Evidence Against a Role of Insulin in Hypertension in Spontaneously Hypertensive Rats

CS-045 Does Not Lower Blood Pressure Despite Improvement of Insulin Resistance

Shigehiro Katayama, Mari Abe, Hideyuki Kashiwabara, Itaru Kosegawa, Jun Ishii

Abstract

Hyperinsulinemia resulting from peripheral insulin resistance has been demonstrated both in spontaneously hypertensive rats (SHR) and in humans with essential hypertension. A new class of anti-diabetic drugs, thiazolidinediones, which can improve insulin resistance, may be able to lower not only blood glucose levels but also blood pressure. The present study was therefore designed to clarify the proportion of SHR that are insulin resistant by the euglycemic hyperinsulinemic clamp technique. In this sense, hypertension, NIDDM, and obesity share a common pathophysiological characteristic of insulin resistance. Hyperinsulinemia as a result of insulin resistance in peripheral tissue, mainly skeletal muscle, may enhance sodium reabsorption from the kidneys and/or increase sympathetic nervous activity, causing an increase in blood pressure.

Sulfonylurea, now used widely as a hypoglycemic agent for patients with NIDDM, appears to stimulate pancreatic insulin secretion. Thiazolidinediones are a new class of anti-diabetic compounds being developed for the treatment of NIDDM patients. These drugs have been reported to improve peripheral tissue insulin sensitivity and to lower the levels of plasma glucose and insulin in experimental diabetes models and NIDDM patients. If hyperinsulinemia causes high blood pressure in hypertensive rodent models as well as in patients with essential hypertension, such drugs may ameliorate not only the underlying metabolic derangements but also hypertension. In fact, drugs such as ciglitazone and CS-045 (troglitazone) have been reported to decrease blood pressure in the obese Zucker rat. The present study was therefore designed to test whether or not insulin resistance and hyperinsulinemia contribute to hypertension in spontaneously hypertensive rats (SHR, a model of human essential hypertension), which also have been reported to have glucose intolerance, hyperinsulinemia, and hence, insulin resistance.

Methods

Male SHR (n=67) and Wistar rats (n=5) aged 8 weeks obtained from Charles River Japan were housed with free access to food and tap water. Systolic blood pressure was measured by a tail-cuff method. Background data are shown in Table 1. Blood samples were obtained from a jugular vein before and 60 and 120 minutes after glucose loading (2 g/kg IP) to determine plasma glucose and insulin concentrations after an overnight fast.

In a separate group (n=13) of male SHR aged 10 weeks, CS-045 (Sankyo Co, Ltd) was administered orally by gavage at 70 mg/kg for 2 weeks. Controls (n=12) received only 2.5 mL/kg vehicle (polyethylene glycol to glycerol to water, 194:12:20 wt/wt). Before and after the 2 weeks of treatment, systolic blood pressure and levels of plasma glucose and insulin before and after glucose loading were determined (see Table 2). In addition, steady-state plasma glucose (SSPG) levels were determined according to the method of Mondon and Reaven. Briefly, rats anesthetized by pentobarbital received a continuous infusion (1 mL/h) of epinephrine (0.08 μg/kg per minute), propranolol (1.7 μg/kg per minute), glucose (8 mg/kg per minute), and insulin (1.25 mU/kg per minute) though a
TABLE 1. Body Weight, Systolic Blood Pressure, and Heart Rate in Wistar Rats and SHR With or Without Glucose Intolerance

<table>
<thead>
<tr>
<th></th>
<th>Normal Glucose Tolerance</th>
<th>Impaired Glucose Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>BW, g</td>
<td>200±1.9</td>
<td>184±3.0*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>111±4.7*</td>
<td>169±2.2</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>360±2.6*</td>
<td>412±7.3</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; BW, body weight; SBP, systolic blood pressure; HR, heart rate. Values are mean±SEM.

*P<.01 vs the other two groups by ANOVA followed by Duncan's multiple range test.

Results

Among the 67 SHR, 50 (74.6%) demonstrated a plasma glucose response to intraperitoneal glucose loading higher than the mean+2 SD for age-matched Wistar rats (5.66 mmol/L at 60 minutes and 7.97 mmol/L at 120 minutes). As shown in Fig 1, SHR with higher plasma glucose levels demonstrated significantly higher plasma insulin levels. Regression analysis revealed no significant correlation between systolic blood pressure and fasting or postloading plasma glucose and insulin levels. Systolic blood pressure was not correlated with the area under the curve for glucose and insulin levels as illustrated in Fig 2.

Treatment with CS-045 had no effect on body weight gain (296±2.7 versus 290.3±3.0 g in controls). Fig 3 illustrates plasma glucose and insulin levels before and after glucose loading. In the group treated with CS-045, the plasma glucose and insulin levels were significantly lower than in controls. As a result, the area under the curve for glucose and insulin levels was significantly lower in the treated group than in the controls.

Discussion

The present study clearly demonstrated glucose intolerance associated with hyperinsulinemia, a characteristic feature of insulin resistance, in SHR in comparison to control Wistar rats. This is consistent with the previous studies by Yamori et al12 and Mondon and...
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Reaven. However, it should be noted that there is a great heterogeneity of glucose metabolism in SHR; that is, not all SHR are glucose intolerant and hyperinsulinemic, thus calling into question whether or not hyper-insulinemia is in fact involved in the pathogenesis of hypertension in SHR.

To clarify this point, we attempted to improve insulin resistance by CS-045 administration and to observe any change in blood pressure. As evidenced by a lower response and area under the curve of plasma glucose and insulin levels after glucose loading, CS-045 treatment improved insulin resistance. This was supported by the observation that SSPG tended to decrease and was significantly diminished in the group with impaired glucose tolerance before treatment. However, systolic blood pressure was not lowered by the treatment with CS-045. This is in contrast to previous reports in which thiazolidinediones such as ciglitazone and CS-045 lowered blood pressure in male obese Zucker rats. In both studies, the drugs were administered for 4 to 8 weeks. The doses of CS-045 administered were about 15 and 67 mg/kg per day, the latter tending to lower blood pressure as early as 1 week after the start of treatment and producing a significant decrease in blood pressure after 6 weeks that was associated with marked natriuresis despite a lack of any difference in body weight. In the present study, the dose used was almost the same as that in the former study, although the 2-week treatment period was shorter than the 8-week period in the former study. However, insulin resistance appeared to be improved by CS-045 treatment. Thus, the discrepancy in the blood pressure response between our study and the previous one may be attributed to the difference in the strains that were used. The obese Zucker fatty rat (fa/fa) is a well-documented animal model of insulin resistance characterized by hyperinsulinemia, obesity, hyperlipidemia, and glucose intolerance in association with mild hypertension. The peripheral insulin resistance observed in these animals results from an impairment of insulin-stimulated glucose uptake into skeletal muscle, the major site of insulin-mediated glucose disposal. Recent studies have demonstrated that the major defect may be a failure of translocation of glucose transporter (GLUT) 4 to the plasma membrane, although gene expression of GLUT 4 and its synthesis in adipocytes may be augmented. The other case in which insulin may be involved in the pathogenesis of hypertension may be that in fructose-fed rats, in which somatostatin inhibition of insulin secretion has been reported to decrease blood pressure. This is the case in humans if they are obese and hyperinsulinemic. Very recently, CS-045 administration to fructose-fed rats for 3 weeks was reported to decrease fructose-induced hypertension from 130±5 to 104±6 mm Hg. In SHR, however, hyperinsulinemia and/or insulin resistance may not be essential for maintenance of high blood pressure, as demonstrated in the present study. Although plasma insulin levels at fasting or after glucose loading in SHR treated with CS-045 was significantly lower than control levels in normal Wistar rats, glucose tolerance or SSPG was not completely normalized, indicating the possibility that there may be a threshold effect of insulin resistance on the regulation in blood pressure. SHR are very homogeneous in terms of blood pressure. However, they are very often heterogeneous with regard to glucose metabolism even if obtained from a single supplier. Furthermore, wide differences from one

Fig 3. Line plots show plasma glucose (left) and insulin (right) levels in response to glucose loading (2 g/kg IP) in spontaneously hypertensive rats treated with CS-045 at 70 mg/kg per day for 2 weeks and in controls. Treatment effect was significant for plasma glucose (P<.05) and insulin levels (P<.01) by repeated-measures ANOVA. Time after glucose loading had a significant effect on plasma glucose and insulin levels (P<.0001). Treatment-by-time interaction was not significant.

Fig 4. A, Steady-state plasma glucose (SSPG) level (left) and systolic blood pressure (SBP) (right) in control and CS045-treated spontaneously hypertensive rats (SHR). B, Same parameters in six SHR from each of the control and treated groups, which had plasma glucose levels higher than the mean ± 2 SD for normal Wistar rats.
colony to another may be evident. Therefore, care must be exercised in using SHR for research in the field of hypertension and insulin resistance.

References
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