Nicotine infusion alters leptin and uncoupling protein 1 mRNA expression in adipose tissues of rats

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Arai, Keiko, Kyongsong Kim, Katsumi Kaneko, Mitsue Iketani, Asuka Otagiri, Naoko Yamauchi, and Tamotsu Shibasaki. Nicotine infusion alters leptin and uncoupling protein 1 mRNA expression in adipose tissues of rats. Am J Physiol Endocrinol Metab 280: E867–E876, 2001.—We attempted to clarify whether leptin and uncoupling protein 1 (UCP1) are involved in the action of nicotine on the energy balance. Male Wistar rats were infused subcutaneously with nicotine (12 mg·kg⁻¹·day⁻¹) for 4 or 14 days. At the end of the 4-day period, the plasma concentrations of leptin of the nicotine-treated and pair-fed rats were lower than those of the freely fed rats, although the levels of leptin mRNA expression in various white adipose tissues did not differ among the three groups. At the end of the 14-day nicotine infusion period, plasma concentrations of leptin were higher, and leptin mRNA expression in the omentum and epididymal and retroperitoneal adipose tissues was stronger in the nicotine-treated rats than in the pair-fed and freely fed rats. UCP1 mRNA expression in the brown adipose tissue of nicotine-treated was stronger than that of the pair-fed rats. These results suggest that continuous nicotine infusion differentially affects the synthesis and secretion of leptin according to the duration of infusion and stimulates UCP1 mRNA expression, probably in a manner independent of leptin.

food intake; body weight; energy balance

Epidemiological studies have demonstrated that the body weight of smokers is lower than that of nonsmokers (24, 46). The reduction in body weight is induced by increased energy expenditure and decreased energy intake. Lines of evidence indicate that tobacco smoking increases the metabolic rate and energy expenditure associated with slightly increased activity in humans and that smokers have a lower caloric intake than nonsmokers (29, 34, 35). Nicotine, which is the main component of tobacco, is primarily responsible for the effects of smoking on body weight. Chronic administration of nicotine reportedly inhibits the synthesis of neuropeptide Y (NPY), a potent stimulator of feeding behavior, in the arcuate nucleus of the hypothalamus, resulting in the reduction in food intake that blunts weight gain in rats (15). Although this report describes body weight by nicotine, details of the mechanism by which nicotine influences energy balance are not fully understood.

The cloning and characterization of the leptin gene have shown that leptin is synthesized in white and brown adipose tissues (30, 50), pituitary gland (23), skeletal muscle (47), stomach (2), and placenta (27). Leptin reduces food intake as a circulating satiety factor by acting on the hypothalamus (7, 42), where leptin receptors are expressed (38, 43). The various peptides that affect food intake exist in the hypothalamus. Leptin increases the synthesis of peptides that inhibit food intake, such as α-melanocyte-stimulating hormone, derived from proopiomelanocortin, and corticotropin-releasing hormone (CRH) (38, 44) and decreases the synthesis of peptides that stimulate food intake, such as NPY, agouti-related protein, and orexin (4, 28, 42). These changes in the hypothalamic result in reducing food intake. Leptin also regulates energy availability in peripheral tissues and affects various functions, such as body temperature, activity level, and metabolic rate (7, 16, 33). Uncoupling protein 1 (UCP1) also plays a role in energy expenditure by inducing nonshivering thermogenesis in the brown adipose tissue (22). Levels of UCP1 mRNA expression decrease under fasting conditions in normal rats (8, 18, 36, 40) and increase under nicotine treatment in genetically obese mice (49).

In the present study, we tested the effects of continuous infusion of nicotine on the plasma concentrations of leptin and on the levels of leptin and UCP1 messenger ribonucleic acid (mRNA) expression in the adipose tissues to determine whether nicotine reduces food intake and body weight gain via leptin and UCP1 in normal rats.

Materials and Methods

Animals. Male Wistar rats weighing 190–200 g were housed under conditions of controlled temperature and illumination (0800–2000). Animals were maintained according to the guidelines of the Animal Experimental Ethical Review Committee of Nippon Medical School.

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Experimental protocol and drug administration. Nicotine (12 mg·kg\(^{-1}\)·day\(^{-1}\)) or saline (12 μl/day) was infused into rats by means of an osmotic minipump (Alzet, model 2001 or 2002, Alza, Palo Alto, CA) implanted subcutaneously in the back for 4 or 14 days. After a 14-day period of nicotine infusion, some rats were allowed ad libitum access to food and water for another 14 days. Two control groups infused with saline, freely fed rats and pair-fed rats, were used in this study. We measured the food intake of the nicotine-treated rats and fed that amount to the pair-fed rats the next day. We started the pair-fed rats 1 day behind the nicotine-treated and freely fed rats. Each group comprised five to seven rats. The body weight and food intake of each rat were measured daily at 0900.

Fig. 1. Changes in daily food intake and body weight during continuous infusion of nicotine for 14 days and subsequent free feeding for another 14 days. ○, △, and □ indicate the freely fed, pair-fed, and nicotine-treated rats, respectively. A: changes in daily food intake. Continuous infusion of nicotine significantly suppressed daily food intake between days 1 and 6 compared with that of freely fed rats (\(*P < 0.01, \text{nicotine-treated and pair-fed vs. freely fed rats on day 1}; \text{**}P < 0.05, \text{nicotine-treated and pair-fed vs. freely fed rats between days 2 and 4}; \text{†}P < 0.05, \text{nicotine-treated vs. freely fed rats on day 5}; \text{††}P < 0.01, \text{nicotine-treated vs. freely fed rats on day 6}). B: changes in body weight. Mean body weight of nicotine-treated rats was significantly lower than that of freely fed rats between days 2 and 14 (\(\text{††P} < 0.05, \text{nicotine-treated vs. freely fed rats between days 2 and 4}; \text{*P} < 0.05, \text{nicotine-treated and pair-fed vs. freely fed rats between days 5 and 14}).
RNA extraction. After a 4-day period of nicotine infusion, a 14-day period of nicotine infusion, and a 14-day period of free feeding after the 14-day infusion of nicotine, rats were killed by decapitation, and the omentum and retroperitoneal, epididymal, subcutaneous, and brown adipose tissues were immediately removed and stored at −80°C. Total RNA was extracted from each tissue by the guanidium thiocyanate-phenol-chloroform method by use of Isogen (Nippon Gene, Toyama, Japan) (9).

Preparation of complementary RNA probes and sense RNA standards. RNA probes complementary to mRNA (cRNA) were produced by RT-PCR by using total RNA from rat subcutaneous adipose tissue for leptin (sense primer: 5'-CCGGATCCGACCATGTCACCCAGTAGTCA-3', antisense primer: 5'-CCGAATTCGGTCTCGCAGGTTCTCCAG-3'), and for β-actin (sense primer: 5'-CCGGATCCAGGCTTCCAGTACTAT-3', antisense primer: 5'-CCGAATTCGCCACCCGTCAT-3'), and for T7 RNA polymerase (Stratagene Cloning Systems, La Jolla, CA), 100 mCi [32P]UTP-labeled rat leptin, UCP1, or β-actin mRNA were standardized by the levels of β-actin mRNA. Assay of plasma leptin concentrations. Rats were killed, and trunal blood samples were collected. After centrifugation, plasma samples were stored at −20°C until use. Plasma concentrations of leptin were measured using the Rat Leptin Kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Statistical analysis. The data of body weight and daily food intake were subjected to a repeated-measurements analysis of variance (ANOVA), and other data were subjected to an ANOVA followed by Fisher’s protected least significant difference test for multiple comparisons. Statistical significance was established at the P < 0.05 level. All results are expressed as means ± SE.

RESULTS

Body weight and food intake. A continuous infusion of nicotine for 14 days significantly decreased the amount of daily food intake between days 1 and 6 (Fig. 1A). The mean body weight of nicotine-treated rats was significantly lower than that of the freely fed rats between days 2 and 14. The mean body weight did not differ significantly between the nicotine-treated and pair-fed rats (Fig. 1B). During the 14-day period of free feeding after the 14-day treatment of nicotine, no significant difference in mean body weight or daily food intake was found among the three groups (Fig. 1, A and B). The total food intake for 4 or 14 days of the nicotine-treated rats was significantly less than that of the freely fed rats, whereas there was no difference in the total food intake for 28 days among the three groups (Table 1).

Table 1. Total food intake

<table>
<thead>
<tr>
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<th>Freely Fed Rats</th>
<th>Pair-Fed Rats</th>
<th>Nicotine-Treated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Day period of nicotine infusion</td>
<td>103.01 ± 1.20</td>
<td>91.91 ± 1.54$</td>
<td>89.75 ± 2.51$</td>
</tr>
<tr>
<td>14-Day period of nicotine infusion</td>
<td>273.80 ± 6.65</td>
<td>254.91 ± 0.69*</td>
<td>253.00 ± 3.76†</td>
</tr>
<tr>
<td>14-Day period of free feeding and 14-day period of nicotine infusion</td>
<td>687.48 ± 22.02</td>
<td>660.95 ± 4.11</td>
<td>671.80 ± 14.71</td>
</tr>
</tbody>
</table>

Values are means ± SE in grams. $P < 0.05, †P < 0.01, ‡P < 0.001 vs. freely fed rats.
leptin mRNA expression in the brown adipose tissue of the nicotine-treated and pair-fed rats was significantly weaker than that of the freely fed rats (Fig. 4).

Leptin mRNA expression in the omentum and retroperitoneal and epididymal adipose tissues of the rats treated with nicotine for 14 days was significantly stronger than that of the freely fed and pair-fed rats, whereas that in the subcutaneous and brown adipose tissues showed no difference among the three groups (Fig. 5).

After the 14-day washout period, the mean leptin mRNA expression in the brown adipose tissue of the nicotine-treated and pair-fed rats was significantly weaker than that of the freely fed rats (Fig. 4).

Leptin mRNA expression in the omentum and retroperitoneal and epididymal adipose tissues of the rats treated with nicotine for 14 days was significantly stronger than that of the freely fed and pair-fed rats, whereas that in the subcutaneous and brown adipose tissues showed no difference among the three groups (Fig. 5). After the 14-day washout period, the mean leptin mRNA expression...
expression in the omentum of rats treated with nicotine was significantly stronger than that of freely fed rats, whereas that in the retroperitoneal, epididymal, subcutaneous, or brown adipose tissue of the nicotine-treated rats did not differ significantly from that of the freely fed or pair-fed rats (Fig. 6).

**UCP1 mRNA expression in brown adipose tissue.** Figure 3, B and C, shows typical autoradiograms of PAGE produced by the RNAase protection assay for UCP1 mRNA in the brown adipose tissue of the freely fed rats, pair-fed rats, and rats treated with nicotine for 4 and 14 days. As shown in Fig. 7, UCP1 mRNA expression in the brown adipose tissue of the pair-fed rats was significantly weaker than that of the freely fed rats and the rats treated with nicotine for both 4 and 14 days. UCP1 mRNA expression showed no significant difference among the three groups after the 14-day period of free feeding after the 14-day infusion of nicotine or vehicle.

**DISCUSSION**

The present study showed that the amount of daily food intake was significantly suppressed by nicotine only 6 days after the start of nicotine infusion, and the mean body weight of nicotine-treated rats was significantly lower than that of freely fed rats throughout the entire period of nicotine infusion. These results are similar to those previously reported by another research group (15).

The present study also showed that the 4-day period of nicotine infusion significantly reduced the plasma levels of leptin, an inhibitor of feeding behavior synthesized and secreted by adipose tissue, and the levels of leptin mRNA expression in the brown adipose tissue. However, this infusion did not significantly influence levels of leptin mRNA expression in white adipose tissues compared with freely fed rats at the end of the 4-day period of nicotine infusion, when the total food eaten and body weight were significantly lower. These results suggest that leptin is not involved in the mechanism underlying the inhibition of food intake induced by continuous infusion of nicotine during the first 4 days of the infusion. Nicotine is known to inhibit the expression of NPY mRNA in the arcuate nucleus of the hypothalamus (15) and to activate CRH-containing neurons in the paraventricular nucleus of the hypothalamus (25, 45). Because NPY is a potent stimulator and CRH is an inhibitor of feeding behavior (11), these changes in the two peptidergic neurons are probably involved in the mechanism underlying the inhibitory effect of nicotine on food intake in a manner independent of leptin.

The weight of white adipose tissue was not measured in the present study. However, it is reported that the mass of white adipose tissue changes in parallel with food intake (3), and the reduction in plasma concentrations of leptin at the end of the 4-day period of nicotine infusion appears to be induced by the de-
crease in total mass of white adipose tissue that produces and secretes leptin. This possibility is supported by the result that the plasma concentrations of leptin in the pair-fed rats were also reduced. Another explanation for the decrease in plasma concentrations of leptin may reflect the decrease in the secretion of leptin from adipose tissues. Insulin stimulates the release of leptin from rat adipocytes without affecting leptin mRNA (5), and catecholamines suppress the release of leptin from human adipocytes (39). Food restriction induces the decrease in plasma concentrations of insulin (3, 15). Nicotine increases the plasma concentrations of catecholamines in humans and rats (10, 19). These reports suggest that the secretion of leptin from adipose tissues of nicotine-treated and pair-fed rats at the end of 4-day period of nicotine infusion may be decreased by the decreased plasma concentrations of insulin or the increased plasma concentrations of catecholamines. The difference in the plasma concentrations of leptin among the freely fed rats at the end of the 4- and 14-day periods of vehicle infusion and the 14-day period of free feeding that followed the 14-day period of vehicle infusion seems to depend on the difference in the body weight of each group.

At the end of the 14-day period of nicotine infusion, plasma concentrations of leptin were significantly increased, and leptin mRNA expression was significantly stronger in the omentum and retroperitoneal and epididymal white adipose tissues of nicotine-treated rats compared with those of the freely fed and pair-fed rats. It has been reported that the plasma concentrations of leptin were higher in smokers than in nonsmokers (13). In conjunction with that report, our results indicate that nicotine stimulates the synthesis and secretion of leptin in the omentum and retroperitoneal and epididymal white adipose tissues when it is continuously infused for 14 days. The levels of leptin mRNA expression in the subcutaneous adipose tissue at the end of the 14-day period of nicotine infusion did not change significantly, whereas those in other white adipose tissues were significantly increased by nicotine treatment. Furthermore, the mean leptin mRNA expression in the omentum of rats treated with nicotine for 14 days was still stronger than that of the freely fed rats after the 14-day period of free feeding, whereas that in other adipose tissues showed no difference among the three groups. These results suggest that the regulatory mechanism of leptin mRNA expression is variable among tissues. This tissue specificity for the expression of leptin mRNA has been previously reported by several groups (26, 31).

A bolus intraperitoneal injection of nicotine induces interleukin (IL)-1α mRNA in the adrenal gland 90 min after injection in rats (1), and an intracerebroventricular injection of nicotine increases plasma levels of IL-6 through the sympathetic nervous system in mice (41).
Cytokines such as tumor necrosis factor and IL-1β are known to induce the expression of leptin mRNA in epididymal adipose tissue when they are administered intraperitoneally to mice and hamsters (14, 20). These findings suggest that nicotine may stimulate the expression of leptin mRNA in the white adipose tissue through cytokines, although there are no reports on the action of IL-1α and IL-6 on the expression of leptin mRNA.

Fig. 6. Leptin mRNA expression in adipose tissues at the end of the 14-day period of free feeding after the 14-day period of nicotine infusion. Mean leptin mRNA expression in omentum of nicotine-treated rats was significantly stronger than that of freely fed rats, whereas that in retroperitoneal, epididymal, subcutaneous, or brown adipose tissue of nicotine-treated rats did not differ significantly from that of freely fed and pair-fed rats.

Fig. 7. UCP1 mRNA expression in brown adipose tissue. Left, middle, and right: levels of UCP1 mRNA expression in brown adipose tissue of freely fed, pair-fed, and nicotine-treated rats, respectively, at the end of the 4- and 14-day periods of nicotine infusion and the 14-day period of free feeding after the 14-day period of nicotine infusion. UCP1 mRNA expression in brown adipose tissue of pair-fed rats was significantly weaker than that of freely fed and nicotine-treated rats at the end of the 4- and 14-day periods of nicotine or vehicle infusion.
E874  EFFECTS OF NICOTINE ON LEPTIN AND UCP1 mRNA

Receptors for leptin are present in adipose tissues (6), and leptin is reported to induce lipolysis and regulate the content of triglyceride in adipocytes by modifying the expression of enzymes involved in the oxidation of free fatty acid in the white adipose tissue and muscle by the autocrine and/or paracrine mechanism (17, 32, 48, 51). However, we observed no significant difference in mean body weight between the nicotine-treated rats and the pair-fed rats whose food intake was almost the same as that of the nicotine-treated rats during the 14-day period of nicotine infusion. Furthermore, the changes in body weight of the nicotine-treated rats were almost the same as those of pair-fed rats during the 14-day period of free feeding after the 14-day period of nicotine or vehicle infusion. The concentration of leptin that induced nonketotic lipolysis was reported to be 20 ng/ml (48). However, the plasma concentration of leptin of nicotine-treated rats at the end of the 14-day period of nicotine infusion was 3.93 ± 1.61 ng/ml. Therefore, these results suggest that the increased plasma concentrations of leptin in the rats treated with nicotine for 14 days might not sufficiently induce a reduction in body weight through its lipolytic effect, although there is a possibility that increased leptin modifies fat metabolism in peripheral tissues.

Reduced UCP1 mRNA expression in brown adipose tissue is induced by decreased energy intake (8, 18, 36, 40). However, in the present study, significantly stronger UCP1 mRNA expression was found in the brown adipose tissue of the nicotine-treated rats compared with pair-fed rats, although it did not differ significantly from that of the freely fed rats at the end of the 4- and 14-day periods of nicotine infusion. This indicates that nicotine induced a disinhibition of UCP1 mRNA expression in the brown adipose tissue when energy intake was reduced. A peripheral administration of leptin increases the expression levels of UCP1 mRNA in the brown adipose of rats (36, 37, 40). Taken together, these results suggest that leptin stimulates the expression of UCP1 mRNA. Because the plasma concentrations of leptin in the nicotine-treated rats were significantly higher than those in the pair-fed rats at the end of the 14-day period of nicotine infusion, leptin might be involved in the higher levels of UCP1 mRNA in the nicotine-treated rats compared with those in the pair-fed rats.

In contrast, the plasma concentrations of leptin in the nicotine-treated rats were almost the same as those in the pair-fed rats at the end of the 4-day period of nicotine infusion, whereas the UCP1 mRNA expression in the brown adipose tissue of the nicotine-treated rats was significantly stronger than that of the pair-fed rats. These results therefore suggest that leptin does not play a major role in the stimulatory effect of nicotine on UCP1 mRNA expression in the brown adipose tissue during the 4-day period of nicotine infusion. The activity of the sympathetic nervous system is also known to increase UCP1 mRNA expression in brown adipose tissue (12, 21). A 10-min period of tobacco smoking increases the plasma level of catecholamines in humans (10), and a 7-day period of continuous infusion of nicotine increases plasma catecholamines in rats (19). Therefore, the sympathetic nervous system activated by nicotine infusion may be involved in the mechanism underlying disinhibition of UCP1 mRNA expression in the brown adipose tissue under low energy intake.

The increased UCP1 mRNA expression in the brown adipose tissue of the nicotine-treated rats does not appear to induce a reduction in body weight gain, because the mean body weight of the nicotine-treated rats did not differ from that of the pair-fed rats throughout the entire period of nicotine infusion. The significant difference in mean body weight between the nicotine-treated rats and the freely fed rats between days 7 and 14 is probably the result of the reduction in food intake between days 1 and 6 after the start of the nicotine infusion.

In summary, our results indicate that nicotine differentially affects the synthesis and secretion of leptin by adipose tissues according to the time course of nicotine infusion and that leptin is not involved in the suppression of food intake by nicotine. These results also suggest that the expression of UCP1 mRNA in the brown adipose tissue is stimulated by nicotine independently of the levels of leptin under reduced energy intake, although the enhanced UCP1 expression may not play an important role in blunting weight gain by nicotine. Nicotine may modify energy availability in peripheral tissues through leptin, although its effect is not evident in body weight gain.

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