Central melanocortin system modulates energy intake and expenditure of obese and lean Zucker rats

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Central melanocortin system modulates energy intake and expenditure of obese and lean Zucker rats. The genetically obese Zucker rat (Leprfa/Leprfa) has been used extensively as an animal model to study factors associated with dysfunctional energy balance (6) and type II diabetes (37). Adult obese Zucker rats exhibit overt obesity, hyperphagia, hypercholesterolemia, hyperlipidemia, and hyperglycemia as an autosomal recessive trait (6). The Leprfa mutation that is responsible for obesity in the Zucker rat is a missense mutation (Gln269→Pro) in the gene encoding the leptin receptor (23). Leptin is an adipocyte-derived hormone whose serum level is directly proportional to body-fat mass (8). Leptin decreases energy intake and increases energy expenditure via activation of leptin receptors in the hypothalamus (30). The long isoform of the leptin receptor with the (Gln269→Pro) mutation leads to reduced leptin binding affinity and defective leptin signaling through the JAK-STAT pathway in vitro (11). Unlike the Koletsky obese rats, which have a null mutation of all leptin receptors, Zucker obese (Leprfa/Leprfa) rats have inconsistent in vivo responses to exogenous intracerebroventricular (icv) administration of leptin (45).

Recent evidence suggests that the hypothalamic melanocortin system is directly influenced by leptin to regulate food intake and body weight. Leptin activates neuropeptide Y (NPY)/agouti-related peptide (AGRP)-containing neurons and proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART)-containing neurons of the ventromedial and ventrolateral arcuate nucleus, respectively (4, 9, 12), via activation of the long isoform of the leptin receptor that is expressed in these neurons. Central infusion of leptin stimulates expression of the POMC gene while reducing the expression of the AGRP gene in the arcuate nucleus (31, 39, 40). Furthermore, animals with deficiencies in leptin or leptin-signaling pathways have elevated AGRP mRNA but reduced POMC mRNA in the arcuate nucleus (26, 43, 46).

POMC- and AGRP-containing neurons have been demonstrated to regulate energy homeostasis through modulation of melanocortin receptors (10, 42, 44). There are five melanocortin receptors (MC1–MC5), three of which (MC3–MC5) have been shown to be expressed in the brain (28, 32, 33). α-Melanocyte-stimulating hormone (α-MSH), a POMC gene product, stimulates central MC4 receptors to reduce food intake and weight gain (2, 29), whereas AGRP inhibits central MC4 and/or MC3 receptors to increase food intake and weight gain (17, 47).

Several studies show evidence that the degree of MC4-receptor activation was determined by the balance of α-MSH and AGRP neurotransmission, which plays a key role in the maintenance of energy balance in vivo. For example, deletion of the MC4 receptors in mice leads to hyperphagia, hyperinsulinemia, hyperglycemia, and delayed-onset obesity (21). Mice with ectopic expression of agouti protein, which is homolo-
gous to AGRP and antagonizes the central MC₄ receptors and the peripheral MC₃ receptors, develop hyperphagia, hyperinsulinemia and obesity (29, 34). In addition, a selective MC₄-receptor agonist (Ro27–3225) reduces food intake and weight gain in rodents without producing any nonspecific aversive effect (5). Furthermore, mutations of the MC₄ receptor and genetic defects in the POMC gene are associated with marked obesity in humans (19, 20, 27, 41). These studies support the contention that the MC₄ receptor plays an important role in weight regulation in animals and humans. In contrast, central administration of γ-MSH, an MC₃ agonist, did not alter food intake or weight gain (1). However, mice with MC₃-receptor deletion develop mild obesity with elevated fat deposition (7). These data suggest that MC₃ may be a factor regulating nutrient partitioning in mice.

The present study compares the central effects of a melanocortin receptor agonist and antagonist on feeding and energy expenditure regulation in lean vs. obese Zucker rats. Although several studies have reported effects of the melanocortin system on feeding behavior (14, 25, 36), the effects on energy metabolism have not been explored in detail. Our data demonstrate that central administration of a melanocortin receptor agonist (MTII) promotes weight loss in both lean and obese Zucker rats by affecting both food intake and energy metabolism. These data also demonstrate that obese Zucker rats have significantly reduced endogenous melanocortin tone that may contribute to the obese/diabetic syndrome of these animals.

METHODS

Animals. Age-matched male Lepr<sup>fa</sup>/Lepr<sup>fa</sup> and lean (+/+ or +/Lepr<sup>fa</sup>) Zucker rats (Charles River, MA), were housed individually and maintained in a temperature- and light-controlled environment on a 12:12-h light-dark cycle (lights on at 4:00 AM). Animals had free access to food and water. All studies were conducted in an American Association for Accreditation of Laboratory Animal Care accredited facility following protocols approved by the Schering-Plough Research Institute’s Animal Care and Use Committee. The procedures were performed in accordance with the principles and guidelines established by the National Institutes of Health for the care and use of laboratory animals.

Surgery. A single 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was chronically implanted into the lateral ventricle of rats under ketamine-xylazine (100:10 mg/kg ip) anesthesia using the following coordinates: 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 3.6 mm ventral to dura (22). The cannula was secured to the surface of the skull with jeweler’s screws and dental cement, and a 28-gauge obturator was inserted into the cannula to maintain patency. After a 3-wk recovery period, all animals were tested for cannula placement by central infusion of 0.3 nmol of NPY. Only animals demonstrating a prompt and robust feeding effect (>2.0-g intake within 60 min of infusion) were retained for the study.

Peptide and injections. MTII [Ac-Nle⁴-c[Asp⁵, d-Phe⁷, Ly⁵⁰c-MSH(₄–₁₀)-NH₂] and SHU9119 [Ac-Nle⁴-c[Asp⁵, d-Nal(₂)/₇, Ly⁵⁰c-MSH(₄–₁₀)-NH₂] were obtained from Phoenix Pharmaceuticals (Belmont, CA). Sterile saline (0.9% NaCl) or MT-II/SHU9119 in saline was infused icv in a total volume of 2.5 µl over 1 min using a Bioanalytical Systems Bee Syringe Pump (West Lafayette, IN). The 28-gauge infusion cannulus (Plastics One) projected to 4.6 mm below the surface of the skull. The infusion cannulus were left in place for an additional minute following the infusion.

Indirect calorimetry. Oxygen consumption (V<sub>O₂</sub>) and carbon dioxide production (V<sub>CO₂</sub>) were monitored every 15 min (settle time: 155 s; measure time: 45 s) for 22 h with the use of an indirect calorimeter (Oxymax, Columbus Instruments, Columbus, OH). Measurements were taken in an airtight Oxymax chamber (10.5 l) with an air flow rate of 2.75 l/min. Respiratory quotient (RQ) was calculated as the molar ratio of V<sub>CO₂</sub> to V<sub>O₂</sub>. Water and food were available ad libitum in

Fig. 1. Differential effects of MTII (0.01–0.1 nmol icv; A) and SHU9119 (0.1–1 nmol icv; B) on daily food intake of lean and obese Zucker rats. Values are means ± SE (<i>n</i> = 8). **Significantly different from saline control group (<i>P</i> < 0.05). **Significantly different between lean and obese control group (<i>P</i> < 0.05).
the calorimeter chamber. Percent energy fuel derived from carbohydrate or fat oxidation was determined from \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) using the methods of Elia and Livesey (13).

**Animal activity monitor.** Physical activities of the rats were monitored by an infrared photocell beam-interruption method (Opto-varimex-minor, Columbus Instruments, Columbus, OH). The monitor interfaced with a computer and recorded the horizontal, vertical, and ambulatory activities every 15 min.

**Experimental design.** Age-matched male lean and obese Zucker rats were used in the study. In the MTII study, the obese and lean Zucker rats were \( 604 \pm 5 \) and \( 410 \pm 17 \) g, respectively. In the SHU9119 study, the obese and lean Zucker rats were \( 751 \pm 5 \) and \( 504 \pm 15 \) g, respectively. Each subject was adapted to the experimental conditions by being placed in a calorimeter chamber for 3 days before testing and continuously monitored in the same calorimeter chamber during the experiment. icv-Cannulated obese and lean Zucker rats were infused with MTII/saline at 1000 or SHU9119/saline at 1500. The nocturnal cycle extended from 1600 to 0400. The effects of either saline, SHU9119 (0.1–1 nmol) or MTII (0.01–1 nmol) icv infusion on feeding and body weight were monitored 23 h after the icv infusion. \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were recorded every 15 min for 22 h.

**Statistical analysis.** Results are given as means \( \pm SE \). All statistical analyses were performed with JMP software (version 3.1.6) from SAS Institute. For each endpoint, a mixed model of ANOVA was performed. Two-factor ANOVA was used to assess significant interaction between drug effect and the obese phenotype. If there was significant interaction between drug effect and the obese phenotype, the drug effect on each phenotype was analyzed independently by one-way ANOVA. Dunnett’s adjustment was used to account for multiple comparisons with the type 1 error rate of 0.05 partitioned into equal amounts for comparisons within each animal type. Statistical significance (\( P < 0.05 \)) of a comparison was assessed by comparing the difference in least-squares means with the appropriate estimate of variability from the ANOVA. The dose that reduced daily food intake to 50% (ID\(_{50}\)) data were analyzed by linear regression analysis of log drug doses and drug responses by Excel 97.

**RESULTS**

**Effects of MTII and SHU9119 on food intake.** Obese Zucker rats had significantly higher daily food intake than the lean Zucker rats (Fig. 1, A and B). However, when the food intake was normalized to the metabolic body size (body weight\(^{0.75}\)), both lean and obese Zucker rats had similar food consumption per metabolic body mass (42.5 ± 3.7 and 42.1 ± 3.8 g/kg\(^{0.75}\) for lean and obese rats, respectively). icv Administration of the melanocortin receptor agonist MTII (0.01–0.1 nmol) significantly reduced daily food intake of both obese and lean Zucker rats. A significant interaction between MTII doses and phenotypes was identified by two-factor ANOVA. The ID\(_{50}\) dose of MTII in obese Zucker rats was 0.028 nmol (logID\(_{50}\) = −1.56 ± 0.03 nmol),

![Fig. 2. Metabolic effects of MTII (0.01–0.1 nmol icv) on average O_2 consumption rate (A) during the first 3 h after dosing, hourly O_2 consumption rate (B) in saline vs. MTII (0.1 nmol)-treated obese Zucker rats, and average total physical activity (C) during the first 3 h after dosing. Values are means ± SE (n = 8). *Significantly different from saline control group. **Significant difference between lean and obese control group (P < 0.05).](http://ajpregu.physiology.org/)

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which was significantly lower than that of the lean Zucker rats (0.1 nmol, logID\(50 = -0.92 \pm 0.20\) nmol). In contrast, icv administration of the melanocortin receptor antagonist SHU9119 (1 nmol) caused a 49% increase in daily food intake in the lean Zucker rats but reduced the daily food intake of obese Zucker rats to the level of lean Zucker rats (Fig. 1B).

**Effects of MTII and SHU9119 on energy metabolism.** Whole body energy metabolism was continuously monitored using an open-circuit calorimeter after icv administration of MTII or SHU9119. The daily averaged \(V_O^2\) of the obese Zucker rats was significantly lower than that of the lean Zucker rats (Fig. 2). Icv Administration of MTII dose dependently elevated average \(V_O^2\) for 3 h in the obese Zucker rats but not in the lean Zucker rats (Fig. 2, A and B). The transient increase in \(V_O^2\) of the obese Zucker rats correlated well with the increase in 3-h total activity recorded concomitantly (Fig. 2C). Six hours after MTII or saline infusion, MTII-treated obese Zucker rats had \(V_O^2\) similar to the saline-treated obese Zucker rats (Fig. 2B). Therefore, the 22-h average \(V_O^2\) of both lean and obese Zucker rats treated with various doses of MTII was not statistically different from their saline controls (Table 1). To dissociate the effect of MTII on food intake from its effects on energy metabolism, we pair fed the obese Zucker rats with the same amount of food consumed during the MTII (0.1 nmol) experiment. The pair-fed lean and obese Zucker rats both had significantly lower \(V_O^2\) than the MTII-treated rats (Table 1). After icv administration of SHU9119 (0.1–1 nmol) to obese or lean Zucker rats (Table 1), we did not observe any significant change in \(V_O^2\) despite significant elevation of food intake of lean Zucker rats with 0.1 nmol of SHU9119. However, there was a trend for SHU9119-treated lean and obese rats to reduce \(V_O^2\) during the dark cycle.

In addition to changes in \(V_O^2\) rate, we also monitored the RQ after icv infusion of MTII or SHU9119 in the lean and obese Zucker rats. The obese Zucker rats had a significantly higher 22-h RQ than the lean Zucker rats (Fig. 3A). After icv infusion of MTII (0.01–0.1 nmol), the RQ of both lean and obese Zucker rats declined in a dose-dependent manner with a significant interaction between MTII doses and the obese phenotype in two-factor ANOVA analysis (Fig. 3A). The obese Zucker rats were more sensitive to the MTII-induced reduction of RQ than the lean Zucker rats, with a minimal effective dose of 0.03 and 0.1 nmol for the obese and lean rats, respectively (Fig. 3A). Analysis of energy fuel usage showed that MTII decreased the proportion of expended energy derived from carbohydrate and increased the proportion derived from fat in both lean and obese Zucker rats (Fig. 3, B and C). Moreover, icv administration of SHU9119 (1 nmol) significantly increased RQ of lean Zucker rats but decreased the RQ of the obese Zucker rats (Fig. 4).

**Effects of MTII and SHU9119 on weight gain.** A single icv infusion of MTII (0.01–0.1 nmol) to both the lean and obese Zucker rats resulted in a dose-dependent reduction of 24-h weight gain (Fig. 5A). Body weight was significantly reduced in lean and obese Zucker rats after icv infusion of MTII (0.1 nmol) by 3.2 ± 0.3% and 4.4 ± 0.4% reduction, respectively. Icv infusion of SHU9119 (0.1–1 nmol) increased weight gain dose dependently in both lean and obese Zucker rats (2-factor ANOVA, \(P < 0.01\)). Body weight was increased in lean and obese Zucker rats after icv infusion of SHU9119 (0.1 nmol) by 2.3 ± 0.8% (\(P < 0.05\)) and 0.54 ± 0.17% (\(P = 0.053\)), respectively (Fig. 5B).

**DISCUSSION**

In this study, we have demonstrated that acute icv administration of MTII, a melanocortin receptor agonist, induced weight loss in both obese and lean Zucker rats by reducing food intake, increasing \(V_O^2\) and promoting the usage of fat as energy substrate. Obese Zucker rats were more sensitive to the effects of MTII than lean Zucker rats. These data not only indicate that both lean and obese Zucker rats have functional melanocortin receptors, but they also suggest that the effects of melanocortin receptor activation by MTII on energy homeostasis may be independent of the leptin receptor. We also demonstrated that inhibition of melanocortin receptors by SHU9119 selectively increased weight gain, food intake, and RQ of the lean Zucker rats. However, SHU9119 normalized food intake and RQ of obese Zucker rats to the level of lean Zucker rats. The observation that obese Zucker rats

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**Table 1. Effect of MTII and SHU9119 on oxygen consumption of lean vs. obese Zucker rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, nmol</th>
<th>Lean</th>
<th>Obese</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC</td>
<td>DC</td>
<td>22 h</td>
<td>LC</td>
</tr>
<tr>
<td>MTII</td>
<td>0</td>
<td>949 ± 24</td>
<td>1,033 ± 22</td>
<td>991 ± 15</td>
<td>816 ± 29</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>920 ± 28</td>
<td>942 ± 17*</td>
<td>931 ± 15</td>
<td>939 ± 38</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>1,023 ± 58</td>
<td>970 ± 19</td>
<td>997 ± 33</td>
<td>993 ± 24</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>999 ± 24</td>
<td>1,020 ± 32</td>
<td>977 ± 18</td>
<td>997 ± 41*</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>0</td>
<td>855 ± 12†</td>
<td>930 ± 15†</td>
<td>892 ± 11†</td>
<td>820 ± 25†</td>
</tr>
<tr>
<td>SHU9119</td>
<td>0</td>
<td>713 ± 24</td>
<td>861 ± 38</td>
<td>787 ± 26</td>
<td>706 ± 14</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>715 ± 20</td>
<td>848 ± 21</td>
<td>785 ± 18</td>
<td>686 ± 8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>758 ± 27</td>
<td>811 ± 31</td>
<td>784 ± 27</td>
<td>676 ± 25</td>
</tr>
</tbody>
</table>

Values are means ± SE. \* \(P < 0.05\) compared with control (0 nmol) group. † \(P < 0.05\) compared with MTII (0.1 nmol) group. LC, light cycle; DC, dark cycle; pair-fed, rats dosed with saline and given the same amount of food consumed during MTII (0.1 nmol) experiment.
are more sensitive to the effects of MTII and less sensitive to the effects of SHU9119 suggests that obese Zucker rats have less endogenous melanocortin tone than lean Zucker rats. Thus reduction of melanocortin tone may contribute to the development of the obese phenotype in the obese Zucker rats.

The differential sensitivity between lean and obese Zucker rats to MTII and SHU9119 could be due to differences in the expression of the melanocortin receptor(s) or variation in the production of the endogenous agonist (α-MSH) or endogenous antagonist (AGRP). Recent evidence has shown that obese Zucker rats have an increased density of MC4 receptors in various hypothalamic regions (dorsomedial hypothalamic nucleus, ventromedial hypothalamic nucleus, arcuate nucleus of hypothalamus, and medial eminence) crucial to feeding and energy regulation compared with lean Zucker rats (16). In addition, obese Zucker rats have less α-MSH peptide in the PVN than lean Zucker rats (24). These data suggest that reduced endogenous melanocortin agonist production and elevated MC4 receptor levels in the obese Zucker rats may contribute to the increase in sensitivity to the MTII and the decrease in sensitivity to SHU9119 in these animals.

Direct administration of MTII to the paraventricular nucleus of the hypothalamus has been shown to increase VO2 of lean mice (10). In this study, we have shown that icv administration of MTII not only reduced energy intake but also affected energy expenditure and energy substrate usage in both lean and obese Zucker rats. By monitoring VO2 and by an indirect
calorimetric method, we have shown that MTII significantly reduced the RQ of both lean and obese Zucker rats. These data suggest that activation of melanocortin receptors promotes the use of fat as the preferred energy substrate while reducing carbohydrate usage (13). Despite the drastic 83% and 50% reductions in energy intake induced by MTII (0.1 nmol icv) in obese and lean Zucker rats, the 22-h averaged VO₂ in both obese and lean Zucker rats was not significantly different from the saline control group. The MTII (0.1 nmol icv)-treated rats had a significantly higher VO₂ rate than pair-fed rats, suggesting that MTII had direct effects on energy expenditure.

Numerous factors can influence the energy expenditure of an animal. Basal metabolic rate, body temperature, physical activity, and diet-induced thermogenesis all contribute to the regulation of energy expenditure (13). Central administration of α-MSH or MTII has been shown to induce transient hyperthermia in the brown fat, renal, and lumbar regions (18). Monitoring physical activity by infrared-photocell sensors, we have observed a simultaneous augmentation of physical activity in the obese Zucker rats during the first 3 h after MTII infusion. These data are consistent with the excessive grooming behavior in rodents after central activation of MC₄ receptors that was previously reported (3, 15). This activity-associated thermogenesis of obese Zucker rats probably was the predominant thermogenic source after MTII infusion. However, we cannot completely rule out the involvement of brown fat thermogenesis on energy expenditure after MTII infusion in obese Zucker rats, because brown fat is the major organ contributing to nonshivering thermogenesis in rodents. Hence, increases in physical activity, body temperature, and brown fat thermogenesis all may underlie the effect of MTII on energy expenditure.

RQ is an indication of the proportion of energy expenditure derived from fat and carbohydrate oxidation (13). In addition, the lipogenesis processes from both carbohydrate and protein require gas exchanges. The RQ of glucose and protein conversion into fat are 5.56 and 1.12, respectively. Therefore, when lipogenesis contributes to a significant part of the overall gas exchange, the RQ can reach higher than 1. The obese Zucker rats had a significantly higher RQ than lean Zucker rats, indicating a relatively low fat oxidation rate or a high rate of lipogenesis (13). It has been demonstrated that high 24-h RQ is one of the risk factors for body weight gain in the Pima Indians (48). Activation of melanocortin receptors by MTII reduced body weight by stimulating fat usage while decreasing carbohydrate usage in both lean and obese Zucker rats. However, inhibition of melanocortin receptors by SHU9119 increased weight gain by reducing fat usage and increasing carbohydrate usage specifically in the lean rats.

In conclusion, the hypothalamic melanocortin system plays a key role in the regulation of body weight, food intake, and energy metabolism. Central administration of the melanocortin receptor antagonist SHU9119 increases weight gain by elevating food intake and promoting fat deposition, especially in the lean Zucker rats. The weight loss induced by central infusion of MTII in both lean and obese Zucker rats is mediated by reducing food intake, lowering RQ, and elevating metabolic rate.

Perspectives

Obesity is a disorder of energy balance, arising from a chronic disequilibrium between energy intake and energy expenditure. Thus the optimal treatment for...
obesity would be one that both suppresses food intake and increases energy expenditure. Energy intake and energy expenditure are closely regulated processes, as reflected in the relative stability of body weight in the presence of large daily fluctuations in caloric intake. Energy balance is controlled by a complex system of metabolic pathways, integrated at the level of the central nervous system by a series of neurotransmitter signals. The central melanocortin system has been demonstrated to act downstream of leptin in the regulation of energy balance. We have demonstrated that obese and lean Zucker rats display differential sensitivities to exogenous melanocortin receptor agonists and antagonists. The apparent reduction in endogenous melanocortin tone in the obese rats that can be inferred from these data may contribute to the energy imbalance in these obese rats. Administration of melanocortin receptor agonist MTII not only reduces food intake and weight gain in both lean and obese Zucker rats but also elevates energy expenditure and fat usage in the obese Zucker rats. As mentioned above, this represents an ideal profile for an antiobesity agent. These data indicate that the obese rats have the potential to respond well to melanocortin agonists and suggest that the hypothalamic melanocortin system may be a potential target for pharmacological intervention in the treatment of obesity.

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REFERENCES


