ± 6</td>
<td>7.45 ± 0.20</td>
</tr>
<tr>
<td>Control + bosentan + insulin</td>
<td>51 ± 6a</td>
<td>6.54 ± 0.20</td>
</tr>
<tr>
<td>Study 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>7.52 ± 0.04</td>
</tr>
<tr>
<td>Control + bosentan + insulin (100 mU/L)</td>
<td>74 ± 4*</td>
<td>6.87 ± 0.14*</td>
</tr>
<tr>
<td>Control + bosentan + insulin + DAHP</td>
<td>96 ± 5</td>
<td>7.78 ± 0.22</td>
</tr>
</tbody>
</table>

*P < 0.05, different from all other groups; aP < 0.05, different from Control, Control + insulin, and Control + Bosentan (Study 4).
vasodilation. Fourth, the component of insulin-mediated vasodilation observed after ET blockade was BH4-dependent because these responses were prevented by inhibition of BH4 synthesis by DAHP (during concurrent ET receptor blockade). Taken together, these data support the notion that at low/subthreshold concentrations, insulin-induced stimulation of ET-1 production may serve to inhibit vasorelaxation by antagonizing the effects of insulin on endothelium-dependent NO production in a BH4-dependent fashion. Because the effects of insulin on endothelial and vascular function represent the net balance of NO versus ET-1 production, we suggest that in rat aortas, this balance is tilted in favor of ET-1 at low concentrations. It is possible that as the concentrations of insulin are increased (to millimolar levels), the effects of NO (versus ET-1) predominate. This possibility would help to explain the observed vasorelaxant effects of pharmacological insulin concentrations in rat aortas.1,8,9 Even at these high concentrations, the vasodepressor actions of insulin are augmented by ET receptor blockade (Figure 5), further supporting the notion of a functional interaction between insulin, ET-1, and NO at the level of the endothelium.

The vascular effects of insulin in rats are dose dependent and vessel specific.1 For example, in rat mesenteric arteries, low concentrations of insulin (100 mU/L) evoke vasoconstrictor responses; this response is exaggerated in hyperinsulinemic states and mediated by the production of ET-1.1 Indeed, in the presence of ET receptor blockade, insulin-induced contraction of mesenteric arteries is attenuated, probably due to a relative enhancement of NO production. Thus the balance of current data (in rats) suggests that, although low concentrations of insulin stimulate NO and ET-1 simultaneously, the net effect appears to favor the production of ET-1.

It is important to note that the relative roles of NO and ET-1 toward net vascular tone are different in humans versus rats. Although insulin-induced ET-1 release appears to predominate in rats (and leads to vasoconstriction and/or a lack of vasorelaxation), in humans, physiological doses of insulin consistently evoke NO-dependent vasodilation. Insulin-induced vasorelaxation is augmented by ET blockade in the human forearm, suggesting that in the human vasculature, insulin stimulation of NO production outweighs the effects exerted by ET-1.

Two issues from the present study require elaboration. First, the use of DAHP to inhibit BH4 and NO production. In the endothelial cell, NO is synthesized from l-arginine by a constitutive NO synthase (NOS). BH4 is an essential cofactor for the proper flow of electrons to oxidize l-arginine, and hence endothelial NO production is highly dependent on the presence of adequate amounts of this agent.10,11 DAHP is a specific GTP cyclohydrolase-I inhibitor that blocks tetrahydrobiopterin synthesis and subsequent NO production.9 Because insulin-mediated vasodilation in rat arteries is dependent on BH4 synthesis,9 we chose to use DAHP to characterize whether ET blockade uncovered BH4-dependent insulin-mediated vasorelaxation. Control experiments were performed that demonstrated the ability of DAHP to inhibit endothelium-dependent dilation to ACh (Figure 7).

The second point that merits brief discussion relates to the mechanism(s) of insulin-induced ET-1 production. Insulin stimulates the synthesis, secretion, and gene expression of the potent vasoconstrictor ET-1.12–14 In turn, ET-1 mediates vasoconstriction via ET A and ET B receptors on vascular smooth muscle cells.15 In certain vascular beds, the endothelial ET B receptor is linked to NO production and vasorelaxation.13 The observation that the effects of insulin in the presence of either ET A or ET B blockade are similar suggests that the ET B receptor may not play a significant role in mediating the effects of insulin on vascular tone, consistent with reports in the human forearm.3

The vascular actions of insulin have implications for the pathogenesis of both insulin resistance and hypertension.1–16 Vasodilation per se has been suggested to account for a significant proportion of insulin-mediated glucose uptake.2,16 In states of insulin resistance, the effects of insulin on vascular tone are blunted (vascular insulin resistance), which may contribute towards the development of increased vascular tone and hypertension.2,9 In addition, because vasodilation is an important determinant of insulin sensitivity, loss of insulin-mediated vasodilation may serve to exaggerate and/or
reinforce the insulin-resistant state per se. Thus, if this cycle is broken by either enhancing insulin sensitivity or promoting vasodilation, the net effect is a reduction in insulin resistance and a decrease in blood pressure.

As highlighted earlier, endothelial dysfunction can be viewed as the net balance of endothelium-derived vasoconstriction and vasodilation; derangements in either segment (or both) may predispose to increased tone and eventual vasospasm. Given this preamble, the beneficial effects noted in the present study may be due to (1) antagonism of ET-1 action on vascular smooth muscle ET receptors, (2) improvement in NO-mediated vasodilation (by release of tonic inhibition of ET-1 on NO production/release), or (3) a combination of the above mechanisms. Alternatively, it may be hypothesized that ET receptor blockade causes insulin-mediated vasorelaxation merely by restoring the balance of NO/ET-1. Said differently, ET-1 may not be primarily inmediated vasorelaxation merely by restoring the balance of NO/ET-1. Said differently, ET-1 may not be primarily increased in response to insulin, but in the face of ET receptor blockade, the relative contribution of NO toward endothelial homeostasis may change (in favor of vasodilation).

Perspective

Because we demonstrate that ET inhibits the vascular relaxation to subthreshold concentrations of insulin, it is logical to ask whether ACh responses are altered following short-term and/or long-term ET receptor blockade. In vascular tissue from control (non-diseased) rats, we have previously demonstrated that ET receptor blockade does not alter ACh-induced relaxation or systemic blood pressure. However, in hyperinsulimemic rats, long-term ET blockade with bosentan exhibits antihypertensive effects and improves endothelium-dependent relaxation to ACh. Furthermore, the vascular content of ET is increased in the face of long-standing hyperinsulinemia. Therefore, although ET may serve to antagonize the effects of insulin on vascular tone in normal arteries (present study), endothelial function is preserved unless chronic hyperinsulinemia/insulin resistance and hypertension are superimposed.

In summary, the present study demonstrates that endothelin receptor blockade uncovers BH4-dependent, insulin-mediated vasodilation, suggesting that subthreshold concentrations of insulin simultaneously stimulate the production of ET-1 and NO in rat aortas. Understanding the functional relationship between insulin, ET, and NO may uncover strategies aimed at improving vascular tone and reactivity in states of insulin resistance.

Acknowledgments

Supported by grants from the Medical Research Council of Canada and the Heart & Stroke Foundation of BC and Yukon (J.H.M.). Subodh Verma is the recipient of Fellowship Awards from the Medical Research Council of Canada, Heart and Stroke Foundation of Canada, Alberta Heritage Foundation for Medical Research, and Burroughs Wellcome Foundation. Todd J. Anderson is a Scholar of the Alberta Heritage Foundation for Medical Research and supported by the Heart and Stroke Foundation of Alberta.

References
