Obesity Induced by Neonatal Monosodium Glutamate Treatment in Spontaneously Hypertensive Rats: An Animal Model of Multiple Risk Factors

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The present study was designed to develop an animal model of multiple risk factors, including obesity, hypertension, non-insulin-dependent diabetes mellitus, and hyperlipidemia. Hypothalamic obesity was induced by neonatal monosodium glutamate (MSG) treatment in spontaneously hypertensive rats (SHR). Female newborn SHR were treated intraperitoneally with 2 or 4 mg/kg body weight of MSG for 5 days. Obesity developed in SHR treated with 4 mg/kg of MSG but not in SHR treated with 2 mg/kg of MSG. Obese SHR had impaired glucose tolerance, hyperinsulinemia, and hypertriglyceridemia. However, the severity of hypertension was attenuated in obese SHR as compared with control SHR. The degree of obesity was closely related to the metabolic abnormalities, but inversely correlated with the blood pressure level. Macrovascular changes were investigated in obese SHR at 14 months of age. Intimal thickening was accelerated in the carotid artery of obese SHR as compared with that of nonobese SHR. Aortic contents of DNA and total cholesterol were significantly increased in obese SHR. SHR associated with MSG-induced obesity showed major manifestations of metabolic syndrome X. This animal model may be useful to study the clustering of risk factors for the development of macrovascular diseases. (Hypertens Res 1998; 21: 1-6)

Key Words: insulin resistance, diabetes mellitus, syndrome X, atherosclerosis, animal model

Obesity, hypertension, non-insulin-dependent diabetes mellitus (NIDDM), and hyperlipidemia frequently exist in the same individual, and clustering of these closely related risk factors markedly increases the incidence and prevalence of atherosclerotic diseases (1-7). Insulin resistance and consequent hyperinsulinemia or visceral fat accumulation may play a pathogenetic role in the clustering of risk factors, i.e., metabolic syndrome X. However, the relationship among these risk factors, especially that between hypertension and other metabolic diseases, is controversial (5-8). Our understanding of metabolic syndrome X may be enhanced by the development of an animal model of this syndrome. We therefore designed the present study to induce experimental obesity in spontaneously hypertensive rats (SHR) by treatment with small or large doses of monosodium glutamate (MSG). MSG induces hypothalamic damage when given during neonatally, leading to stunted growth and obesity (9). We studied the effects of MSG-induced obesity on hypertension, glycemia, lipemia, and the development of macrovascular changes in the obese SHR.

Materials and Methods

SHR were from our inbred colony and were bred in specific-pathogen-free conditions at Kyushu University Animal Center, where both temperature and lighting (on from 8:00 am to 8:00 pm) were controlled. They had free access to tap water and a standard chow diet, which contained 51.6% carbohydrate, 24.8% protein, 7.0% minerals, 4.4% fat, and 3.5% cellulose (Clea Japan Inc., Tokyo, Japan). Animals were cared for as directed by guidelines of Kyushu University. Female newborn SHR were treated intraperitoneally with 2 or 4 mg/kg body weight of MSG at the ages of 1, 2, 3, 4, and 5 d (n = 11 or 10, respectively), or 4 mg/kg body weight of MSG at the ages of 1, 3, 5, 7, and 9 d (n = 8). Control rats (n = 6) received 10% NaCl at the ages of 1, 2, 3, 4, and 5 d. The injection volume was 8 μl/g body weight. The pups were left with their own mothers and were weaned at 4 wk of age.

Obesity was evaluated on the basis of the Lee index, calculated as 3√body weight/nasoanal length. Food intake was measured by using metabolic cages.
for 24 h. Blood was collected from a tail vein after an overnight fast at 6 months of age. Serum glucose was measured with a Beckman glucose analyzer 2 (Beckman Instruments, Fullerton, Calif, USA). Serum immunoreactive insulin (IRI) was measured by radioimmunooassay (Pharmacia Co., Uppsala, Sweden) with rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark). Glycated hemoglobin (GHB) was measured by aminophenylboronic acid affinity chromatography (Isolab Inc., Akron, Ohio, USA). Triglyceride and total cholesterol were measured by an enzymatic method (Wako Co., Osaka, Japan). Awake systolic blood pressure was measured by the tail-cuff method at 4:00 to 7:00 pm by the same investigator (Muromachikikai Co., Tokyo, Japan). Rats were preheated at 37°C for 10 min, and then three stable consecutive measurements of blood pressure were averaged.

Five control SHR and seven SHR treated with 4 mg/kg MSG were maintained until 14 mo of age. Body weight was significantly greater in the MSG-treated groups than control. For statistical analysis, the analysis of variance (ANOVA) was used to compare multiple groups. Differences between two groups were tested by Scheffe's F-test only when found to be significant by ANOVA. Two-tailed unpaired Student's t-test was used to compare variables between two groups. Correlation coefficients were determined by simple linear regression analysis. Differences were considered significant when the p value was less than 0.05. Values are expressed as means ± SEM.

Table 1. Effects of Neonatal Monosodium Glutamate (MSG) Treatment in Female 6-Month-Old SHR.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control</th>
<th>2 mg/kg×5 daily</th>
<th>4 mg/kg×5 daily</th>
<th>4 mg/kg×5 alt day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>6</td>
<td>11</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>205±2</td>
<td>181±4</td>
<td>190±5</td>
<td>209±8</td>
</tr>
<tr>
<td>Nasoanal length (cm)</td>
<td>19.6±0.1</td>
<td>18.7±0.1**</td>
<td>17.8±0.1***</td>
<td>17.9±0.1***</td>
</tr>
<tr>
<td>Lee index</td>
<td>301±2</td>
<td>303±1</td>
<td>322±2***</td>
<td>331±4***</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>15.6±1.0</td>
<td>15.4±0.8</td>
<td>12.8±0.9</td>
<td>13.1±0.8</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>101±8</td>
<td>112±8</td>
<td>111±4</td>
<td>98±2</td>
</tr>
<tr>
<td>Serum IRI (µU/ml)</td>
<td>65±6</td>
<td>63±2</td>
<td>72±8</td>
<td>90±11</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>4.3±0.1</td>
<td>4.4±0.1</td>
<td>4.8±0.1*</td>
<td>5.0±0.1**</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>114±13</td>
<td>106±9</td>
<td>251±29**</td>
<td>297±19***</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>108±8</td>
<td>107±6</td>
<td>111±8</td>
<td>104±7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>212±4</td>
<td>191±3*</td>
<td>185±3***</td>
<td>182±4***</td>
</tr>
</tbody>
</table>

×5 daily, MSG was injected at 1, 2, 3, 4, and 5 d of age; ×5 alt day, MSG was injected at 1, 3, 5, 7, and 9 d of age; Lee index, \( \sqrt{\text{body weight} \times \text{nasoanal length}} \); Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control.

Results

As shown in Table 1, body weight did not differ significantly between the control and MSG-treated groups at 6 mo of age. However, nasoanal length was significantly shorter in the MSG-treated groups than in control. Thus, the Lee index, calculated as \( \sqrt{\text{body weight} \times \text{nasoanal length}} \), was significantly higher in the 4 mg/kg MSG treated groups than control. Food intake did not significantly differ among the groups. Overnight fasted serum glucose and IRI did not significantly differ between the control and MSG-treated groups, but GHB was significantly higher in the 4 mg/kg MSG-treated groups than control. Serum triglyceride was also significantly higher in the 4 mg/kg MSG-treated groups than control, whereas total cholesterol was similar. Systolic blood pressure was significantly lower in both the 2 mg/kg and 4 mg/kg MSG-treated groups than control.

As shown in Fig. 1, Lee index positively correlated with GHB (Fig. 1A, \( r = 0.60, p = 0.0002 \)), serum IRI (Fig. 1B, \( r = 0.64, p = 0.0001 \)), and triglyceride (Fig. 1C, \( r = 0.83, p = 0.0001 \)). On the other hand, Lee index negatively correlated with systolic blood pressure (Fig. 1D, \( r = -0.52, p = 0.0015 \)). No significant correlation was seen between systolic blood pressure and serum IRI.

Table 2 shows some characteristics of 14-mo-old female SHR treated with 4 mg/kg MSG. Body weight was significantly greater in MSG-treated
SHR than control SHR. Lee index and mesenteric and retroperitoneal fat depositions were markedly higher in MSG-treated SHR than control. Overnight fasting serum glucose and serum IRI and GHb were significantly elevated in the MSG-treated group. Pancreatic IRI content tended to be higher in MSG-treated SHR (p = 0.08). Triglyceride was significantly higher in MSG-treated SHR, but total cholesterol did not differ between the two groups. Systolic blood pressure was significantly lower in MSG-treated SHR than in control SHR.

Figure 2 shows the contents of DNA and total cholesterol in the aortas of 14-mo-old SHR with or without obesity. The aortic contents of both DNA and total cholesterol were significantly higher in MSG-treated SHR than in control SHR. Histologically, there were no atheromatous lesions in the carotid artery of control or obese SHR. However, intimal thickening of most of the circumference of the carotid artery was seen in MSG-treated SHR (Fig. 3) and was associated with cell migration (Fig. 4), whereas such lesions were not evident in control SHR. The average grade of intimal thickening was higher in MSG-treated SHR than in control SHR (2.6 ± 0.2 vs. 4.0 ± 0.4, p < 0.05).
The present study demonstrated that neonatal MSG treatment with a dose of 4 mg/kg induced obesity without hyperphagia, impaired glucose tolerance, hyperinsulinemia, and hypertriglyceridemia in female SHR. However, the severity of hypertension was attenuated, and blood pressure levels inversely correlated with the degree of obesity. The obesity persisted up to 14 mo of age, resulting in the development of macrovascular lesions despite no hypercholesterolemia with attenuated hypertension.

Although a number of obese rat models are available, few are associated with overt hypertension (12). Zucker rats and Wistar fatty rats are mildly hypertensive. However, they do not have macrovascular disease (13, 14). It is well known that arterial lesions tend to be disappointingly meager in rats even though hyperlipidemia is inducible in this species (15). However, JCR:LA corpulent rats have occlusive thrombi in coronary arteries (16). JCR:LA rats have marked hypercholesterolemia, glucose intolerance, and insulin resistance, but not hypertension. In contrast, MSG-induced obese SHR had major manifestations of metabolic syndrome X, including hypertension, impaired glucose tolerance, hyperinsulinemia, and hypertriglyceridemia.

Hypertension has been induced in obese rats by bilateral electrolytic lesions in the satiety center of the ventromedial hypothalamus (17) and occurs in genetically obese rats, such as Zucker rats (13) and Wistar fatty rats (14). Although the exact mechanisms by which obesity increases blood pressure are not fully understood, peripheral insulin resistance and hyperinsulinemia have been suggested to play a causal role (18). In the present study, however, blood pressure did not correlate with serum IRI and inversely correlated with the Lee index. In contrast, metabolic abnormalities, including hyperinsulinemia, hypertriglyceridemia, and glucose intolerance, were closely related to the degree of obesity. These disparate associations of MSG-induced obesity with blood pressure and metabolic changes are intriguing, because controversy exists over whether insulin resistance and the resultant hyperinsulinemia are really
the causes of hypertension (5–7). The present study suggests that hyperinsulinemia per se is not always associated with increased blood pressure. This is in agreement with our previous findings that hyperinsulinemia did not increase blood pressure in SHR or in patients with functioning insulinoma (19, 20). It was reported that SHR are heterogeneous with respect to glucose tolerance and insulin resistance and that troglitazone, an insulin sensitizing agent, does not lower blood pressure in SHR. These results suggest that insulin resistance and hyperinsulinemia may not be involved in the pathogenesis of hypertension in SHR (21). This may explain why hyperinsulinemia associated with MSG-induced obesity failed to increase blood pressure in our colony of SHR, which were not glucose intolerant or hyperinsulinemic (22). Parental administration of MSG during the neonatal period induces specific lesions in the central nervous system that are mainly confined to the hypothalamus. In the mediobasal hypothalamus, MSG causes local acute, irreversible degeneration of 80% to 90% of the neuronal cell bodies in the arcuate nucleus, whereas glial cells or axons passing through the nucleus remain unaffected (23). Complete loss of opioid peptide neurons in the arcuate nucleus may contribute to the attenuation of hypertension in MSG-treated obese SHR. Obesity is associated with plasma volume expansion, which may be one of the pathogenetic factors for obesity-induced hypertension (24). The plasma volume expansion may be attributed in part to hyperphagia, including excess salt intake, although hyperphagia was absent in our MSG-induced obesity model.

It was reported that intimal thickening of large vessels develops with aging in male SHR due to increased deposition of extracellular matrix and increased migration of mononuclear cells (25). This is compatible with the histological changes of the carotid artery seen in our female obese SHR. Female animals are known to be less susceptible to cardiovascular disease than males. The aortic DNA content is increased in obese SHR. DNA synthesis is elevated in smooth muscle of large arteries in SHR (26), resulting in medial hypertrophy (27). The increased aortic DNA content may reflect increased cellularity or polyplody in medial smooth muscle cells. In addition, the increased aortic content of total cholesterol suggests early atherogenesis in MSG-treated SHR. Capron et al. (28) reported that when isolated rat aorta was perfused in situ at high or low pressure, glucose-derived lipid synthesis in the aortic media was stimulated by insulin only at high perfusion pressure, suggesting the importance of the combination of hypertension and hyperinsulinemia in atherogenesis. The present study showed that the clustered risk factors of glucose intolerance, hyperinsulinemia, hypertriglyceridemia, and hypertension might accelerate macrovascular changes in the rat, which is a species resistant to experimental atherosclerosis (15).

In conclusion, neonatal treatment with 4 mg/kg of MSG induces obesity, impaired glucose tolerance, hyperinsulinemia, hypertriglyceridemia, and attenuated hypertension in female SHR. These multiple risk factors result in enhanced macrovascular lesions at 14 mo of age. MSG-induced obese SHR may be a useful model to study the clustering of risk factors for the development of macrovascular diseases.

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References