Effects of Chronic Administration of Sibutramine on Body Weight, Food Intake and Motor Activity in Neonatally Monosodium Glutamate-Treated Obese Female Rats: Relationship of Antiobesity Effect with Monoamines

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Abstract: When the hypothalamic ventromedial nucleus and arcuate nucleus were destroyed in rats by treatment with monosodium glutamate in the neonatal stage, increase in the Lee index (body weight $1/3$ /body length) and in retroperitoneal fat as well as decreases in spontaneous motor activity, food consumption and growth hormone secretion function associated with hypothalamic low body length obesity (monosodium glutamate-treated obesity; MSG-OB) were observed as these rats grew. Treatment with sibutramine at 3 and 10 mg/kg p.o. once a day continuously for 14 days improved these parameters, and the degree of improvement was dose related. The plasma lipid values in MSG-OB rats, which were the same as those in normal rats, were decreased by consecutive administration of sibutramine. Levels of hypothalamic monoamines (MAs) such as norepinephrine, 5-HT (serotonin) and dopamine and their metabolites DOPAC, HVA and 5-HIAA were decreased in MSG-OB rats, and further decrease in them, though slight, was observed with consecutive daily administration of sibutramine, probably as a result of the feedback attributable to an increase in MA in synapses caused by inhibition of MA uptake by sibutramine. These results suggest that sibutramine can activate the MA nervous system by MA uptake inhibition in regions of the brain such as the lateral hypothalamic area and the paraventricular nucleus, which control food intake and sympathetic nerve activity, and the nigrostriatal area related to the extrapyramidal motor system, and thereby exhibit anti-obesity effects in the MSG-OB rat.

Key words: body weight, food intake, hypothalamic monoamine level, sibutramine, spontaneous motor activity

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Introduction

Obesity is defined as excessive accumulation of fat tissue (i.e., of somatic fat). When energy balance is positive, that is, the number of calories contained in ingested food greater than the energy released through exercise, body temperature increase, etc., calories are stored in the body as fat, glycogen and protein, resulting in an increase in body weight [14]. Bray proposed “The Mona Lisa hypothesis in the time of leptin”, and indicated that inhibition of appetite and increased sympathetic nervous system activity have anti-obesity effects [4]. Food intake is controlled through complex interactions of nervous systems in the brain which are related to appetite, exercise, reward and memory. Above all, various nuclei in the hypothalamus play central roles in controlling food intake. According to the so-called dual center hypothesis, which attracted attention in the past, the ventromedial nucleus (VMH), the satiety center, and the lateral hypothalamic area (LH), the food intake center, adjust appetite in the hypothalamus [14]. But recent studies on leptin, which is secreted from peripheral fat cells and which suppresses food intake, and studies on the mechanism of action of neuropeptide Y (NPY), reported that the paraventricular nucleus (PVN), perifornical hypothalamus (PFH), arcuate nucleus (ARC) and other brain areas are also involved in food intake behavior [4].

Obesity results in rats and cats when the VMH is electrically destroyed, but this method requires special devices and techniques and requires much time. On the other hand, intraperitoneal administration of monosodium glutamate (MSG) to rats in the neonatal stage destroys the VMH and ARC in the hypothalamus, producing neonatally MSG-treated obese (MSG-OB) rats whose sympathetic nerve activity decreases with growth [10, 34, 45, 46]. Hypothalamic obesity animal models may thus be easily prepared through destruction of the VMH by systemic administration of MSG. On the other hand, destruction of the lateral hypothalamus has effects opposite those of destruction of the ventromedial hypothalamus, with reduction in food intake, decrease in body fat, and increase in sympathetic nerve system activity [2].

In the presence of anxiety and mental tension, the sympathetic nervous system is activated. Even when resting, secretion of norepinephrine and epinephrine is increased, whereas blood glucose and free fatty acid concentrations increase. As a result, the fat and glycogen stored in the body as energy are oxidized and decomposed, resulting in body weight decrease [14].

During development of sibutramine (N-1-(1-[4-chlorophenyl]-cyclobutyl)-3-methylbutyl-N, N-dimethylamine hydrochloride monohydrate :BTS 54-524, ® Meridia) as an antidepressant, it was found to have a body weight-reducing effect in clinical studies, and it has recently been developed as an anti-obesity drug [3]. In the mechanisms of centrally acting anorectic drugs, monoamines (MAs) such as norepinephrine, dopamine and 5-hydroxytryptamine (5-HT, serotonin) play important roles [41].

The results of in vivo acute administration tests and of in vitro tests have indicated the norepinephrine, 5-HT and dopamine reuptake-suppressing effects of sibutramine [28].

It is of an interest to determine whether the food intake suppression by acute administration of sibutramine to normal rats is attributable to effects of this agent mediated by the VMH and ARC or effects of this agent in other brain areas, e.g., the LH and PVN, etc., and whether sibutramine acts on these hypothalamic nuclei to activate the sympathetic nervous system to increase energy expenditure [16, 19, 20, 44].

Activation of the nigrostriatal dopaminergic system is important because it increases the amount of motor activity, thereby expending energy. In this regard, we have reported that sibutramine increased rat motor activity due to activation of the dopaminergic system in the nigro-striatum [28]. Otherwise, there is also a report that sibutramine does not have dopaminergic effects [18]. It is still unclear whether continued administration of sibutramine maintains the increase in spontaneous motor activity, as observed after acute administration, which contributes to the anti-obesity effect of sibutramine by energy expenditure.

Although the anti-obesity effect of sibutramine has been clinically confirmed, few studies have examined the anti-obesity effects of daily administration of sibutramine on food intake or body weight (or Lee index = body weight / body length) in animal models of obesity [9, 39, 40]. In the present study, sibutramine was chronically administered to normal and MSG-OB rats, and the relationship of behavioral pharmacological changes to hypothalamic MA levels was examined.
Materials and Methods

Drugs

Sibutramine HCl and growth hormone (GH) secretagogue (growth hormone releasing peptide (GHRP:KP-102)) were synthesized at Kaken Pharmaceutical Co. (Tokyo). Methamphetamine (Philopon®) was purchased from Dainippon Pharmaceutical Co. (Osaka). Other drugs were obtained from Sigma-RBI (St. Louis, MO, USA).

Animals

Monosodium glutamate-treated obesity (MSG-OB) rats were obtained by intraperitoneal administration of MSG to female neonates (born to female Sprague-Dawley strain rats purchased from SLC Inc. (Shizuoka) on Day 16 of gestation) at 2 mg/g body weight 5 times, i.e., 1, 3, 5, 7 and 9 days after birth. Physiological saline was administered in similar fashion 5 times intraperitoneally to the control animals. The animals were raised normally thereafter, and studied at the age of 10 weeks. The animals were kept through the experiments on a 12-hr light-dark cycle at a room temperature of 21 ± 1°C.

Experiment 1: Effects on body weight, food intake, and levels of hypothalamic MAs and plasma GH and lipid levels of normal and MSG-OB rats

Three days before drug administration, rats in the neonatally physiological-saline-treated group (normal group) underwent measurement of retroperitoneal fat with a body-fat measuring apparatus for rats (Biotex, Kyoto) by the impedance method. On the basis of observed values, rats were distributed to a distilled water p.o. group, sibutramine 3 mg/kg p.o. group, and sibutramine 10 mg/kg p.o. group by the replication method (block design); each group included 10 rats. MSG-OB rats were similarly divided into 3 groups, each group with 10 rats. In each group, distilled water was given orally for 3 days before the start of drug administration.

Body weight and food consumption were measured at 1–2 p.m. every day. The drug was orally administered at 4–5 p.m. daily for 14 days, from Day 0 through Day 13. Sixteen hours after the final administration, body-fat impedance, body weight (g) and body length (nasal-anal distance in cm) were measured, and the Lee index (body weight $1/3$/body length) was obtained. A dose of 100 µg/kg (0.1 ml/100 g) of GHRP was injected into the tail vein, and 15 min later the rats were decapitated and blood was collected. Immediately after blood collection, the hypothalamus and retroperitoneal fat were extracted and weighed. The blood samples were immediately centrifuged at 1,500 G at 4°C for 15 min, and the plasma was stored at –20°C or below until use.

For measurement of hypothalamic MAs, each sample was homogenized in 0.06 M perchloric acid solution containing an internal standard, 3,4-dihydroxybenzylamine (DHBA, 100 ng/ml), and centrifuged with a high-speed refrigerated centrifuge (4°C, 20,000 G, 20 min). The supernatant was filtered, and MAs and their metabolites in the filtrate were measured by means of HPLC with an electrochemical detector.

Plasma levels of total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were quantified with a biochemical auto-analyzer (Fuji Drychem 3030). Free fatty acid was measured by the ACS-ACOD method (NEFA C Test, Wako), and GH was measured by radio-immunoassay.

Experiment 2: Effects of sibutramine and methamphetamine administered for 14 consecutive days on spontaneous motor activity rhythm of normal and MSG-OB rats

Normal rats and MSG-OB rats were grouped by the replication procedure mentioned above, and groups of 4 rats each were prepared. Body weight and food intake were also measured by the methods described above. Spontaneous motor activity of the rats was monitored by the Rhythmer system with 16 channels for automatic simultaneous measurement of spontaneous motor activity, feeding and drinking (Biotex, Kyoto).

Motor activity rhythm was depicted in a figure by double-plotting for each rat with measurement of motor activity every 10 min. During the course of drug administration for 14 days, 336 hourly data points were collected for each rat. The data were subjected to rhythm analysis (lag 202 points (60%), 5-point moving average, 0.027–0.25 frequency/hr) by the maximum entropy method (MEM) to obtain cycle, amplitude, phase and mean motor activity parameters [30].

Statistical analysis

Experiments were performed according to the MSG
time-course data for body weight, food intake and spontaneous motor activity were tested by analysis of variance (ANOVA) for repeated measurements. Other data were tested by two-way ANOVA of MSG treatment-sibutramine treatment-replication. Thereafter, 2 × 3 point parallel line bioassay, i.e., MSG treatment effect, sibutramine treatment effects (linearity and quadratic curve) and effects of their interaction (linear non-parallelism and quadratic non-parallelism), was performed [7].

When either one of the two non-parallelism tests indicating interaction was significant, interaction was judged to be present and expressed as “I” for p<0.05. Then multiple comparison testing of the corresponding control group and sibutramine treatment at each dose was performed by Dunnett’s method. The finding of significant difference between the saline treatment control and the MSG treatment control was expressed as “#” for p<0.05 or “##” for p<0.01 (two-tailed test). When neither of the two interactions was significant, multiple comparison testing with the crossed control group, which places the saline treatment control group and MSG treatment control group together, and crossed sibutramine treatment group at each dose was performed by Dunnett’s method. Significances of p<0.05 and p<0.01 were expressed as “$” and “$$” for the repeated measurement design, and by “*” and “**” for others. Significances of p<0.05 and p<0.01 were expressed as “#” and “##”, respectively, in comparisons of the crossed saline treatment group and crossed MSG treatment group.

## Results

Experiment 1: Effects of sibutramine administered for 14 consecutive days on body weight, food intake, hypothalamic MAs, and plasma GH and lipid levels of normal and MSG-OB rats

Compared with the control physiological saline group, rats in the neonatally MSG 2 mg/g i.p.-treated group exhibited lower body weight, shorter body length, higher Lee index, and more retroperitoneal fat, and thus demonstrated successful preparation of a growth-suppressed obese rat model (Fig. 1, Table 1). During daily administration of sibutramine at 3 and 10 mg/kg p.o., which inhibited MA reuptake in vivo, body weights of normal and MSG-OB rats decreased daily for the first 4 days but were steady thereafter until the end of administration (after 13 days of administration) (Fig. 1) [16, 28, 33]. The decreases in body weight and the Lee index in the normal and MSG-OB rats were dose-dependent, but body length was independent of the dose (Fig. 1, Table 1). Food intake in the MSG-OB group was significantly less than in the normal group (Fig. 2). Administration of sibutramine at 3 and 10 mg/kg p.o. decreased food intake by both normal and MSG-OB rats (Fig. 2). The amount of retroperitoneal fat in the MSG-OB group rats was significantly greater than that in the normal group rats. Sibutramine at 3 and 10 mg/kg p.o. decreased retroperitoneal fat dose-dependently in both normal and MSG-OB rats (Table 1). Impedance values obtained with the body fat measuring apparatus were closely correlated with the amount of retroperitoneal fat (r=0.93, p<0.01).

No significant differences between the MSG-OB group and the normal group were found in plasma levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol or free fatty acid. Sibutramine at 3 and 10 mg/kg p.o. dose-dependently and significantly lowered plasma levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol and free fatty acid in both normal rats and MSG-OB rats (Table 2).

GH secretion into plasma induced by loading of 100 µg/kg i.v. of GHRP was significantly less in the MSG-OB group than in the normal group (Table 2). Sibutramine at 3 and 10 mg/kg p.o. did not affect GH secretion into plasma induced by GHRP loading in normal rats, but significantly accelerated GH secretion into plasma in MSG-OB rats (Table 2).

Hypothalamic levels of norepinephrine, dopamine, 5-HT and their metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA), were significantly lower in the MSG-OB group than in the normal group, but the turnover rates of DOPAC/dopamine and 5-HIAA/5-HT were similar in between these two groups (Table 3). Sibutramine at 3 and 10 mg/kg p.o. significantly lowered the norepinephrine level in both normal rats and MSG-OB rats (Table 3). Sibutramine at 3 and 10 mg/kg p.o. tended to lower the dopamine level but affected neither the levels of its metabolites, DOPAC and HVA, nor the turnover rate of DOPAC/dopamine, in either normal rats or MSG-OB rats (Table 3). Sibutramine at 3 and 10 mg/kg p.o.
tended to lower the 5-HT level, lowered the level of its metabolite, 5-HIAA, and decreased the turnover rate of 5-HIAA/5-HT in both normal rats and MSG-OB rats (Table 3).

Experiment 2: Effects of sibutramine and methamphetamine administered for 14 consecutive days on spontaneous motor activity rhythm of normal and MSG-OB rats

Sibutramine at 3 and 10 mg/kg p.o. decreased body weight and food consumption in the MSG-OB group,

**Table 1.** Effects of chronic systemic administration of sibutramine on body weight, body length, Lee index and retroperitoneal fat weight in normal and MSG-OB female rats

<table>
<thead>
<tr>
<th>Neonatal Treatment</th>
<th>Drugs</th>
<th>Dose (mg/kg, p.o)</th>
<th>Body Weight (g)</th>
<th>Nasal-anal Length (cm)</th>
<th>Lee Index</th>
<th>Retroperitoneal Fat Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated</td>
<td>Control</td>
<td></td>
<td>253 ± 14</td>
<td>21.5 ± 0.363</td>
<td>0.294 ± 0.004</td>
<td>9.951 ± 3.950</td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>248 ± 12</td>
<td>21.3 ± 0.262</td>
<td>0.294 ± 0.004</td>
<td>7.863 ± 2.974</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>234 ± 7.5*</td>
<td>21.4 ± 0.386</td>
<td>0.288 ± 0.005**</td>
<td>3.711 ± 1.694**</td>
<td></td>
</tr>
<tr>
<td>MSG-treated</td>
<td>Control</td>
<td>229 ± 29*</td>
<td>19.8 ± 0.911*</td>
<td>0.309 ± 0.008*</td>
<td>16.82 ± 6.209*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>218 ± 24</td>
<td>19.5 ± 0.774</td>
<td>0.308 ± 0.012</td>
<td>14.71 ± 6.057</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>203 ± 25*</td>
<td>19.8 ± 0.675</td>
<td>0.296 ± 0.009**</td>
<td>11.26 ± 4.296**</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD for 10 rats. The Lee index is (body weight (g))^{1/3}/(nasal-anal length (cm)).

*: p<0.05 compared with the neonatally saline-treated group, since interaction was not significant on ANOVA.

**: p<0.01, **: p<0.01 compared with the crossed-control group, since interaction was not significant on ANOVA.

**Fig. 1.** Effects of chronic systemic administration of sibutramine on the body weight of normal and MSG-OB female rats. Normal rats: ○ control, △ sibutramine 3 mg/kg and ■ sibutramine 10 mg/kg. MSG-OB rats: ● control, ▲ sibutramine 3 mg/kg and ■ sibutramine 10 mg/kg. Each value is the mean ± SD for 10 rats. ##: p<0.01 compared with the saline-treated crossed group, since interaction was not significant on ANOVA. $$: p<0.01 compared with the crossed control group, since interaction was not significant on ANOVA.
Methamphetamine at 10 mg/kg p.o. decreased body weight and food consumption in the MSG-OB group to the same degree as did sibutramine 10 mg/kg p.o. (data not shown). The mean spontaneous motor activity in the MSG-OB group was significantly less than that in the normal group for 14 consecutive days, and the mean amplitude of the activity rhythm in the MSG-OB group tended to decrease, although not to a significant extent.

No significant difference between the normal and MSG-OB control groups in this group was found in either the diurnal motor activity rhythm or phase (Table 4). Sibutramine at 3 mg/kg p.o. increased the mean spontaneous motor activity in the MSG-OB group, but no changes were found in either the diurnal motor activity rhythm or phase in this group (Table 4). Sibutramine at 10 mg/kg p.o. and methamphetamine at 10 mg/kg p.o. increased both mean spontaneous motor activity

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**Table 2.** Effects of chronic systemic administration of sibutramine on total cholesterol, triglyceride, HDL-cholesterol and free fatty acid levels, and GHRP-loaded GH secretion in plasma of normal and MSG-OB female rats

<table>
<thead>
<tr>
<th>Neonal treatment</th>
<th>Drugs</th>
<th>Dose (mg/kg, p.o.)</th>
<th>GH* (log ng/ml)</th>
<th>T-Cho (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-Cho (mg/dl)</th>
<th>NEFA (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated</td>
<td>Control</td>
<td>2.077 ± 0.643</td>
<td>89 ± 13</td>
<td>120 ± 72</td>
<td>54 ± 12</td>
<td>0.638 ± 0.226</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>1.801 ± 0.602</td>
<td>82 ± 11</td>
<td>100 ± 28*</td>
<td>45 ± 5**</td>
<td>0.406 ± 0.160**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>1.980 ± 0.450</td>
<td>66 ± 10**</td>
<td>76 ± 12**</td>
<td>39 ± 6**</td>
<td>0.419 ± 0.132**</td>
<td></td>
</tr>
<tr>
<td>MSG-treated</td>
<td>Control</td>
<td>1.539 ± 0.245†</td>
<td>85 ± 14</td>
<td>161 ± 90</td>
<td>48 ± 11</td>
<td>0.561 ± 0.121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>1.883 ± 0.500†</td>
<td>82 ± 12</td>
<td>109 ± 45*</td>
<td>46 ± 21**</td>
<td>0.513 ± 0.119**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>1.867 ± 0.633†</td>
<td>68 ± 10**</td>
<td>92 ± 41**</td>
<td>38 ± 7**</td>
<td>0.425 ± 0.091**</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD for 10 rats. *: loaded with GHRP 100 µg/kg, i.v., GH: growth hormone, T-Cho: total cholesterol, TG: triglyceride, HDL-Cho: high-density lipoprotein cholesterol, NEFA: free fatty acid. †: p<0.05 compared with the neonatally saline-treated group, since interaction was significant on ANOVA.

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Fig. 2. Effects of chronic systemic administration of sibutramine on food intake by normal and MSG-OB female rats. Each value is the mean ± SD for 10 rats. #: p<0.05 compared with the saline-treated group. **: p<0.01 compared with each control group.
**Table 3.** Effects of chronic systemic administration of sibutramine on hypothalamic norepinephrine, DOPAC, dopamine, DOPAC/ dopamine, 5-HIAA, 5-HT, 5-HIAA/5-HT and HVA levels in normal and MSG-OB female rats

<table>
<thead>
<tr>
<th>Neonatal treatment</th>
<th>Drug</th>
<th>Dose (mg/kg, p.o.)</th>
<th>NE</th>
<th>DOPAC</th>
<th>DA</th>
<th>DOPAC/DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
<th>5-HIAA/5-HT</th>
<th>HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated</td>
<td>Control</td>
<td>1143 ± 227</td>
<td>104 ± 26</td>
<td>558 ± 124</td>
<td>0.187 ± 0.032</td>
<td>309 ± 80</td>
<td>581 ± 105</td>
<td>0.541 ± 0.160</td>
<td>37 ± 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>930 ± 185*</td>
<td>127 ± 38</td>
<td>536 ± 134</td>
<td>0.244 ± 0.073</td>
<td>275 ± 59</td>
<td>547 ± 95</td>
<td>0.523 ± 0.174</td>
<td>39 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>927 ± 142**</td>
<td>117 ± 49</td>
<td>525 ± 236*</td>
<td>0.246 ± 0.115</td>
<td>239 ± 58*</td>
<td>548 ± 79*</td>
<td>0.437 ± 0.103*</td>
<td>38 ± 11</td>
<td></td>
</tr>
<tr>
<td>MSG-treated</td>
<td>Control</td>
<td>843 ± 224*</td>
<td>78 ± 21#</td>
<td>420 ± 97#</td>
<td>0.191 ± 0.055</td>
<td>265 ± 22#</td>
<td>475 ± 81#</td>
<td>0.574 ± 0.113</td>
<td>31 ± 9#</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>680 ± 227*</td>
<td>63 ± 21</td>
<td>352 ± 103</td>
<td>0.178 ± 0.027</td>
<td>222 ± 28</td>
<td>437 ± 54</td>
<td>0.512 ± 0.069</td>
<td>30 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>624 ± 164**</td>
<td>87 ± 22</td>
<td>332 ± 139*</td>
<td>0.215 ± 0.081</td>
<td>207 ± 39**</td>
<td>429 ± 85*</td>
<td>0.506 ± 0.165*</td>
<td>29 ± 11</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD (ng/g wet weight of tissue) for 10 rats. #: p<0.05 compared with saline-treated and the MSG-treated crossed group, since interaction was not significant on ANOVA. +: p<0.1, *: p<0.05, **: p<0.01 compared with the crossed-control group, since interaction was not significant on ANOVA.

**Table 4.** Effects of chronic systemic administration of sibutramine and methamphetamine on spontaneous motor activity rhythms of normal and MSG-OB female rats

<table>
<thead>
<tr>
<th>Neonatal treatment</th>
<th>Drugs</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Period (hr)</th>
<th>Amplitude (logarithm)</th>
<th>Phase (hr)</th>
<th>Mean activity (logarithm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated</td>
<td>Control</td>
<td>23.58 ± 0.27</td>
<td>0.661 ± 0.257</td>
<td>2.61 ± 1.03</td>
<td>5.25 ± 0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>23.44 ± 0.06</td>
<td>0.985 ± 0.402*</td>
<td>3.22 ± 0.26</td>
<td>6.16 ± 0.37**</td>
<td></td>
</tr>
<tr>
<td>MSG-treated</td>
<td>Control</td>
<td>24.01 ± 0.62</td>
<td>0.506 ± 0.346</td>
<td>0.63 ± 3.45</td>
<td>4.40 ± 0.28#</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 3</td>
<td>23.71 ± 0.52</td>
<td>0.645 ± 0.285</td>
<td>1.37 ± 1.80</td>
<td>5.66 ± 0.41**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sibutramine 10</td>
<td>23.67 ± 0.33</td>
<td>1.089 ± 0.236*</td>
<td>0.97 ± 3.49</td>
<td>5.33 ± 0.20**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methamphetamine 10</td>
<td>23.94 ± 0.21</td>
<td>1.070 ± 0.184*</td>
<td>-1.72 ± 4.08</td>
<td>5.46 ± 0.39**</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD for 4 rats. **: p<0.01 compared with the control group treated with saline neonatally. *: P<0.05, **: P<0.01 compared with each control.

**Fig. 3.** Representative double plot of effects of sibutramine and methamphetamine on spontaneous motor activity rhythm of normal and MSG-OB female rats. Control (distilled water), administration of sibutramine 10 mg/kg p.o. and administration of methamphetamine 10 mg/kg p.o. for 14 days to MSG-OB rats.
and movement (amplitude) in the active phase, but affected neither the diurnal rhythm nor the phase in either the normal or the MSG-OB group (Fig. 3, Table 4).

**Discussion**

In the present study we found that 12-week-old MSG-OB female rats neonatally treated with MSG exhibited obesity with reduced body weight and body length and increased Lee index, compared with control normal rats neonatally treated with saline. An increase in retroperitoneal white adipose tissue weight was also found in MSG-OB rats. Food intake by MSG-OB rats during the 14-day period from 10 through 12 weeks of age was smaller than that by saline-treated rats. Hypothalamic levels of norepinephrine, dopamine and 5-HT, and their metabolites, DOPAC, HVA and 5-HIAA, in the MSG-OB group were lower than in the normal group. These observations are consistent with the results of many studies [21, 31, 34–36, 46].

Centrally-acting antiobesity drugs now clinically used can be classified into serotonergic drugs, such as fluoxetine and fenfluramine, and adrenergic drugs, such as mazindol, and these drugs are thought to exert antiobesity effects in the hypothalamus [41]. Acute or chronic administration of MA uptake inhibitors increases MA in the synapse by inhibiting MA uptake. As a result, metabolic turnover of MA is decreased by reduction and feedback of MA receptors, with a decrease in tissue concentrations of MA and its metabolites. Methamphetamine, which releases MA, and pargyline, which suppresses MA oxidase, change MA and MA metabolite levels severalfold [18, 28]. On the other hand, the changes caused by MA uptake inhibitors in the MA level in cerebellar tissue were statistically almost insignificant [17, 22, 24, 26, 28]. Wortley et al. and we have already reported that sibutramine strongly inhibited reuptake of 5-HT, norepinephrine and dopamine in mice *in vivo*, and caused large increases in extracellular 5-HT and norepinephrine levels and a small increase in the dopamine level, as measured by microdialysis, in the hypothalamus and striatum of rats [28, 33, 44]. Inhibitory effects of sibutramine on this MA reuptake have also been confirmed by *in vitro* studies [5]. It was reported that sibutramine significantly reduced adrenaline $\beta_1$- and $\alpha_2$- receptors and tended to decrease the number of dopamine D2-receptors [5, 17, 26]. The decreases in MA and its metabolite concentrations in the tissue of MSG-OB rats appear to be attributable to the destruction of the VMH and ARC in the hypothalamus. Slight decreases in the concentrations of norepinephrine, 5-HT and dopamine and its metabolites in hypothalamic tissue observed after chronic administration of sibutramine to normal and MSG-OB rats were assumed to be due to the MA uptake inhibition by sibutramine in the hypothalamic areas which had not been destroyed, which promoted function of the MA nervous system by increasing MA levels in synapses.

Consecutive daily administration of sibutramine at 3 and 10 mg/kg p.o. for 14 days induced body weight loss and improved the Lee index in normal and MSG-OB female rats. According to Bray, food intake and sympathetic nerve activity play important roles in obesity. These activities are controlled by various nuclei in the hypothalamus [4]. Food intake was dose-dependently suppressed by systemic administration of a dopamine release drug, amantadine, or by microinjection of this drug into the LH. Furthermore, chronic administration of amantadine decreased the body weight of obese rats administered sulpiride, a dopamine antagonist [1]. Systemic administration of fluoxetine, which specifically suppresses 5-HT uptake, not only suppressed food intake but also increased the extracellular 5-HT concentration in the PVN [37]. Leibowitz reported that microinjection of dopamine and norepinephrine into the LH strongly suppressed food intake, but that food intake was increased by injection of these agents into the PVN [23–25]. This increase in food intake caused by norepinephrine was antagonized by microinjection of 5-HT and fluoxetine into the PVN [13, 42]. It was reported that microinjection of neuropeptide Y into the PVN increased food intake, but did not affect food intake when administered into the LH and VMH [8].

Sakaguchi and Bray recorded the firing rate of sympathetic nerves innervating interscapular brown adipose tissue (BAT) after microinjection of 5-HT or norepinephrine into the VMH or PVN [38]. They found that these drugs produced marked dose-dependent increases in the firing rate of sympathetic nerves. It has been reported that in VMH-lesioned MSG-OB rats, lipolysis and thermogenesis are decreased in association with weakened sympathetic activity by lesions in the VMH; these changes are considered to be reasons for obesity in MSG-OB rats despite decreased food intake [10, 45].
It was reported that microinjection of neuropeptide Y into the PVN suppressed sympathetic nerve activity in BAT, but that injections of this agent into the LH and VMH did not do so [12]. Nevertheless, the results of experiments have not always reached consistent conclusions, since food intake and sympathetic nerve activity are controlled through various mechanisms including MAIs such as 5-HT, dopamine and norepinephrine as well as peptides. The mechanism of control of various nuclei in the hypothalamus therefore requires further investigation.

Edwards et al. reported that chronic systemic administration of 5-HT, which does not cross the blood-brain barrier, and must therefore act peripherally, induced body weight losses in male and female rats, but that a food intake-suppressing effect of 5-HT was found in male rats but not female rats [11]. For this reason, we used female rats in the present study but a marked decrease in food consumption caused by the administration of sibutramine was observed for both normal and MSG-OB female rats. This suggests that peripheral and central effects of some factor other than 5-HT are involved in the suppression of food intake due to sibutramine. Similar findings of suppression of food intake by sibutramine have been observed for obese rats fed a high-fat diet, genetically obese Zucker rats, obese-diabetic ob/ob mice and normal rats [9, 20, 39, 40].

The monoaminergic effects of sibutramine also explain the finding that suppression of food consumption by sibutramine is antagonized by pretreatment with blockers of 5-HT_{2A/2C} receptors, β_{1} adrenoreceptors, and particularly α_{1} adrenoreceptors [19]. Interestingly, Jackson et al. reported that low-dose administration of either the selective 5-HT reuptake inhibitor fluoxetine or the selective norepinephrine reuptake inhibitor nisoxetine did not suppress food intake, but that combined administration of the two noticeably suppressed food intake [20]. The food intake-suppressing effect of sibutramine therefore appears to be due to a synergistic effect of noradrenergic and serotonergic systems resulting from norepinephrine and 5-HT reuptake inhibition. Tolerance did not occur in food intake suppression since interaction of sibutramine x day on ANOVA for food intake was not significant. We believe that the food intake-suppressing effect of sibutramine mediated via norepinephrine, 5-HT and dopamine reuptake inhibition continued for 14 days and caused body weight losses via stimulation of energy expenditure.

It was reported that systemic administration of sibutramine antagonized the food intake increase induced by administration of neuropeptide Y into the PVN [15]. This also suggested the possibility of antagonism by sibutramine of the suppression of the sympathetic nervous system activity in BAT induced by microinjection of neuropeptide Y into the PVN [12]. In fact, sibutramine, similarly to β_{1} adrenoreceptor agonists, enhances oxygen uptake, glucose utilization and thermogenesis in BAT [6]. Studies of microinjection of sibutramine into hypothalamic areas and electrophysiological determination of the sympathetic nervous system activity in BAT should be performed in order to elucidate further the elevation of sympathetic nerve activity caused by sibutramine in future.

Corresponding to previous report, the results of this experiment indicated a decrease in spontaneous motor activity by MSG-OB rats [34]. Daily administration of sibutramine at 10 mg/kg p.o. or of methamphetamine (MA releaser) at 10 mg/kg p.o. for 14 consecutive days increased the amplitude of the diurnal motor activity rhythm and the mean motor activity of normal and MSG-OB rats. These findings suggest that sibutramine enhanced motor activity in the active phase rather than in the resting phase. Many studies have indicated the participation of MA in the motor activity rhythm. Methamphetamine increases spontaneous motor activity via release of dopamine from the corpus striatum [33]. We reported that sibutramine has dopaminergic effects, since it increased the extracellular dopamine level in the rat corpus striatum as measured by a microdialysis method, induced ipsilateral circling behavior in rats in which the corpus striatum had been lesioned unilaterally by means of 6-hydroxydopamine, and antagonized reserpine-induced suppression of conditioned avoidance behavior [19, 28, 32, 33].

These findings suggest that the reversal by sibutramine of the decrease in rat motor activity caused by neonatal MSG treatment is due to dopamine reuptake inhibition in the striatum in addition to inhibition of MA reuptake in the hypothalamus. Tolerance to sibutramine did not occur in the increase in spontaneous motor activity, since the interaction of sibutramine x day on ANOVA for motor activity was not significant. We believe that the increase in spontaneous motor activity caused by sibutramine via MA reuptake inhibition in rat striatum
and hypothalamus continued for 14 days and caused body weight losses via an increase in energy expenditure.

The reaction of the blood GH level to GH secretagogue loading is low in obese patients, but is said to be restored when such patients lose body weight and decrease their plasma lipid levels by dieting [43]. Therefore, the improvement in secretion of GH into plasma in GHRP loading tests and the decrease in the plasma lipid level after consecutive administration of sibutramine to MSG-OB rats appear to be secondary effects accompanying improvements such as decreased body fat.

These results suggest that chronic administration of sibutramine inhibited MA uptake in the nigrostriatal area and the hypothalamus which was not destroyed in MSC-OB rats, which increased function of the MA nervous system by increasing MA levels in synapses and thereby suppressed food intake and increased sympathetic nerve activity as well as spontaneous motor activity. In this way sibutramine decreased the Lee index, body weight and retroperitoneal white adipose tissue weight, and improved the secretion of GH into plasma in GHRP loading tests and decreased plasma lipid levels.

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References


