Leptin Inhibits Food Intake without Affecting Brain NOx Levels in Food-Deprived Mice

Yumi Sugimoto,*a Hiroshi Hirose,* Tatsuo Yagura,b and Jun Yamadaa

*Department of Pharmacology, Kobe Pharmaceutical University; 4–19–1 Motomakita-machi, Higashinada-ku, Kobe 658–8538, Japan; and b Department of Bioscience, Faculty of Science and Technology, Kwansei-Gakuin University; 2–1 Gakuen, Sanda 669–1337, Japan. Received August 29, 2002; accepted October 22, 2002

Effects of leptin on food intake and nitric oxide (NO) metabolites (nitrite and nitrate, NOx) levels of brain were investigated in mice. Leptin dose-dependently decreased milk intake in food-deprived mice. Administration of leptin at a dose of 1 mg/kg, which induces an apparent hypophagia, did not affect NOx levels in the hypothalamus and frontal cortex. These results suggest that leptin reduces food intake in food-deprived mice without altering NO production in the hypothalamus, which plays an important role in regulation of feeding.

Key words nitric oxide; food intake; NOx; hypothalamus; frontal cortex

Leptin, the ob gene product, is secreted from white adipose tissues.1,2 Leptin potently induces anorexia and increases energy expenditure in animals.1–5 Leptin suppresses food intake in both obese and normal animals.1–3 It was reported that leptin decreases food intake in genetically obese ob/ob mice, which are deficient in leptin.4,5 It has been suggested that leptin-induced hypophagia is elicited by activation of leptin receptors in the central nervous system.1–3 Hypophagic effects of leptin have been investigated regarding several neurotransmitters and hormones. For instance, it was reported that corticotropin releasing hormone (CRH), melanin stimulating hormone (MSH), neuropeptide Y or histamine may be targets of leptin for expressing anorexia.1,2

Nitric oxide (NO) plays a role in several physiological functions including regulation of vascular tone and blood pressure.6 Previous reports demonstrated that NO may participate in the regulation of food intake. It was reported that NO synthase (NOS) inhibitors such as Nω-nitro-L-arginine or Nω-nitro-L-arginine methyl ester (L-NAME) decreases food intake in obese and normal animals.7–9 NO synthase inhibitors also inhibit hyperphagia elicited by 2-deoxy-D-glucose, chloridrazepoxide or morphine.10–13 Squadrito et al. reported that the NOS inhibitor induces anorexia by modifying brain serotonin, which is an anorectic neurotransmitter.8

These previous findings raise the possibility that there may be an interaction between NO and leptin in the regulation of food intake. Previously it was reported that leptin may decrease NOS activity in the brain.14 Thus, following the administration of leptin, NO production may be decreased in the brain. However, it has not yet been clarified whether leptin alters NO production. In the present study, therefore, we investigated the effects of leptin on food intake of food-deprived mice and NO metabolites (nitrite and nitrate, NOx) levels in brain.

MATERIALS AND METHODS

Animals Male ddY mice weighing 28–32 g were obtained from SLC Japan Inc. (Japan). Mice were given free access to food and water and they were housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature at 23±1 °C and humidity at 55±5%. Mice werestarved for 24 h before experiments. Water was always provided during experiments. After starvation for 24 h, about 3–5 g body weights decreased.

Drugs and Treatment Mouse recombinant leptin was obtained from PeproTech EC (U.K.). Leptin was dissolved in citrate-Na buffer (pH 4.0) and injected i.p. at a volume of 0.1 ml/10 g. Leptin was administered between 1:00 and 2:00 p.m.

Measurement of Food Intake Mice were placed into individual stainless wire cages. Milk (Eva milk, Snow Brand, Japan) diluted with purified water three times, was given to mice and milk intake over 120 min was measured. Milk was given to mice 30 min after the injection of leptin. Milk intake was expressed as ml per 10 g of body weight.

Determination of NOx in the Brain NO metabolites (nitrite and nitrate, NOx) levels in the hypothalamus and frontal cortex were extracted and determined following the previously described method using the HPLC-diazotization detection system15–17 (HPLC-Griess method, NO analyzing system, ENO-20, Eicom, Japan). Nitrite and nitrate were separated by a reverse-phase column (10 µmolystyrene polymer, NO-PAK, 4.6×50 mm, Eicom, Japan). Nitrate was reduced to nitrite by a copperized column (NO-RED, Eicom). Nitrate was mixed with Griess reagent (1.25% HCl, 5 g/l sulfuric acid and 0.25 g/l N-naphthylenediamine) to form a purple azo dye in a reaction coil placed in a column oven at 35 °C and the absorbance of the dye product was measured at 547 nm. The mobile phase was 10% methanol containing 0.15 M NaCl, 0.15 M NH4Cl and 0.5 g/l EDTA-Na and the flow rate was 0.33 ml/min. The Griess reagent was delivered by a pump at a rate of 0.1 ml/min.

The mouse brain was rapidly removed after decapitation and the frontal cortex and hypothalamus were dissected on dry ice. The frontal cortex and hypothalamus were sonicated in 3 volumes of methanol and centrifuged at 12000 rpm for 10 min, then the supernatant was diluted with an equal volume of the mobile phase. The diluted supernatant of brain was injected into the HPLC. The total NO metabolite (NOx) levels were calculated by summing the nitrite and nitrate levels.

Statistical Analysis Dose related effects of leptin and time courses changes were analyzed by analysis of variance (ANOVA), that were followed by Dunnet’s test at each time point. Data between two groups was analyzed by Student’s t-
RESULTS

Figure 1 shows the effects of leptin on milk intake in food-deprived mice. Milk intake gradually increased during 120 min. As shown in the results, administration of leptin at 0.5 and 1 mg/kg significantly reduced milk intake.

Figures 2 and 3 show the effects of leptin on NOx levels of the hypothalamus and frontal cortex of food-deprived mice. NOx levels were determined 30 and 60 min after the injection of leptin. As shown in the results, leptin did not NOx levels of both the hypothalamus and frontal cortex of mice.

DISCUSSION

The product of the ob gene, leptin, is circulated into the blood and enters the brain, thereby controlling appetite and body weight. The present result demonstrated that a single administration of leptin i.p. 0.5 or 1 mg/kg decreases milk intake in food-deprived mice. Hypophagic effects of leptin lasted for at least 120 min. Continuous injection of leptin at lower dosages than those in this study, reduces the food intake and body weight of ob/ob mice with leptin deficiency and normal mice. However, the previous report showed that after a single administration of leptin at 1 mg/kg, it induced the anorectic effects in fasted rats, which is consistent with the present result. Therefore, the present results demonstrated that a single administration of leptin decreases food intake in food-deprived mice, although a higher dose is required than those of continuous treatment.

It has been reported that NO may be a factor in regulating food intake. Since inhibition of NO formation by NOS inhibitors decrease feeding, NO is recognized as an endogenous orexigenic factor. Calapai et al. postulated that leptin may decrease neuronal NOS activity of brain. However, there is no report examining brain NO production after treatment with leptin. It is well known that the hypothalamus is a significant brain area controlling appetite and that frontal cortex plays an important role in behavior including feeding behavior. Thus, we investigated NO metabolites, NOx levels in the hypothalamus and frontal cortex.

As shown in results, NOx levels are higher in the hypothalamus. Following the injection of leptin at 1 mg/kg, NOx levels in the hypothalamus were not changed at all. Leptin did not affect NOx levels in the frontal cortex either. Thus, leptin can elicit anorexia in food-deprived mice without decreasing NO production in the brain. A previous report indicated that administration of leptin for 5 consecutive days decreases brain NOS activity in non-fasted mice. The present result demonstrated that a single administration of leptin did not affect NOx levels in fasted mice. Thus, differences between those results and our result may be due to the experimental condition. Since NOS or NOS mRNA levels are not yet clear in this condition, further studies are required.

Treatment with NOS inhibitors decreases food intake in rats and mice. It was reported that the NOS inhibitor, L-nitro-arginine decreases feeding in food-deprived rats. This suggests that NO production may be increased by food deprivation. However, investigation on NOS levels in food-deprived rats did not show elevation in the brain. Isse et al. reported that starvation for 48 h decreases neuronal NOS mRNA levels in the rat hypothalamus. Outkonyong also demonstrated that food deprivation reduces NADPH diaphorase-positive neurons in the brain, which reflect the NOS levels. In addition, we recently found that food deprivation for 24 h did not alter hypothalamus NOx levels, while that for 48 h decreased NOx levels. Therefore, under food
deprivation, there is no elevation of NOS and NOx levels in
the brain, although the appetite is facilitated. Our present re-
sults have shown that leptin did not alter NOx levels in the
hypothalamus and frontal cortex of fasted mice, while it sup-
pressed food intake. Therefore, in food deprivation, the
anorectic effects of leptin are not associated with NO produc-
tion.

In conclusion, the present results demonstrate that periph-
erally administered leptin inhibited food intake in food-de-
prived mice. However, leptin did not alter NOx levels in the
hypothalamus and frontal cortex of food-deprived mice. Hy-
pophagia elicited by leptin has been reported to involve sev-
eral factors such as neuropeptide Y or CRH. Therefore, lep-
tin-induced anorexia in food-deprived mice may be related to
these substances rather than NO.

Acknowledgements This work was supported in part by
a Grant-in-Aid for Scientific Research (C) from the Ministry
of Education, Science, Sports and Culture of Japan and The
Science Research Promotion Fund of The Japan Private
School Promotion Foundation.

REFERENCES

2) Pellymounter M. A., Cullen M. J., Baker M. B., Hecht R., Winters D.,
3) Campfield M. A., Smith F. J., Gulsez Y., Devos R., Burn P., Science,
4) Halaas J. L., Gajiwala K. S., Maffei M., Cohen S. L., Chait B. T., Rabi-
nowitz D., Lallone R. L., Burley S. K., Friedman J. M., Science, 269,
5) Harris R. B., Redman S. M., Jr., Smagin G. N., Smith S. R., Rodgers
8) Squadrito F., Calapai G., Altavilla D., Cucinotta D., Zingarelli B., Ar-
(1994).
9) Yamada J., Sugimoto Y., Yoshikawa T., Horisaka K., Eur. J. Pharma-
10) Calignano A., Persico P., Mancuso F., Sorrentino L., Eur. J. Pharma-
12) Yamada J., Sugimoto Y., Yoshikawa T., Horisaka K., NeuroReport, 8,
2097—2100 (1997).
14) Calapai G., Corica F., Corsonello A., Sautelin L., Di Rosa M., Campo
G. M., Buemi M., Maruo V. N., Caputi A. P., J. Clin. Invest., 104,
975—982 (1999).
15) Matsumoto K., Yohimoto K., Thu Huang N. T., Abdel-Fattah M., Van
16) Uchida M., Nagatomo I., Akasaki Y., Tominaga M., Hashiguchi W.,
Kuchiwi S., Nakagawa S., Takigawa M., Brain Res. Bull., 50, 223—
227 (1999).
(2002).
18) Buysse M., Bado A., Dauge V., Neuropharmacology, 40, 818—825
20) Squadrito F., Calapai G., Altavilla D., Cucinotta D., Zingarelli B., Ar-
coraci V., Campo G. M., Caputi A. P., Neuropharmacology, 33, 83—
86 (1994).
21) Isse T., Ueta Y., Serino R., Noguchi J., Yamamoto Y., Nomura M.,
Sibuya I., Lightman S. L., Yamashita H., Brain Res., 846, 229—235
(1999).
22) Otukonyong E. E., Okutani F., Takahashi S., Murata T., Morioka N.,