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European Journal of Pharmacology 406 (2000) 159–162

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Short communication

Serum leptin levels after central and systemic injection of a serotonin precursor, 5-hydroxytryptophan, in mice

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Received 12 July 2000; received in revised form 10 August 2000; accepted 15 August 2000

Abstract

Effects of peripheral and central injections of a serotonin (5-hydroxytryptamine, 5-HT) precursor, 5-hydroxytryptophan (5-HTP), on serum leptin levels were studied in mice. Intracerebroventricular (i.c.v.) injection of 5-HTP or 5-HT did not increase serum leptin levels, although the peripheral injection of 5-HTP elicited an apparent hyperleptinemia. The elevation of serum leptin levels in mice induced by the peripheral injection of 5-HTP was inhibited by pretreatment with the peripheral aromatic amino acid decarboxylase inhibitor, benserazide. Furthermore, the peripheral injection of 5-HT increased serum leptin levels. These results suggest that the hyperleptinemia following systemic injection of 5-HTP is elicited by 5-HT formed in the peripheral system.

Keywords: Leptin; 5-HTP (5-Hydroxytryptophan); 5-HT (5-hydroxytryptamine, serotonin); Benserazide; (Mouse)

1. Introduction

It is well known that serotonin (5-hydroxytryptamine, 5-HT) regulates feeding behaviour and decreases food intake in humans and rodents (Dourish, 1995). It has been suggested that 5-HT-induced anorexia is mediated by the 5-HT receptors, including the central 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} subtypes, since agonists of these receptors, including 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) or 1-(3-chlorophenyl)piperazine (mCPP), elicit hypophagia in rats (Curzon, 1990; Dourish, 1995). Moreover, 5-HT receptors located in the peripheral system relate to hypophagic effects of 5-HT, as the peripheral 5-HT receptor agonists decrease food intake (Simansky, 1996; Sugimoto et al., 1996). Many neurotransmitters or hormones, such as opioid, neuropeptide or glucocorticoid, are related to the regulation of food intake (Bernardis and Bellinger, 1996). Leptin has been newly identified as the product of the obese gene and it is mainly released from adipose tissues (Flier, 1997; Fruhbeck et al., 1998). Leptin strongly suppresses food intake and is a powerful satiation signal and can reduce body weight (Pellymounter et al., 1995; Fruhbeck et al., 1998; Auwerx and Staels, 1998).

We previously demonstrated that the systemic injection of a 5-HT precursor, 5-hydroxytryptophan (5-HTP), induces hyperleptinemia in mice (Yamada et al., 1999). This result first demonstrated that the serotonergic mechanism is involved in secretion of leptin and that leptin and 5-HT may interact in food intake regulation. Since 5-HTP can cross the blood–brain barrier, it may cause hyperleptinemia through its central and/or peripheral actions. We now aimed to clarify whether hyperleptinemia induced by 5-HTP originated centrally or peripherally. To this end, we studied the effects of central and peripheral injections of 5-HTP on serum leptin levels in mice.

2. Materials and methods

2.1. Animals

Male ddY mice weighing 28–32 g were obtained from SLC Japan (Japan). Mice were given free access to food and water and housed under a controlled 12-h/12-h light–dark cycle (light from 0700 to 1900 h), with a room temperature of 23 ± 1°C and humidity of 55 ± 5%.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00624-5

2.2. Drug treatment

5-HTP and 5-HT creatinine sulfate were obtained from Nakarai Tesque (Japan) and Merck (Germany), respectively. 5-HTP and 5-HT were dissolved in saline. Benserazide was purchased from RBI (USA) and dissolved in saline. 5-HTP and 5-HT were injected intraperitoneally (i.p.) at a volume of 0.1 ml/10 g or i.c.v. at 5 μl/mouse according to the method of Haley and McCormik (1957).

2.3. Determination of serum leptin levels

Mice were decapitated and blood was collected in plastic tubes. Serum leptin was measured using a commercially available ELISA kit (Morinaga mouse leptin kit, Japan).

2.4. Statistics

Dose-related effects of 5-HTP or 5-HT on serum leptin levels were evaluated by one-way analysis of variance

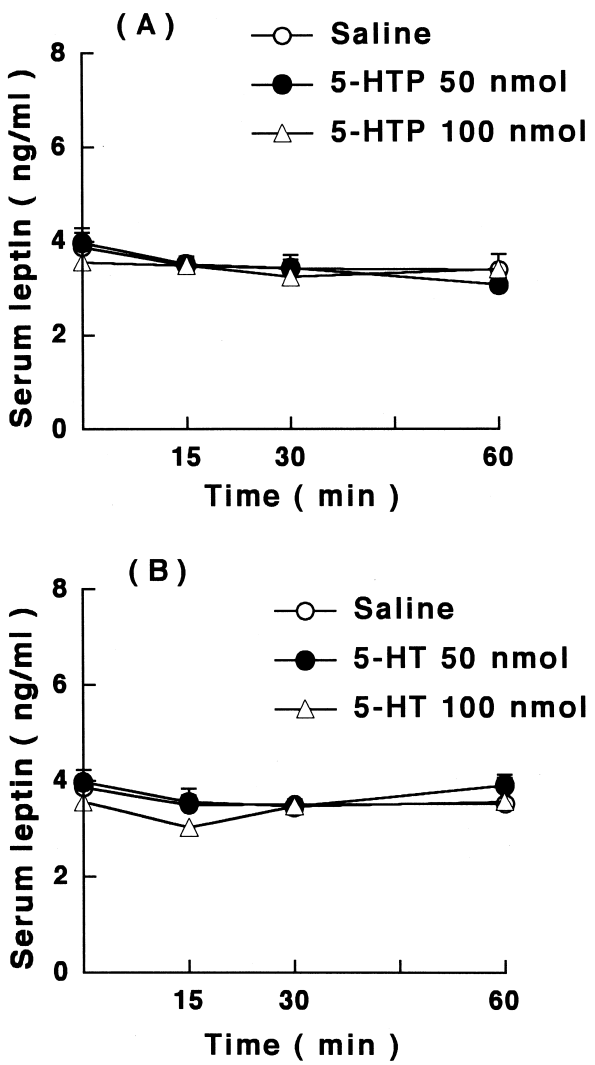

Fig. 1. Serum leptin levels in mice after intracerebroventricular injections of 5-HTP and 5-HT. Results are shown as means ± S.E. ($N = 5–8$). 5-HTP and 5-HT were given i.c.v.

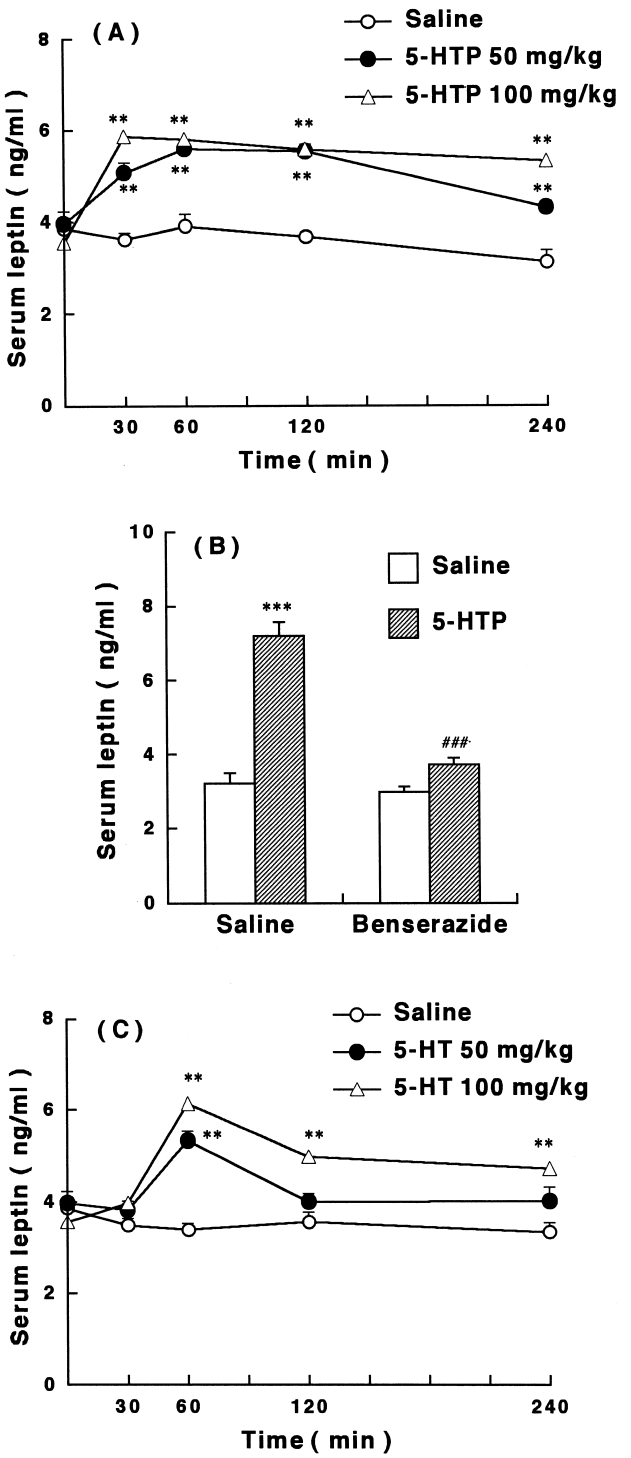

Fig. 2. Effects of 5-HTP and 5-HT i.p. on serum leptin levels and effects of benserazide on 5-HTP-elicited hyperleptinemia in mice. (A) Effects of 5-HTP i.p. on serum leptin levels of mice. Results are shown as means ± S.E. ($N = 5–8$). 5-HTP was injected i.p.; $^{**}P < 0.01$. (B) Effects of benserazide on 5-HTP-induced hyperleptinemia in mice. Results are shown as means ± S.E. ($N = 5–7$). 5-HTP was injected i.p. at 100 mg/kg. Benserazide at 50 mg/kg was given i.p. 30 min before the injection of 5-HTP. Serum leptin levels were determined 30 min after the injection of 5-HTP. $^{***}P < 0.001$ vs. saline of respective group; $\#\#\#P < 0.01$ vs. saline + 5-HTP-treated group. (C) Effects of 5-HT i.p. on serum leptin levels of mice. Results are shown as means ± S.E. ($N = 5–8$); $^{**}P < 0.01$.

(ANOVA) followed by Dunnett's test. Effects of benserazide on 5-HTP-elicited hyperleptinemia were analyzed by two-way ANOVA followed by Tukey's test.

3. Results

Fig. 1A shows the effects of 5-HTP i.c.v. on serum leptin levels in mice. Neither 5-HTP i.c.v. nor 5-HT i.c.v. modified serum leptin levels (Fig. 1B).

Fig. 2A shows the time course of changes in the serum leptin levels following the administration of 5-HTP i.p. An apparent hyperleptinemia is elicited by 5-HTP i.p., which persisted for over 240 min. Effects of the peripheral aromatic amino acids decarboxylase inhibitor, benserazide, on 5-HTP i.p. (100 mg/kg)-induced hyperleptinemia are demonstrated in Fig. 2B. Pretreatment with benserazide significantly reduced the elevation of serum leptin levels elicited by 5-HTP i.p. As shown in Fig. 2C, 5-HT i.p. increased serum leptin levels in the mice.

4. Discussion

Leptin, which is released from adipose tissue into circulating blood, reduces food intake and increases energy expenditure, leading to reduced body weight (Campfield et al., 1995; Pellymounter et al., 1995; Fruhbeck et al., 1998). Elevated leptin in blood acts on the leptin receptor located in the hypothalamus (Schwartz et al., 1996). We previously demonstrated that systemic administration of the 5-HT precursor, 5-HTP, apparently elevates serum leptin levels in mice (Yamada et al., 1999). This result suggested that there may be interactions between anorectic factors, 5-HT and leptin. Peripherally administered 5-HTP can enter the brain and is converted to 5-HT in the brain as well as in the periphery. However, it remains unclear whether hyperleptinemia induced by systemic injection of 5-HTP is related to central or peripheral mechanisms.

As we showed previously, 5-HTP at 100 mg/kg elicited significant hyperleptinemia (Yamada et al., 1999). It has been demonstrated that following the peripheral injection of 5-HTP (100 mg/kg), brain 5-HT increased by about 5–10 nmol in mice (Hyttel and Fjalland, 1972; Everett, 1974). Previous studies indicated that doses of 5-HTP or 5-HT i.c.v. near 50 or 100 nmol would induce several pharmacological effects, such as hypothermia or cardiovascular effects (Krstic and Djurkovic, 1981; Yamada et al., 1988). Thus, we used doses of 50 and 100 nmol of 5-HTP or 5-HT by i.c.v. and studied these effects on serum leptin levels. Our results demonstrate that the central injection of 5-HTP did not affect serum leptin levels of mice and 5-HT i.c.v. had no effect on leptin levels either. These results suggest that elevation of 5-HTP or 5-HT in the central nervous system is not related to hyperleptinemia induced by the peripheral injection of 5-HTP. Recently, Dryden et al. (1999) reported that the single and i.p. injection of a selective serotonin reuptake inhibitor, fluoxetine, caused a small but significant reduction of plasma leptin levels in lean rats 4 h after the injection, while this was not observed in Zucker fatty rats. Chronic treatment with fluoxetine for 7 days induces significant decreases in plasma leptin levels of both lean and fatty rats. Since fluoxetine increases 5-HT levels at the synaptic cleft in brain by inhibition of 5-HT transporter, the central serotonergic mechanisms may suppress leptin secretion.

In contrast to central administration, peripheral injection of 5-HTP increased serum leptin levels, which is consistent with our previous report (Yamada et al., 1999). We previously demonstrated hyperleptinemia induced by 5-HTP i.p. up to 60 min after the treatment. The present study showed that hyperleptinemia induced by 5-HTP i.p. lasts for 240 min. Hyperleptinemia elicited by 5-HTP i.p. is inhibited by the peripheral aromatic amino acids decarboxylase inhibitor, benserazide, which blocks 5-HT formation from 5-HTP in the periphery. This result is in agreement with results of a previous study using another inhibitor, carbidopa (Yamada et al., 1999). Furthermore, the systemic injection of 5-HT increased serum leptin levels. Therefore, these findings suggest that the hyperleptinemia induced by the systemic injection of 5-HTP is elicited by 5-HT formed in the periphery but not in the central nervous system.

It has been reported that the systemic injection of 5-HTP suppresses food intake in rats and that its effects are partially prevented with carbidopa (Blundell and Latham, 1979). Taken together with previous findings, our results raise the possibility that hyperleptinemia induced by 5-HTP is associated with hypophagic effects.

In summary, the present results demonstrated that central injections of either 5-HTP or 5-HT did not increase serum leptin levels of mice, while the peripheral injection of 5-HTP- and 5-HT-induced hyperleptinemia. The results of coadministration of 5-HTP and benserazide further suggest that 5-HT formed in the periphery is strongly associated with hyperleptinemia. It is known that leptin is synthesized as an obese gene product in adipocytes and is released into the blood (Flier, 1997; Fruhbeck et al., 1998). This suggests that the increases in serum leptin levels elicited by 5-HTP i.p. may be related to the mechanism of leptin formation from the *ob* gene and the secretion process from adipocytes.

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