Involvement of Leptin in Hypophagia Induced by the Serotonin Precursor 5-Hydroxytryptophan (5-HTP) in Mice

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We previously demonstrated that a serotonin (5-HT) precursor 5-hydroxytryptophan (5-HTP) increases serum leptin levels in mice. It was reported that administration of 5-HTP elicits hypophagia in rodents and humans. In the present study, we examined involvement of leptin in 5-HTP-elicited decreases in the milk intake of fasted mice. Serum leptin levels increased with increases in milk intake in mice, while 5-HTP strongly decreased milk intake in fasted mice compared to that in the control group. Serum leptin levels in fasted mice treated with 5-HTP were similar to those control mice after milk intake. As leptin is a powerful anorectic signal, 5-HTP-induced anorexia may be mediated by facilitation of leptin secretion.

Key words leptin; 5-hydroxytryptophan (5-HTP); 5-hydroxytryptamine (serotonin, 5-HT); food intake; mice; hypophagia

MATERIALS AND METHODS

It is well recognized that 5-hydroxytryptamine (serotonin, 5-HT) plays an important role in the central nervous system and that 5-HT participates in neurological disorders such as depression, anxiety or schizophrenia. Several drugs affecting serotonergic neurons are administered as therapy of these diseases. 5-HT is also a significant factor of regulation of food intake. A serotonin-releasing drug fenfluramine and selective serotonin reuptake inhibitors (SSRIs) reduce food intake and modify bulimia in humans. Furthermore, the 5-HT receptor agonists such as the 5-HT2A receptor agonist DOI or 5-HT2C receptor agonist mCPP can elicit hypophagia in rats and mice. Anorectic effects of 5-HT may involve both the central and peripheral nervous system, since central and peripheral injection of 5-HT or the peripheral 5-HT receptor agonist can decrease food intake.

It has been reported that administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP) decreases food intake in rodents. It is considered that the anorectic effects of 5-HTP are caused by 5-HT formation. Supplementation of 5-HTP tablets for reducing body weights have been used in Europe and U.S.A.

Leptin is an adipocytokine, released from adipose tissue. Leptin is a strong anorectic factor that affects the hypothalamic, which controls food intake. It is well known that mice deficient in leptin display marked hyperphagia and obesity. Leptin secretion is controlled by several hormones or peptides such as insulin, corticosterone or neuropeptide Y. We previously demonstrated that systemic injection of a 5-HT precursor, 5-HTP, increases serum leptin levels in mice. We further reported that hyperleptinemia elicited by 5-HTP is mediated by insulin but not corticosterone. Since both 5-HTP and leptin cause hypophagia, it is suggested that 5-HTP-induced anorexia may be related to its leptin-releasing effects.

In this study, to clarify the involvement of leptin in 5-HTP-induced anorexia, we examined the correlation between serum leptin levels and food intake in normal mice and 5-HTP-treated mice.

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 housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 am to 7:00 pm), with room temperature at 23 ± 1 °C and humidity at 55 ± 5%. Before the experiments, mice were food-deprived for 24 h, although water was allowed. After 24 h, there was a decrease of about 3—5 g of body weights.

Drugs and Treatment 5-Hydroxytryptophan (5-HTP) was obtained from Nakalai Tesque (Japan) and dissolved in saline. 5-HTP was administered i.p. at a volume of 0.1 ml/10 g body weight.

Determination of Food Intake Experiments were performed according to the method previously described. Mice were placed into individual stainless wire cages. During experiments, water was not given. Food intake was measured using milk (Eva milk, Snow Brand, Japan) diluted 3 fold with purified water. Milk was given to the mice and milk intake over 90 min was measured. Milk was given to mice 30 min after injection of 5-HTP. Milk intake was expressed as ml per 10 g of body weight.

Determination of Serum Leptin Levels Experiments were performed according to the method previously described. Mice were decapitated and blood was collected in saline. Serum leptin levels were measured using a commercially available ELISA kit (Morinaga mouse leptin kit, Japan).

Statistics Statistical significance in the dose-dependent study was evaluated by Dunnett’s test. Other results were analyzed by two-way ANOVA followed by Tukey’s test.

RESULTS

Figure 1 shows milk intake 30 min after the injection of 5-HTP. 5-HTP apparently decreased milk intake dose-dependently.

Figure 2 shows the time course changes in effects of 5-HTP on serum leptin levels of mice after milk intake. In saline-treated mice, serum leptin levels elevated after milk intake. 5-HTP increased the serum leptin levels in both fasted and milk-fed mice.
We previously reported that the 5-HT precursor, 5-HTP increases serum leptin levels in mice.\textsuperscript{15,16} We examined the mechanism of leptin secretion induced by 5-HTP. We found that hyperleptinemia induced by 5-HTP was caused by formation of 5-HT in the periphery.\textsuperscript{16} We demonstrated that 5-HTP did not affect leptin mRNA levels of white adipose tissues nor leptin release from isolated fat pads.\textsuperscript{19} This indicates that leptin secretion caused by 5-HTP are mediated by leptin-secretory factors. It is reported that insulin may enhance 5-HTP-induced hyperleptinemia and insulin deficiency induced by streptozotocin decreased it.\textsuperscript{17,19}

It is well recognized that 5-HT participates in appetite regulation.\textsuperscript{2,20} It was reported that administration of the 5-HT precursor, 5-HTP decreases food intake in rats.\textsuperscript{8} Injection of 5-HT itself either centrally and peripherally induces hypophagia in rats.\textsuperscript{2,20} Therefore, it was suggested that 5-HT formed from 5-HTP in the both central and peripheral system is involved in anorectic effects observed after administration of 5-HTP.

Leptin is an \textit{ob} gene product, which is mainly produced in the white adipose tissue and it potently induces anorexia.\textsuperscript{10,12} The ob/ob mice, lacking leptin and the db/db mice, deficient in leptin receptor both display hyperphagia and marked obesity.\textsuperscript{21} Since leptin is a powerful anorectic factor,\textsuperscript{10} hypophagic effects of 5-HTP may be related to leptin.

The present findings demonstrate that feeding increases serum leptin levels, which is in agreement with previous findings.\textsuperscript{22} Increases in food intake are correlated with elevation of serum leptin levels.\textsuperscript{22} It further supports findings that leptin provides a signal to stop ingestion.

Administration of 5-HTP increases serum leptin levels in fasted mice. In previous reports, we showed that 5-HTP elevated serum leptin levels in non-fasted mice.\textsuperscript{15,16} Thus, it indicates that 5-HTP can increase leptin secretion in both fasted and non-fasted mice. Serum leptin levels after administration of 5-HTP are similar to those after milk intake in mice.

As shown in the results, 5-HTP significantly decreased food intake in fasted mice. This result is in agreement with previous reports,\textsuperscript{23} showing that 5-HT at a dose of 100 mg/kg powerfully inhibited food intake and almost abolished feeding. As shown in results, 30 min after the injection of 5-HTP, that is, before milk intake (0 min), serum leptin significantly increased. Furthermore, serum leptin levels in mice treated with 100 mg/kg of 5-HTP are high, comparable to the levels in saline-treated, milk-fed mice. Thus, 5-HTP increased serum leptin levels regardless of whether food was eaten. Therefore, these findings suggest that the leptin secretory effects of 5-HTP may be involved in its anorectic effects.

A previous report indicated that changes in gastric motility may be related to the anorectic effects of 5-HTP.\textsuperscript{24} However, the mechanisms of anorexia induced by 5-HTP have not yet been established. Our findings demonstrate that serum leptin levels in fasted mice treated with 5-HTP were similar to those levels of control mice after milk intake. It is well known that leptin is a powerful anorectic signal.\textsuperscript{10} Therefore, our findings demonstrate that 5-HTP-induced anorexia may be mediated by its facilitation of leptin secretion.

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**REFERENCES**


