A Pair-Feeding Study Reveals That a Y5 Antagonist Causes Weight Loss in Diet-Induced Obese Mice by Modulating Food Intake and Energy Expenditure


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ABSTRACT

Neuropeptide Y (NPY) is thought to have a significant role in the physiological control of energy homeostasis. We recently reported that an NPY Y5 antagonist inhibits body weight gain in diet-induced obese (DIO) mice, with a moderate reduction in food intake. To clarify the mechanism of the antiobesity effects of the Y5 antagonist, we conducted a pair-feeding study in DIO mice. The Y5 antagonist at 100 mg/kg produced a moderate feeding suppression leading to an 18% decrease in body weight, without altering body temperature. In contrast, the pair-fed group showed only a transient weight reduction and a reduced body temperature, thus indicating that the Y5 antagonist stimulates thermogenesis. The Y5 antagonist-treated mice showed an up-regulation of uncoupling protein mRNA in brown adipose tissue (BAT) and white adipose tissue (WAT), suggesting that both BAT and WAT contribute to energy expenditure. Thus, the Y5 antagonist induces its antiobesity effects by acting on both energy intake and expenditure.

Several lines of evidence have shown that the hypothalamus plays a key role in energy homeostasis, and a number of hypothalamic neuropeptides are involved in energy balance. Of these, neuropeptide Y (NPY), a 36-amino acid peptide neurotransmitter (Tatemoto et al., 1982), is one of the most potent orexigenic peptides identified. The physiological functions of NPY are mediated by activation of G protein-coupled receptors. Five NPY receptors have been cloned (Y1, Y2, Y4, Y5, and mY6) and Y1, Y2, and Y5 are abundantly expressed in the hypothalamus (Parker et al., 2002).

Accumulating evidence indicates that multiple NPY receptors regulate energy homeostasis. i.c.v.-injected Y1- or Y5-selective agonists stimulate feeding, whereas long-term treatments with both classes of agonists increase body weight and adiposity (Gerald et al., 1996; Mullins et al., 2001; Mashiko et al., 2003). In contrast, intraperitoneally injected Y2-selective agonists inhibit food intake and exhibit antiobesity effects (Batterham et al., 2002; Pöttner et al., 2004). Since Y2 acts as an inhibitory autoreceptor, Y2 activation results in reduced NPY release and attenuates the orexigenic effects of NPY (King et al., 1999). As expected, Y2 receptor-deficient mice exhibit an obese phenotype (Naveilhan et al., 1999). Selective Y1 receptor antagonists have been reported to suppress both NPY-induced and spontaneous food intake (Ishihara et al., 1998; Wieland et al., 1998; Kanatani et al., 2001), and NPY-induced food intake is significantly suppressed in Y1-deficient mice (Pedrazzini et al., 1998; Kanatani et al., 2000b). In addition, long-term treatment with a selective Y1 antagonist suppresses body weight gain in Zucker fatty rats (Ishihara et al., 1998). However, there are conflicting reports regarding the antiobesity effects of long-term treatment with Y5 antagonists. Two Y5 antagonists, CGP71683A and GW438014A, sup-
pressed body weight gain in DIO and genetically obese models (Criscone et al., 1998; Daniels et al., 2002), whereas NPY5RA-972 had no effect in DIO rats (Turnbull et al., 2002). We recently demonstrated that a selective Y5 antagonist produces antiobesity effects in mice that were developing DIO (Ishihara et al., 2006). We also demonstrated that the efficacy of the Y5 antagonist was Y5 mechanism-based using Y5 knockout mice and suggested that high and sustained brain Y5 receptor occupancy was required for the antiobesity effects. Thus, the differing results could be explained by non-Y5-mediated off-target actions or by differences in the pharmacodynamic profiles of these compounds. Interestingly, the antiobesity effects of the Y5 antagonist are specific to the DIO model; the Y5 antagonist was not effective in genetically obese models, such as Lepr<sup>ob/ob</sup> mice and Zucker fatty rats. Thus, the NPY-Y5 pathway might be specifically activated and play a key role in energy homeostasis in DIO mice due to the high-fat feeding conditions.

In this study, we further investigated the antiobesity mechanism of the Y5 antagonist by conducting a pair-feeding experiment in another DIO mouse model. For these studies, we used an established DIO mouse model, which had been fed a high-fat diet for about 10 months. It is difficult to evaluate the precise efficacy of an antiobesity agent in a developing DIO mouse model, since developing DIO mice show a large variation in body weight gain, due to differences in DIO sensitivity. In contrast, the established DIO mice show a stable obesity state and enable us to better define the efficacy of an antiobesity agent here. We show that the Y5 antagonist clearly reduced body weight in this established DIO mouse model, affecting both energy intake and expenditure.

**Materials and Methods**

**Materials.** Y5 antagonist 3,3-dimethyl-9-(4,4-dimethyl-2,6-dioxocyclohexyl)-1-oxo-1,2,3,4-tetrahydroxanthene (Kanatani et al., 2000a) was synthesized by Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan).

**Animals.** Male C57BL/6J mice (age 18 weeks; CLEA Japan Inc., Tokyo, Japan) were housed individually in plastic cages and were kept at 23 ± 2°C and 55 ± 15% relative humidity on a 12-h light/dark cycle (7:00 PM, lights off) for about 2 weeks before high-fat diet exposure. Water and regular chow (CE-2; CLEA Japan Inc.) were available ad libitum. All experimental procedures followed the Japanese Pharmacological Society Guidelines for Animal Use.

**Experimental Design.** Mice were fed a moderately high-fat (MHF) diet (Oriental BioService Co., Tokyo, Japan) for about 10 months before the drug treatment started. MHF diet provides 52.4% energy as carbohydrate, 15.0% as protein, and 32.6% as fat (4.4 kcal/g). MHF diet-fed mice were then divided into three groups, and each group was orally administered either vehicle (0.5% methylcellulose) or the Y5 antagonist at a dose of 100 mg/kg once daily for 1 month. Dosing was performed 1.5 h before the beginning of the dark period and after measurement of daily food intake and body weight.

In the pair-fed group, animals were fed the same amount of MHF diet as that consumed by the Y5 antagonist-treated animals over the preceding 24 h. This was divided into two meals and given at 8:00 AM and 6:00 PM to avoid long durations of fasting. Rectal temperature was measured on the 10th, 18th, 25th, and 30th days at 1:00 PM by insertion of thermoprobe.

After the final dosing, mice were fasted for 2 h, and blood samples were collected from infrarenal veins for leptin and insulin measurement, then the mice were euthanized by collecting whole blood under isoflurane anesthesia. Plasma biochemical and lipidic parameters were measured. Liver, white adipose tissue (WAT) (epididymal, retroperitoneal, and mesenteric), brown adipose tissue (BAT), and soleus muscle were collected for measurement of mRNA expression levels, and TG contents.

**Measurement of Hormone and Blood Chemistry.** Plasma glucose, TG, free fatty acid (FFA), and total cholesterol (TC), HDL-cholesterol (HDLC), and non-HDL-C levels were measured using the respective commercial kits (Determiner GL-E kit and Determiner L TGII, Kyowa Medex; Tokyo, Japan; NEFA-HA testwako(II), Wako Pure Chemicals, Tokyo, Japan; and Determiner L TCII, Determiner L HDL-C, and Determiner L LDL-C, Kyowa Medex). Insulin and leptin levels were measured by enzyme-linked immunosorbent assay (Moringa, Yokohama, Japan). T3 and T4 were measured by radioimmunoassay (Nihon Schering, Osaka, Japan).

**Measurement of TG Contents.** Total lipids in the liver were extracted by the procedure of Folch et al. (1957). After drying, the extracts were dissolved in isopropanol, and the TG content in the samples was measured enzymatically using a commercial kit (Determiner L TG II; Kyowa Medex).

**Measurement of Motor Activity.** Another set of DIO and lean C57BL/6J mice were prepared for measurement of spontaneous mo-

**Fig. 1.** Effects of the Y5 antagonist or pair feeding on body weight (a) and food intake (b) in DIO mice during 30-day treatment period. The Y5 antagonist at a dose of 100 mg/kg was administered once daily. In the pair-fed group, animals were fed the same amount of diet that was consumed by the Y5 antagonist-treated animals over the preceding 24 h. Values are means ± S.E.M. of 9–11 mice. **##,** P < 0.01 versus vehicle-treated ad libitum or pair-fed group.
tor activity. After the baseline measurement, mice were orally administered either vehicle or the Y5 antagonist at a dose of 100 mg/kg at 1.5 h before the beginning of the dark period. After the drug administration, motor activity was measured for 24 h using an activity monitoring system (NS-AS01; Neuroscience, Tokyo, Japan). The activity monitor was composed of an infrared ray sensor placed over each cage, a signal amplification circuit, and a control unit. The sensor detected the movement of the animal on the basis of the released infrared radiation associated with its body temperature. The motor activity data were collected at 10-min intervals and analyzed with a computer-associated analyzing system (AB system-24A; Neuroscience).

**TaqMan Analysis.** TaqMan assays using an ABI Prism 7900HT sequence detector (Applied Biosystems, Foster City, CA) was performed to determine mRNA levels of uncoupling protein (UCP)-1, -2, and -3, β3-adrenergic receptor (β3AR) in BAT and WAT, sterol regulatory element binding protein (SREBP)-1c in liver and WAT, muscle type carnitine palmitoyltransferase I (Cpt1b) in muscle, and type 1 iodothyronine deiodinase (Dio1) in liver. Total RNA was isolated from the liver, soleus muscle, mesenteric WAT, and BAT samples using ISOGEN reagent (Nippongene, Toyama, Japan). Internal standards were adapted from 18S ribosomal RNA for liver, WAT, and BAT transcripts and from β-actin for soleus muscle transcripts. Polymerase chain reaction and analysis were performed according to the manufacturer’s protocols. Sequences of primers and probes were as follows: Adrb3 (GenBank accession no. NM_013462): forward, CAAATCGGCGCTGCCTTG; reverse, AGAAAAGAGCAGGAGGAGGAGAG; TaqMan probe, 5’-6-carboxyfluorescein-TGCCCTCAACA TGCCCTATGGG-5’-6-carboxytetramethylrhodamine-3’; Dio1 (GenBank accession no. NM_007860): forward, CGAGTTCAAGAGACTGCTGATA GATGACT; reverse, GGCCTAGCTCCTTAATGTTA; and TaqMan probe, 5’-6-carboxyfluorescein-TGCCCTACAAGCCGGATT TCCCTA-5’-6-carboxytetramethylrhodamine-3’. Detailed conditions including additional sequences and fluorogenic probes for UCP-1, -2, -3; SREBP-1c; and Cpt1b were as described previously (Ito et al., 2003; Mashiko et al., 2003).

**Statistical Analysis.** Data are expressed as means ± S.E.M. Body weight changes were compared between groups using repeated measured two-way analysis of variance coupled with a post hoc Bonferroni/Dunn test. For food intake, blood parameters, tissue weights, and mRNA levels, one-way analysis of variance coupled with a post hoc Bonferroni/Dunn test. For food intake, blood parameters, tissue weights, and mRNA levels, one-way analysis of variance coupled with a post hoc Bonferroni/Dunn test was performed. P values of <0.05 were considered significant.

**Results**

**Effects of the Y5 Antagonist and Pair Feeding on Food Intake, Body Weight, and Rectal Temperature.** We administered the Y5 antagonist to DIO C57BL/6J mice that had been fed a MHP diet for 10 months and that exhibited stable obesity with body weights of 48.6 ± 0.7 g, whereas age-matched regular diet-fed mice weighed 33.8 ± 0.9 g. Oral administration of the Y5 antagonist, at a dose of 100 mg/kg once daily, significantly reduced body weight (p < 0.01) and food intake by about 10% (p < 0.01), compared with vehicle-treated mice (Fig. 1, a and b). In the pair-fed group, which were fed the same amount of food as the Y5 antagonist-treated group, similar body weight reductions were initially observed (p < 0.01). However, body weight reductions in the pair-fed group reached a plateau within a week, and body weight remained at a constant, reduced level during the rest of the experimental period (Fig. 1a). During this period, an approximately 0.5°C reduction in rectal temperature was observed in the pair-fed group compared with vehicle-treated animals (Fig. 2). In contrast, Y5 antagonist-treated mice showed no reductions in rectal temperature throughout the treatment period. After 30 days of treatment, the Y5 antagonist-treated mice weighed 41.4 ± 0.9 g, whereas the pair-fed mice weighed 47.9 ± 1.6 g, the vehicle-treated mice weighed 50.3 ± 1.3 g, and the regular diet-fed mice weighed 36.7 ± 2.6 g.

**Effects of the Y5 Antagonist and Pair Feeding on Liver and Adipose Tissue Weights and Liver TG Content.** At the end of drug treatment, we measured tissue weights and plasma biochemical parameters. The Y5 antagonist significantly decreased mesenteric adipose tissue weight (p < 0.01; Fig. 3a). Similar results were observed in epididymal and retroperitoneal fat pads (data not shown). In contrast to the Y5 antagonist-treated group, no significant reduction of adipose tissue weight was observed in the pair-fed group, even though their body weight was significantly reduced. Although liver weight was not affected by treatment with the Y5 antagonist, TG content in the liver was significantly decreased (p < 0.05; Fig. 3, b and c). The pair-fed group tended to show reductions in both liver weight and TG content.

**Effects of the Y5 Antagonist and Pair Feeding on Plasma Biochemical Parameters.** Treatment with the Y5 antagonist significantly decreased plasma TC and non-HDL-C levels (p < 0.01), but it did not affect plasma TG or FFA levels. These lipid parameters were not affected by pair feeding. Treatment with the Y5 antagonist significantly reduced plasma leptin levels (p < 0.01), whereas pair feeding had no significant effect (Table 1). Plasma insulin levels were also significantly reduced in Y5 antagonist-treated mice (p < 0.05).
0.01), whereas the pair-fed group did not show a significant reduction (Table 1). Plasma glucose levels tended to be reduced by treatment with the Y5 antagonist but not by pair feeding. Pair feeding produced a significant reduction in plasma T3 levels relative to the vehicle-treated group ($p < 0.05$), but it did not produce a significant change in plasma T4 levels. In contrast, the Y5 antagonist reduced plasma T4 levels by about half ($p < 0.01$), while maintaining plasma T3 levels.

**Effects of the Y5 Antagonist and Pair Feeding on mRNA Expression Levels in White and Brown Adipose Tissue, Liver, and Skeletal Muscle.** In BAT, Y5 antagonist treatment increased expression levels of UCP-1 ($p < 0.05$) and UCP-3 mRNA ($p < 0.01$). Pair feeding had no effect on these mRNA expression levels. $\beta_3$AR expression levels were comparable in all groups (Table 2).

In WAT, treatment with the Y5 antagonist significantly increased expression levels of UCP-3, $\beta_3$AR, and SREBP-1c mRNA levels ($p < 0.05$). UCP-1 mRNA expression level tended to increase with Y5 antagonist treatment. In contrast, pair feeding had no effect on any of these mRNA expression levels in WAT (Table 2).

In liver, treatment with the Y5 antagonist significantly decreased SREBP-1c ($p < 0.01$), and it significantly increased Dio1 expression levels ($p < 0.01$). Pair feeding had no effect on these mRNAs expression levels. In skeletal muscle, neither the Y5 antagonist nor pair feeding had any effect on expression of UCPs or the $\beta$-oxidation-related gene Cpt1b (Table 2).

**Effect of the Y5 Antagonist on Motor Activity in DIO and Lean Mice.** We measured the motor activity to evaluate whether the prevention of hypothermia was due to the increased motor activity. In another set of regular chow-fed lean mice or DIO mice, the Y5 antagonist at 100 mg/kg was orally administered, and the motor activity was measured for 24 h. The Y5 antagonist did not affect motor activity either in the light or the dark cycle for both lean and DIO mice (Fig. 4).

**Discussion**

Long-term treatment with the Y5 antagonist significantly decreased body weight by 18%, compared with the vehicle-treated group, with a moderate feeding suppression in the established DIO mice model used here. This body weight reduction lasted throughout the 30-day treatment period and was accompanied by reductions in fat weight and improvements in plasma biochemical/hormonal parameters. In addition, TG content in liver was significantly decreased, indicating that treatment with the Y5 antagonist ameliorated obesity-related fatty liver in DIO mice. These results clearly demonstrate that the Y5 antagonist has potent antiobesity effects in DIO mice and corroborate our previous findings that the Y5 antagonist inhibits body weight gain during the early stages of development of DIO (Ishihara et al., 2006). In contrast, the pair-fed group showed body weight reductions only in the beginning of the treatment period. Thereafter, body weight reductions stopped, and the reduced body weight was maintained throughout the experimental period. Also in the pair-fed group, a reduced rectal temperature was observed after body weight had reached a plateau, and this mild hypothermia persisted throughout the experimental period. It is known that feeding suppression in rodents causes reductions in body temperature and energy expenditure, as a compensatory mechanism to conserve energy (Gavrilova et al., 2006).

![Fig. 3. Effects of the Y5 antagonist or pair feeding on adipose tissue (a) and liver weight (b), and liver TG content (c) in DIO mice. Values are means ± S.E.M. of 9–11 mice. #, $p < 0.05$; ##, $p < 0.01$ versus vehicle-treated ad libitum or pair-fed group.](image-url)
al., 1999; Severinsen and Munch, 1999). Therefore, the reduction in rectal temperature observed in the pair-fed group is thought to compensate for the reduced food intake, and it may have assisted in maintaining the body weight in the pair-fed group. In contrast, reductions in rectal temperature were not observed in the Y5 antagonist-treated animals, despite the same reductions in food intake. This may account for the greater body weight reduction in the Y5 antagonist-treated group. In addition, the Y5 antagonist at 100 mg/kg did not affect motor activity in mice. Thus, the present pair-feeding study indicates that the antiobesity effectiveness of the Y5 antagonist treatment is due to both a moderate feeding suppression and an alteration in energy expenditure.

As target tissues for the Y5 antagonist-mediated thermogenic function, both BAT and WAT are likely to be important. Long-term treatment with the Y5 antagonist significantly increased mRNA levels of UCP-1 and UCP-3 in BAT and UCP-3 in WAT, and it tended to increase UCP-1 mRNA levels in WAT, whereas expression of UCPs and oxidation-related genes in skeletal muscle and liver were not affected. UCP-3 is thought to be involved in thermogenesis and fatty acid oxidation (Dulloo et al., 2004). Transgenic mice that overexpress UCP-1 showed resistance to obesity (Li et al., 2000), and transgenic mice that overexpress UCP-3 had a lean phenotype (Clapham et al., 2000). Thus, the increases in UCP-1 and UCP-3 mRNA expression levels suggest that energy expenditure in BAT and WAT was elevated by long-term treatment with the Y5 antagonist. It has been reported that centrally injected NPY, or a Y5-selective agonist, decreases BAT thermogenesis (Billington et al., 1991; Hwa et al., 1999), and long-term central infusion of a selective Y5 agonist significantly decreases UCP1 mRNA expression in BAT (Mashiko et al., 2003). These data suggest that the NPY-Y5 pathway may modulate a thermogenic function in BAT. However, since these data were obtained after a supractivation of the Y5 receptor, the physiological functions of the Y5 receptor to mediate energy expenditure remained undefined. Thus, the present findings obtained with the Y5 antagonist indicate a role for the Y5 receptor in energy expenditure under physiological conditions. In the present study, we demonstrated that blockade of the Y5 receptor prevented the compensatory reduction of rectal temperature, but it did not increase rectal temperature above the vehicle-treated group. Since the Y5 antagonist did not increase rectal temperature above normal levels, the Y5 receptor may not alter energy expenditure during normal conditions of energy balance. The Y5 receptor may sense a negative energy balance associated with hypophagia, function to suppress energy expenditure, and prevent further weight loss. In addition to the thermogenic function of UCP-1, recently Yamada et al. (2006) reported that ectopic expression of low levels of UCP-1 in epididymal fat decreased food intake and improved glucose tolerance in DIO mice via afferent nerve signals or humoral factors. Thus, this novel function of UCP-1 in WAT might also contribute to the antiobesity effects of the Y5 antagonist.

The Y5 antagonist also increased \( \beta_3 \)AR and SREBP-1c expression levels in WAT. \( \beta_3 \)AR is the most abundant adrenergic receptor in WAT and plays a key role in lipolysis via sympathetic stimulation. The reduction of \( \beta_3 \)AR is considered to be one of the causes of DIO development. Collins et al. (1997, 1999) reported that \( \beta_3 \)AR expression was reduced in genetic and dietary obesity in mice, and the degree of obesity was correlated with the extent of lost \( \beta_3 \)AR expression in adipocytes. Long-term treatment with the selective \( \beta_3 \)AR

### TABLE 1
Plasma biochemical parameters of Y5 antagonist-treated or pair-fed DIO mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>Y5 Antagonist</th>
<th>Pair-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>156.1 ± 6.4</td>
<td>116.7 ± 7.8**</td>
<td>136.9 ± 7.8</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>123.0 ± 4.1</td>
<td>104.1 ± 6.6</td>
<td>110.4 ± 6.0</td>
</tr>
<tr>
<td>Non-HDL-C (mg/dl)</td>
<td>24.2 ± 2.0</td>
<td>12.2 ± 1.2***</td>
<td>19.1 ± 1.4</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>20.4 ± 1.3</td>
<td>25.7 ± 2.1</td>
<td>22.7 ± 1.9</td>
</tr>
<tr>
<td>FFA ((\mu)Eq/l)</td>
<td>412.6 ± 23.2</td>
<td>456.2 ± 31.4</td>
<td>450.9 ± 19.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>205.5 ± 7.1</td>
<td>186.8 ± 10.9</td>
<td>195.8 ± 4.6</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>5.07 ± 0.50</td>
<td>1.74 ± 0.19***</td>
<td>4.00 ± 0.37</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>45.58 ± 2.63</td>
<td>19.25 ± 3.73***</td>
<td>38.07 ± 3.36</td>
</tr>
<tr>
<td>T3 (mg/ml)</td>
<td>0.74 ± 0.03</td>
<td>0.67 ± 0.04</td>
<td>0.63 ± 0.03*</td>
</tr>
<tr>
<td>T4 ((\mu)g/dl)</td>
<td>4.43 ± 0.17</td>
<td>2.02 ± 0.10***</td>
<td>4.15 ± 0.12</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \), ** \( p < 0.01 \) vs. ad libitum-fed vehicle-treated group.

### TABLE 2
mRNA expression levels of Y5 antagonist-treated or pair-fed mice in liver, BAT, WAT, and skeletal muscle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vehicle</th>
<th>Y5 Antagonist</th>
<th>Pair-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP-1</td>
<td>1.97 ± 0.22</td>
<td>2.75 ± 0.25***</td>
<td>1.72 ± 0.12</td>
</tr>
<tr>
<td>UCP-2</td>
<td>2.99 ± 0.22</td>
<td>3.18 ± 0.37</td>
<td>2.40 ± 0.23</td>
</tr>
<tr>
<td>UCP-3</td>
<td>0.53 ± 0.04</td>
<td>0.91 ± 0.05***</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>( \beta_3 )AR</td>
<td>1.83 ± 0.16</td>
<td>1.85 ± 0.11</td>
<td>1.58 ± 0.14</td>
</tr>
<tr>
<td>WAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP-1</td>
<td>12.91 ± 3.25</td>
<td>34.68 ± 14.43</td>
<td>9.32 ± 4.12</td>
</tr>
<tr>
<td>UCP-2</td>
<td>0.56 ± 0.05</td>
<td>1.08 ± 0.34</td>
<td>0.57 ± 0.07</td>
</tr>
<tr>
<td>UCP-3</td>
<td>0.21 ± 0.02</td>
<td>0.43 ± 0.08*</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>( \beta_3 )AR</td>
<td>1.65 ± 0.21</td>
<td>3.23 ± 0.38**</td>
<td>2.03 ± 0.33</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>0.72 ± 0.08</td>
<td>1.54 ± 0.28*</td>
<td>0.93 ± 0.17</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>5.34 ± 0.50</td>
<td>3.30 ± 0.25**</td>
<td>4.46 ± 0.41</td>
</tr>
<tr>
<td>Dio1</td>
<td>1.07 ± 0.07</td>
<td>1.54 ± 0.11***</td>
<td>0.99 ± 0.11</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP-2</td>
<td>0.60 ± 0.06</td>
<td>0.75 ± 0.08</td>
<td>0.51 ± 0.07</td>
</tr>
<tr>
<td>UCP-3</td>
<td>0.20 ± 0.02</td>
<td>0.16 ± 0.03</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Ctrlb</td>
<td>1.93 ± 0.12</td>
<td>1.60 ± 0.11</td>
<td>1.76 ± 0.12</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \), ** \( p < 0.01 \) vs. ad libitum-fed vehicle-treated group.

** \( p < 0.05 \), *** \( p < 0.01 \) vs. pair-fed vehicle-treated group.
agonist CL316,243 prevented the development of DIO and the decline in $\beta_3$AR mRNA levels in WAT (Collins et al., 1997). Thus, the increase of $\beta_3$AR expression may produce TG mobilization in WAT and contribute to reduced adiposity. However, the increased SREBP-1c expression level was unexpected. Since SREBP-1c is a transcriptional factor that regulates expression of genes related to lipogenesis, the increase of SREBP-1c seems to be inconsistent with reduced WAT weight. The physiological meanings of these contradictory changes are unknown at present. Perhaps there is compensatory up-regulation of SREBP-1c due to body weight reduction, or alternatively, perhaps there is simultaneous activation of lipogenesis and lipolysis leading to energy dissipation within WAT. Further study will be needed to elucidate the precise functions of these changes in WAT.

Plasma T3 and T4 levels are key factors in regulating metabolic rate (Silva, 1995). In the present study, pair feeding resulted in decreased plasma T3 levels. This is in agreement with reports showing that fasting reduces plasma thyroid hormone levels (Ahima et al., 1996). In contrast, the Y5 antagonist maintained plasma T3 levels similar to those in the vehicle-treated group. In addition, plasma T4 levels were significantly reduced in the Y5 antagonist-treated group, suggesting that the Y5 antagonist might stimulate the conversion of T4 to T3. In support of this observation, Dio1 mRNA levels in liver, which is the major site of conversion outside the thyroid, were significantly increased in the Y5 antagonist-treated group compared with the control and pair-fed groups. A similar phenomenon was reported for leptin; long-term i.c.v. administration of leptin in rats prevented the feeding reduction-induced decrease in plasma T3 levels by stimulating conversion of T4 to T3 in liver (Cusin et al., 2000). Therefore, as with leptin infusion, thyroid hormones may mediate the effects of the Y5 antagonist on energy expenditure.

We recently reported that the antiobesity effects of the Y5 antagonist are specific to the DIO model; the antagonist was not effective in lean rodents or genetically obese models, such as Leprdb/db mice and Zucker fatty rats (Ishihara et al., 2006). The variability in the pattern of NPY expression and its receptor in various rodent models may account for the DIO-specific effects. A DIO rodent shows increased expression of NPY mRNA in the dorsomedial hypothalamic and ventromedial hypothalamic nuclei (Guan et al., 1998), and increased density of Y2/Y5-like receptors in hypothalamus (Widdowson et al., 1997), whereas genetically obese rodents showed increased expression of NPY in the arcuate nucleus (Sanacora et al., 1990) and decreased density of Y2/Y5-like receptors (Widdowson, 1997; Xin and Huang, 1998). We hypothesize that the Y5 antagonist may elicit its antiobesity effects by improving leptin sensitivity, since the Y5 antagonist is ineffective in Zucker fatty rats and Leprdb/db mice in which leptin function is disrupted; thus, the Y5 antagonist produces DIO-specific effects (Ishihara et al., 2006). In support of this hypothesis, the Y5 antagonist produced some leptin-like peripheral metabolic effects in present study, such as increased conversion of T4 to T3 and stimulation of BAT and WAT thermogenesis. However, further investigation is necessary to elucidate the DIO-specific mechanisms of the Y5 antagonist.

In conclusion, our study demonstrates that the Y5 antagonist affects both energy intake and expenditure to produce potent antiobesity effects in the DIO mouse model. The Y5 antagonist may increase metabolic activity both in BAT and WAT via neural pathways or hormonal action. The present data indicate that the NPY-Y5 receptor pathway plays a key role in energy homeostasis under pathophysiological conditions and that a Y5 antagonist may have potential as an antiobesity agent in humans. Indeed, very recently, Erondu et al. (2006) reported that an NPY Y5 antagonist does actually produce body weight reductions in obese patients, although the magnitude of induced weight loss was not clinically meaningful. Species differences in energy homeostasis as well as different causes of obesity may pose a significant challenge to develop effective antiobese agents.

References


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