Effects of Insulin and Adrenalectomy on Elevation of Serum Leptin Levels Induced by 5-Hydroxytryptophan in Mice

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We previously reported that a serotonin precursor 5-hydroxytryptophan (5-HTP) increases serum leptin levels in mice. In this study, we studied the effects of insulin and adrenalectomy on hyperleptinemia induced by 5-HTP. Co-administration of insulin significantly increased hyperleptinemia elicited by 5-HTP. 5-HTP itself increased serum insulin levels. Adrenalectomy, which depletes corticosterone, did not abolish hyperleptinemic effects of 5-HTP. These results suggest that insulin may participate in hyperleptinemic effects of 5-HTP and that the involvement of corticosterone in effects of 5-HTP may be probably small.

Key words leptin; 5-hydroxytryptophan (5-HTP); 5-hydroxytryptamine (5-HT); insulin; adrenalectomy; corticosterone

5-Hydroxytryptamine (serotonin, 5-HT) plays a role in several physiological functions such as regulation of mood, behavior or body temperature. 5-HT is involved in feeding behavior via both central and peripheral systems. The roles of 5-HT in these effects have been investigated using 5-HT receptor agonists or the 5-HT precursor, 5-hydroxytryptophan (5-HTP). For example, systemic administration of 5-HT receptor agonists, such as the 5-HT2A receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)2-aminopropan (DOI) or the 5-HT2C receptor agonist m-chlorophenylpiperazine (mCPP) decreases food intake in mice and rats. The 5-HT precursor 5-HTP also suppresses feeding in rodents. 5-HT also plays a role in secretion of several hormones. It was reported that 5-HTP increases secretion of prolactin or corticosterone in rats.

Leptin, a product of the obese gene is released from adipose tissues into the blood and it strongly suppresses appetite. Mice lacking with leptin or leptin receptors display a marked obesity and increased food intake. It has been demonstrated that leptin release and blood leptin levels can be altered by several factors. Cytokines including TNF-α and IL-1 increase leptin secretion. Several hormones such as insulin, growth hormone or prolactin are also known to amplify leptin synthesis and secretion.

We previously demonstrated that the systemic injection of a 5-HT precursor, 5-HTP, induces hyperleptinemia in mice. Since insulin amplifies the secretion of leptin, insulin may be involved in hyperleptinemia induced by 5-HTP. We recently demonstrated that 5-HTP increases serum insulin levels and that, in mice treated with streptozotocin, which depletes insulin, 5-HTP did not increase the serum leptin level. This suggests that hyperinsulinemia may participate in the elevation of serum leptin levels elicited by 5-HTP. In the present paper, to examine the involvement of insulin, we further studied administration of insulin on hyperleptinemia elicited by 5-HTP. Corticosterone is a factor involved in leptin secretion. However, it is not yet clear whether corticosterone is related to hyperleptinemia elicited by 5-HTP. Therefore, we studied the effects of adrenalectomy, which depletes corticosterone, on 5-HTP-induced hyperleptinemia.

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 a.m. to 7:00 p.m.), with a room temperature of 23±1 °C and humidity of 55±5%.

Drugs and Treatment 5-HTP was obtained from Nacalai Tesque (Japan) and dissolved in saline. 5-HTP was administered i.p. Injectable form of insulin solution (Novorin R, Novo Nordisk) was diluted with saline and injected s.c. at a dose of 1 U/kg. Insulin and 5-HTP were given simultaneously.

Adrenalectomy Adrenalectomy was performed under anesthesia with pentobarbital Na at 50 mg/kg. 0.5% NaCl was given to adrenalectomized mice to maintain mineral balance. Experiments were carried out 1 week after the operation. After the experiments, adrenalectomized mice were dissected to confirm that the adrenal gland had been completely resected.

Determination of Serum Leptin and Insulin Levels Mice were decapitated and blood was collected in plastic tubes. Serum leptin and insulin levels were measured using a commercially available ELISA kits (Morinaga mouse leptin kit and Morinaga insulin kit, Japan).

Statistics Statistical significance between two groups was evaluated by Student’s t-test. Other results were analyzed by two-way ANOVA followed by Tukey’s test.

RESULTS

Effects of Insulin on Serum Leptin Levels and Hyperleptinemia Induced by 5-HTP Figure 1 shows the effects of insulin on serum leptin levels of mice. Insulin at a dose of 1 U/kg did not alter serum leptin levels 30 min after the injection, but caused significant elevation after 60 min. Effects of insulin on 5-HTP-induced hyperleptinemia are shown in Fig. 2. Insulin at a dose of 1 U/kg significantly enhanced 5-HTP (100 mg/kg)-induced hyperleptinemia in mice.

Effects of 5-HTP on Serum Insulin Levels Effects of 5-HTP on serum insulin levels are shown in Fig. 3. Following the administration of 5-HTP at 100 mg/kg increased
serum insulin levels of mice.

Effects of Adrenalectomy on Hyperleptinemia Induced by 5-HTP Figure 4 shows the effects of 5-HTP on serum leptin levels of adrenalectomized mice. Adrenalectomy significantly decreased serum leptin levels. In adrenalectomized mice, 5-HTP increased serum leptin levels.

DISCUSSION

Our previous studies demonstrated that a 5-HT precursor, 5-HTP elevates serum leptin levels in mice. Leptin mainly secreted from white adipose tissue, is circulated to the blood and entered the brain, leading to stimulation of leptin receptors in the hypothalamus and anorexia. It is well recognized that 5-HT participates in regulation of appetite. Since leptin is a powerful anorectic factor, anorectic effects of 5-HT may be related to leptin.

We recently demonstrated that 5-HTP increases serum leptin levels without affecting leptin mRNA levels of white adipose tissues nor leptin release from isolated fat pads. Thus, hyperleptinemia elicited by 5-HTP may be mediated by leptin secretory factors. It has been reported that several drugs or hormones modify leptin secretion. Acute administration of prolactin or TNF-α rapidly increases blood leptin levels of rats. Insulin is known to increase both leptin synthesis and secretion. Our recent finding suggests that 5-HTP increases serum insulin levels and that streptozotocin which destroy pancreatic β cells and decreases insulin, strongly suppresses 5-HTP-induced hyperleptinemia. Thus, 5-HTP-induced hyperleptinemia is caused by its insulin-elevating effects. In the present study, to clarify the influence of insulin, we examined the effects of insulin on hyperleptinemia elicited by 5-HTP.

As shown in the results, insulin treatment significantly increased serum leptin levels 60 min after the injection, although it did not alter the level at 30 min. A previous report has shown that administration of insulin increased circulating leptin levels of rats within at least 4 h, which is consistent with the present results. Co-administration of insulin amplified hyperleptinemia elicited by 5-HTP. Stimulatory effects of insulin were observed 30 min after the injection, which time insulin itself did not affect basal serum leptin levels.

Our recent report indicated that in streptozotocin-treated mice, that is insulin-depleted mice, 5-HTP did not increase serum leptin levels at all. As shown in results, 5-HTP significantly increased serum insulin levels 30 and 60 min after the injection, which time insulin itself did not affect basal serum leptin levels.

Fig. 1. Effects of Insulin on the Serum Leptin Levels of Mice

Results are shown as mean±S.E. (n=5—6). Insulin was given s.c. *p<0.05.

Fig. 2. Effects of Insulin on 5-HTP-Induced Hyperleptinemia in Mice

Results are shown as mean±S.E. (n=5—6). 5-HTP at 100 mg/kg was given i.p. Insulin at 1 IU/kg was given s.c. Insulin and 5-HTP were administered simultaneously. **p<0.01, ***p<0.001 vs. saline of respective group. ###p<0.001 vs. saline + 5-HTP-treated mice. §p<0.05 vs. saline-treated mice.

Fig. 3. Effects of 5-HTP on the Serum Insulin Levels of Mice

Results are shown as mean±S.E. (n=6). 5-HTP at 100 mg/kg was given i.p. ***p<0.001.

Fig. 4. Effects of 5-HTP on the Serum Leptin Levels of Adrenalectomized Mice

Results are shown as mean±S.E. (n=5—6). 5-HTP at 100 mg/kg was given i.p. Serum leptin levels were determined 30 min after the injection of 5-HTP *p<0.05, ***p<0.001 vs. saline of respective group. ###p<0.001 vs. 5-HTP of sham-operated mice. §p<0.05 vs. saline of sham-operated mice.
corticosterone can raise serum leptin levels, corticosterone may be involved in hyperleptinemia induced by 5-HTP. Thus, we examined the effects of 5-HTP on serum leptin levels in adrenalectonized mice. Tan et al.\textsuperscript{17} reported that adrenalectomy inhibited leptin mRNA and serum leptin levels. As shown in results, adrenalectomy reduced basal serum leptin levels of mice, which is in agreement with a previous report. In adrenalectomized mice, administration of 5-HTP significantly increased serum leptin levels. The ratio of serum leptin levels increment elicited by 5-HTP was similar between that of sham and adrenalectomized mice. However, after adrenalectomy, the absolute increment amount of leptin levels elicited by 5-HTP decreased, compared to that of sham group. Therefore, although corticosterone may not play a major role in 5-HTP-induced hyperleptinemia, it may be partially involved in effects of 5-HTP. Adrenalectomy diminishes both the adrenal cortex and medulla, resulting in abolition of corticosterone and adrenaline. It was reported that catecholamine decreases serum leptin levels, since catecholamine synthesis inhibitor α-methyl-p-tyrosine increases serum leptin levels.\textsuperscript{21} 5-HTP-induced elevation of serum leptin levels was not enhanced by adrenalectomy. Therefore, 5-HTP-induced hyperleptinemia is not associated with adrenal catecholamines.

In conclusion, our results demonstrate that insulin amplifies hyperleptinemia induced by 5-HTP. Therefore, the present finding further suggests that insulin may be important to the appearance of hyperleptinemic effects elicited by 5-HTP. Adrenalectomy did not abolish 5-HTP-induced hyperleptinemia, although the increment of leptin levels decreased. This indicates that the involvement of corticosterone in 5-HTP-induced hyperleptinemia may be smaller than that of insulin.

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

REFERENCES