Enhanced endothelin-1 response and receptor expression in small mesenteric arteries of insulin-resistant rats

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1University of Georgia College of Pharmacy, Augusta 30912-3910; 2Vascular Biology Center, Departments of Pharmacology and Toxicology, Surgery, Physiology, and Endocrinology, School of Medicine, Medical College of Georgia, Augusta 30912-3910; and 3Augusta Veterans Affairs Medical Center, Augusta, Georgia 30904-6285

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Katakam, Prasad V. G., Jennifer S. Pollock, David M. Pollock, Michael R. Ujhelyi, and Allison W. Miller. Enhanced endothelin-1 response and receptor expression in small mesenteric arteries of insulin-resistant rats. Am J Physiol Heart Circ Physiol 280: H522–H527, 2001.—Hyperinsulinemia, a primary feature of insulin resistance, is associated with increased endothelin-1 (ET-1) activity. This study determined the vascular response to ET-1 and receptor binding characteristics in small mesenteric arteries of insulin-resistant (IR) rats. Rats were randomized to control (C) (n = 32) or IR (n = 32) groups. The response to ET-1 was assessed (in vitro) in arteries with (Endo+) and without (Endo−) endothelium. In addition, arteries (Endo+) were pretreated with the ETB antagonist A-192621 or the ETA antagonist A-127722. Finally, binding characteristics of [125I]ET-1 were determined. Results showed that in Endo+ arteries the maximal relaxation (E_{max}) to ET-1 was similar between C and IR groups; however, the concentration at 50% of maximum relaxation (EC_{50}) was decreased in IR arteries. In Endo− arteries, the E_{max} to ET-1 was enhanced in both groups. Pretreatment with A-192621 enhanced the E_{max} and EC_{50} to ET-1 in both groups. In contrast, A-127722 inhibited the ET-1 response in all arteries in a concentration-dependent manner; however, a greater ET-1 response was seen at each concentration in IR arteries. Maximal binding of [125I]ET-1 was increased in IR versus C arteries although the dissociation constant values were similar. In conclusion, we found the vasoconstrictor response to ET-1 is enhanced in IR arteries due to an enhanced expression of ET receptors and underlying endothelial dysfunction.

hyperinsulinemia; ETA; ETB

One important aspect of determining whether ET-1 is involved in the development of insulin-resistance-induced hypertension and vascular dysfunction is to know whether the vascular response to ET-1 is altered in arteries from insulin-resistant (IR) animals. Three studies to assess the vascular response to ET-1 in arteries from fructose-induced IR rats have been performed; however, these studies provide conflicting results (7, 11, 17). Thus the question of whether the vascular response to ET-1 is augmented in the setting of insulin resistance and hyperinsulinemia has not been adequately answered. In addition, the response to ET-1 has not been assessed in isolated small mesenteric arteries, a more appropriate venue to determine the response to ET-1 because these arteries have a greater involvement in dictating peripheral vascular resistance.

This study determined 1) the ET-1 response in small mesenteric arteries from IR and control rats in the presence and absence of endothelium, 2) the ETA response in small mesenteric arteries from IR and control rats in the presence of ETA- or ETB-receptor antagonists, and 3) the ETA receptor binding characteristics in small mesenteric arteries from IR and control rats.

METHODS

The Animal Care Committees at the Medical College of Georgia and the Augusta Veterans Affairs Medical Center approved the current protocol. Male Sprague-Dawley rats were obtained at age 6 wk and randomized into one of two groups: IR (n = 32) or control (n = 32). IR rats were fed a fructose-rich diet (containing as percentage of total calories: 66% fructose, 22% casein, and 12% lard, plus essential vitamins and minerals) (Teklad Labs, Madison, WI) and control animals received standard rat chow. Fructose-fed rats develop insulin resistance and hyperinsulinemia within 7 days of diet therapy, endothelial dysfunction within 14 days, and borderline hypertension within 20–28 days (8, 9). Each group of animals was continued on its respective diet for a period of 4 wk so that endothelial dysfunction was consistently established (8, 9).

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The intraluminal diameter) were isolated and removed for vascular studies. Blood vessels were isolated from the superior mesenteric artery (SMA) of control and insulin-resistant (IR) rats. 

**Materials.** A-127792, A-192621, and [125I]ET-1 were purchased from New England Nuclear (Boston, MA), and ET-1 was obtained from American Peptide (Sunnyvale, CA). Abbott Laboratories (Arlington Heights, IL) supplied A-127792 and A-192621. 

**Methods.** Vascular preparations were pooled from several animals because the quantity of membrane protein obtained from a single rat was not sufficient to determine a binding curve. 

**Results.** Mean body wt (303 ± 8 g for control and 310 ± 6 g for IR) and fasting glucose (149 ± 11 mg/dl for control and 383 ± 24 mg/dl for IR) were similar between control and IR groups. 

**Conclusion.** Insulin-resistant rats have an enhanced ET-1 response when compared with control rats.
142 ± 8 mg/dl for IR) were similar between control and IR rats. In contrast, fasting plasma insulin (97 ± 27 pM for control and 234 ± 37 pM for IR, P < 0.05) and MAP (116 ± 2 mmHg for control and 132 ± 4 mmHg for IR, P < 0.05) were significantly elevated in IR rats compared with control rats.

The resting intraluminal diameters of the small mesenteric arteries (both endothelium intact and denuded) did not differ between groups (231 ± 5 µm for control vs. 238 ± 4 µm for IR rats). ET-1 elicited a concentration-dependent vasoconstriction of arteries with endothelium from both groups. The E_max to ET-1 was similar between the groups of arteries, whereas the EC_{50} for the IR group was significantly lower versus control arteries (Fig. 1, Table 1).

After endothelium denudation, the E_max to ET-1 was enhanced in both IR and control arteries (Fig. 1) compared with arteries with endothelium. In contrast, the EC_{50} was not significantly affected by the removal of endothelium in either group (Table 1, Fig. 1) compared with arteries with endothelium.

Pretreatment of arteries with the ET_B antagonist A-192621 markedly increased both E_max and EC_{50} to ET-1 in IR and control arteries compared with untreated endothelium-intact arteries (Table 1, Fig. 2). However, the difference in EC_{50} measurements before and after pretreatment with A-192621 were 3.8 ± 0.2 nM and 0.4 ± 0.2 nM for control and IR rats, respectively. Thus the absolute change in the concentration-response curve after ET_B blockade was greater for control than IR (P < 0.001) arteries, suggesting a lesser role of ET_B receptors in IR arteries.

In contrast, pretreatment of arteries with the ET_A antagonist A-127722 inhibited the ET-1-induced vasoconstriction in arteries from both groups in a concentration-dependent manner (Fig. 3). It should be noted that ET-1 induced a greater vasoconstriction at each concentration of A-127722 (0.05 and 0.1 nM) in IR arteries compared with control arteries (Table 1, Fig. 3). The EC_{50} values of A-127722 were 0.02 ± 0.01 and 0.08 ± 0.01 nM for control and IR arteries, respectively (P < 0.001). Additionally, the concentration required to completely abolish the response to ET-1 was greater in IR than control arteries (Fig. 3). These data suggest that ET_A-mediated vasoconstriction is greater in IR versus control arteries.

Receptor binding experiments showed that maximal binding (B_max) of [125I]ET-1 was significantly increased in IR arteries (232 ± 10 fmol/mg protein) compared with control (136 ± 7 fmol/mg protein) (P < 0.05). In contrast, the dissociation constant (K_d) was similar for control (0.049 ± 0.014 nM) and IR (0.034 ± 0.008 nM) arteries (Fig. 4). These data suggest a greater number of endothelin receptors in arteries from IR compared with control rats.

Table 1. E_max and EC_{50} values of ET-1 dose-response curves

<table>
<thead>
<tr>
<th>ET-1 Dose Response</th>
<th>Control Arteries</th>
<th>Insulin-Resistant Arteries</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>E_max, %constriction</td>
<td>EC_{50}, nM</td>
</tr>
<tr>
<td>Endothelium intact</td>
<td>58 ± 2</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>Endothelium denuded</td>
<td>71 ± 2*</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>ET_A block (A-192621)</td>
<td>86 ± 1*</td>
<td>0.6 ± 0.2*</td>
</tr>
<tr>
<td>ET_B block (A-127722)</td>
<td>0.01 ± nM</td>
<td>39 ± 4*</td>
</tr>
<tr>
<td></td>
<td>0.05 ± nM</td>
<td>22 ± 7*</td>
</tr>
<tr>
<td></td>
<td>0.1 nM</td>
<td>0 ± 2*</td>
</tr>
<tr>
<td></td>
<td>1 nM</td>
<td>0 ± 2*</td>
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</table>

Values are means ± SE. ET-1, endothelin-1; E_max, maximal relaxation; P < 0.05; *P < 0.05 vs. respective ET-1 response in endothelium-intact arteries; †P < 0.05 vs. respective control.
DISCUSSION

The current study demonstrates an enhanced response to ET-1 in resistance arteries from IR rats. This enhanced response may be explained by two mechanisms. First, ET-1-induced vasoconstriction occurs via the activation of \( \mathrm{ET}_\alpha \) receptors. According to the receptor binding studies, it is likely that these receptors are overexpressed in vascular tissue from IR rats. Second, impaired \( \mathrm{ET}_\beta \)-activated production or release of endothelium-derived relaxing factors results in an imbalance between endothelium-derived vasodilating and contracting factors, leading to enhanced vasoconstriction by ET-1.

Two distinct endothelin receptor subtypes, \( \mathrm{ET}_\alpha \) and \( \mathrm{ET}_\beta \), mediate the vascular response to ET-1 (12, 14). \( \mathrm{ET}_\alpha \) receptors are located on the vascular smooth muscle and their stimulation results in vasoconstriction (10, 13). \( \mathrm{ET}_\beta \) receptors, expressed predominantly on endothelial cells, have also been reported on vascular smooth muscle (10, 13). \( \mathrm{ET}_\beta \) receptors located on the endothelium are responsible for inducing the release of endothelium-derived relaxing factors, whereas \( \mathrm{ET}_\beta \) receptors on vascular smooth muscle induce vasoconstriction (10, 13).

The current data illustrate that in small mesenteric arteries from control and IR rats vasoconstriction is mediated by the \( \mathrm{ET}_\alpha \) receptor because the presence of the \( \mathrm{ET}_\alpha \)-receptor antagonist A-127722 was able to abolish vasoconstriction in arteries from both groups. In addition, experiments to assess the response to ET-1 in the presence of various concentrations of A-127722 suggest that \( \mathrm{ET}_\alpha \)-receptor expression is enhanced in IR arteries because the maximal vasoconstriction to ET-1 was significantly greater in IR arteries at each concentration of the receptor antagonist (0.05 and 0.1 nM). Moreover, the concentration of A-127722 necessary to abolish the ET-1 response in IR arteries was markedly higher than that to eliminate the response in control arteries. To confirm these findings, we estimated total \([^{125}\mathrm{I}]\)-ET-1 binding in vascular membrane preparations from small mesenteric arteries of control and IR rats. The receptor binding experiments demonstrated a significant increase in maximal binding of \([^{125}\mathrm{I}]\)-ET-1 in IR arteries compared with control, suggesting an increase in expression of total endothelin receptors. In contrast, no difference was found in the \( K_d \) for \([^{125}\mathrm{I}]\)-ET-1 binding.

**Fig. 3.** Cumulative dose-response curve to ET-1 in small mesenteric arteries of control (A) and IR (B) rats in presence and absence of various concentrations (0.01, 0.05, 0.1, and 1 nM) of \( \mathrm{ET}_\alpha \)-receptor antagonist A-127722. *Concentrations where ET-1 responses in presence of A-127722 were significantly different from respective ET-1 responses in IR (Endo+) and control (Endo+) arteries without A-127722 pre-treatment (P < 0.05).

**Fig. 4.** Saturation-binding isotherms and Scatchard analysis (insets) of \([^{125}\mathrm{I}]\)-labeled ET-1 in membrane preparations of small mesenteric arteries of control (A) and IR (B) rats.
curves between control and IR arteries. Taken together, these data suggest that the increased response to ET-1 in IR arteries is due to upregulation of ET\(_A\) receptors in IR arteries.

Enhanced expression of ET\(_A\) receptors in the presence of insulin resistance or hyperinsulinemia has been previously shown. In rat aortic vascular smooth muscle cells, incubation with a supraphysiological concentration of insulin stimulated a selective upregulation of ET\(_A\) receptors as measured by both saturation binding and mRNA expression (5). Moreover, in rat tail arteries from fructose-fed IR rats, mRNA for ET\(_A\) receptors was increased approximately threefold compared with control rats (7). The current data confirm that endothelin (likely ET\(_A\)) receptors are overexpressed in vascular tissue from IR rats and are the first to demonstrate this in small mesenteric (near resistance) arteries.

The current data also suggest that in this arterial bed stimulation of the ET\(_B\) receptor enhances the production and release of endothelium-derived relaxing factors, because vasothrombosis was enhanced in the presence of the ET\(_B\)-receptor antagonist (A-192621) or with removal of the endothelium. Interestingly, the difference in EC\(_{50}\) between the control and IR groups was abolished after pretreatment with A-192621, with the EC\(_{50}\) for the control group decreasing dramatically and the EC\(_{50}\) for the IR group changing minimally. These data illustrate the impaired ability of the IR arteries to produce endothelium-derived relaxing factors in response to ET\(_B\) stimulation. This finding is not surprising because we and others have previously demonstrated impaired endothelium-dependent relaxation in mesenteric arteries from IR rats (8, 9, 16).

Similar to ET\(_B\) antagonist studies, endothelial denudation also induced a significant increase in maximal vasoconstriction in both groups compared with endothelium-intact arteries. However, the EC\(_{50}\) value was not altered with endothelium denudation compared with endothelium-intact arteries. It may seem contradictory that the EC\(_{50}\) was reduced with the ET\(_B\) antagonist but not by endothelium denudation. However, endothelial denudation removes the ability to produce both endothelium-derived relaxing factors and contracting factors; blocking the ET\(_B\) receptors on the endothelium only affects production of relaxing factors. Thus the resultant ET-1 response after endothelium denudation is represented entirely by the stimulation of vascular smooth muscle endothelin receptors, although the ET-1 response after ET\(_B\)-receptor blockade may be contributed to by endothelium-derived contracting factors. Importantly, the fact that the difference in EC\(_{50}\) remained between IR and control groups after endothelium denudation demonstrates that the enhanced response to ET-1 in IR arteries cannot be completely explained by impaired production of endothelium-derived relaxing factors.

Several other laboratories have assessed the vascular response to ET-1 in fructose-fed IR rats in a variety of vascular preparations; however, the results of these studies are conflicting. Enhanced maximal contraction was observed in aortic rings from IR rats (7), although a diminished (17) or normal (11) response was observed in superior mesenteric artery rings and the mesenteric vascular bed. It should be noted that the normal response reported by Navarro-Cid and colleagues (11) was elicited at 10 pM ET-1, which was ineffective in our experiments. None of these studies demonstrated a difference in the EC\(_{50}\) to ET-1. The apparent variation in these observations, compared with one another and to our own, may be explained by differences in artery size, vascular bed, or methodology. The current data differs from all of the above studies because we assessed the response to ET-1 in isolated small mesenteric arteries and measured intraluminal diameter under constant pressure.

Several studies in IR humans have reported increased ET-1 serum concentrations that directly correlated with the levels of hyperinsulinemia (2, 3, 12). In the current study we did not measure ET-1 serum concentrations; therefore, we are unsure whether this could also contribute to the hypertension or vascular dysfunction seen in this model. Two previous studies using the fructose-fed IR rat have failed to demonstrate increased serum ET-1 concentrations (7, 11); however, one study did show that vascular ET-1 content was elevated (15). Thus elevated tissue levels of ET-1 may also be a contributing factor to the vascular dysfunction seen in IR rats.

In summary, the response to ET-1 is enhanced in small mesenteric arteries from IR rats, as shown by a significantly decreased EC\(_{50}\). This enhanced response appears to be due to both increased expression of ET\(_A\) receptors on vascular smooth muscle and to underlying endothelial dysfunction.

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