LONG-TERM EFFECTS OF HIGH CALORIE SUCROSE-ENRICHED DIET AND STREPTOZOTOCIN-INDUCED DIABETES ON INSULIN RESISTANCE IN SPONTANEOUSLY HYPERTENSIVE RATS§

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SUMMARY

1. The effects of two experimental manipulations on insulin resistance were compared in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Rats were fed a high calorie sucrose-enriched diet (high calorie diet) or were made diabetic by the injection of streptozotocin (STZ).

2. After treatment with the high calorie diet for 8 weeks, blood pressure increased in SHR, but not in WKY rats. In contrast, STZ treatment decreased blood pressure in SHR, but increased it in WKY rats.

3. Plasma glucose levels during oral glucose loading were higher in SHR than in WKY rats. Glucose tolerance was impaired to a greater extent by both the high calorie diet and STZ in SHR than in WKY rats. Hyperinsulinaemia induced by the high calorie diet was severe in SHR compared with WKY rats.

4. Abnormalities in lipid metabolism induced by a high calorie diet or STZ-induced diabetes were more marked in SHR than in WKY rats.

5. Steady-state plasma glucose levels in the insulin suppression test were higher in SHR than in WKY rats, both of which were treated by either the high calorie diet or STZ. These findings indicate that insulin-stimulated glucose uptake by high calorie diet or STZ-induced diabetes was impaired to a greater extent in SHR than in WKY rats.

6. It is concluded, therefore, that SHR fed on high calorie diet or SHR with STZ-induced diabetes are suitable models to study the effects of antihypertensive treatment on glucose tolerance, insulin resistance or lipid metabolism as well as blood pressure.

Key words: high calorie sucrose-enriched diet, hypertension, insulin resistance, lipid metabolism, spontaneously hypertensive rats, streptozotocin-induced diabetes mellitus, Wistar-Kyoto rats.

INTRODUCTION

Essential hypertension in humans is known to be associated with insulin resistance. However, the exact relationship between hypertension and insulin resistance remains to be elucidated. Spontaneously hypertensive rats (SHR) are generally used as the best available model of genetic hypertension, because they have numerous similarities to the human counterpart of essential hypertension. We have shown that SHR have impaired glucose tolerance and are insulin resistant. However, the degree of insulin resistance was so mild that we could not demonstrate the beneficial effects of antihypertensive treatment on glucose intolerance or insulin resistance in SHR.

Insulin resistance can be seen in diabetes, obesity or simple carbohydrate feeding. The present study was initiated to evaluate the suitability and to define the effect of two experimental methods for inducing insulin resistance in SHR and normotensive Wistar-Kyoto (WKY) rats. One method used was the feeding of a high calorie sucrose-enriched diet (high calorie diet) to rats and the other was streptozotocin (STZ)-induced diabetes mellitus.

METHODS

Animals

Male normotensive WKY rats (280–300 g) and SHR (275–300 g) were obtained from Izumo colonies (Shimane Institute of Health Science, Izumo, Japan). Animals at 12–14 weeks of age were divided into three groups. The first group (n = 18) was fed conventional chow (Funabashi SP; Funabashi Farm, Chiba, Japan), the second group (n = 12) was fed a high calorie diet (Funabashi Farm) and the third group (n = 12) was made diabetic by the i.v. injection of STZ (Sigma Chemical Co., St Louis, MO, USA) and was fed conventional chow. Considering the different diabetogenic effect of STZ in WKY rats and SHR, 50 and 35 mg/kg STZ diluted in 0.01 mol/L citrate buffer (pH 4.5) was injected into WKY rats and SHR, respectively. Blood was obtained from the tail vein 2 days later and analysed for blood glucose (BM TEST Blood Sugar 20–800R and Refloux II; Boehringer-Mannheim, Mannheim, Germany). Mean (± s.d.) blood glucose values were found to be 15.4 ± 0.4 and 15.1 ± 0.6 mmol/L in WKY rats and SHR, respectively, and similar levels were achieved in both strains.

The conventional normal diet contained 25% protein, 5% fat and 70% vegetable carbohydrate as per cent weight. The high calorie diet contained 25% protein, 25% fat and 50% sucrose, the total calories of which were 1.3-times higher than those of the normal diet. The
conventional diet contained 4.0 g/kg sodium and 7.5 g/kg potassium; in contrast, the high calorie diet contained 1.9 g/kg sodium and 3.3 g/kg potassium. Animals had free access to tap water and chow and were exposed to a 12 h light–dark cycle (lights on 0700–1900).

Bodyweight (BW), blood pressure (BP) and non-fasting blood glucose were measured in animals monthly. Blood pressure was indirectly measured without anaesthesia by a tail-cuff method (UR-1000; Ueda Electric Works Co. Ltd, Tokyo, Japan). The tail-cuff method was performed as follows: initially a rat was warmed in a hot box at 38°C for 10 min and then placed in a restraining apparatus that was also kept at 38°C. The tail was inserted through the cuff, which contained a photoelectric pulse detector, and systolic BP was recorded when the first oscillation appeared during the gradual reduction of the cuff pressure.

**Insulin secretion**

After 8 weeks, an oral glucose tolerance test (OGTT) was performed on WKY rats and SHR. On the experimental day after a 24 h fast, glucose solution (2 g/kg) was administered by stomach gavage through a metal catheter attached to a syringe and blood samples were collected into heparinized haemocrit tubes after cutting the tip of the tail without anaesthesia. Plasma glucose and immunoreactive insulin (IRI) levels were determined at fasting and 30, 60 and 120 min after glucose administration. Plasma glucose was determined by a glucose-oxidase method. Plasma IRI was determined by a double-antibody radio-immunoassay.

Plasma total cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride and non-esterified fatty acid (NEFA) levels were analysed in fasting samples. Plasma total cholesterol and triglyceride were measured by an enzymatic method. High density lipoprotein-cholesterol was measured after precipitating other lipoproteins. Plasma NEFA concentrations were measured by a microfluorometric method.

**Insulin sensitivity**

Insulin sensitivity was evaluated using the insulin suppression test, which was performed 2 weeks after the OGTT. The procedure started with the withdrawal of food at 0800 h on the morning of the experiment. All infusions started at midday, under sodium thiamylal anaesthesia (6.0 mg/100 g BW, i.p.). Rats were continuously infused with adrenaline (0.08 mg/kg per min), propranolol (1.7 μg/kg per min), glucose (8 mg/kg per min) and porcine insulin (2.5 mU/kg per min) for 160 min. Blood samples were obtained from the tip of the tail at 0, 60, 120, 130, 140, 150 and 160 min and plasma glucose and insulin levels were determined. Steady state plasma insulin (SSPI) and steady state plasma glucose (SSPG) concentrations were calculated from the values determined between 130 and 160 min and studies in which the coefficient of variation exceeded 10% were excluded. Insulin radioimmunoassays used rat insulin as the standard when determining insulin concentration in the basal state, while porcine insulin was used for the measurement of insulin levels during infusion. Hypothermia was prevented by the use of a heat lamp.

**Statistical analysis**

All values are expressed as the mean ± s.d. Statistical differences were evaluated by one-way analysis of variance (ANOVA) and Fisher's multiple comparison test by using STATVIEW 512+ software (Brainpower, Calabasas, CA, USA) on an Apple Macintosh SE computer (Cupertino, CA, USA).

**RESULTS**

Figure 1 shows changes in BW during the experiment. After 8 weeks, BW was significantly higher ($P<0.001$) in the high calorie diet groups (379 ± 4 and 382 ± 5 g in WKY rats and SHR, respectively) and lower ($P<0.001$) in the diabetic groups (296 ± 10 and 304 ± 6 g in WKY rats and SHR, respectively) than in the normal diet groups (342 ± 4 and 340 ± 4 g in WKY rats and SHR, respectively). In the diabetic groups, BW was significantly ($P<0.05$) lower in SHR than in WKY rats.

Figure 2 shows changes in BP in WKY rats and SHR during the experiment. Blood pressure was consistently higher in SHR than in WKY rats during the experiment. Blood pressure was significantly higher in high calorie diet-fed SHR than in normal diet-fed SHR (190 ± 6 vs 184 ± 4 mmHg, respectively; $P<0.05$) after 8 weeks, whereas BP was not affected in WKY rats by the high calorie diet (126 ± 5 vs 125 ± 5 mmHg for the high calorie vs normal diet, respectively). In contrast, STZ treatment significantly ($P<0.001$) suppressed BP in SHR (172 ± 6 mmHg), but elevated ($P<0.001$) BP in WKY rats (140 ± 4 mmHg).

Figure 3 shows changes in non-fasting blood glucose in WKY rats and SHR during the experiment. Blood glucose was significantly ($P<0.001$) greater in the diabetic groups (15.3 ± 0.8 and 16.4 ± 0.7 mmol/L in WKY rats and SHR, respectively) and the high calorie diet groups (8.0 ± 0.3 and 8.4 ± 0.3 mmol/L in WKY rats and SHR, respectively) than in the normal diet.
groups (6.8±0.3 and 6.9±0.2 mmol/L in WKY rats and SHR, respectively). Furthermore, blood glucose was significantly (P<0.001) higher in the diabetic groups than in the high calorie diet groups. In the diabetic groups, blood glucose was significantly (P<0.05) higher in SHR than in WKY rats after 8 weeks.

The effects of high calorie diet or STZ-induced diabetes on plasma glucose and insulin responses to oral glucose loading are shown in Fig. 4. In normal diet groups, plasma glucose levels were significantly (P<0.01) higher in SHR than in WKY rats during the 120 min period following oral glucose administration, whereas plasma insulin levels were not so different.

High caloric diet feeding and STZ-induced diabetes mellitus resulted in a greater impairment of glucose tolerance in SHR than in WKY rats. Plasma insulin responses to glucose by high caloric diet feeding were higher in SHR than in WKY rats. In contrast, STZ blunted insulin responses to glucose in both WKY rats and SHR.

Table 1 shows the plasma lipid profiles in WKY rats and SHR after 8 weeks. Plasma total cholesterol and HDL-cholesterol levels were significantly higher in SHR than in WKY rats. Plasma triglyceride and NEFA levels were significantly higher in diabetic groups compared to normal diet groups. In SHR, triglyceride levels were significantly higher than in WKY rats, while NEFA levels were significantly higher in WKY rats compared to SHR.

Table 1. Mean (± s.d.) plasma total cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride and non-esterified fatty acids (NEFA) in six groups of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>No. rats</th>
<th>Total cholesterol (mmol/L)</th>
<th>HDL-Cholesterol (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
<th>NEFA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>WKY</td>
<td>18</td>
<td>2.38±0.07</td>
<td>1.76±0.10</td>
<td>0.85±0.05</td>
<td>0.73±0.05</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>18</td>
<td>1.95±0.08*</td>
<td>1.48±0.12*</td>
<td>1.29±0.07**</td>
<td>0.78±0.05</td>
</tr>
<tr>
<td>High calorie diet</td>
<td>WKY</td>
<td>12</td>
<td>2.30±0.09</td>
<td>1.52±0.08†</td>
<td>0.97±0.07†</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>12</td>
<td>1.88±0.10*</td>
<td>1.30±0.10†</td>
<td>2.00±0.15**</td>
<td>0.81±0.06</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>WKY</td>
<td>12</td>
<td>2.24±0.10</td>
<td>1.44±0.12†</td>
<td>1.07±0.12†</td>
<td>1.07±0.09‡</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>12</td>
<td>1.80±0.09*</td>
<td>1.21±0.09*</td>
<td>2.12±0.19**</td>
<td>1.24±0.10‡</td>
</tr>
</tbody>
</table>

*P<0.01, **P<0.001 vs corresponding WKY rats; †P<0.05, ‡P<0.01, ‡‡P<0.001 vs corresponding normal diet group.
terol concentrations were consistently lower in SHR than in WKY rats, whereas plasma triglyceride concentrations were significantly higher in SHR than in WKY rats. Plasma HDL-cholesterol levels were significantly decreased, whereas plasma triglyceride was significantly increased in both the high calorie diet and diabetic groups than in normal diet groups. Plasma NEFA levels were significantly higher in diabetic groups than in normal diet and high calorie diet groups in both strains. Abnormalities in lipid metabolism induced by a high calorie diet or STZ-induced diabetes were more prominent in SHR than in WKY rats.

Figure 5 shows SSPG and SSPI levels in the insulin suppression test. As shown in Fig. 5b, there was no difference in SSPI levels among all six subgroups. These results indicate that the glucose disposal rates reflect the ability of insulin to stimulate glucose utilization. In normal diet animals, SSPG was significantly higher in SHR than in WKY rats (12.5 ± 1.0 vs 11.7 ± 0.6 mmol/L, respectively; \( P < 0.05 \)). SSPG levels were significantly \( (P < 0.01) \) increased by either high calorie diet feeding or STZ in both WKY and SHR. SSPG levels in SHR were significantly \( (P < 0.001) \) higher than those in corresponding WKY rats (18.6 ± 1.2 vs 15.5 ± 0.7 mmol/L in high calorie diet groups and 26.1 ± 0.9 vs 20.3 ± 0.5 mmol/L in diabetic groups, respectively).

**DISCUSSION**

In the present study, a sucrose-enriched high calorie diet resulted in an increase in BP in SHR, while the same diet failed to elevate BP in WKY rats. Although WKY rats lived longer and became heavier and more insulin resistant and hyperinsulinaemic with age, they did not develop high BP. It has been previously reported that BP is not invariably increased by sucrose feeding in the rat. Different BP responses to sucrose may reflect differences in sodium intake, strain and model of hypertension. Johnson et al. reported that sucrose (7% sucrose in drinking solution)-induced elevations of BP could not be achieved in the normotensive Sprague-Dawley rat maintained on a low salt (0.45% NaCl) diet, suggesting that the presence of NaCl was an absolute requirement for BP to be increased by sucrose in this strain. Kotchen et al. also reported that sucrose-drinking (7% sucrose) did not increase BP in one-kidney, one-clip hypertensive rats or Dahl salt-sensitive rats on either a 0.45 or 3% NaCl intake, in contrast to the Dahl salt-resistant rats, in which BP was increased by the high sucrose intake. These results indicate that the underlying mechanisms of 'sucrose sensitivity', which was originally mentioned by Kotchen et al., are already present in these models and, consequently, sucrose produced no additional effect on BP.

In contrast, STZ treatment suppressed BP in SHR, but elevated it in WKY rats. Several researchers have reported incompatible results on BP response to STZ. It was previously reported that STZ treatment induced not only diabetes but also hypertension in rats, whereas STZ had a hypotensive effect in SHR. Somani et al. reported that STZ treatment lowered BP in SHR and raised it in WKY rats. Fluckiger et al. reported that STZ treatment attenuated the development of hypertension in SHR and had a mild hypotensive effect in WKY rats. The mechanisms of different BP responses to STZ treatment between WKY rats and SHR still remain obscure.

High calorie diet feeding and STZ-induced diabetes worsened impaired glucose tolerance in SHR compared with WKY rats. Plasma insulin responses to glucose by high calorie diet feeding were higher in SHR than in WKY rats. These findings indicate that WKY rats are more sensitive to insulin than SHR.
Abnormalities in lipid metabolism induced by high calorie diet feeding or STZ-induced diabetes were more prominent in SHR than in WKY rats. Similarly, the institution of fructose-glucose and triglyceride concentrations in SHR than in WKY rats. SPI-glucose intolerance, hyperinsulinaemia, and hypertriglyceridaemia, hypertension and obesity, will be suitable animal models for the syndrome of insulin resistance, the deadly quartet or syndrome X. STZ-induced diabetic SHR will also be a suitable model for non-obese diabetic hypertension. These animal models will be useful tools to study the effect of antihypertensive treatment on insulin resistance in each condition.

In summary, plasma glucose levels during oral glucose loading were higher in SHR than in WKY rats. Glucose intolerance induced by either a high calorie diet or STZ was more severe in SHR than in WKY rats. Hyperinsulinaemia induced by a high calorie diet was more prominent in SHR than in WKY rats. SSPG levels were consistently higher in SHR than in WKY rats, both strains being treated by either a high calorie diet or STZ. The results of our study show that SHR are insulin resistant and more sensitive to the induction of insulin resistance than WKY rats. Spontaneously hypertensive rats with enhanced insulin resistance by high calorie diet or STZ treatment are good models to study the effects of antihypertensive agents on glucose tolerance, insulin resistance or lipid metabolism as well as BP.

REFERENCES


