Original Article

Effects of Monosodium Glutamate-Induced Obesity in Spontaneously Hypertensive Rats vs. Wistar Kyoto Rats: Serum Leptin and Blood Flow to Brown Adipose Tissue

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We compared the effects of hypothalamic obesity induced by neonatal monosodium glutamate (MSG) treatment between spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY). Newborn WKY and SHR were injected intraperitoneally with 4 mg/kg body weight of MSG daily for 5 days. At 6 months of age, the obesity of SHR was more advanced than that of WKY, but at 14 months of age the severity of obesity was similar between the two strains. Hypertriglyceridemia was enhanced in MSG-treated SHR as compared with MSG-treated WKY. Systolic blood pressure measured by the tail-cuff method was consistently lower in MSG-treated SHR than in control SHR, whereas blood pressure was not affected by neonatal MSG treatment in WKY. Food restriction reduced body weight more in control SHR than in control WKY, with the former also showing enhanced ketogenesis. Neonatal MSG treatment abolished the accelerated reduction of body weight in SHR. Serum leptin concentration was markedly increased in MSG-treated obese rats, though no differences were seen between WKY and SHR in the control or MSG-treated groups. Serum leptin was closely correlated with both Lee obese index and mesenteric fat weight over the strain. Blood flow in interscapular brown adipose tissue (BAT) measured by Laser Doppler flowmetry was significantly increased in response to β3-adrenoceptor agonist BRL26830A in both the control and MSG-treated rats. However, the response of blood flow was not affected by MSG treatment or strain difference. The present study demonstrated some strain differences in response to neonatal MSG treatment between WKY and SHR. These differences could not be explained by the difference in serum leptin level or β3-adrenergic reactivity in BAT. (Hypertens Res 2000; 23: 503-510)

Key Words: leptin, obesity, blood pressure, animal model, β3-adrenergic receptor

Introduction

The recent discovery of the ob gene product leptin has led to a new era of intense research into the mechanisms of body weight regulation (1). Obesity is a metabolic abnormality fundamental to the development of hypertension in many hypertensive patients, and hypertension can be effectively controlled by a reduction of body weight (2, 3). Recently, it has been reported that plasma leptin concentration is higher in hypertensive patients than in normotensive subjects of the same gender or comparable body mass index (4). However, it remains to be clarified whether the mechanisms of body weight regulation differ
between hypertensive and normotensive subjects. Monosodium glutamate (MSG) treatment of neonatal rats produces various endocrine and behavioral abnormalities, resulting from neurotoxicity to the arcuate nucleus in the hypothalamus (5). MSG-treated rats develop obesity without hyperphagia as adults (6). In a previous study, we applied this obesity model to spontaneously hypertensive rats (SHR), which are frequently used as a model of human hypertension, and found that atherosclerotic lesions were accelerated although hypertension was attenuated (7). In the present study, we attempted to compare the effects of MSG-induced obesity on blood pressure and metabolic parameters between SHR and Wistar Kyoto rats (WKY) as a normotensive control. We studied serum leptin concentration, the effect of food restriction on body weight, and the reactivity of blood flow to brown adipose tissue in response to the $\beta_3$-adrenoceptor agonist, which is a promising agent for the treatment of obesity (8).

Materials and Methods

Animals

WKY were purchased from Charles River Laboratories (Atsugi, Japan). SHR were from an inbred colony maintained in our facility since 1973. Animals were bred in specific-pathogen-free conditions at the Kyushu University Animal Center, where both temperature and lighting (on from 8:00 AM to 8:00 PM) were controlled. They had ad libitum access to tap water and a diet of standard chow, which contained 50.3 g carbohydrate, 25.4 g protein, 8.9 g water, 6.9 g minerals, 4.4 g fat, and 4.1 g fiber per 100 g diet according to information supplied by the manufacturer (Clea Japan Inc., Tokyo, Japan). Animals were treated according to the guidelines set out by Kyushu University.

Induction of MSG-Induced Obesity

Newborn female and male WKY and SHR ($n=16$ females and 10 males in WKY; $n=19$ females and 11 males in SHR) were injected intraperitoneally with 4 mg/kg body weight of MSG at the ages of 1, 3, 5, 7, and 9 days. Control rats received 10% NaCl ($n=8$ females and 10 males in WKY; $n=10$ females and 5 males in SHR). The injection volume was 8 $\mu$l/g body weight. The pups remained with their respective mothers until they were weaned at 4 weeks of age. Nasoanal length, body weight, and systolic blood pressure were measured in female and male rats at 3 and 6 months of age. Because MSG-treated rats showed overall shorter body length due to impaired secretion of hypothalamic GH-RH (9), and because SHR are originally smaller than WKY (10), obesity was evaluated on the basis of the Lee index, calculated as $\sqrt{3}$body weight $\times$10/nasoanal length (11), which is well correlated with adiposity in rats (12). At 14 months of age, when marked obesity developed in MSG-treated rats, blood was obtained from the tail vein in the nonfasting state, and then the food restriction study was performed as follows.

Food Restriction

Food intake was determined at 14 months of age by placing rats in metabolic cages for 24 h after a several-day acclimatization period. Acclimatization to metabolic cages was considered sufficient when the difference of body weight between before and after placement in a metabolic cage was negligible. Food was restricted to 30% of ad libitum food consumption for 7 days. Food was given at 6:00 PM and body weight was measured daily. Blood was collected from a tail vein at 10:00 AM on the last day of food restriction for the determination of serum total ketone body concentration (Ketone test; Sanwa Kagaku Co., Nagoya, Japan) (13).

Measurement of Blood Flow in Interscapular Brown Adipose Tissue (BAT)

Fourteen-month-old female rats were anesthetized with pentobarbital (50 mg/kg i.p.) and set on a body temperature control table. A catheter was inserted into the femoral vein for an infusion of the $\beta_3$-adrenoceptor agonist BRL26830A (SmithKline Beecham Pharmaceuticals, Surrey, UK). The interscapular brown adipose tissue was carefully exposed and its blood flow was measured by Laser Doppler flowmetry (LDF) (ALF21; ALF Advance Co., Tokyo, Japan). The LDF probe (tip diameter, 10 mm) was positioned above the left lobe surface of BAT (14, 15). BRL26830A dissolved in sterile water was intravenously administered at a dose of 0.01 mg/kg/min, 0.1 mg/kg/min, and 1.0 mg/kg/min for 10 min at each dose. After BAT blood flow measurements had been obtained, animals were killed by bleeding, and then mesenteric fats were weighed.

Measurements

Awake systolic blood pressure was measured at the proximal part of the tail by a tail-cuff method (Muromachikai Co., Tokyo, Japan). MSG-treated rats have shorter tails, but in this study, the size of the proximal portion was similar to that of the controls. The measurement was performed from 4:00 to 7:00 PM by the same investigator. Rats were warmed at 37°C for 10 min, then three stable consecutive measurements were taken and averaged. Blood was collected from a tail vein after an overnight fast at 14 months of age. Serum glucose was measured with a Beckman glucose analyzer 2 (Beckman Instruments, Fullerton, CA); serum immunoreactive insulin
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(IRI) by radioimmunoassay (RIA) (Pharmacia Co., Uppsala, Sweden) using rat insulin as a standard (Novo Research Inst., Bagsvaerd, Denmark); serum triglyceride and total cholesterol by an enzymatic method (Wako Co., Osaka, Japan); and serum leptin by a rat leptin RIA kit (Linco Research Inc., St. Charles, MO).

Statistical Analysis

The analysis of variance (ANOVA) was used to compare multiple groups. Differences between the two groups were tested by Scheffe's F-test only when found to be significant by ANOVA. Correlation coefficients were determined by univariate Spearman correlation. A difference was considered significant at a p value less than 0.05. Values are expressed as means ± SEM.

Results

Since MSG-treated rats as well as SHR showed shorter body length (Table 1), the degree of obesity was evaluated by Lee index (11). In female rats (Table 1), the Lee index was significantly higher in MSG-treated SHR than in controls at both 3 and 6 months of age, whereas no difference was observed between MSG-treated WKY and controls. MSG-treated SHR developed mild obesity at 6 months of age. Systolic blood pressure was lowered in SHR but not in WKY by neonatal MSG treatment (3 months, 150±2 mmHg in control WKY, 144±1

Table 1. Characteristics of Female WKY and SHR Treated Neonatally with Monosodium Glutamate (MSG)

<table>
<thead>
<tr>
<th></th>
<th>3 months of age</th>
<th>6 months of age</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MSG</td>
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<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Nasoanal length (cm)</td>
<td>19.3±0.2</td>
<td>17.6±0.1***</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>18.5±0.2†</td>
<td>16.7±0.1*** ††</td>
</tr>
<tr>
<td>WKY</td>
<td>197±5</td>
<td>154±2***</td>
</tr>
<tr>
<td>SHR</td>
<td>174±3††</td>
<td>148±2***</td>
</tr>
<tr>
<td>Lee index</td>
<td>301±1</td>
<td>305±1</td>
</tr>
<tr>
<td>WKY</td>
<td>303±3</td>
<td>317±1*** ††</td>
</tr>
<tr>
<td>SHR</td>
<td>140±3</td>
<td>135±1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>192±6††</td>
<td>178±2*** ††</td>
</tr>
</tbody>
</table>

Lee index, $\sqrt[3]{\text{body weight}} \times 10/$nasoanal length. The number of animals is 6 in control WKY, 16 in MSG-treated WKY, 6 in control SHR, and 19 in MSG-treated SHR. Mean±SEM. *p<0.05, ***p<0.001 vs. control, †p<0.05, ††p<0.01, †††p<0.001 vs. WKY.

Table 2. Characteristics of 14-Month-Old Female WKY and SHR Treated with MSG

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MSG</td>
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<tr>
<td></td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>263±5</td>
<td>286±4</td>
</tr>
<tr>
<td>Lee index</td>
<td>304±2</td>
<td>341±2***</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>15.0±0.6</td>
<td>13.0±0.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131±9</td>
<td>131±2</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>93±1</td>
<td>94±2</td>
</tr>
<tr>
<td>Serum IRI (U/ml)</td>
<td>24±3</td>
<td>53±6**</td>
</tr>
<tr>
<td>Pancreatic IRI content (U/g)</td>
<td>1.60±0.10</td>
<td>2.03±0.22</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>113±3</td>
<td>217±10***</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>128±2</td>
<td>146±6</td>
</tr>
<tr>
<td>Mesenteric fat (g)</td>
<td>3.90±0.27</td>
<td>7.43±0.33***</td>
</tr>
</tbody>
</table>

Mean±SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control of each strain, ††p<0.001 vs. WKY.
mmHg in MSG-treated WKY, ns; 209±3 mmHg in control SHR, 193±4 mmHg in MSG-treated SHR, p<0.01; 6 months, 140±2 mmHg in control WKY, 143±2 mmHg in MSG-treated WKY, ns; 222±3 mmHg in control SHR, 207±4 mmHg in MSG-treated SHR, p<0.05).

At 14 months of age (Table 2), both WKY and SHR developed marked obesity as evaluated by Lee index, and values were similar in MSG-treated WKY and SHR. Food intake was reduced in MSG-treated rats as compared with that in WKY and SHR controls, respectively. Systolic blood pressure did not differ between MSG-treated and control WKY rats, whereas hypertension was attenuated in MSG-treated SHR. Serum glucose did not significantly differ among the groups, but values for serum IRI and pancreatic IRI content were higher in MSG-treated than control rats in both WKY and SHR. Serum triglyceride was significantly increased in MSG-treated rats, and the increase was greater in MSG-treated SHR than in MSG-treated WKY. Serum cholesterol did not differ among the groups. Mesenteric fat weight was markedly higher in MSG-treated obese rats than controls in both WKY and SHR. No difference was observed in mesenteric fat weight between MSG-treated WKY and SHR.

Serum leptin was significantly higher in MSG-treated rats than in controls, though no differences were observed between WKY and SHR (7.7±1.4 ng/ml in control WKY (n=8), 53.3±4.8 ng/ml in MSG-treated WKY (n=10), 2.4±0.4 ng/ml in control SHR (n=5), 47.6±11.5 ng/ml in MSG-treated SHR (n=6)). Serum leptin was closely correlated with Lee index (Fig. 1A) and mesenteric fat weight (Fig. 1B) over the strain (r=0.92, p<0.0001; r=0.90, p<0.0001, respectively). However, no significant correlation was found between serum leptin and food consumption (Fig. 1C, r=−0.23, ns).

Since body weight and Lee index differed significantly among the groups before food restriction, changes of body weight or Lee index were expressed as a percentage change in order to compare the effects of 7-days’ 70% food restriction among the groups (Table 3). Body weight reduction was significantly greater in control SHR than in control WKY. MSG-treated SHR showed a smaller reduction of body weight than that in the control SHR. Serum total ketone body concentration during food restriction was markedly more increased in SHR than in WKY, although no difference was seen between control and MSG-treated rats in either strain.

Basal BAT blood flow did not differ among the groups (9.8±1.3 ml/min/100 g in control WKY, 9.5±0.9 ml/min/100 g in MSG-treated WKY, 8.4±1.7 ml/min/100 g in control SHR, 8.0±0.9 ml/min/100 g in MSG-treated SHR). BAT blood flow rose significantly in response to the β2-adrenergic agonist BRL26830A in both WKY and SHR (p<0.001). No significant difference in the percentage increase of BAT blood flow was observed between the control and MSG-treated rats in either strain (control WKY 226±30% at 0.01 mg/kg/min, 328±39% at 0.1 mg/kg/min, 340±35% at 1.0 mg/kg/min; MSG-treated WKY 209±30% at 0.01 mg/kg/min, 300±42% at 0.1 mg/kg/min, 296±38% at 1.0 mg/kg/min; control SHR 161±16% at 0.01 mg/kg/min, 265±46% at 0.1 mg/kg/min, 275±43% at 1.0 mg/kg/min; MSG-treated SHR 205±20% at 0.01 mg/kg/min, 284±30% at 0.1 mg/kg/min, 291±35% at 1.0 mg/kg/min).
The present study demonstrated several differences between the WKY and SHR strains with respect to response to neonatal MSG treatment. First, SHR developed obesity earlier than did WKY, although the degree of obesity was similar in WKY and SHR at 14 months of age. Hypertriglyceridemia was enhanced in MSG-treated SHR as compared with MSG-treated WKY. Secondly, hypertension was attenuated in SHR throughout the experimental period, whereas blood pressure was not affected by MSG treatment in WKY. No gender-related differences in the response to MSG were observed in either WKY or SHR. Thirdly, food restriction reduced body weight more in control SHR than in control WKY, and MSG treatment abolished an accelerated reduction of body weight in SHR. However, no strain-related differences were observed among the groups in terms of serum leptin levels or BAT blood flow in response to the β3-adrenergic agonist BRL26830A.

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The interpretation of the results in the present study is somewhat complicated by the facts that SHR are initially smaller than WKY (10) and that gender-related differences exist in the body weights of rats of either strain. However, the Lee index was found to be well-correlated with adiposity (12) and to be closely correlated with mesenteric fat weight in the present study (n=40, r=0.95, p < 0.0001). Thus, these differences in body weight or nasoanal length could be corrected for by using the Lee index for a comparison of obesity between the groups.

Neonatal MSG treatment almost completely destroys neuronal cell bodies in the arcuate nucleus of the hypothalamus (16), and MSG-treated rats display a characteristic syndrome of obesity, blindness, stunted growth, hypogonadism, and short tail (5). The occurrence of hypertriglyceridemia is probably due to the increased hepatic VLDL synthesis induced by hyperinsulinemia. Although MSG-treated rats did not appear cachexic, body weight was lower in MSG-treated rats than in controls at 3 and 6 months of age probably due to shorter body length. Olney (5) and Bunyan et al. (6) found that MSG-treated mice were rather hypophagic and weighed less than the controls before the development of marked obesity. The absence of hyperphagia is the most outstanding feature of MSG-induced obesity, as seen in our 14-month-old MSG-treated rats. MSG treatment eliminated neuropeptide Y mRNA, which is the most potent stimulator of food intake, in the arcuate nucleus (17). Therefore, it is likely that suppressed energy expenditure due to the altered hypothalamic-pituitary axis contributes to MSG-induced obesity. In a study by Moss et al. (18), thermoregulatory thermogenesis was impaired in MSG-treated mice. It has been reported that BAT is a major site for energy expenditure and thermogenesis (19). Regarding BAT function in SHR, Hayashi et al. (20) showed that there were no strain-related differences in BAT activity or whole body oxygen consumption in either a basal or catecholamine-stimulated state, suggesting that the peripheral reactivity of BAT was not impaired in SHR. However, it has been reported that SHR are less able to maintain body temperature at a low ambient temperature (21), and that BAT thermogenic response activated by the hypothalamus is attenuated in SHR as compared with WKY (22). Thus SHR may demonstrate altered regulation of BAT function by the hypothalamus. BAT has a very high perfusion rate because increased blood flow through BAT is necessary not only to supply oxygen and substrates for thermogenesis but also to transfer heat from tissue (23). BAT is richly innervated by sympathetic nerves, and norepinephrine induces marked vasodilation by an increased production of inducible nitric oxide in BAT (24). The β3-adrenoceptor agonist BRL-26830A has been shown to induce vasodilation in BAT (25). In the present study, there were no differences in the increase of BAT blood flow in response to BRL-26830A between SHR and WKY, or between MSG-treated and control rats, suggesting that sensitivity to β3-adrenergic stimulation may not be affected by either the strain or MSG treatment. This finding is compatible with those of Dulloo et al. (26), who found that neonatal MSG treatment did not affect sympathetic nervous activity as assessed by norepinephrine turnover in BAT. The mechanism for the strain-related difference in body weight regulation between WKY and SHR remains to be clarified.

The attenuation of hypertension in SHR by neonatal MSG treatment was a somewhat unexpected finding, because obesity usually elevates blood pressure (2, 3). However, MSG-induced obesity does differ from the com-
common type of obesity seen in humans, in that MSG disrupts the hypothalamic-pituitary-adrenal, thyroid, and gonadal axis (27, 28). Hypertension in SHR has been shown to be attenuated by each of hypophysectomy, adrenalectomy, thyroidectomy, or gonadectomy (29, 30). Full development of hypertension in SHR may require intact endocrine functions. Although the arcuate nucleus may not directly participate in the regulation of blood pressure, hypothalamic neuropeptide Y may contribute to such regulation (31), and MSG insult may affect the function of other brain neurons regulating blood pressure. The possible strain-related difference in susceptibility to MSG neurotoxicity may contribute to the difference in blood pressure response to MSG treatment.

It has been reported that the fat cell membrane of SHR have a lower density of \( \beta \)-adrenergic receptors than those of WKY, resulting in lower catecholamine-induced lipolysis in adipocytes isolated from SHR (32). However, findings in the present study showed that the reduction of body weight induced by food restriction was greater in control SHR than in control WKY, and that ketogenesis was also enhanced in the former. It is tempting to speculate that sympathetic overactivity in SHR (33) may be responsible for the accelerated body weight reduction and that MSG may suppress the sympathetic activity, resulting in the termination of the accelerated body weight reduction as well as the attenuation of hypertension. In addition, SHR have been reported to show an increased circulating free fatty acid level (34) and increased hepatic synthesis of carnitine, with the latter increase being essential for the transport of fatty acid across the inner mitochondrial membrane (35). These findings may partly explain the increased ketogenesis in SHR.

Leptin is an adipostatic hormone secreted mainly by adipocytes. It has been demonstrated that serum level is well correlated with body fat mass, and that leptin secretion is regulated by insulin (36). In the present study, serum leptin was well correlated with both Lee index and mesenteric fat weight (Fig. 1) and with serum IRI level \((r = 0.72, p < 0.0001)\). Leptin diminishes food intake and increases heat production by activating thermogenesis in BAT through brain leptin receptors, which are abundantly distributed in the arcuate nucleus (37). Serum leptin was markedly increased in both MSG-treated WKY and SHR to a level comparable to that in obese humans (38). Satoh et al. (39) reported that rats with hypothalamic obesity induced by ventromedial hypothalamus (VMH) lesions exhibited hyperleptinemia, but lacked leptin action by intracerebroventricular or intravenous injection of leptin. Therefore, it is likely that leptin has no central effects in MSG-treated rats whose arcuate nucleus has been damaged by neonatal MSG treatment. This may explain why hyperleptinemia did not affect the BAT blood flow response to BRL-26830A in MSG-treated rats. Transgenic mice overexpressing leptin have recently been developed (40). In this model, blood pressure was increased by 10–15 mmHg as compared with that in control mice, and the \( \alpha_1 \)-adrenoceptor antagonist bunazosin was found to normalize high blood pressure. Leptin activates the sympathetic nervous system and possibly increases blood pressure. In a study by Agata et al. (4), plasma leptin concentration was higher in hypertensive patients than in normotensive subjects, despite these groups being matched by gender and body mass index, suggesting that leptin may contribute to the pathogenesis of hypertension. In the present study, however, serum leptin was found to be at similar levels in control WKY and SHR, and its correlation with adiposity was exhibited across the strain. Inversely, hypertension was attenuated in MSG-treated obese SHR and blood pressure was not affected by neonatal MSG treatment in WKY. The present findings do not directly contradict the hypothesis that leptin may contribute to the pathogenesis of hypertension through the hypothalamus, although they do suggest that leptin has no peripheral effect to elicit hypertension.

In conclusion, MSG-induced obesity developed earlier in SHR than in WKY. Food restriction reduced body weight more in SHR than in WKY, and MSG treatment abolished the accelerated body weight reduction. These differences could not be explained by differences between the groups in serum leptin level or \( \beta_3 \)-adrenergic reactivity in BAT. In addition, neonatal MSG treatment lowered blood pressure in SHR but not in WKY. It remains to be clarified whether these strain-related differences may be due to a difference in susceptibility to MSG neurotoxicity or to gene(s) related to hypertension in SHR.

Acknowledgements

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References

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