Original Article

Effects of Angiotensin II Receptor Antagonists on Insulin Resistance Syndrome and Leptin in Sucrose-Fed Spontaneously Hypertensive Rats

Mamoru Umeda, Tsugiyasu Kanda, and Masami Murakami

In order to investigate the usefulness of angiotensin II type 1 receptor (AT1) antagonists (ARA) in the treatment of hypertension with insulin resistance syndrome, we studied the effects of a high dose sucrose diet and ARA on insulin sensitivity, plasma lipids, and leptin in spontaneous hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). SHR and WKY were divided into three groups and treated for 12 weeks: those fed a standard chow, those given a sucrose-rich chow or those given a sucrose-rich chow and ARA. While in SHR the weight of both subcutaneous and mesenteric adipose tissue was greater in the sucrose-rich chow fed animals than in the standard chow fed animals, ARA treatment significantly decreased the weights of both subcutaneous and mesenteric adipose tissue. ARA treatment decreased free fatty acid and triglyceride in SHR, and increased high density lipoprotein cholesterol in SHR and WKY. Homeostasis model assessment-insulin resistance (HOMA-IR) index, plasma levels of leptin, and leptin mRNA in mesenteric adipose tissue were significantly greater in the sucrose-rich chow fed animals than in the standard chow fed animals, and significantly lower in the ARA-treated sucrose-rich chow fed animals than in the sucrose-rich chow fed animals in both SHR and WKY. ARA improved insulin resistance, and reduced plasma leptin and leptin mRNA in adipose tissue. These results suggest that the improvement of insulin resistance by ARA may be attributed, at least in part, to the reduction of adipose tissue weight. It is concluded that ARA is useful in the treatment of patients with hypertension and concomitant insulin resistance syndrome.

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Key Words: insulin resistance, angiotensin II type 1 receptor antagonists, leptin, hypertension, adipose tissue

Introduction

Insulin resistance syndrome has been proposed as a possible metabolic link among hypertension, non insulin-dependent diabetes mellitus (NIDDM), obesity, dyslipidemia, and atherosclerotic cardiovascular disease (1). Humans with a genetic predisposition to hypertension tend to develop insulin resistance and hyperinsulinemia (2, 3). It is suggested that hypertension may be associated with hyperinsulinemia in part via glucose intolerance-induced alterations such as sodium retention, vasculopathy, and nephropathy (4). Therefore, the effects of antihypertensive agents on insulin sensitivity may have important benefits. Although some antihypertensive drugs, such as thiazide diuretics and β-adrenergic blockers, have been reported to impair insulin sensitivity (5, 6), angiotensin-converting enzyme (ACE) inhibitors have been shown to improve the insulin sensitivity in hypertensive patients (7), and increase the insulin sensitivity in spontaneous hypertensive rats (SHR) (8).

Recently, angiotensin II type 1 receptor (AT1) antagonists (ARA) have been introduced into clinical practice for the treatment of hypertension and congestive heart failure (9). Results of experimental and clinical studies on the effect of...
ARA on insulin sensitivity have been conflicting, with some studies showing no influence on insulin sensitivity (10–12) and others demonstrating an improvement of insulin sensitivity (13–15). Therefore, it appears important to examine the effect of ARA on insulin sensitivity in genetic hypertensive animals with insulin resistance.

In addition to genetic factors, alterations in lifestyle are also important as potential causes of insulin resistance. Reduced physical activity, dietary indiscretion with excessive food intake, and overconsumption of fat and sugar may promote insulin resistance syndrome, which is characterized by such conditions as hypertension, hyperlipidemia, and diabetes mellitus. Adipose tissue is a rich source of metabolically active molecules, ranging from free fatty acids to tumor necrosis factor-α and leptin, the product of ob gene (16). Insulin resistance has been shown to be linked to elevated plasma leptin levels (17). Leptin is produced almost exclusively in fat cells (18). Plasma leptin levels correlate strongly with body mass index and fat cell volume (19), and are closely related to the amount of lipids stored in the fat cells (20). It has been recently reported that the abundant expression of angiotensinogen, the precursor of the potent vasoconstrictor angiotensin II, in human adipose tissue is derived from omental and subcutaneous fat deposits (21). Therefore the influence of leptin and angiotensin II may play a key role in modulating the effects of hypertension and lifestyle on cardiovascular disease.

Because of these considerations, the present study was conducted to determine whether insulin resistance can be induced in genetic hypertensive rats by dietary means, and whether ARA treatment is able to improve insulin sensitivity in hypertensive animals with insulin resistance.

**Methods**

**Study Design and Experimental Protocol of Angiotensin II Type 1 Receptor (AT1) Antagonists Administration**

Age- and sex-matched Wistar-Kyoto rats (WKY) and male SHR 6 weeks of age (Charles River Japan, Yokohama, Japan) were used in this study. All animals were housed according to institutional guidelines for 12 weeks in climate-controlled metabolic cages with a 12-h light/12-h dark cycle, and food (as specified below) and water provided ad libitum. All experiments were approved by the Animal Care and Experimentation Committee of Gunma University, Showa Campus.

SHR and WKY were divided into three groups of six rats each at the start of the study: those fed a standard chow (MF: 81% carbohydrates, 13% protein, and 6% fat; Oriental Yeast Co., Tokyo, Japan), those given a sucrose-rich chow (50% sucrose and 50% standard chow), or those given a sucrose-rich chow and ARA. The ARA-treated animals were given L-158,809 (Merck Banyu Co., Ltd., Tokyo, Japan; 1 mg/kg body weight in 0.3 ml of saline) orally once a day for 8 weeks, beginning on week 4 of this study. After 12 weeks the systolic blood pressure (SBP) was measured by tail plethysmography (PS-600; Riken Kaimatsu, Tokyo, Japan). Animals were fasted for 15 h, then anesthetized with sodium amobarbital (100 mg/kg, i.p.). Blood samples were taken from the portal vein. Adipose tissue was removed immediately from the right abdominal region as described previously (22). The weight of subcutaneous and mesenteric adipose tissue was measured, and then the specimens were frozen in liquid nitrogen and stored at -70 °C until processed.

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

Total RNA was extracted from the adipose tissue using Isogen reagent (Nippon Gene, Tokyo, Japan). Reverse transcription was performed on 2 μg total RNA with an RT-PCR kit (Takara, Tokyo, Japan) using oligo-dT primer. The samples were incubated for 30 min at 45 °C, for 5 min at 99 °C and for 5 min at 5 °C. The primers for leptin were: forward 5'-CCAAACACCTCATCAAGACC-3' and reverse 5'-GTCCA ACTGTGGAAGAATGTC-3'. The cDNAs were amplified using primers for β-actin: forward 5'-GGACCCAGATCATGTTGAAGAATGTCCC-3' and reverse 5'-CTCATACCCAAAGAGGGAAAGG-3'. As a quality and quantity control, the PCR profile consisted of 1 cycle of 94 °C for 1 min, 34 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and 1 cycle of 72°C for 10 min. PCR products were separated by electrophoresis on a 1% agarose gel, visualized by ethidium bromide staining and analyzed by scanning densitometry. The results for the expression of leptin mRNA were presented relative to the expression of the control β-actin gene.

**Assays**

Blood glucose (BG) was measured by the glucose oxidase H2O2 electrode method (ANTOSENSE; Bayer-Sankyo, Tokyo, Japan). Immunoreactive insulin (IRI) was measured by a double antibody radioimmunoassay (Insulin Radioimmunoassay Kit, Eiken Chemical Co., Tokyo, Japan) using rat crystalline insulin as a standard (Novo Nordisk Biolabs, Inc., Copenhagen, Denmark). Measurement of plasma leptin levels by radioimmunoassay was performed as previously described (23). Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), and free fatty acid (FFA) were assayed by automated enzymatic methods (Hitachi Medical System, Tokyo, Japan).

**Data Analysis and Statistics**

Insulin resistance was assessed by homeostasis model assessment-insulin resistance (HOMA-IR) index (24), which was calculated as: [fasting BG (mg/dl) × fasting IRI (ng/ml)]/405. All data are expressed as the means ± SD. Student’s t-test was used to determine significance in comparison with...
the unpaired data. To compare the six groups, the analysis of variance (ANOVA) was used for multiple comparisons. Levels of $p < 0.05$ were considered to indicate statistical significance.

**Results**

In the present study, there were no significant differences among the six groups with regard to body weight (Table 1). Both the standard chow fed and the sucrose-rich chow fed SHR had severe hypertension. There was no significant difference in SBP between the standard chow fed and the sucrose-rich chow fed SHR, but the SHR given a sucrose-rich chow and ARA showed a significantly lower SBP. In the groups of WKY there were no significant differences in SBP (Table 1).

In SHR the weight of both subcutaneous and mesenteric adipose tissue was greater in the sucrose-rich chow fed animals than in the standard chow fed animals, and this increase was significant for mesenteric adipose tissue ($p < 0.05$) (Fig. 1). However, in the SHR receiving sucrose-rich chow and ARA treatment the weights of both subcutaneous and mesenteric adipose tissue were significantly lower than those of the group receiving sucrose-rich chow alone ($p < 0.05$) (Fig. 1). No significant changes were observed in WKY (Fig. 1).

There were alterations in FFA, TG, and HDL-C in these animals (Fig. 2). Significant changes in FFA and TG were limited to SHR, in which serum levels in both the standard chow fed and the sucrose-rich chow fed groups were significantly higher than in the corresponding WKY groups. FFA and TG levels were also higher in the SHR fed sucrose-rich chow than in those fed standard chow ($p < 0.05$). However, FFA and TG levels were significantly reduced in SHR receiving sucrose-rich chow and ARA treatment than in those receiving sucrose-rich chow alone ($p < 0.05$). There were significant alterations in the serum level of HDL-C in both WKY and SHR, and the HDL-C level was increased for both groups ($p < 0.05$) in the animals receiving sucrose-rich chow and ARA treatment compared to that in the animals receiving sucrose-rich chow alone. The serum level of TC showed no significant change (Fig. 2).

In SHR a significant increase of fasting BG and IRI was noted compared with corresponding WKY in the standard chow fed, sucrose-rich chow fed and sucrose-rich chow fed

### Table 1. Profile of 18-Week-Old Spontaneously Hypertensive Rats (SHR) and Wistar-Kyoto Rats (WKY)

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
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<tr>
<td></td>
<td>STD</td>
<td>SUC</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>BW at baseline (g)</td>
<td>68 ± 3</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>BW at 12 weeks (g)</td>
<td>338 ± 7</td>
<td>343 ± 12</td>
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<tr>
<td>BW gain (g)</td>
<td>270 ± 5</td>
<td>274 ± 8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>153 ± 8</td>
<td>160 ± 10</td>
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Values are the means ± SD. STD, standard chow fed rats; SUC, sucrose-rich chow fed rats; ARA, angiotensin II type I receptor antagonist-treated rats; N, number of samples; BW, body weight; SBP, systolic blood pressure. $^a p < 0.05$ vs. STD in WKY, $^b p < 0.05$ vs. ARA in SHR, $^c p < 0.05$ vs. SUC in WKY, $^d p < 0.05$ vs. ARA in WKY.
and ARA-treated groups ($p < 0.05$) (Table 2). The serum level of IRI was significantly greater in the sucrose-rich chow fed animals than in the standard chow fed animals, and significantly lower in the ARA-treated sucrose-rich chow fed animals than in the sucrose-rich chow fed animals ($p < 0.05$) in both SHR and WKY (Table 2). The HOMA-IR index was significantly higher in SHR than in WKY. In both SHR and WKY, the HOMA-IR index in the sucrose-rich chow fed animals was significantly increased compared with that in the standard chow fed animals, and that in the ARA-treated sucrose-rich chow fed animals was significantly reduced compared with that in the sucrose-rich chow fed animals ($p < 0.05$) (Fig. 3). The HOMA-IR index was strongly correlated to adipose tissue weight in both SHR and WKY (SHR: subcutaneous adipose tissue, $r = 0.914$, $p < 0.05$; mesenteric adipose tissue, $r = 0.941$, $p < 0.05$; WKY: subcutaneous adipose tissue, $r = 0.833$, $p < 0.05$; mesenteric adipose tissue, $r = 0.895$, $p < 0.05$).

![Fig. 2](image-url) **Fig. 2.** Values of free fatty acid (a), triglyceride (b), total cholesterol (c) and high density lipoprotein (HDL)-cholesterol (d) in standard chow fed, sucrose-rich chow fed, and angiotensin II type 1 receptor antagonist-treated rats. Values are the means $\pm$ SD. Abbreviations are as shown in Fig. 1. $^a p < 0.05$ vs. STD in WKY, $^b p < 0.05$ vs. SUC in WKY, $^c p < 0.05$ vs. STD in SHR, $^d p < 0.05$ vs. ARA in SHR, $^* p < 0.05$ vs. SUC in SHR.

**Table 2. Effect of Angiotensin II Type 1 Receptor Antagonists on Fasting Blood Glucose (FBG) and Plasma Insulin in Spontaneously Hypertensive Rats (SHR) and Wistar-Kyoto Rats (WKY)**

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
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<th>SHR</th>
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<tbody>
<tr>
<td></td>
<td>STD</td>
<td>SUC</td>
<td>ARA</td>
</tr>
<tr>
<td>$N$</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>107 $\pm$ 4.3</td>
<td>134 $\pm$ 6.1 $^a$</td>
<td>125 $\pm$ 5.0 $^a$</td>
</tr>
<tr>
<td>IRI (ng/ml)</td>
<td>14 $\pm$ 1.1</td>
<td>24 $\pm$ 1.9 $^{ace}$</td>
<td>18 $\pm$ 1.8 $^a$</td>
</tr>
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</table>

Values are the means $\pm$ SD. STD, standard chow fed rats; SUC, sucrose-rich chow fed rats; ARA, angiotensin II type 1 receptor antagonist-treated rats; $N$, number of samples; IRI, immunoreactive insulin. $^a p < 0.05$ vs. STD in WKY, $^b p < 0.05$ vs. SUC in WKY, $^c p < 0.05$ vs. STD in SHR, $^d p < 0.05$ vs. ARA in SHR, $^* p < 0.05$ vs. ARA in SHR.
The plasma level of leptin was significantly greater in SHR than in WKY. Plasma leptin was also significantly greater in the sucrose-rich chow fed animals than in the standard chow fed animals, and significantly lower in the ARA-treated sucrose-rich chow fed animals than in the sucrose-rich chow fed animals (p < 0.05) in both SHR and WKY (Fig. 4). Plasma leptin was strongly correlated to adipose tissue weight in both SHR and WKY (SHR: subcutaneous adipose tissue, \( r = 0.942, p < 0.05 \); mesenteric adipose tissue, \( r = 0.924, p < 0.05 \); WKY: subcutaneous adipose tissue, \( r = 0.897, p < 0.05 \); mesenteric adipose tissue, \( r = 0.934, p < 0.05 \)) and to HOMA-IR index in both SHR and WKY (SHR: \( r = 0.889, p < 0.05 \); WKY: \( r = 0.833, p < 0.05 \)).

Leptin mRNA expression in mesenteric adipose tissue in the sucrose-rich chow fed animals was significantly increased compared with that in the standard chow fed animals and with the group receiving sucrose-rich chow and ARA treatment in SHR but not in WKY. No significant differences were observed in leptin mRNA expression in subcutaneous adipose tissue (Fig. 5).

**Discussion**

The present study showed that sucrose feeding was able to induce hyperinsulinemia and insulin resistance in genetic hypertensive rats. Hypertension-induced insulin resistance has been demonstrated to accompany increased skeletal muscle vascular resistance (25). Additionally, it has been reported that sucrose feeding increases norepinephrine excretion, turnover, and plasma concentration and enhances sympathetic nerve responses in rats (26, 27). Thus, sympathetic overactivity may be involved in the pathogenesis of this model, and may be responsible at least in part for the impairment of blood flow to skeletal muscle, which in turn would favor the development of insulin resistance (28). The finding that
changes in adipose tissue weight, HOMA-IR, and leptin were more pronounced in SHR than in WKY may be attributed to the effect of hypertension, as increased blood pressure is considered to be one of the major risk factors for insulin resistance syndrome. These results suggest that both aberrations in lifestyle—e.g., overeating, and particularly overeating diets high in sucrose-containing foods—and a genetic predisposition to hypertension may contribute to the development of insulin resistance.

Our results demonstrated that ARA (L-158,809) could improve insulin sensitivity and reduce both plasma leptin levels and expression of the ob gene in mesenteric adipose tissue of SHR fed a sucrose-rich diet. Furthermore, L-158,809 improved lipids metabolism, and led to a particularly pronounced increase in HDL-C. The increase in HDL-C by ARA has not previously been reported, although a significant reduction in serum triglyceride by ARA has been observed in studies carried out in patients (29).

As noted above, reports on the effects of ARA on insulin sensitivity are conflicting. ACE inhibitors may improve insulin resistance by reducing vasoconstriction and/or sympathetic overactivity (30, 31). ACE inhibitors stimulate the bradykinin system, which has been considered to be a mechanism contributing to improvement in insulin sensitivity (7). Kinins induce vasodilation, increase vascular permeability, and prevent vascular rarefaction (32), which may increase glucose and insulin delivery to tissue. However, since ARA does not stimulate the bradykinin system, this pathway cannot contribute to changes of insulin sensitivity by L-158,809 treatment.

It is also known that tissue renin activity or angiotensin II production can be elevated in SHR without alteration of systemic levels of these substances (33, 34). Such locally generated angiotensin II could induce peripheral vasoconstriction, and could contribute to blood pressure elevation and insulin resistance. Consequently, it is possible that ARA exerts its beneficial effects by the suppression of the vascular actions of angiotensin II via AT1 receptor antagonism (28). This would reduce elevated blood pressure levels and restore a normal flow to the skeletal muscle, which would facilitate glucose uptake. Henriksen et al. (15) have reported that AT1 receptor antagonism improved insulin resistance due to the effect on type 1 skeletal-muscle glucose uptake associated with an increase in glucose transporter-4 (GLUT-4) protein expression. Giacchetti et al. (35) have demonstrated that AT1 receptor mRNA levels in adipose tissue were increased in rats fed a fructose-enriched diet. They have suggested a possible link between an increase in the number of AT1 receptors and reduced sensitivity to insulin. Although we have not studied AT1 receptor mRNA levels, a similar phenomenon could be hypothesized to occur in rats fed a sucrose-enriched diet.

The blockade of AT1 receptors by ARA increases angiotensin II by a feedback mechanism, which results in overstimulation of AT2 receptors (36). It remains to be studied whether the stimulation of AT2 receptors by ARA is involved in the improvement of insulin sensitivity observed in the present study.

Adipose tissue weight was increased in the SHR fed a sucrose-rich diet, especially in mesenteric adipose tissue. Adipose tissue is known to be an important source of leptin and angiotensinogen. It is also known that plasma leptin levels correlate with blood pressure (37), and that visceral white adipose tissue contains the components of the rennin-angiotensin system (38) that gives rise to angiotensin II from angiotensinogen. These facts suggest that adipose tissue may play a pivotal role in blood pressure control. ARA treatment of the SHR fed a sucrose-rich diet reduced adipose tissue weight. Angiotensin II inhibits adipogenic differentiation of human adipocytes via the AT1 receptor (39), and the expression of angiotensin II-forming enzymes in adipose tissue is inversely correlated with insulin sensitivity (40). Since rennin-angiotensin system blockade by ARA and ACE inhibitors promotes the differentiation of adipocytes (39), the reduction of adipose tissue weight by ARA may be attributed, at least in part, to the function of AT1 receptors. Because of the strong correlation between HOMA-IR and adipose tissue weight observed in our study, the improvement of insulin resistance by ARA may be due to the reduction of adipose tissue weight.

As with ARA and ACE inhibitors, calcium channel blockers and α-blockers have been reported to improve insulin sensitivity (5). However, the improvements of insulin sensitivity by these agents are not superior to that by ARA or ACE inhibitors. Moreover, some antihypertensive drugs, such as thiazide diuretics and β-adrenergic blockers, have been reported to impair insulin sensitivity (5, 6). Thus, the improvement of insulin sensitivity by ARA or ACE inhibitors may be attributed to mechanisms other than antihypertensive effects.

In the present study, ARA decreased plasma leptin and expression of ob gene in adipose tissue. There have not been any reports on the relationship between ARA and plasma leptin levels. Both the reduction of adipose tissue weight and the decreased expression of ob gene lead to the reduction of plasma leptin. The mechanism responsible for the reduced expression of ob gene observed in the present study remains to be elucidated.

Plasma leptin has been reported to correlate with HOMA-IR (19, 41) and leptin has been shown to improve insulin resistance (42). However, other studies have reported that there was no relationship between leptin and HOMA-IR (43). We found that plasma leptin was strongly correlated to the HOMA-IR index in both SHR and WKY. Thus, the reduction of leptin level may be related to the improvement of insulin resistance by ARA. Further studies will be needed to elucidate the relationship between leptin and insulin sensitivity.

In conclusion, we found that a sucrose-enriched diet induced insulin resistance in genetically hypertensive rats, and
that ARA improved insulin resistance, and reduced the levels of plasma leptin and leptin mRNA in adipose tissue. The improvement of insulin resistance by ARA may be attributed, at least in part, to the reduction of adipose tissue weight. Further studies will be needed to elucidate the mechanism of the reduction of leptin mRNA in adipose tissue by ARA. ARA also improved lipid metabolism, and induced a particularly pronounced increase in serum HDL-C. These results suggest that ARA is useful in the treatment of patients with hypertension and concomitant insulin resistance syndrome.

References

29. Lerch M, Teuscher AU, Beissner P, Schneider M, Shaw SG, Weidmann P: Effects of angiotensin II-receptor block-


