Selective Angiotensin II Receptor Antagonism Reduces Insulin Resistance in Obese Zucker Rats

Erik J. Henriksen, Stephan Jacob, Tyson R. Kinnick, Mary K. Teachey, Michael Krekler

Abstract—Effects of oral administration of the angiotensin II receptor antagonist (selective AT₁-subtype) irbesartan on glucose tolerance and insulin action on skeletal-muscle glucose transport were assessed in the insulin-resistant obese Zucker rat. In the acute study, obese rats received either vehicle (water) or irbesartan 1 hour before the experiment. Although irbesartan had no effect on glucose transport (2-deoxyglucose uptake) in the epitrochlearis muscle, which consists mainly of type IIb fibers, acute angiotensin II receptor antagonism led to a dose-dependent increase in insulin action in the predominantly type I soleus muscle. Irbesartan at 25 and 50 mg/kg induced significant increases (41% and 50%, respectively; \( P<0.05 \)) in insulin-mediated glucose transport. Moreover, these acute irbesartan-induced improvements in soleus-muscle glucose transport were associated with enhancements in whole-body insulin sensitivity \( (r=-0.732; P<0.05) \), as assessed during an oral glucose tolerance test. After chronic administration of irbesartan (21 days at 50 mg · kg\(^{-1}\) · d\(^{-1}\)), glucose tolerance was enhanced further, and insulin-mediated glucose transport was significantly elevated in both epitrochlearis (32%) and soleus (73%) muscle. Chronic angiotensin II receptor antagonism was associated with significant increases in glucose transporter-4 (GLUT-4) protein expression in soleus (22%) and plantaris (20%) muscle and myocardium (15%). Chronic irbesartan-induced increases in whole-body insulin sensitivity were associated with increased insulin-mediated glucose transport in both epitrochlearis \( (r=-0.677; P<0.05) \) and soleus \( (r=-0.892; P<0.05) \) muscle. In summary, angiotensin II receptor (AT₁-subtype) antagonism, either acutely or chronically, improves glucose tolerance, at least in part because of an enhancement in skeletal-muscle glucose transport, and the effect of chronic angiotensin II receptor antagonism on type I skeletal-muscle glucose uptake is associated with an increase in GLUT-4 protein expression. \( \text{(Hypertension. 2001;38:884-890.)} \)

Key Words: irbesartan ■ glucose ■ muscle, skeletal ■ transport, glucose ■ rats, Zucker ■ receptors, angiotensin

Individuals with essential hypertension frequently display a clustering of additional atherogenic risk factors, including insulin resistance of skeletal-muscle glucose uptake, hyperinsulinemia, dyslipidemia, and central adiposity, in a condition described as “insulin resistance syndrome.” Angiotensin (Ang) II is a potent vasconstrictor and can contribute to the pathogenesis of hypertension. Reductions in formation of Ang II, as results from treatment with ACE inhibitors or inhibition of the cellular actions of Ang II by the use of specific Ang II (AT₁-subtype) antagonists, are effective interventions for lowering blood pressure. Although both animal model studies and clinical investigations have demonstrated that ACE inhibitor treatment can ameliorate peripheral insulin resistance, the role of a specific reduction in Ang II action on whole-body and skeletal-muscle insulin responsiveness remains controversial.

We have recently shown that acute oral administration of an Ang II (AT₁-subtype) receptor antagonist (eprosartan) has no significant effect on in vitro insulin-stimulated glucose transport activity in type IIb muscle (epitrochlearis) of the insulin-resistant obese Zucker rat. In contrast, chronic administration of Ang II receptor antagonists to insulin-resistant fructose-fed rats or spontaneously hypertensive rats or to human subjects with essential hypertension leads to significant improvements in whole-body insulin sensitivity. Moreover, Rao and Richey et al have reported that acute infusion of Ang II leads to substantial reduction in glucose disposal in normal skeletal muscle, which could not be attributed to hemodynamic alterations. To our knowledge, neither the acute effects of specific Ang II receptor antagonism on insulin-stimulated glucose transport in insulin-resistant type I skeletal muscle (eg, soleus) nor the chronic effects of Ang II receptor antagonism on the skeletal-muscle glucose transport system in conditions of insulin resistance have been investigated.

In this context, the purpose of the present study was to assess in an animal model of skeletal-muscle insulin resistance, hyperinsulinemia, glucose intolerance, and dyslipidemia (obese Zucker fafa rats) the effects of acute or chronic treatment with a specific Ang II receptor (AT₁-subtype) antagonist.
antagonist, irbesartan, on whole-body glucose disposal and on in vitro insulin-stimulated skeletal-muscle glucose transport activity. This latter approach has the advantage of allowing for assessment of adaptive responses in the skeletal-muscle glucose transport system independent of any potential hemodynamic influences. In addition, the potential role of alterations in expression of skeletal-muscle glucose transporter-4 (GLUT-4) protein in the regulation of insulin-stimulated glucose transport activity after chronic antagonism of Ang II receptors was investigated.

**Methods**

**Animals and Treatments**
Female obese Zucker rats (HsdOla:ZUCKER-fa; Harlan World Headquarters, Indianapolis, Ind) were received at 6 to 7 weeks of age. Starting at 8 to 9 weeks of age, animals received acutely (1 hour) by gavage either vehicle (water) or irbesartan (5, 25, or 50 mg/kg). In a separate chronic study, animals received 50 mg \( \cdot \) kg \( \cdot \) d \( \cdot \) 1 \( \cdot \) of either vehicle or irbesartan by gavage for 21 consecutive days.

**Oral Glucose Tolerance Tests**
Animals were food restricted (4 g of chow given at 5:00 PM) the evening before the experiment. Between 8:00 and 10:00 AM, 1 hour (acute study) or \( \sim \) 12 hours (chronic study) after the most recent treatment, animals underwent an oral glucose tolerance test (OGTT) with a 1-g/kg body wt glucose feeding by gavage. Blood was drawn from a cut at the tip of the tail at 0, 30, 60, 90, and 120 minutes after the glucose feeding. Whole blood was mixed thoroughly with EDTA (18 mmol/L final concentration) and centrifuged at 13 000 g to separate the plasma. Plasma samples were analyzed for glucose (Sigma Chemical Co), insulin (Linco Research Inc), and free fatty acids (Wako Chemicals USA Inc). Immediately after completion of the OGTT, all animals received 2.5 mL SC of sterile 0.9% saline to compensate for plasma loss. In the acute study, animals remained untreated for 3 days after OGTT and then received a second acute administration of the same dosage level of irbesartan. In the chronic study, treatments resumed for 3 days.

**Glucose Transport Activity**
In the acute study, animals were again food restricted as described above before receiving a second acute dose of irbesartan. In the chronic study, animals were likewise food restricted. Between 8:00 and 10:00 AM, 1 hour after the second acute dose or \( \sim \) 12 hours after final chronic treatment with irbesartan, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg IP). Both epimyocardial muscles and 1 soleus muscle were surgically removed and prepared for in vitro incubation. Epimyocardial muscles were incubated intact, whereas soleus muscles were prepared in strips (weight, \( \sim \) 25 mg).\(^{24}\) Glucose transport activity, assessed as 2-deoxyglucose (2-DG) uptake, was determined in the absence of presence of insulin (13.3 nmol/L) exactly as described previously.\(^{7-10,19}\)

**Muscle GLUT-4 Protein**
In the chronic study, the contralateral soleus and plantaris and the heart were removed, quickly frozen in liquid N\(_2\), weighed, and stored at \(-80^\circ\)C until processed. Muscles and left ventricle of the heart were homogenized in 20 vol of ice-cold buffer containing 20 mmol/L HEPES, 1 mmol/L EDTA, and 250 mmol/L sucrose, pH 7.4. Total protein concentration was determined by use of the bicinchoninic acid method (Sigma). GLUT-4 protein was assayed as described previously.\(^{10}\)

**Statistical Analysis**
All data are presented as mean\(\pm\)SE. Significance of differences between multiple groups was assessed by ANOVA with a post hoc Fisher Protected Least Significant Difference Test. Differences between groups were determined by an unpaired Student \(t\) test. Statistical significance was set at the 0.05 probability level.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Results**

**Acute Administration of Irbesartan**
Final body weights were similar in the obese, vehicle-treated group (310\(\pm\)6 g) and in the obese groups treated acutely with irbesartan at 5 (319\(\pm\)8 g), 25 (313\(\pm\)8 g), or 50 (308\(\pm\)4 g) mg/kg. Glucose and insulin responses during an OGTT in the acutely treated groups are shown in Figure 1, top. The lowest dose of irbesartan (5 mg/kg) did not alter these responses. Compared with the vehicle-treated group, acute irbesartan treatment at 25 mg/kg resulted in a 14% lower \((P<0.05)\)
glucose value at the 120-minute point, whereas acute treatment with 50 mg/kg caused lower (10% to 19%; \( P < 0.05 \)) glucose values at 30, 90, and 120 minutes. Only acute irbesartan treatment at 50 mg/kg caused significantly lower (17% to 21%; \( P < 0.05 \)) insulin responses during the course of the OGTT.

Total areas under the curve (AUC) for these glucose and insulin responses and the glucose-insulin index (the product of glucose AUC and insulin AUC; a reduction in this value reflects an increase in whole-body insulin sensitivity\(^2\)) are presented in Figure 1, bottom. Whereas the reductions in the obese group treated with 25 mg/kg irbesartan did not reach statistical significance, animals treated with 50 mg/kg irbesartan displayed significant reductions in glucose (13%; \( P < 0.05 \)) and insulin (15%; \( P < 0.05 \)) AUC. The glucose-insulin index was significantly reduced (17%; \( P < 0.05 \)) in the 25 mg/kg irbesartan-treated group, with the greatest diminution (26%; \( P < 0.05 \)) in this variable observed in the 50 mg/kg irbesartan-treated group.

To identify the potential cellular locus for the improvements in whole-body insulin sensitivity brought about by acute irbesartan treatment, insulin action on isolated skeletal muscle from the obese animals was assessed (Figure 2). In the epitrochlearis, which consists primarily of less-insulin-sensitive type IIb fibers,\(^2\)\(^4\)\(^6\) no increases were seen either in the rate of insulin-stimulated 2-DG uptake (Figure 2, top left) or in the insulin-mediated increase (increase over basal) in 2-DG uptake (Figure 2, top right) after acute irbesartan treatment. In contrast, acute treatment of the obese animals with irbesartan was associated with enhanced insulin action in the soleus, which consists mainly of more-insulin-responsive type I fibers.\(^2\)\(^4\)\(^7\) After animals were treated with either 25 or 50 mg/kg of irbesartan, both the rates of insulin-stimulated 2-DG uptake (18% for both dosages; \( P < 0.05 \)) and insulin-mediated 2-DG uptake (41% and 50%, respectively; both \( P < 0.05 \)) were increased in the soleus. Enhancement of insulin-mediated 2-DG uptake in soleus but not epitrochlearis after acute treatment with irbesartan was correlated significantly \((r = 0.732; \ P < 0.05)\) with improvement in whole-body insulin sensitivity (reduction in glucose-insulin index; Figure 3).
Chronic Administration of Irbesartan
The highest effective acute dose, 50 mg/kg, was chosen to assess the effect of chronic irbesartan treatment on insulin action in the obese Zucker rat. Final body weights did not differ between groups (Table). The positive systemic influence of chronic irbesartan treatment was demonstrated by a 18%-lower heart weight in the irbesartan-treated versus the vehicle-treated group. Whereas chronic irbesartan treatment elicited a significant lowering (14%; \( P < 0.05 \)) of plasma glucose in food-restricted obese animals, this treatment regimen did not significantly alter plasma insulin or free fatty acid levels (Table).

Chronic irbesartan treatment lowered significantly both glucose (17% to 21%; \( P < 0.05 \)) and insulin (25% at 90 minutes and 29% at 120 minutes, \( P < 0.05 \)) responses during OGTT (Figure 4, top). Likewise, the glucose (19%; \( P < 0.05 \)) and insulin (21%; \( P < 0.05 \)) AUC and the glucose–insulin index (34%; \( P < 0.05 \)) were all significantly less in the chronic irbesartan-treated versus the vehicle-treated obese groups (Figure 4, bottom).

Insulin-stimulated rate of 2-DG uptake and insulin-mediated increase in 2-DG uptake in both epitrochlearis (21% and 32%, respectively; \( P < 0.05 \)) and soleus (40% and 73%, respectively; \( P < 0.05 \)) muscles were significantly enhanced after chronic irbesartan treatment (Figure 5). These improvements in insulin-mediated 2-DG uptake in skeletal muscle after chronic irbesartan treatment were significantly correlated with enhancement of whole-body insulin sensitivity, as assessed by reduction in the glucose-insulin index (epitrochlearis, \( r = -0.677 \); soleus, \( r = -0.892 \); both \( P < 0.05 \); Figure 6). Importantly, chronic treatment with irbesartan elicited a significant enhancement (22%; \( P < 0.05 \)) in GLUT-4 protein level in the soleus muscle (Figure 7). Similarly small but significant increases in GLUT-4 protein expression after chronic Ang II receptor antagonism were observed in the mixed-type IIa/IIb plantaris muscle (20%; \( P < 0.05 \)) and in myocardium (15%; \( P < 0.05 \)).

Discussion
In the present investigation, we assessed the potential role of Ang II receptor antagonism using irbesartan in the modulation of whole-body and skeletal-muscle glucose disposal in an animal model of obesity-associated insulin resistance, the obese Zucker rat. We have confirmed our previous observation\(^{19}\) that acute oral administration of an AT\(_1\)-specific Ang II receptor antagonist does not appreciably enhance insulin action on glucose transport activity in skeletal muscle comprised primarily of less-insulin-responsive type IIb fibers (epitrochlearis) from the obese Zucker rat. However, we report here for the first time that acute administration of an

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Values are mean±SE for 5 to 6 animals per group. Irbesartan (50 mg/kg) was administered by gavage daily for 21 days. *\( P < 0.05 \) vs obese vehicle-treated group.

Figure 4. Effect of chronic treatment of obese Zucker rats with 50 mg/kg irbesartan for 21 consecutive days on glucose and insulin responses during OGTT, glucose (mmol/L · min) and insulin (nmol/L · min) AUC, and glucose-insulin index (mmol/L · mmol/L · min² · 10²). Data are mean±SE for 5 to 6 animals per group. *\( P < 0.05 \) vs obese vehicle-treated group.
Ang II receptor antagonist increases, in a dosage-dependent fashion, insulin action on glucose transport in skeletal muscle made up of more-insulin-responsive type I fibers from soleus (Figure 2). Chronic administration of irbesartan clearly elicited the expected systemic effects, as evidenced by the substantial regression of cardiac mass in these obese animals (Table), consistent with previous clinical findings. Moreover, chronic administration of this Ang II receptor antagonist elicited significant increases in insulin action on glucose transport in both epitrochlearis and soleus muscles (Figure 5), with the absolute effect of this intervention being greater in the latter muscle. These findings underscore the importance of assessing the effectiveness of antihypertensive interventions on metabolic variables in muscles that consist of a variety of fiber-type compositions, given that fiber type–dependent alterations in these metabolic responses may be apparent.

Whole-body insulin sensitivity, as reflected in the reduced glucose-insulin index by use of glucose and insulin responses during OGTT, was significantly enhanced in obese Zucker rats by acute administration of irbesartan (Figure 1) and, to an even greater extent, after chronic treatment with this Ang II receptor antagonist (Figure 4). At least part of these increases in whole-body glucose disposal was the result of an enhanced capacity for insulin-mediated glucose transport in skeletal muscle, given that significant correlations between the glucose-insulin index and the insulin-mediated increase in 2-DG uptake were found for the soleus after acute irbesartan treatment (Figure 3) and for both the epitrochlearis and soleus after chronic Ang II receptor antagonism (Figure 6). Whereas a portion of these improvements in whole-body insulin action can be attributed to the well-documented hemodynamic modifications elicited by Ang II receptor antagonists, both the acute and chronic treatments with irbesartan resulted in upregulation of the skeletal-muscle glucose transport system: increases observed in insulin-stimulated 2-DG uptake in the epitrochlearis (chronic treatment only) and soleus (acute and chronic treatments) were assessed in vitro and therefore were independent of hemodynamic influences. In addition, in light of the small but significant effects of chronic irbesartan treatment for reduction of fasting plasma glucose (Table), the possibility exists that Ang II receptor antagonism may en-
hance hepatic insulin sensitivity and diminish hepatic glucose production in the fasting state.

Present findings support and extend previous investigations that demonstrate that inhibition of Ang II formation, either through administration of ACE inhibitors or through specific inhibition of Ang II action at AT1 receptors, frequently is associated with enhancement of whole-body insulin sensitivity in a variety of insulin-resistant animal models or in insulin-resistant humans with essential hypertension. Whereas the vasodilatory effects of these interventions account for some of the whole-body metabolic improvements, the present results point toward an additional important contribution of the insulin-dependent glucose transport system in skeletal muscle to the beneficial metabolic effects of these compounds. Reports of the metabolic neutrality of Ang II receptor antagonists in conditions of hypertension may be related to the apparent dosage-dependency of the effects of these compounds on insulin action (see Figures 1 through 3); dosages that elicit lowering of blood pressure were ineffective for bringing about increased insulin sensitivity. Future investigations should be designed to address this possibility specifically.

The role of the whole-muscle expression of GLUT-4 protein in the capacity of that muscle for insulin-mediated glucose transport activity has long been recognized. In the present investigation, we found that chronic antagonism of Ang II receptors caused small (15% to 22%) but significant increases in GLUT-4 protein expression in both oxidative (soleus) and substantially glycolytic (plantaris) skeletal muscle and in the highly oxidative myocardium (Figure 7). At least in the soleus muscle, this increase in GLUT-4 protein could have contributed to enhancement of insulin action on glucose transport after chronic irbesartan treatment. This increase in muscle GLUT-4 protein after chronic Ang II receptor antagonism is consistent with the concept that Ang II may function as a negative modulator of GLUT-4 gene expression in these tissues, in parallel with its known effects on gene expression and cellular phenotypic alterations in the vascular endothelium, smooth muscle cells, and cardiac myocytes.

We have observed that chronic administration of the ACE inhibitor trandolapril can induce small (25% to 30%) increases in GLUT-4 protein expression in skeletal muscle of the obese Zucker rat. This induction of GLUT-4 protein in insulin-resistant rat skeletal muscle could not be reproduced with chronic administration of bradykinin, the level of which is increased by ACE inhibitors. However, the present findings support the hypothesis that the ability of the ACE inhibitor trandolapril to enhance GLUT-4 protein expression in muscle of the obese Zucker rat may be reliant on the ability of this compound to effectively diminish formation and action of Ang II.

Chronic administration of ACE inhibitors to obese Zucker rats results in a significant diminution of the elevated level of fasting plasma free fatty acids, and this decrease in free fatty acids may be mechanistically linked to the improvement in insulin action on skeletal-muscle glucose transport. Interestingly, a lowering of plasma free fatty acids can be achieved with chronic administration of bradykinin but not with chronic administration of an Ang II receptor antagonist (Table). These findings are consistent with the interpretation that the lipid-lowering effects of ACE inhibitors are mediated by an enhancement of bradykinin action and not by a reduction in Ang II action. Furthermore, it is clear that the enhancement of insulin action brought about by chronic Ang II antagonism is not mediated by a reduction in the inhibitory effects of circulating free fatty acids.

Results of the present investigation are consistent with and complement the findings of Richey et al. These investigators demonstrated that acute infusion of Ang II into normal dogs during euglycemic, hyperinsulinemic clamp led to a significant decrease in insulin sensitivity and that this Ang II-induced insulin resistance probably developed because of direct inhibition of glucose transport by the myocyte. Our findings that the acute administration of irbesartan, which would prevent any negative action of Ang II on cellular glucose transport, led to an enhancement of insulin-stimulated skeletal-muscle glucose transport (at least in type I muscle), is entirely consistent with this hypothesis. What remains to be determined is the exact cellular mechanism for this deleterious effect of Ang II on insulin action. Acute administration of Ang II is known to cause inhibition of insulin-stimulated phosphatidylinositol-3-kinase activity in rat heart. However, whether this mechanism for Ang II-
induced impairment of insulin signaling is functional in skeletal muscle and whether the antagonism of Ang II receptors removes this inhibitory effect of Ang II in insulin-resistant skeletal muscle of the obese Zucker rat are presently unknown.

In summary, the present investigation has demonstrated that acute administration of irbesartan, a specific Ang II (AT1-subtype) receptor antagonist, to the insulin-resistant obese Zucker rat induces dose-dependent improvement of whole-body insulin sensitivity, at least in part because of an enhancement of glucose transport in type I skeletal muscle. Moreover, chronic administration of irbesartan elicited an even greater improvement in whole-body insulin sensitivity, which was associated with increased glucose transport in both type I and type IIb skeletal muscles. This chronic Ang II receptor antagonism also elicited significant increases in GLUT-4 protein expression in skeletal muscle and myocardium. The results support the use of Ang II receptor antagonists to treat the hypertension and insulin resistance of skeletal-muscle glucose metabolism associated with insulin-resistance syndrome.

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References


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