Responsiveness to Peripherally Administered Melanocortins in Lean and Obese Mice

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To elucidate mechanisms of melanocortin action, we investigated the effects of a melanocortin receptor agonist (melanotetan II [MTII]) in lean C57BL/6J and obese (DIO, ob/ob, UCP1-DTA) mice. MTII administration (100 µg q.i.d. i.p.) for 24 h results in similar weight loss but a more pronounced decrease of food intake in DIO mice. After 4 and 8 days of MTII treatment, however, the reduction in both food intake and body weight is more pronounced in DIO mice than in lean mice. MTII administration for 24 h prevents food deprivation-induced alterations in hypothalamic neuropeptide Y (NPY) and liver adiponectin receptor 1 and adiponectin receptor 2 mRNA expression, but does not alter hypothalamic mRNA expression of melanocortin 4 receptor or adiponectin serum and mRNA expression levels. NPY and agouti gene-related protein (AgRP) mRNA expression after 8 days of MTII is increased to levels comparable to pair-fed mice. In summary, 1) MTII is an effective treatment for obesity and related metabolic defects in leptin-resistant (DIO, UCP1-DTA) and leptin-sensitive (ob/ob) mouse models of obesity; 2) the effects of MTII on food intake and body weight are more pronounced in DIO mice than in lean mice; 3) the tachyphylactic effect after prolonged MTII administration appears to be, at least in part, caused by a compensatory upregulation of NPY and AgRP mRNA levels, whereas decreasing leptin levels may play a very minor role in mediating tachyphylaxis; and 4) alterations in adiponectin receptor mRNA expression after fasting or MTII treatment may contribute to altered insulin sensitivity and needs to be studied further.

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The melanocortin pathway, one of the direct targets of leptin action in the brain (1), plays an important role in energy homeostasis. Mice with targeted deletion of the pro-opiomelanocortin (POMC) gene (2) or the melanocortin 4 receptor (MC4R) gene develop obesity associated primarily with hyperphagia and hyperinsulinemia (3). In addition, MC3 receptor knockout mice have increased fat mass and reduced lean body mass, but normal food intake, suggesting defects in energy partitioning (4).

Peripheral or central administration of the synthetic nonselective melanocortin receptor agonist melanotetan II (MTII) to fasted or neuropeptide Y (NPY)-treated mice as well as obesity-prone or genetically obese animals, such as Sprague-Dawley rats or rhesus macaques, acutely and chronically suppresses food intake and increases sympathetic nervous system activity (5–9), whereas melanocortin antagonism has opposite effects (10–13). We recently reported that MTII treatment decreases body weight, primarily by suppressing food intake and secondarily by increasing energy expenditure, and improves insulin resistance in mice (14). However, the mechanisms underlying improvement of weight loss and insulin resistance, as well as the development of tachyphylaxis after prolonged MTII administration, remain largely unknown.

RESEARCH DESIGN AND METHODS

Male C57BL/6J (3-week-old) and ob/ob (6-week-old) mice were purchased from The Jackson Laboratories (Bar Harbor, ME). Male UCP1-DTA mice (6–8 weeks old; FVB background) were obtained from a colony maintained at the Beth Israel Deaconess Medical Center. All animals were individually caged and handled, as previously described (15).

Regular mouse chow (Purina Rodent Chow 5008;Ralston-Purina, St. Louis, MO) and water were available to all animals ad libitum, unless noted otherwise. For the long-term studies, a group of C57BL/6J mice received a Western diet for 12 weeks (WD1) (D12451; Research Diets, New Brunswick, NJ), and for the short-term study, C57BL/6J mice were fed either chow or a Western diet (WD2) (TD 88137; Harlan Teklad, Madison, WI) for 18 weeks before the experiments, as previously described (16,17).

Short-term studies (24 h)

Effects of short-term MTII administration in lean (C57BL/6J) mice (experiment A). A group of lean C57BL/6J mice (normal chow, n = 8/group) were injected with MTII (100 µg) four times a day (q.i.d.) intraperitoneally for 24 h. A PBS-treated (100 µg q.i.d. i.p.) group, fed ad libitum, and a placebo group (100 µl PBS q.i.d. i.p.), pair-fed to the MTII-treated group, were included as controls.

Comparative evaluation of short-term MTII administration in lean (C57BL/6J) and obese (DIO) mice (experiment B). To investigate whether MTII has similar effects on acute weight loss and reduction in food intake in lean (chow-fed) and DIO mice (WD2 for 18 weeks), MTII (100 µg q.i.d. i.p.) was injected for 24 h in both groups of mice. Control groups (PBS-treated and pair-fed to each of the MTII-treated groups) were also
TABLE 1
Effect of MTII administration on body weight and food intake

<table>
<thead>
<tr>
<th></th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight loss (g)</th>
<th>Cumulative food intake (g)</th>
<th>Cumulative food intake (kcal)</th>
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<tr>
<td>C57Bl/6J lean and obese</td>
<td></td>
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<tr>
<td>PBS/chow</td>
<td>32.20 ± 0.89</td>
<td>32.33 ± 1.09</td>
<td>0.30 ± 0.21</td>
<td>4.09 ± 0.30</td>
<td>14.30 ± 1.00</td>
</tr>
<tr>
<td>MTII/chow</td>
<td>32.10 ± 0.76</td>
<td>30.33 ± 0.75</td>
<td>1.42 ± 0.23‡</td>
<td>2.55 ± 0.21†</td>
<td>8.89 ± 0.72§</td>
</tr>
<tr>
<td>Pair-fed to MTII/chow</td>
<td>32.06 ± 0.61</td>
<td>30.18 ± 0.56</td>
<td>1.57 ± 0.32‡</td>
<td>2.55 ± 0.03‡</td>
<td>8.89 ± 0.08§</td>
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<tr>
<td>PBS/high-fat</td>
<td>44.07 ± 0.42</td>
<td>42.51 ± 0.72</td>
<td>1.43 ± 0.23¶†</td>
<td>2.83 ± 0.3</td>
<td>12.83 ± 1.36</td>
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<tr>
<td>MTII/high-fat</td>
<td>43.81 ± 0.76</td>
<td>41.30 ± 0.71</td>
<td>2.51 ± 0.21⁺‡</td>
<td>1.15 ± 0.22§‡‡</td>
<td>5.22 ± 1.00††</td>
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<tr>
<td>Pair-fed to MTII/high-fat</td>
<td>44.27 ± 1.08</td>
<td>42.20 ± 0.94</td>
<td>2.07 ± 0.19†‡</td>
<td>1.15 ± 0.01§</td>
<td>5.22 ± 0.03§</td>
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<td>Ob/ob</td>
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<tr>
<td>PBS</td>
<td>69.74 ± 10.5</td>
<td>69.38 ± 1.22</td>
<td>0.36 ± 0.28</td>
<td>9.7 ± 3.2</td>
<td>33.76 ± 11.14</td>
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<td>MTII</td>
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<td>67.45 ± 1.82</td>
<td>2.26 ± 0.24‡</td>
<td>6.4 ± 0.6</td>
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<td>UCPI-DTA</td>
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<td>PBS</td>
<td>40.04 ± 2.6</td>
<td>36.39 ± 2.6</td>
<td>3.65 ± 0.22†</td>
<td>3.09 ± 0.43</td>
<td>10.75 ± 1.50</td>
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<td>MTII</td>
<td>39.27 ± 2.2</td>
<td>34.48 ± 2.1</td>
<td>4.79 ± 0.22‡‡</td>
<td>1.78 ± 0.42‡</td>
<td>6.19 ± 1.46††</td>
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<td>Pair-fed</td>
<td>39.55 ± 2.0</td>
<td>34.01 ± 2.0</td>
<td>5.54 ± 0.22§</td>
<td>1.79 ± 0.01†</td>
<td>6.19 ± 0.03†‡</td>
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<td>DIO-C57Bl/6J 8-day MTII + leptin vs. MTII treatment</td>
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<td>PBS</td>
<td>34.62 ± 0.93</td>
<td>32.63 ± 0.99</td>
<td>1.99 ± 0.40†</td>
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<td>5.3 ± 0.36§</td>
<td>17.71 ± 0.70</td>
<td>83.76 ± 3.32</td>
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<td>MTII + leptin</td>
<td>34.33 ± 1.46</td>
<td>27.94 ± 1.14‡‡</td>
<td>6.39 ± 0.45**</td>
<td>17.40 ± 0.57</td>
<td>82.28 ± 2.69</td>
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<tr>
<td>Pair-fed</td>
<td>33.59 ± 0.94</td>
<td>29.32 ± 0.58‡</td>
<td>4.27 ± 0.43§</td>
<td>17.30 ± 0.01</td>
<td>82.25 ± 0.04</td>
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Data are means ± SE. Short-term studies: Effect of 24 h of MTII administration on body weight and cumulative food intake in lean C57Bl/6J, DIO, ob/ob, and UCPI-DTA mice. Long-term studies: effects of 4 days of MTII administration in lean C57Bl/6J and DIO mice as well as 8 days of either MTII or MTII plus leptin administration in DIO mice on body weight and cumulative food intake. †P < 0.05, ‡P < 0.01, §P < 0.001 vs. the respective PBS-treated group; ¶P < 0.05, ¶¶P < 0.01, §§P < 0.001 vs. the pair-fed group; ††P < 0.05, †¶P < 0.01, §§§P < 0.001 vs. the MTII-treated group on chow; ||P < 0.01 vs. the PBS-treated group on chow; &&P < 0.01 vs. both groups on chow.

Ob/ob mice were killed, and hypothalami were isolated. Serum, white adipose tissue, and UCP1-DTA mice as well as 8 days of either MTII or MTII plus leptin administration in DIO mice on body weight and cumulative food intake. 

**RESULTS**

**Short-term (24-h) MTII administration results in a similar decrease in body weight in lean and obese mice but a more pronounced decrease in caloric intake in obese mice.**

_Lean and DIO-C57Bl/6J mice._ We observed an average weight loss of 3.7% in both DIO and lean MTII-treated mice.
beyond weight loss seen in the respective PBS-treated control mice after 24 h of MTII treatment (Table 1). DIO mice, however, have a more significant decrease in cumulative caloric intake (Table 1), which, if sustained for a longer period of time, would be expected to result in a more pronounced decrease in body weight.

**Obese leptin-sensitive ob/ob and leptin-resistant UCP1-DTA mice.** Similar to lean and obese C57BL/6J mice, both ob/ob and UCP1-DTA mice exhibited a significant decrease in body weight after 24 h of MTII treatment (Table 1). MTII-treated lean C57BL/6J, obese ob/ob, and obese UCP1-DTA mice all exhibit an ~38% decrease in cumulative caloric intake in comparison to PBS-treated controls, whereas MTII-treated DIO-C57BL/6J mice exhibit a 59.3 ± 7.8% decrease in cumulative caloric intake after 24 h of MTII treatment when compared with baseline levels ($P < 0.001$).

**Long-term (4-day) MTI administration results in a more pronounced reduction in food intake and body weight in DIO versus lean C57BL/6J mice.** MTII treatment for 4 and 8 days decreases both caloric intake and body weight but is more effective in decreasing caloric intake and body weight in DIO versus lean C57BL/6J mice (Table 1, Fig. 1A and B, and Fig. 2A and B). MTII-treated DIO mice exhibited a significant decrease of ~7.9% in body weight, whereas lean MTII-treated mice exhibited an ~5.1% decrease in body weight beyond the respective PBS-treated control mice (Table 1, Fig. 1A, and Fig. 1B).

**Normalization of circulating leptin levels has only minimal effects on the tachyphylactic effect of prolonged MTII administration.** We then investigated whether falling leptin levels after several days of MTII treatment are responsible for the development of tachyphylaxis (14). After 8 days, MTII-treated DIO-C57BL/6J mice exhibited a significant decrease in circulating leptin levels when compared with their PBS-treated controls (18.5 ± 1.8 ng/ml in the MTII-treated group vs. 26.9 ± 2.0 ng/ml in the PBS-treated group, $P < 0.01$), whereas MTII plus leptin–treated DIO mice did not exhibit a significant decrease in circulating leptin levels (30.8 ± 1.6 ng/ml in the MTII plus leptin–treated group vs. 26.9 ± 2.0 ng/ml in the PBS-treated group). MTII-treated DIO mice also exhibited a significant decrease in body weight during the 8-day period (5.3 ± 0.4 g in the MTII-treated group vs. 2.0 ± 0.4 g in the PBS-treated group, $P < 0.0001$), but the effects of MTII appeared to plateau after 4 days of treatment (Fig. 3A and B). Once replacement doses of leptin were administered in conjunction with MTII, however, DIO mice continued to lose weight and exhibited a more significant percent decrease in body weight after 8 days of treatment.
either food deprivation or MTII treatment (1.24 ± 0.35 ng/ml in the MTII-treated group and 1.35 ± 0.26 ng/ml in the pair-fed group vs. 2.90 ± 1.01 ng/ml in the PBS-treated group, P < 0.05 for both groups). Ob/ob mice responded to 24 h of MTII treatment similarly to lean C57BL/6J and DIO mice, with an ~59.4% decrease in insulin levels (3.17 ± 1.36 ng/ml in the MTII-treated group vs. 7.81 ± 0.94 ng/ml in the PBS-treated group, P < 0.05). In UCP1-DTA mice, the effect of short-term MTII administration on circulating insulin levels was even more pronounced. Although pair-fed UCP1-DTA mice had an ~55% decrease in insulin levels, insulin levels were more significantly decreased by ~78.8% in MTII-treated UCP1-DTA mice (0.62 ± 0.04 ng/ml in the MTII-treated group and 1.32 ± 0.38 ng/ml in the pair-fed group vs. 2.92 ± 0.81 ng/ml in the PBS-treated group, P < 0.05 for MTII-treated vs. PBS-treated mice and NS for pair-fed vs. PBS-treated mice).

**Long-term studies (4–8 days).** The effects of MTII on insulin levels persisted after 4 days of treatment in DIO mice only (lean C57BL/6J: 1.38 ± 0.06 ng/ml in MTII-treated mice vs. 1.44 ± 0.02 ng/ml in PBS-treated mice, NS; DIO: 1.42 ± 0.12 ng/ml in MTII-treated mice vs. 2.44 ± 0.41 ng/ml in PBS-treated mice, P < 0.001). After 8 days of treatment, serum insulin levels remained significantly decreased in DIO mice, but this difference was most pronounced in the MTII plus leptin–treated group (1.2 ± 0.3 ng/ml in the MTII plus leptin–treated group, 1.9 ± 0.6 ng/ml in the MTII-treated group, and 1.8 ± 0.2 ng/ml in the pair-fed group vs. 2.7 ± 0.6 in the PBS group, P < 0.05 between the MTII plus leptin– and PBS-treated group only).

Serum thyroxine concentrations were not significantly altered in lean, DIO, or ob/ob mice after 24 h, 4 days, or 8

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**FIG. 3.** Comparative evaluation of weight loss after 8 days of MTII plus leptin versus MTII administration in DIO mice. Arrows indicate initiation and discontinuation of treatment (means ± SE). *P < 0.05 between the MTII- and the PBS-treated groups; @@P = 0.02, @@@P < 0.01 between the MTII plus leptin and PBS-treated groups; 0.09 > P > 0.05, #P < 0.05 between the pair-fed and PBS-treated groups; ###P < 0.001 vs. the PBS-treated group; ###P = 0.001 vs. the pair-fed group; +P = 0.076 between the MTII plus leptin and the MTII-treated groups.

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**FIG. 4.** Comparative evaluation of total percent body fat and triglycerides after 8 days of MTII plus leptin vs. MTII administration in DIO mice (means ± SE). *P < 0.05 vs. the PBS-treated group; ###P < 0.01, ###P < 0.001 vs. the pair-fed group; @P < 0.05 vs. the MTII-treated group.
days of MTII administration or pair-feeding (data not shown).

**Effects of short-term (24-h) MTII treatment on the mRNA expression of hypothalamic neuropeptides in lean C57BL/6J, DIO, and ob/ob mice.** To gain a better understanding of why tachyphylaxis occurs after prolonged MTII treatment, we investigated the expression pattern of key hypothalamic orexigenic (NPY, AgRP) and anorexigenic (POMC, orexin) neuropeptides as well as hypothalamic MC4R mRNA expression. Hypothalamic NPY mRNA expression increased by ∼40–50% after 24 h of pair-feeding–induced food deprivation (P < 0.01; Fig. 5A). The expression pattern of AgRP followed that of NPY, with elevated AgRP mRNA expression levels in pair-fed, but not MTII-treated or PBS-treated mice, after 24 h of treatment, but these differences failed to reach statistical significance.

Hypothalamic POMC mRNA expression decreased in both MTII-treated and pair-fed lean mice when compared with PBS-treated controls (86.4 ± 11.8% of baseline levels in MTII-treated lean [P < 0.05] and 64.1 ± 8.2% in pair-fed lean mice [P < 0.01 vs. the PBS-treated group]). There was a similar POMC mRNA expression response to MTII treatment in DIO mice, with a more significant decrease in pair-fed mice (79.5 ± 8.9% of baseline levels in MTII-treated DIO mice [P < 0.05 vs. the PBS-treated group and P < 0.08 vs. the pair-fed group] and 56.4 ± 7.5% in pair-fed DIO mice [P < 0.001 vs. the PBS-treated group]). Similar short-term changes in NPY, AgRP, and POMC mRNA expression were also observed in ob/ob mice. Neither MTII nor food deprivation, however, had short-term effects on hypothalamic MC4R mRNA expression or orexin mRNA expression in lean, DIO, and ob/ob mice.

**Long-term (8-day) effects of MTII or MTII plus leptin administration on mRNA expression of hypothalamic neuropeptides in DIO mice.** Prolonged MTII treatment failed to prevent food deprivation–induced increases in hypothalamic NPY expression. Both pair-fed and MTII-treated DIO mice exhibited a significant increase in hypothalamic NPY mRNA expression after 8 days when compared with PBS-treated controls (Fig. 5B). Similarly, mRNA expression of AgRP increased by 55.5 ± 7.8% in pair-fed mice (P < 0.05) and 20.2 ± 5.8% in MTII-treated DIO mice, but this compensatory increase was prevented by a combination treatment of MTII plus leptin (1.1 ± 10.4% in MTII plus leptin–treated DIO mice). Hypothalamic POMC mRNA expression was similarly decreased in both MTII-treated and pair-fed mice (88.7 ± 12.1% of baseline levels in MTII-treated DIO mice and 79.0 ± 5.2% in pair-fed DIO mice). Replacement doses of leptin in conjunction with MTII treatment failed to alter the effects observed after MTII alone (76.1 ± 4.5% of baseline levels). MCH mRNA expression was significantly decreased after prolonged MTII plus leptin treatment, but failed to change after prolonged MTII treatment or pair-feeding alone (77.1 ± 3.0% of baseline levels in MTII plus leptin–treated mice [P < 0.05] vs. 91.3 ± 2.7% in MTII-treated and 90.2 ± 5.1% in pair-fed mice). Hypothalamic mRNA expression of MC4R was not altered after prolonged administration of MTII or MTII plus leptin (data not shown), implying that previously observed decreases in MC4R protein (19) is probably due to posttranscriptional regulation. Orexin mRNA expression was not altered after prolonged food deprivation, MTII, or MTII plus leptin administration (data not shown).

**Effects of MTII on serum adiponectin concentrations, adiponectin mRNA expression in WAT, and AdipoR1 and AdipoR2 mRNA expression in liver of lean C57BL/6J and DIO mice.**

**Short-term studies (24 h).** Because the molecular pathways mediating adiponectin’s ability to improve insulin secretion and sensitivity remain largely unknown, we investigated the patterns of adiponectin secretion and mRNA expression in WAT and the regulation of AdipoR1 and AdipoR2 in the liver of lean C57BL/6J and DIO mice. Although there was a trend toward elevated circulating adiponectin levels in both lean and obese mice after 24 h of MTII treatment, increased variability prevented these data from reaching statistical significance (data not shown). Similarly, after 24 h of MTII treatment, there were no significant differences in adiponectin mRNA expression in the WAT of lean C57BL/6J and DIO mice when compared with respective PBS-treated controls. As previously described in DIO rats (38), however, DIO mice exhibited a significant decrease in adiponectin mRNA expression when compared with the lean PBS-treated group (64.9 ± 12.5% of baseline levels in PBS-treated DIO mice and 64.1 ± 16.0% in pair-fed DIO mice, P < 0.0001 for both groups vs. lean PBS-treated mice).

We found significant changes in liver AdipoR1 and AdipoR2 mRNA expression after food deprivation but not MTII treatment. Specifically, in lean C57BL/6J mice, there were no significant differences in AdipoR1 mRNA expression after 24 h of MTII treatment (Fig. 6A), but mice pair-fed to the MTII-treated group exhibited a significant
increase of 187.1 ± 24.6% from baseline levels in AdipoR1 mRNA expression (P < 0.05 vs. the PBS-treated group; Fig. 6A). However, both MTII-treated and pair-fed lean C57BL/6J mice exhibited a significant decrease in AdipoR2 mRNA expression after 24 h of treatment or pair-feeding (37.4 ± 4.8% of baseline levels in MTII-treated mice and 25.5 ± 5.6% in pair-fed mice, P < 0.0001 vs. the PBS-treated group; Fig. 7A). DIO mice exhibited significantly increased liver AdipoR1 mRNA expression and decreased liver AdipoR2 mRNA expression in all three groups (PBS-treated, MTII-treated, and pair-fed mice) when compared with the respective lean PBS-treated mice (Fig. 6A and Fig. 7A), but it remains to be clarified by future studies whether this difference is due to a high-fat diet per se or the more pronounced weight loss of DIO mice seen in this study. We observed no significant differences in both AdipoR1 and AdipoR2 mRNA expression in liver after 24 h of MTII treatment, despite a significant increase in liver AdipoR1 RNA expression and decrease in liver AdipoR2 mRNA expression in mice pair-fed to the MTII-treated group (Fig. 6A and 7A).

**DISCUSSION**

Peripheral and central MTII treatment decreases body weight by suppressing food intake and increasing energy expenditure (14,21). However, it remains unknown 1) whether MTII is effective in leptin-resistant DIO-, UCP1-DTA−, and leptin-sensitive ob/ob obese mice, 2) whether leptin-resistant DIO mice are more sensitive to MTII, and 3) what the mechanisms are that underlie the development of metabolic syndrome.

**Long-term studies.** Serum adiponectin did not change in DIO mice after 8 days of MTII administration, MTII plus leptin administration, or pair-feeding (data not shown). Moreover, there were no significant changes in AdipoR1 or AdipoR2 mRNA expression after 8 days of MTII treatment (Fig. 6B and 7B). However, DIO mice pair-fed for 8 days to the MTII-treated group, as well as DIO mice administered a combination of MTII plus leptin for 8 days, exhibited a significant increase in liver AdipoR1 mRNA expression and a decrease in liver AdipoR2 mRNA expression compared with the PBS-treated group (Fig. 6B and 7B).
of tachyphylaxis and increased insulin sensitivity after MTII treatment.

**MTII decreases food intake and body weight in both leptin-sensitive and leptin-resistant mice.** We first evaluated DIO, a model of acquired leptin and insulin resistance (22) that results in central and peripheral changes in hormonal and neuropeptide levels and/or their signaling pathways (23), and UCP1-DTA mice, which have significantly decreased brown fat (~20% of control animals) and thus deficient adaptive thermogenesis, leading to an obese phenotype (16,24). UCP1-DTA mice are resistant to the effects of endogenous and exogenously administered leptin on body weight, food intake, and glucose and insulin levels (24,25). We found that food intake, body weight, and insulin levels in both DIO and UCP1-DTA mice decrease after MTII administration, suggesting that this agent acts downstream of the putative point of leptin resistance. Further studies are necessary to assess the relative contribution of increased thermogenesis, lipolysis, and anorexia in the weight-reducing effect of the melanocortin system in these mice. The 24 h of MTII administration also resulted in suppression of cumulative food intake, decreases in body weight, and decreases in circulating insulin levels (59.5%) in leptin-sensitive ob/ob mice (26). Our data are consistent with previous studies demonstrating that a single intraperitoneal dose of MTII to ob/ob mice induces anorexia, shows similar kinetics with a single intracerebroventricular dose to those mice, inhibits feeding potently for the first 4 h (5), and decreases circulating insulin levels by 58% (5).

**The effects of MTII are more pronounced in DIO mice than in lean mice.** MTII administration for 24 h resulted in similar weight loss in lean and DIO mice, but cumulative caloric intake was significantly lower in MTII-treated DIO versus lean mice, suggesting that DIO mice are more sensitive to the hypophagic effects of MTII in the short term. This would be expected to potentially result in more pronounced weight loss after more prolonged administration. Indeed, 4 days of MTII treatment resulted in a more pronounced weight loss and a decrease in caloric intake in DIO mice. Previous studies have shown that food intake is significantly more decreased after a single central MTII injection (27) or peripheral administration for 3 days in obese Zucker (fa/fa) rats with impaired leptin signaling due to defective leptin receptors than in lean controls (28). In addition, DIO Sprague-Dawley rats have significantly

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**FIG. 7.** Liver AdipoR2 mRNA expression as the percent change from the lean PBS-treated group (means ± SE). ****P < 0.0001 vs. the lean PBS-treated group; ####P < 0.0001 vs. the respective PBS-treated group; °0.10 < P < 0.15, ‡‡‡‡P < 0.0001 vs. the respective pair-fed group.
enhanced nocturnal inhibitory feeding responses to a single intracerebroventricular injection of α-melanocyte-stimulating hormone when compared with lean controls in both the short and long term (29). However, we show for the first time that melanocortins are more potent in DIO mice when compared with lean controls by inducing a more pronounced decrease in food intake and body weight in DIO mice. We therefore suggest that leptin-resistant obese rodents may have a reduced endogenous melanocortin tone, probably due to a lack of leptin action, which results in increased MTII sensitivity.

**Hypothalamic NPY levels are suppressed after MTII-induced food deprivation but rise after prolonged administration.** MTII acutely decreases appetite and body weight in rodents, but tachyphylaxis to chronic central or peripheral MTII administration has been observed in mice (14) and rats (30,31). We found that although MTII may prevent food deprivation–induced increases in hypothalamic NPY and possibly AgRP mRNA expression in the short term, both NPY and AgRP mRNA increase toward levels seen in the pair-fed animals after 8 days of MTII administration, suggesting that this increase in hypothalamic NPY and AgRP mRNA levels might mediate, in part, the tachyphylactic effects of prolonged MTII treatment. It has recently been shown that tachyphylaxis is due to downregulation of the MC4R protein (19). Because mRNA expression of MC4R is not altered after short-term or prolonged administration of MTII, this regulation may occur at the translational level, but future studies are needed to investigate this topic in detail as well as to examine expression of hypothalamic neuropeptides or synaptic activity in specific areas of the hypothalamus (29). Our data are consistent with the physiological outcomes of this study, including the fact that MTII-treated mice don’t regain weight 4 days after treatment, nor do they increase their food intake, but just fail to lose additional weight.

A direct effect of melanocortins on the NPYnergic system is also consistent with the presence of MC3 receptors on NPY neurons (32). Because NPY neurons have been shown to have a regulatory effect on POMC neurons (33), it is reasonable to speculate that a prolonged effect of leptin or melanocortins on NPY may result in compensatory changes in the endogenous melanocortin system. Similar to melanocortin agonists, NPY antagonists have been shown to exert stronger effects on reducing food intake in obese leptin-resistant Zucker (fa/ fa) rats than in lean controls (34). However, NPY-deficient mice (NPY−/−) exhibit a normal response to centrally administered MTII (35), suggesting that additional molecules other than NPY may mediate the effects of MTII. We found that changes in both NPY and AgRP may mediate the short-term effects of MTII (36). Because both NPY and AgRP mRNA levels rise after chronic administration toward levels seen in the pair-fed mice, explaining in part why the effect of MTII wanes after prolonged administration, it is possible that a combined treatment of MTII with NPY and AgRP antagonists might provide a valuable drug combination for obesity.

In addition, a combination treatment of MTII and leptin decreases body weight more significantly than MTII treatment or pair-feeding alone because of either the direct lipolytic effects of leptin or altered energy homeostasis. A combination treatment of MTII plus leptin for 8 days, but not MTII administration alone, prevents changes in MCH, NPY, and AgRP mRNA expression, suggesting that leptin may in part act to prevent the development of tachyphylaxis by altering hypothalamic neuropeptide levels. We thus conclude that decreasing leptin levels after MTII-induced weight loss might play a minor role in the development of tachyphylaxis, and a combination treatment of leptin and MTII may prove more effective in causing lipolysis and resulting in an additional decrement of body weight.

**Altered insulin sensitivity and insulin levels after food deprivation or MTII administration may be in part mediated by alterations in adiponectin receptor levels.** MTII acts as a potent insulin-sensitizing agent in mice (14,37), but the mechanisms underlying this effect, although poorly characterized, seem not to include increases in adiponectin expression and secretion (38), for there were no significant changes in adiponectin levels in lean or obese C57BL/6J mice after MTII treatment. After food deprivation and MTII administration, we observed significant changes in the liver mRNA expression of AdipoR1, a high-affinity receptor for globular adiponectin, and AdipoR2, an intermediate-affinity receptor for both globular and full-length adiponectin, both of which appear to mediate adiponectin-stimulated fatty acid oxidation and possibly hepatic glucose uptake (20). Further, we demonstrate that, in contrast to the tachyphylaxis observed in terms of hypothalamic neuropeptide responses to MTII, no peripheral tachyphylaxis is seen with respect to the effects of MTII on adiponectin receptor expression in the liver. Future studies investigating the physiological regulation of adiponectin and its receptors, as well as identification of the signaling pathways downstream of adiponectin receptors in lean and obese mice, may prove to be of significant importance in determining the mechanisms underlying insulin resistance.

In summary, our results suggest that 1) MTII is an effective treatment for obesity and related metabolic defects in leptin-resistant and leptin-sensitive mouse models of obesity; 2) DIO mice appear to be more sensitive to MTII treatment because the effects of MTII on food intake and body weight are more pronounced in DIO than lean mice; 3) the short-term effects of MTII to induce anorexia may, at least in part, be mediated by altered hypothalamic NPY mRNA levels; 4) the tachyphylactic effect after prolonged MTII administration may, at least in part, be caused by a compensatory increase of NPY mRNA concentrations and possibly AgRP mRNA concentrations; 5) alterations in orexin, MCH, and MC4R mRNA expression do not appear to play a role of comparable importance, whereas decreasing leptin levels appear to play a minor role in the development of tachyphylaxis; and 6) alterations in adiponectin receptor mRNA expression after food deprivation, as well as short- and long-term MTII administration, may play a role in altering insulin sensitivity and needs to be studied further. Finally, our data provide further evidence that melanocortin receptor agonists offer a potential therapeutic approach to syndromes of obesity accompanied by leptin resistance and metabolic abnormalities (37) and raise the possibility that a combination treatment with...
leptin or NPY and AgRP antagonists might provide an even more effective treatment option for obesity.

REFERENCES


