Administration of Capsiate, a Non-Pungent Capsaicin Analog, Promotes Energy Metabolism and Suppresses Body Fat Accumulation in Mice

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We investigated the effects of a single oral administration of capsiate, which is found in the fruits of a non-pungent cultivar of pepper, CH-19 Sweet, and has the same structure as capsaicin except for replacement of NH by O in the alkyl chain, on the thermogenesis and fat accumulation in mice. The oxygen consumption and serum adrenalin concentration were higher in both the capsaicin (10 mg/kg-body weight) and capsiate (10 mg/kg-body weight) groups than those in the control group. We also examined the effects of 2 weeks of administration of capsaicin and capsiate on body fat accumulation. Every day for 2 weeks administration of capsiate (10, 50 mg/kg-body weight/day) markedly suppressed body fat accumulation as well as capsaicin (10 mg/kg-body weight/day). These results suggest that capsiate promotes energy metabolism and suppresses body fat accumulation as does capsaicin.

Key words: CH-19 Sweet; capsiate; capsaicin; body fat; oxygen consumption

Capsium species, hot peppers, are important plants and have been used world wide as food, spices, and medicines. Capsaicin, (E)-N-[4-hydroxy-3-methoxyphenyl]methyl]-8-methyl-6-nonenamide, the major pungent component in fruits of Capsium, has been reported to increase the catecholamine secretion and energy expenditure and suppress body fat accumulation by long-term treatment in experimental animal studies. Although hot peppers and capsaicin may be used in a diet therapy for obesity, their usage as a food additive or a drug is limited by its strong pungency and nociceptive activity for humans.

Among many capsaicin analogs reported as components of hot red peppers, that with a C14 to C20 side alkyl chain had no pungency. Furthermore, Watanabe et al. reported that these long-chain non-pungent capsaicin analogs stimulated adrenalin release. Indeed, the chemically synthesized C18 long-chain capsaicin analog did not show any pungency and increased fat metabolism. Unfortunately, these long-chain non-pungent capsaicin analogs are minor components in natural hot pepper fruits and substantially hard to isolate from hot capsaicin. Many species of low-pungent hot red peppers have been analyzed but a fruit body with a higher ratio of the long-chain capsaicin analogs has not been found.

Yazawa et al. reported that the fruit of a non-pungent cultivar of pepper, named CH-19 Sweet, contains only a small amount of capsaicinoids but a considerable amount of capsaicinoid-like substances (CLSs). This new variety of sweet pepper was reported to increase body temperature and oxygen consumption in humans. Kobata et al. characterized one of the CLSs and found a non-pungent capsaicin analog, named capsiate. They also reported that capsiate has a structure similar to capsaicin and no pungency orally.

A single administration of capsiate was reported to increase body temperature in mice. To further investigate the effects of capsiate on energy metabolism, we measured respiratory gas and serum components in mice. Furthermore, we examined the effects of capsiate on body fat accumulation by measuring the tissue weight of mice after 2 weeks administration of capsiate.

Methods

Animals. Five-week-old male Std ddY mice (Japan Shizuoka Laboratory Center, Hamamatsu, Japan)
were used. They were housed in standard cages (33 × 23 × 12 cm) under controlled conditions of temperature (22 ± 0.5°C), humidity (50%), and lighting (lights on from 1800 to 0600). They were given free access to water and a commercial diet (type MF; Oriental Yeast, Tokyo, Japan) containing the following (g/kg diet): water 80, protein 246, fat 56, and carbohydrate 523. The care and treatment of the experimental animals conformed to Kyoto University guidelines for the ethical treatment of laboratory animals.

A single administration of capsaicin and capsiate. The mice were allowed to adapt to the laboratory housing for at least 1 week before starting the operation. To avoid circadian variations in physical activity, experiments were done from 11:00 to 16:00. The mice were prohibited access to diet 3 h before administration of samples to avoid the effect of the components in the diet or its digestion and absorption on serum components and respiratory gas. As a control, we used the solvent of capsaicin or capsiate (vehicle), 0.9% NaCl solution containing 3% ethanol and 10% Tween 80 as described elsewhere. The mice were administered the vehicle (control) or capsaicin (10 mg/kg body weight) or capsiate (10 mg/kg body weight) via a stomach tube. The respiratory gas and serum components were measured when mice were at rest (as a basal value), and after oral administration for 3 hours.

Respiratory gas analysis. The instruments used for the measurement of oxygen consumption and respiratory quotient in the mice consisted of six acrylic metabolic chambers, gas analyzers (model RL-600), and a switching system (model AN16-A-S) to sample gas from each metabolic chamber. Mice were separated into two groups with equal body weights and each mouse was placed into a metabolic chamber designed to measure respiratory gas. The details of methods were described in a previous report. Briefly, room air was pumped through the chambers and expired air was dried in a thin cotton column and then directed to an gas analyzer. The amount of fat and carbohydrate oxidized were calculated from the value of oxygen consumption and respiratory quotients using software for analysis. The data for each chamber were obtained every 7 minutes and stored on a spreadsheet. The instruments and software were obtained from Alco System, Chiba, Japan.

Serum components. Mice were divided into three groups so that the mean body weights of the groups were equal. Blood was taken from the hearts of the mice at 30, 60, 120, and 180 min after administration of capsaicin, capsiate, or vehicle. Each mouse was used only once. Serum was obtained by centrifugation and stored at −20°C until measurement. Serum free fatty acids (FFA) were measured by the acyl CoA-synthetase and acyl CoA oxidase enzyme method with a commercial kit (NEFA C-Test; Wako, Wako Pure Chemical Industries, Kyoto, Japan). Glucose was assayed by a combination of mutase and glucose oxidase with a commercial kit (Glucose CII Test; Wako). Triglycerides were assayed with a commercial kit (Triglyceride G Test; Wako). Noradrenalin and adrenalin were assayed by an HPLC-electrochemical detector (EDC-300, EiCOM, Kyoto, Japan).

Long-term administration of capsaicin and capsiate. The mice were allowed to adapt to the laboratory housing for at least 1 week. Mice were housed in a windowless room with a 12-h light/12-h dark cycle. Diet freshly provided every day was fed ad libitum. Mice were randomly divided into 4 groups so that the mean body weights of the groups were equal and administered vehicle (control) or capsaicin (10 mg/kg body weight) or capsiate (10, 50 mg/kg body weight) via a stomach tube every day for 2 weeks. Then, the mice were killed and the organs were removed and weighed.

Statistical Analysis. Data are expressed as means ± SE. The effects of time, treatment, and time × treatment were evaluated by two-way repeated measures ANOVA; for comparisons between the two groups at certain time points, Student’s t test was used (Fig. 1). Differences in adrenalin, triglyceride, free fatty acids, and glucose in the blood among the three groups (control, capsaicin, and capsiate) for each time point were analyzed by one-way ANOVA with Dunnet’s Multiple Comparison Test (Fig. 2). Body weight, food and water intake and the weight or organ data were analyzed by ANOVA, and Dunnet’s Multiple Comparison Tests were made (Figs. 3, 4, Tables 1, 2). Statistics were calculated with the Stat View software package (Macintosh Version J 5.0, Abacus Concepts, Berkeley, CA), with P<0.05 as statistically significant.

Results

Effect of a single administration

The oxygen consumption was higher in the capsaicin group than in the mice of the control group (time × treatment effect, P<0.05; Fig. 1(A)), the difference being significant at 30–60 min after administration. The same results were obtained in the capsiate group (time × treatment effect, P<0.05; Fig. 1(B)), the difference being significant around 2 hours after administration. The respiratory quotient did not differ among the groups (data not shown).

The concentration of serum adrenalin in the capsaicin group was significantly higher than in the
Fig. 1. Oxygen Consumption of Mice Administered Vehicle (control) or Capsaicin (upper panel) and Vehicle or Capsaicin (lower panel). Values are means ± SE (n = 6–8) Oxygen consumption was significantly higher in the capsaicin and capsiate groups than in the control group (time × treatment effect, P < 0.05 by two-way repeated measures ANOVA). *Significantly different from control group (P < 0.05 by Student’s t test).

control group at 30 min after administration in agreement with the previous reports (Fig. 2(A)). The concentration of serum adrenalin was also significantly higher in the capsaicin group than in the control group at 1 hour after administration. There was no difference in serum noradrenalin among the three groups (data not shown).

The serum triglyceride concentration was significantly lower in both the capsaicin and capsiate group than in the control group 1 and 2 hours after administration (Fig. 2(B)). The serum free fatty acid concentration was significantly higher in both the capsaicin and capsiate group than in the control group 1 hour after administration (Fig. 2(C)). The serum glucose concentration in both the capsaicin and capsiate group was significantly higher than in the control group 3 hours after administration (Fig. 2(D)).

Effects of long-term administration
The body weight in both the capsaicin and capsiate groups tended to be lower than that in the control group throughout the two-week administration period (Fig. 3). There was no difference in food intake. Water intake in both the capsaicin and capsiate group was slightly higher than that in the control group, but not significant (Table 1).

The organ weight of heart, liver, spleen, gastroc- nemius muscle, quadriceps muscle and interscapula brown adipose tissue did not show any difference among the groups (Table 2). The fat accumulation of white adipose tissue was clearly diminished in both the capsaicin and capsiate groups (Fig. 4). A significant difference was detected in the epididymal fat weight between the control group and capsaicin or capsiate (10 mg/kg wt.) group and in the perirenal fat weight between the control group and capsaicin or capsiate (50 mg/kg wt.) group.

Discussion
This study demonstrated that a single administra- tion of capsiate increased the secretion of adrenalin and oxygen consumption in mice. We also show that 2 weeks administration of capsiate suppressed body fat accumulation in mice.

In this, we used originally prepared small highly air-tight acrylic chambers for accurately measuring
oxygen consumption of a mouse. We detected these parameters and converted digital data in a separate room to avoid stresses to the mice. This system clearly reconfirmed that capsaicin increased oxygen consumption in mice. It is unclear which organ is activated by capsaicin. We need to measure uncoupling protein in brown adipose tissue or beta-oxidase enzymes in liver or muscle.

The serum triglyceride and glucose in the control group (Figs. 1(B) and 1(D)) were gradually decreased with time. The reason for this decrease was derived from circadian variation, change in environment, or the prohibition of meals.

Table 1. Food and Water Intake of Mice During Experimental Period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Capsaicin</th>
<th>Capsiate 10 mg/kg wt.</th>
<th>Capsiate 50 mg/kg wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g)</td>
<td>71.8 ± 5.4</td>
<td>71.2 ± 5.4</td>
<td>71.0 ± 5.4</td>
<td>71.4 ± 5.4</td>
</tr>
<tr>
<td>Water intake (g)</td>
<td>91.2 ± 6.8</td>
<td>101.7 ± 7.7</td>
<td>93.2 ± 7.8</td>
<td>102.0 ± 7.6</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6).

Table 2. Relative Organ Weights of Mice Administered Vehicle, Capsaicin and Capsiate for 2 Weeks

<table>
<thead>
<tr>
<th>Muscle/Organ</th>
<th>Control</th>
<th>Capsaicin</th>
<th>Capsiate 10 mg/kg wt.</th>
<th>Capsiate 50 mg/kg wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.37 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>3.95 ± 0.10</td>
<td>4.06 ± 0.10</td>
<td>3.94 ± 0.11</td>
<td>3.93 ± 0.10</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.27 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.27 ± 0.01</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.50 ± 0.03</td>
<td>1.48 ± 0.05</td>
<td>1.41 ± 0.03</td>
<td>1.40 ± 0.09</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>0.77 ± 0.02</td>
<td>0.82 ± 0.05</td>
<td>0.81 ± 0.04</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>0.77 ± 0.04</td>
<td>0.84 ± 0.03</td>
<td>0.81 ± 0.03</td>
<td>0.79 ± 0.05</td>
</tr>
<tr>
<td>BAT</td>
<td>0.47 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>0.39 ± 0.04</td>
<td>0.39 ± 0.05</td>
</tr>
</tbody>
</table>

Fig. 3. Body Weight of Mice Administered Vehicle, Capsaicin (10 mg/kg wt.) or Capsiate (10, 50 mg/kg wt.) during the Experimental Period.

Fig. 4. Epididymal (upper panel) and Perirenal (lower panel) Adipose Tissue Weights of Mice Administered Vehicle (control), Capsaicin (10 mg/kg wt.), or Capsiate (10, 50 mg/kg wt.) for 2 weeks.

Values are means ± SE (n = 6). *Significantly different from control group by one-way ANOVA and Dunnett's multiple comparison test (P < 0.05).

Capsiate had the same effects as capsaicin in the ability of catecholamine release and the oxygen consumption increase. However, there was a difference between capsaicin and capsiate in the time-lag from the administration in the concentration of adrenaline and oxygen consumption. The cause of the difference is unclear, but one possible explanation is a difference in the absorption processes of capsiate and capsaicin in the gastrointestinal tract. This is consistent with the previous report by Kawada et al., that is, each capsaicin analog has a different absorption process in the gastrointestinal tract. Further studies on the absorption of capsiate are needed.

Two-week administration of capsiate indicated that capsiate suppressed the accumulation of body...
fat as well as that of capsaicin. In this, we gave capsaicin and capsiate via a stomach tube, because animals may avoid a diet containing capsaicin because of its pungency. Accumulation of body fat was markedly suppressed in both the capsaicin and capsiate groups, compared with the control group there being no significant difference between the capsaicin and capsiate groups. In humans, suppression of body fat accumulation by capsaicin has not been observed, mainly because only a limited amount of capsaicin can be ingested because of its strong pungency. The amount used in this study, 10 mg/kg-body weight/day, would be too hot, especially for people who do not commonly consume it in large amounts. However capsiate does not have such a pungent taste even at a dose of 50 mg/kg-body weight. This suggests that the non-pungent capsiate may be useful as a therapeutic tool against obesity.

The effect of capsiate on white adipose tissue weight was not different between the mice administered 10 and 50 mg/kg weight. In our preliminary study, the white adipose tissue weight of mice administered 2.5 or 5 mg/kg weight was to be lower than that of control group, but not significant. There results suggest that the mice need to be administered capsiate at a dose of 10 mg/kg weight to suppress their body fat weight, and do not need to be administered more than 10 mg/kg weight.

In spite of the lack of pungency, capsiate had a similar effect on energy consumption, adrenalin release, and body fat accumulation, suggesting that pungency itself is not involved in the various physiological effects of capsaicin. This may provide a new insight into the action of capsaicin and its analogs in the cells. We reported that the rise in body temperature was surpressed by capsazepin, a competitive and specific antagonist of the vanilloid receptor. This result suggests that the effect of capsiate was mediated by the vanilloid receptor.

Recently, information on the vanilloid receptor has been accumulated. Caterina et al. isolated a single cDNA encoding the capsaicin-gated channel and named this receptor vanilloid receptor subtype 1 (VR1). VR1 was expressed in sensory neurons specifically and activated by noxious heat. They also found a vanilloid receptor like protein subtype (VRL-1). VRL-1 was expressed in various organs, activated by noxious heat and did not respond to capsaicin. Liu et al. reported distinct subtypes of vanilloid receptors that respond to capsaicin by whole cell patch-clamp measurement on cultured rat trigeminal ganglion neurons. They used the phorbol 12-phenylacetate 13 acetate 20-homovanillate (PPAHV), non-pungent capsiain analog. They showed that capsazepine did not inhibit the PPAHV-induced currents in some neurons and partially inhibited them in other neurons. This suggested the presence of capsazepine-sensitive and non-sensitive subtypes of the vanilloid receptor. Biochemical studies using these agonists and antagonists have not been done on capsiate. Further studies are needed to elucidate the mechanism underlying the physiological action of capsiate.

References
13) Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Julius, D., The cap-

