The Renin-Angiotensin and Adrenergic Nervous System in Cardiac Hypertrophy in Fructose-Fed Rats

Kei Kamide, Hiromi Rakugi, Jitsuo Higaki, Atsunori Okamura, Michiko Nagai, Kouichi Moriguchi, Mitsuru Ohishi, Noriyuki Satoh, Michael L. Tuck, and Toshio Ogihara

Background: Hyperinsulinemia and insulin resistance are associated with left ventricular hypertrophy (LVH) and cardiovascular complications in hypertensive subjects. The aim of this study was to explore the mechanisms for LVH including activation of the renin-angiotensin system (RAS) and the sympathetic nervous system and their activation by insulin using a rat model of hyperinsulinemia and insulin resistance.

Methods: Male Sprague-Dawley rats were fed a high-fructose or control diet. The fructose-fed rats (FFR) were divided into four subgroups that were administered either vehicle or the following antihypertensive drugs (n=6–8) for 4 weeks: 1) olmesartan, an angiotensin II type 1 (AT₁) receptor antagonist; 2) bunazosin, an α₁-receptor blocker; and 3) hydralazine, a direct vasodilator.

Results: Fructose feeding induced significant increases in mean systolic blood pressure (BP) levels at 4 weeks (control, 117 ± fructose, 131 mm Hg), left ventricular weight, and the sum of the insulin level in response to a glucose tolerance test (2 g/kg). Fructose feeding also increased urinary excretion of epinephrine and norepinephrine, the density of cardiac α₁-adrenergic receptors, and the content of angiotensin II in the left ventricle. All antihypertensive drugs decreased systolic BP, but only the AT₁ receptor antagonist attenuated the development of LVH in FFR. The AT₁ receptor antagonist did not affect glucose-mediated insulin responses, but did suppress urinary catecholamine excretion and cardiac α₁-adrenergic receptor density.

Conclusions: Left ventricular hypertrophy in FFR may be less dependent on systemic elevations of BP and more dependent on the RAS and the sympathetic nervous system. Use of an AT₁ receptor antagonist might be the most beneficial way to prevent progression of LVH through direct effects on tissue RAS and the sympathetic nervous system in FFR. As these changes occur in a rat model with hyperinsulinemia, insulin may have a role in promoting LVH by activating the local RAS and sympathetic nervous system activity. Am J Hypertens 2002;15:66–71 © 2002 American Journal of Hypertension, Ltd.

Key Words: Hyperinsulinemia, fructose feeding, left ventricular hypertrophy, angiotensin II, sympathetic nervous system.
and their inhibition by antihypertensive drugs in the development of fructose diet–induced LVH.

Methods
Animal and Experimental Protocol
Male Sprague-Dawley (SD) rats weighing 160 to 180 g (6 weeks old, Nihon SLC Co., Hamamatsu, Japan) were housed in metabolic cages and given standard rat chow containing 24.6 g protein, 5.6 g fat, 0.26 g sodium, and 0.85 g potassium per 100 g chow (Oriental Yeast Co., Osaka, Japan) ad libitum. After 3 days, rats were divided into two groups according to diet. Control diet was continued for an additional 4 weeks in the normal chow fed rats (NFR), and a high-fructose diet containing 66% fructose was given to the FFR as previously described.8,10 The content of minerals, protein, fat, and vitamins was matched in the control and high-fructose diets. In addition, both groups were given vehicle or three different antihypertensive drugs: 1) olmesartan,11 an angiotensin II type 1 (AT1) receptor antagonist; 2) bunazosin,12,13 an α1-receptor blocker; or 3) hydralazine, a direct vasodilator by gastric gavages daily for 4 weeks from the beginning of the special diet. Six rats in each group in NFR and eight rats for each group in FFR were used.

Body Weight and Blood Pressure Measurement
Body weight was measured before and every week after starting the special feedings. Intake per day was measured in every group. The systolic blood pressure (BP) and pulse rate were measured by the tail-cuff method with the use of a programmed electrophysgmomanometer (model PS-100; Bio-Research Center Co., Tokyo, Japan) before and 2 and 4 weeks after starting the special feedings. The rats were prewarmed at 40°C for 10 min. The mean of three to four consecutive readings was used as the reported value of the systolic BP for each rat.

Urinary Collection
Four weeks after beginning the diet, urine was collected in 1 mL of 6N hydrochloric acid for 24 h for measurement of catecholamine excretion. Epinephrine and norepinephrine in the urine were measured by high performance liquid chromatography.14

Oral Glucose Tolerance Test
The oral glucose tolerance test (OGTT) was performed measuring plasma glucose and insulin levels at the end of the study period in each drug group both in NFR and FFR. The rats were fasted from evening of the day before, and blood samples were obtained by jugular vein puncture. A dose of 2 g/kg (body weight) glucose solution was given by gastric gavages. Blood samples were obtained from a jugular vein puncture at pre- and 30, 60, and 120 min post–glucose intake. Plasma glucose level was measured by the glucose oxidase reaction (Glucoboy; Eiken Chemical Co., Nagoya, Japan) and plasma insulin level was measured by an enzyme immunoassay kit (Morinaga Biochemistry Laboratory Co., Yokohama, Japan), using rat insulin as a standard.

Blood and Tissue Sample Measurements
Blood was obtained by decapitation on the morning after the rats had fasted 2 to 3 h at the end of both control and fructose feedings. After decapitation, the animals were perfused systematically with 0.9% physiologic saline to remove blood from the heart, and the heart was isolated and rinsed by Tris-HCL buffer (50 mmol/L, pH 7.2). Only the left ventricle (LV) was extracted from the whole heart, and the wet weight of the left ventricle was measured. The LV was divided into two parts for measurement of α1-adrenergic and β-adrenergic receptors by binding assays and to measure intraventricular angiotensin II (Ang II). Tissues were frozen in liquid nitrogen and stored at −80°C.

Table 1. Changes in systolic blood pressure during study

<table>
<thead>
<tr>
<th>Group</th>
<th>NFR (n = 6/group)</th>
<th>FFR (n = 8/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Week 2 Week 4 Week</td>
<td>0 Week 2 Week 4 Week</td>
</tr>
<tr>
<td>Vehicle</td>
<td>101.0 ± 2.0 107.0 ± 2.2 117.2 ± 1.6*</td>
<td>98.9 ± 1.9 114.1 ± 2.7† 131.0 ± 1.6*</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>97.8 ± 2.1 98.8 ± 2.0 105.5 ± 1.8†</td>
<td>99.1 ± 2.0 98.5 ± 1.3 107.1 ± 2.1†</td>
</tr>
<tr>
<td>Bunazosine</td>
<td>97.8 ± 3.1 102.5 ± 1.3 106.8 ± 1.9†</td>
<td>95.1 ± 2.1 105.0 ± 2.6 115.5 ± 3.5†</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>96.3 ± 2.5 106.2 ± 4.4 108.0 ± 3.4</td>
<td>99.5 ± 1.8 105.5 ± 2.4 112.5 ± 2.1†</td>
</tr>
</tbody>
</table>

NFR – normal chow-fed rats; FFR – fructose-fed rats.
Data are presented as means ± SEM (all mm Hg).
* P < .05 v 0 week; † P < .05 v vehicle.
was regarded as significant at least. Analysis of variance (ANOVA), followed by Fisher’s protected least significant difference test using StatView (Abacus Concepts Inc., Berkeley, CA). A value of $P < .05$ was regarded as significant.

### Results

#### Effects of Diet and Drugs on Blood Pressure and Pulse Rate

The changes in systolic BP and pulse rate in every treatment group during study periods are shown in Tables 1 and 2. The fructose diet raised systolic BP significantly compared with the control diet group at both 2 weeks and 4 weeks by ANOVA ($P < .0001$). The three antihypertensive agents attenuated the rise in systolic BP significantly in both NFR and FFR groups ($P < .0001$). The pulse rate was significantly higher in the FFR compared with the NFR by ANOVA ($P < .0001$). There were no significant differences in systolic BP and pulse rate between the drug-treated groups at either the 2 or 4 week periods in NFR and FFR.

#### Effects of Diet and Drugs on Plasma Glucose and Insulin During OGTT

The sum of plasma glucose during the OGTT was not changed by the fructose diet compared with NFR (NFR: 26.0 ± 3.8 v FFR: 28.2 ± 1.1 mmol/L, NS). The antihypertensive agents did not change the sum of plasma glucose during OGTT in both the NFR and FFR study groups. The sum of insulin levels during the OGTT was raised significantly by the fructose diet (NFR: 468.7 ± 67.7 v FFR: 508.5 ± 20.7 pmol/L, $P < .05$). None of the three drugs affected the high insulin levels, which were elevated by the fructose diet.

#### Effects of Diet and Drugs on LV Weight and Ventricular Angiotensin II

The LV weight/body weight was significantly increased by the fructose diet (Fig. 1). The AT$_1$-receptor antagonist olmesartan attenuated LV weight/body weight during fructose feeding ($P < .05$). Bunazosin did not affect LV

### Table 2. Changes in pulse rate during study

<table>
<thead>
<tr>
<th>Group</th>
<th>NFR (n = 6/Group)</th>
<th>FFR (n = 8/Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Week</td>
<td>2 Week</td>
</tr>
<tr>
<td>Vehicle</td>
<td>381.8 ± 7.5</td>
<td>348.5 ± 2.2</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>373.8 ± 12.3</td>
<td>323.8 ± 5.1</td>
</tr>
<tr>
<td>Bunazosine</td>
<td>398.5 ± 6.5</td>
<td>330.2 ± 6.4</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>370.2 ± 13.4</td>
<td>348.8 ± 9.9</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Data are presented as means ± SEM (all beats/min).

* $P < .05$ v vehicle; † $P < .05$ v hydralazine.
increased in the FFR (analysis of variance model, *P < .05) and was significantly lower during treatment with olmesartan (P < .05, Fig. 3A). β-Adrenergic receptor density in the ventricles was not changed during the fructose diet or by administration of any of the three antihypertensive drugs (Fig. 3B). There was no significant difference of Kd for α₁-adrenergic and β-adrenergic receptor binding in all study groups.

**Discussion**

Recently, several trials have shown that agents that block the RAS are more effective than antihypertensive drugs that act through other mechanisms in reducing the negative cardiovascular outcomes in patients with hypertension and diabetes. For examples, the Appropriate Blood Pressure Control in Diabetes study and the Fosinopril versus Amlodipine Cardiovascular Events Randomized Trial showed that angiotensin converting enzyme inhibitors (ACEI) had a more beneficial effect than Ca antagonists on cardiovascular disease mortality in hypertensive type 2 diabetes patients. These trials indicate that blocking of the RAS may have a favorable effect in preventing the cardiovascular complications that are often seen in states of insulin resistance and hyperinsulinemia. Thus, the activation of the tissue and circulating RAS and sympathetic nervous system may cause cardiovascular complications that are amplified in patients with insulin resistance and hyperinsulinemia.

Fructose feeding for 2 weeks induces LVH in SD rats that are associated with an activated systemic RAS, and an AT₁ receptor antagonist can prevent the progression of fructose-induced LVH independent of the effects on BP. However, the exact role of the cardiac tissue RAS and adrenergic nervous system and the effect of blockade of these systems in the LVH in the FFR has not been definitively assessed. The present study investigates cardiac tissue RAS and adrenergic nervous system, and their interaction in the progression of fructose-induced LVH.

It should be noted that Iyer et al have also demonstrated that the development of LVH in FFR is accompanied by differences in AT₁ receptor density in the ventricles and the aorta between NFR and FFR, and that AT₁ receptor density was significantly increased in the cardiac ventricles and decreased in the aorta in FFR. It is possible that these systems in the LVH in the FFR have not been definitively assessed. The present study investigates cardiac tissue RAS and adrenergic nervous system, and their interaction in the progression of fructose-induced LVH.

**Effects of Diet and Drugs on Urinary Catecholamine Excretion**

Urinary epinephrine levels compared to vehicle were significantly increased in the FFR. Only olmesartan suppressed urinary epinephrine and norepinephrine excretion in the FFR (P < .05) (Table 3).

**Effects of Diet and Drugs on α₁- and β-Adrenergic Receptor Density in Left Ventricular Tissue**

Cardiac α₁-adrenergic receptor density in the ventricles increased in the FFR (P < .05) and was significantly lowered during treatment with olmesartan (P < .05, Fig. 3A). β-Adrenergic receptor density in the ventricles was not changed during the fructose diet or by administration of any of the three antihypertensive drugs (Fig. 3B). There was no significant difference of Kd for α₁-adrenergic and β-adrenergic receptor binding in all study groups.

**Table 3.** Urinary catecholamine excretion

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine (ng/day)</th>
<th>Norepinephrine (ng/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFR</td>
<td>FFR</td>
</tr>
<tr>
<td></td>
<td>(n = 6/Group)</td>
<td>(n = 8/Group)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>18.8 ± 4.9</td>
<td>70.9 ± 9.8*</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>25.7 ± 5.2</td>
<td>23.9 ± 6.5†</td>
</tr>
<tr>
<td>Bunazosine</td>
<td>22.7 ± 5.5</td>
<td>67.0 ± 13.6*</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>21.7 ± 2.4</td>
<td>63.2 ± 14.2*</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.

Data are presented as means ± SEM.

* P < .05 v normal chow; † P < .05 v vehicle.

P values obtained by ANOVA.
that the hyperinsulinemic state in FFR may alter the RAS activity in cardiovascular tissue, but there is limited information on how insulin might affect the tissue RAS activity. Several potential mechanisms could mediate an effect of insulin on the tissue RAS, as insulin has been shown to induce tissue RAS activation directly \(^{21}\) and to cause increases in the AT\(_1\) receptor in cultured vascular cells. \(^{22}\) Cardiac tissue RAS activity may further induce LVH by various stimuli such as increased pressure loading and catecholamine release. \(^{23-25}\)

Insulin is well known to stimulate sympathetic nervous system activity \(^1\) and activated sympathetic nervous system activity has been shown to induce cardiac hypertrophy. \(^{25}\)

To clarify further the role of the RAS and the sympathetic nervous system in the mechanism of LVH seen in the FFR, we administered antihypertensive agents that have varying actions on the RAS and on the sympathetic nervous system. We selected the AT\(_1\) receptor antagonist olmesartan because it is known to have a more specific and long-lasting blockade of the AT\(_1\) receptor than does losartan. \(^{11}\) \(\alpha\_1\)-adrenergic receptor blockade with agents such as bunazosin is reported to improve insulin sensitivity \(^{12,13}\) and to reduce LVH in hypertensive subjects. \(^{26}\) The direct vasodilator hydralazine was also used, as it acts through systems independent of the RAS and the sympathetic nervous system.

Fructose diet increased LV weight in all drug-treated groups except for the olmesartan treated group, despite equal reductions in systolic BP. These findings suggest that fructose-induced cardiac hypertrophy is affected by factors independent of increasing systolic BP. The hydralazine group showed an even further increase in LV weight with FFR, suggesting that this agent might reflexly activate the sympathetic nervous system as documented by the high excreted level of norepinephrine with hydralazine. Olmesartan attenuated an increase in LV weight in the FFR, suggesting that RAS blockade is important in minimizing LVH in the FFR. In FFR, the LV Ang II content is increased significantly by fructose diet and plasma Ang II level are high, \(^9\) suggesting a specific need for agents that block RAS in this model. As FFR are hyperinsulinemic, it could be proposed that high level of insulin induced by fructose diet might directly activate cardiac tissue RAS and promote cardiac Ang II production, leading to LVH.

Urinary epinephrine excretion was significantly increased by the fructose diet. Moreover, urinary norepinephrine excretion was also increased by the fructose diet, although it was not statistically significant. Olmesartan completely blocked the rise in urinary catecholamines in response to fructose; however, bunazosin and hydralazine did not affect these responses. These findings suggest that the fructose diet activates the systemic adrenergic nervous system. Because olmesartan blocked urinary catecholamine excretion, it would appear that only an AT\(_1\) receptor antagonist compared with other agents is best to blunt adrenergic nervous system activation. In theory, an \(\alpha\_1\)-adrenergic receptor antagonist should block \(\alpha\)-adrenergic action and reduce BP in FFR, but it failed to attenuate the fructose diet-induced LVH. Therefore, blocking of \(\alpha\_1\)-adrenergic receptor might be not sufficient to attenuate LVH in the FFR. The fructose diet increased \(\alpha\_1\)-receptor but not \(\beta\)-receptor density in the LV, and this increases in \(\alpha\_1\)-receptor in LV was reversed by an AT\(_1\) receptor antagonist. These findings show that the fructose diet induces both an elevation in systemic catecholamine levels but also increases cardiac \(\alpha\_1\)-receptor density. Moreover, this increase in \(\alpha\_1\)-receptor density might be mediated through insulin-induced RAS activation. Matsui et al revealed a relationship between cardiac adrenergic receptors and the RAS in the LVH model of rats using aortic banding. \(^{17}\) They reported that pressure overload induced LVH of rats with cardiac \(\alpha\_1\)-receptor upregulation and ACEI blunted this response and, furthermore, that cardiac \(\beta\_1\) receptors were also not changed, yielding results similar to those in our present study. Thus, it appears that the fructose diet has an effect on cardiac adrenergic receptors similar to that of pressure overload. In this mechanism, elevated Ang II induced by fructose diet would be involved in this modulation of cardiac adrenergic receptors.

In summary, LVH in FFR may be less dependent on systemic elevation of BP and more dependent on the...
activation of cardiac tissue RAS and the adrenergic nervous system. Use of an AT$_1$ receptor antagonist might be the most beneficial to prevent progression of LVH through direct blocking effects on RAS and adrenergic nervous system. As these changes occur in a rat model with hyperinsulinemia, a high level of plasma insulin may have a role in promoting LVH and increased local RAS and adrenergic nervous system activity in patients with insulin resistance. The blockade of RAS such as an AT$_1$ receptor antagonist would be very useful for prevention and regression of LVH in patients with hypertension and insulin resistance.

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References


