Proline Decreases the Suppressive Effect of Histidine on Food Intake and Fat Accumulation

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Summary We recently suggested that proline might decrease the suppressive effect of histidine on food intake. Our purpose in the present study was to investigate the influence of proline on the suppressive effect of histidine on food intake and accumulation of body fat. Male Wistar rats were divided into four groups and allowed free access to the following diets for 3 wk: control (C), 5% proline (P), 5% histidine (H), or 5% histidine plus 10% proline (HP) diets. Food intake for 7 d and retroperitoneal fat tissue weight at the end of the experimental period of the HP diet group were greater than those of the H diet group, whereas no significant difference existed between the HP diet group and the C diet group. Our results indicate that proline inhibits the influence of histidine on food intake and accumulation of body fat.

Key Words histidine, proline, food intake, body fat, inhibition

A common factor in metabolic syndrome is visceral obesity, which is associated with hyperlipidemia, hyperpiesia and hyperglycemia. Fat accumulation in adipose tissues is caused by a decreased basal metabolic rate due to a lack of exercise and excessive caloric intake (1). Therefore, the prevention and resolution of obesity may play a role in preventing the development of metabolic syndrome.

Many substances are known to regulate ingestive behavior, including histamine. Hypothalamic neuronal histamine has anti-obesity effects such as suppressed food intake (2–6), increased lipolysis in white adipose tissue (WAT), and increased energy consumption through increased expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) (7–9). Histamine cannot cross the blood-brain barrier (BBB), which is in contrast to histidine, a precursor in the synthesis of histamine (10). When histidine is absorbed and crosses the BBB, it is converted into histamine by histidine decarboxylase (HDC) within neuronal cell bodies located in the tuberomammillary nucleus (TMN) of the hypothalamus (11). We previously reported on the anti-obesity effect of histidine using nutrition surveys in humans and animal experiments (12–16). Oral histidine administration in rats suppressed food intake and the accumulation of body fat (16). In the human nutrition survey, there was a negative correlation between energy intake and histidine/protein intake (17). However, this suppressive effect decreased under conditions of high proline/protein intake. Recently, we also reported the influence of proline on the suppressive effect of histidine on food intake using the cafeteria method, where rats are allowed to choose two diets (18). Intake of the histidine diet was significantly lower than that of the histidine plus proline diet. These results strongly suggested that proline reduced the suppressive effect of histidine on food intake. However in the cafeteria method, the preference of diet influences the selection of diet and food intake. Furthermore, it is difficult to see the influence of each diet, since two different diets were set in the same cage. The purpose of this study was to clarify the influence of proline on suppressive effect of histidine on food intake using a single diet in an animal experiment.

Materials and Methods

Animal experimental procedures. Five-week-old male Wistar rats (n=24) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The animals were housed in individual stainless steel cages with wire mesh bottoms in a temperature- (23±2°C) and light-controlled room (lights on from 2:00 AM to 2:00 PM). The rats were acclimated to the experimental conditions and given free access to the control diet (C diet) for 4 d. The components of diets are shown in Table 1. After the rats were acclimated to the C diet, they were assigned to one of four groups (n=6/group) on the basis of body weight (average initial body weight=140 g, range=134–147 g) and allowed free access to water and the experimental diets for 3 wk: 25% casein (C) diet, 20% casein plus 5% proline (P) diet, 20% casein plus 5% histidine (H) diet and 20% casein plus 5% histidine and 10% proline (HP) diet (Table 1). Food intake and body weight were recorded daily. At the end of the experimental period, the rats were anesthetized using somnopentyl (Kyoritsuseiyaku Co., Ltd., Japan)

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injection, whole blood was collected from the heart, and the retroperitoneal fat and epididymal fat removed and weighed. The animal use committee of Bunkyo University approved the study (approval number: 10-2), and animals were maintained in accordance with university guidelines for the care and use of laboratory animals.

**Statistical analysis.** Data are presented as means± standard error of the mean. The significance of differences among the group was determined by one-way analysis of variance (ANOVA) with Ryan’s multiple-range test. *p*<0.05 was considered a statistically significant difference.

**Table 1. Composition of the experimental diets.**

<table>
<thead>
<tr>
<th>Components (g/kg)</th>
<th>C diet</th>
<th>P diet</th>
<th>H diet</th>
<th>HP diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>—</td>
<td>—</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Proline</td>
<td>—</td>
<td>50</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Casein</td>
<td>250</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>377</td>
<td>377</td>
<td>377</td>
<td>277</td>
</tr>
<tr>
<td>Corn oil</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sucrose</td>
<td>210</td>
<td>210</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Cellulose</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*1 Based on the American Institute of Nutrition dietary allowance for rats (AIN-76).

**Results**

The content of nitrogen sources (protein and free amino acids) in the C diet, P diet and H diet were set to the same level (Table 1). Since a significant suppression effect on food intake of histidine had been observed at a concentration of 5%, the concentration of histidine in this experiment was set at 5% (16). The concentration of proline was also set to the same 5% concentration to see the influence of proline under the same protein content conditions. We also prepared a diet containing both proline and histidine. In previous research, a high proline/histidine content ratio in the diet suggested that proline decreased the suppressive effect of histidine on food intake and proline content added 2 volumes of additive amount of histidine in the histidine diet in the cafeteria method (17, 18); thus, we examined the influence of proline by supplementing with 5% histidine and 10% proline (Table 1).

Food intake was significantly greater in the HP diet group than in the H diet group on days 3 to 6, while food intake in the H diet group was significantly lower than in the C diet group on each day. Moreover, the C and P diet groups did not significantly differ in food intake (Fig. 1). Total food intake and body weight gain over the 7 d showed a similar tendency; the HP diet group values were significantly higher than those of the H diet group, while there were no significant differences between the C and P diet groups (Table 2). However, there was little difference among the four diet groups in food intake and body weight gain beginning on day 8, and both total

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**Table 2. Food intake and body weight gain in rats in each diet regimen.**

<table>
<thead>
<tr>
<th></th>
<th>C diet</th>
<th>P diet</th>
<th>H diet</th>
<th>HP diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/7 d)</td>
<td>104.8±2.8a</td>
<td>97.5±1.9a</td>
<td>81.9±1.3b</td>
<td>87.2±0.8c</td>
</tr>
<tr>
<td>Body weight gain (g/7 d)</td>
<td>56.6±2.1a</td>
<td>52.2±0.6a</td>
<td>43.2±1.1b</td>
<td>47.3±1.3c</td>
</tr>
</tbody>
</table>

*1 Data are expressed as mean±standard error of mean (n=6). Different letters indicate mean values that were significantly different from each other (*p*<0.05) using ANOVA by Ryan’s multiple-range test.
Inhibitory Effect of Proline on Histidine

Food intake and body weight gain over the 3 wk duration did not significantly differ between the H and HP diet groups (data not shown).

The HP, C, and P diet groups did not differ in retroperitoneal fat weight. However, the weight of retroperitoneal fat in the HP diet group was significantly lower than that of the other groups (Fig. 2A). This finding supports that of a previous study in which the retroperitoneal fat weight of the H diet group was significantly less than that of the C diet group (16). The epididymal fat weight of the HP diet group showed an increasing tendency compared to the H diet group; however, this difference was not significant (Fig. 2B).

Discussion

Histidine can pass the BBB and enter the brain, where it is taken up into histamine-containing neurons and is converted to histamine by HDC. The released histamine controls ingestive behavior through histamine H1 receptors, which belong to a family of G protein (guanine nucleotide-binding regulatory proteins) coupled receptors (3, 19, 20), and induces anti-obesity effects such as feeding suppression, lipolysis in white adipose tissue (intracerebroventricular injection of histamine promotes glycerol release from white adipose tissue), and weight reduction (2–4). Moreover, histaminergic neurons are known to participate in regulating heat production (thermoregulation) and energy consumption. Expression of UCP1 in brown adipose tissues of diet-induced obese (DIO) or diabetic (db/db) mice increased when histamine was administered to the cerebral ventricle, and histamine H1 receptor knockout mouse showed a decreased suppressive effect on food intake and expression of UCP1 (7). On the other hand, intake of histidine, which is a precursor of histamine, also affects the histamine neuron system, and the expression level of UCP1 increased and food intake decreased when a histidine diet of 5% concentration was provided to Wistar rats for 8 d (16).

In this study, food intake of the HP diet group, in which the proline content was two-fold greater than the histidine content of the H diet group, was increased compared to the H diet group (Fig. 1), indicating a reduction in the feeding suppression effect of histidine. In our previous study using the cafeteria method, intake of a high proline/histidine ratio diet was significantly increased compared to a low ratio diet, supporting the results of this experiment. However, prior to the second day, it was thought that differences in the protein contents were responsible for the lack of significant differences in food intake between the H and HP diet groups.

Changes in dietary protein contents have been reported to decrease early food intake in animal experiments (21, 22). Therefore, we consider that protein contents may influence the dietary intake of the HP diet group (protein contents 35.3%) because the tested animals in our experiments were initially maintained on the C diet with a protein content of 25.3%.

Furthermore, we consider that the addition of proline had no effect on ingestion behavior because food intake was the same between the C diet and P diet groups. In addition, it is discussed that the proline alone has no food intake enhancement effect since the previous research showed a 0% to 5% proline concentration diet has no influence on the food intake (18, 23). This study also revealed the food intake of the P diet group with 5% proline concentration did not increase day by day. On the other hand, in the P diet, histidine contained in the casein of the diet did not show any obvious food intake enhancement effect despite the high proline/histidine ratio, since the histidine concentration was low. From this result, not only the proline/histidine ratio, but also the histidine and proline concentration is considered to be important.

Moreover, total food intake and total body weight gain over the experimental period did not significantly differ; however, retroperitoneal fat weight differed between groups (Fig. 2). We suggest that proline inhibited the lipolysis action of histidine in the presence of both histidine and proline since a significant increase in fat was
observed in the HP diet group compared to the H diet group. Therefore, we conclude that proline decreases the suppressive effect of histidine on food intake and accumulation of body fat in rats.

The relation between obesity and histamine dynamics in the brain has been studied using various model animals. Hypothalamic histamine content in Zucker fatty (fa/fa) rats was significantly lower than in Wistar rats (24). On the other hand, by chronic infusion of histamine to the cerebral ventricle of DIO mice and db/db mice, food intake was suppressed and WAT decreased significantly (7). Our result showed that the proline inhibited the decrease of both food intake and WAT weight, which are affected by activation of the histamine neuronal pathway. Consequently, proline is expected to exert its inhibition somewhere along the histaminergic pathway. One of the possibilities for proline effects on the histaminergic neuron pathway is the disturbance of histamine supply to the brain. To clarify this point will be a milestone in deciding the direction of this work. Further experiments are necessary to determine the levels of free histidine, histamine and proline in plasma and brain, under the same conditions as in this work.

Acknowledgments

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REFERENCES