Effects of Capsaicin and Isothiocyanate on Thermogenesis of Interscapular Brown Adipose Tissue in Rats

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Summary To clarify whether capsaicin or isothiocyanate, which are found in common spices, activate brown adipose tissue (BAT) function, BAT temperature, mitochondrial guanosine diphosphate (GDP) binding (a thermogenic indicator), and mitochondrial oxygen consumption were measured in interscapular brown adipose tissue (IBAT) of rats. Intramuscular injection of capsaicin (0.7 mg/kg) or isothiocyanate (3.0 mg/kg) increased significantly IBAT temperature without affecting the rectal temperature, a possible side effect, and increased significantly GDP binding and mitochondrial oxygen consumption in IBAT, as did administration of ephedrine (0.375 mg/kg). Therefore, the administration of capsaicin or isothiocyanate activates BAT function in rats.

Key Words capsaicin, isothiocyanate, ephedrine, brown adipose tissue (BAT), BAT temperature, GDP binding, oxygen consumption

Brown adipose tissue (BAT) is a main effector of diet-induced thermogenesis (1–3) as well as non-shivering thermogenesis (4), and a defect in or an absence of BAT would predispose obesity, as shown in genetically obese (ob/ob) mice (5–7), yellow KK mice (8), Japanese KK mice (9), VMH-lesioned weanling rats (10), MSG-induced obese mice (11, 12), ovariectomized rats (13), and dietary obese rats (14), although the relevance of BAT to thermogenesis per se, let alone the pathogenesis of obesity in humans is still unknown.

On the other hand, dietary supplementation of capsaicin, a pungent constituent of hot red pepper, in high fat diets lowers the perirenal adipose tissue weight and serum triglyceride concentration in rats (15) due to the enhancement of energy metabolism (16). However, the effect of capsaicin and isothiocyanate (mustard oil) on BAT thermogenesis is less substantial. Therefore, we determined whether capsaicin or isothiocyanate activates BAT thermogenesis by measuring BAT
temperature, mitochondrial guanosine diphosphate (GDP) binding, which is a thermogenic indicator, and mitochondrial oxygen consumption in BAT in rats. The results were compared with those of ephedrine, a compound which is known to activate BAT function.

MATERIALS AND METHODS

The 145 female Sprague-Dawley rats (approximate weight, 220 g; 8 weeks old) used in these experiments were purchased from Charles River Japan Inc. (Osaka, Japan) and were housed in a temperature-controlled room (22 ± 2°C) with artificial light from 0600 to 1800h. Commercial powdered chow (Charles River Japan) and tap water were available ad libitum for a 7-day period. To minimize the effects of environmental temperature, experiments were carried out in a box at 33 ± 0.3°C.

Experiment 1. Measurement of interscapular brown adipose tissue (IBAT) temperature and its response to intramuscular ephedrine, capsaicin, or isothiocyanate injection.

Fifty-five rats fasted for 24 h were anesthetized by an intraperitoneal injection of urethane (500 mg/kg). A small incision was made above the scapulae, the IBAT was partially separated from the muscle below, and a small thermistor (IT-21, Sensortek, N.J., USA) was placed under the IBAT pad. A similar thermistor was inserted into the rectum. After the rectal temperature reached a steady level, ephedrine was injected intramuscularly at the doses of 0.15, 0.375, and 0.5 mg/kg or saline was injected as a control. Furthermore, capsaicin (0.8, 0.7, and 0.6 mg/kg) dissolved in dimethylsulfoxide (DMSO), isothiocyanate (4.0, 3.0, and 2.0 mg/kg) dissolved in DMSO or DMSO alone as a control was injected intramuscularly. The IBAT and rectal temperature were recorded by a multi-channel digital recorder (TR2724, Advantest, Tokyo) at 30-sec intervals for 60 min after the ephedrine or saline injection, or for 120 min after the capsaicin, isothiocyanate, or DMSO administration.

Experiment 2. Effects of intramuscular injection of ephedrine, capsaicin, and isothiocyanate on GDP binding and mitochondrial oxygen consumption in IBAT.

Ninety rats fasted for 24 h were killed by cervical dislocation 30 min after intramuscular injection of ephedrine (0.375 mg/kg) or saline, and 80 min after the intramuscular administration of capsaicin (0.7 mg/kg), isothiocyanate (3.0 mg/kg), or DMSO (control), respectively. IBAT was rapidly removed and dissected from the connective tissue. IBAT samples were collected from 3 rats in each group for a measurement of the parameters mentioned below. IBAT samples were weighed and homogenized in ice-cold medium (pH 7.2) containing 250 mM sucrose and 5 mM potassium TES [N-tris(hydroxy-methyl)-2-amino-ethane-sulfonic acid]. The mitochondria were isolated by differential centrifugation according to the procedure described by Cannon and Lindberg (17). Tissue protein content was measured by
the method of Lowry et al. (18). Mitochondrial GDP binding was determined by Nicholls' method (19). Mitochondria were incubated at 20°C in 0.5 ml of a medium containing [3H]GDP (1.3 µCi and 10 µM), [14C]sucrose (0.123 µCi), 100 mM sucrose, 100 µM potassium atractyloside, 20 mM TES (pH 7.1), 10 mM choline chloride, and 5 µM rotenone. After 7 min of incubation, 0.4 ml aliquots were withdrawn and filtered through a Sartorius membrane filter (0.6 µm, Göttinger, W. Germany). The filters were counted for [3H] and [14C] by scintillation spectrometry in a Packard TRI CARB 460 liquid scintillation counter (Packard Downers Grove, Ill. USA). The assay mixture contained 0.26 mg of mitochondrial protein, and the assay was carried out in duplicate. [14C]Sucrose was included to calculate the volume of medium trapped on the filter. Mitochondrial respiration was determined polarographically in 2 ml of medium consisting of 100 mM KCl, 20 mM TES (pH 7.2), 4 mM KH2PO4, 2 mM MgCl2, 1 mM EDTA, 4 µM rotenone, and 10% defatted BSA at 20°C using a Rank oxygen electrode apparatus (Rank Brotteus, Cambridge, UK). Ten mM α-glycerophosphate was added as a substrate for mitochondrial respiration.

All data presented are the mean value ± SE. Statistical analysis was conducted using the Student's t-test.

RESULTS

Experiment 1

The IBAT temperature after the intramuscular injection of 0.15 mg/kg ephedrine was similar to that after the injection of saline, but that after the injection of 0.375 or 0.5 mg/kg ephedrine was significantly higher (p < 0.05–0.01) from 10 to 60 min after the injection. The rectal temperature after the injection of 0.5 mg/kg

![Fig. 1. Effects of intramuscular ephedrine injection on IBAT (A) and rectal (B) temperatures. *, p < 0.05; **, p < 0.01 vs. saline.](image-url)
ephedrine was markedly higher than that after the injection of saline, or 0.15 or 0.375 mg/kg ephedrine. The optimal dose of intramuscularly injected ephedrine for the activation of IBAT function in rats was 0.375 mg/kg. Therefore, this dose was used in Experiment 2 (Fig. 1, A and B).

The intramuscular injection of 0.6 mg/kg capsaicin markedly decreased ($p<0.05$–$0.01$) IBAT and rectal temperatures compared to the injection of DMSO alone used as a control. However, administration of 0.7 mg/kg capsaicin significantly increased ($p<0.05$) IBAT temperature 80 min after the injection but not rectal temperature. Injection of 0.8 mg/kg capsaicin increased both IBAT and rectal temperatures.

**Fig. 2.** Effects of intramuscular capsaicin injection on IBAT (A) and rectal (B) temperatures. *, $p<0.05$; †, $p<0.01$ vs. control.

**Fig. 3.** Effects of intramuscular isothiocyanate injection on IBAT (A) and rectal (B) temperatures. *, $p<0.05$; †, $p<0.01$ vs. control.
Table 1. Effects of ephedrine, capsaicin, and isothiocyanate injection (i.m.) on various biochemical parameters of interscapular brown adipose tissue (IBAT).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>DMSO (Control)</th>
<th>Ephedrine</th>
<th>Capsaicin</th>
<th>Isothiocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBAT weight (mg)</td>
<td>151.3 ± 7.6</td>
<td>153.2 ± 8.9</td>
<td>160.7 ± 12.6</td>
<td>156.5 ± 12.9</td>
<td>153.8 ± 10.2</td>
</tr>
<tr>
<td>Tissue protein content (mg)</td>
<td>12.0 ± 0.7</td>
<td>12.2 ± 0.8</td>
<td>12.4 ± 1.0</td>
<td>12.7 ± 0.9</td>
<td>11.6 ± 0.8</td>
</tr>
<tr>
<td>Mitochondrial protein content (mg)</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>O$_2$ consumption (nmol/min·mg)</td>
<td>27.2 ± 1.2</td>
<td>26.9 ± 1.3</td>
<td>36.5 ± 2.3***</td>
<td>35.1 ± 2.0***</td>
<td>31.9 ± 1.6**</td>
</tr>
<tr>
<td>GDP binding (pmol/mg)</td>
<td>121.4 ± 7.6</td>
<td>122.2 ± 5.7</td>
<td>150.3 ± 9.0**</td>
<td>150.3 ± 9.3**</td>
<td>155.7 ± 5.0***</td>
</tr>
</tbody>
</table>

Mean ± SE ($n=6$). * $p<0.05$, ** $p<0.01$; saline vs. ephedrine, capsaicin, and isothiocyanate. † $p<0.05$, ‡ $p<0.01$; control vs. ephedrine, capsaicin, and isothiocyanate.
temperatures. Both IBAT and rectal temperatures after the intramuscular injection of 2.0 mg/kg isothiocyanate were similar to those after the injection of DMSO as a control. However, administration of 3.0 or 4.0 mg/kg isothiocyanate significantly increased IBAT temperature. Although 4.0 mg isothiocyanate increased the rectal temperature significantly, the injection of 3.0 mg/kg had no effect on rectal temperature. The optimal dose for activation of IBAT function in rats by intramuscular injection was 0.7 mg/kg for capsaicin and 3.0 mg/kg for isothiocyanate was considered optimal. Therefore, these doses were used in Experiment 2 (Fig. 2, A and B, Fig. 3, A and B).

Experiment 2
No significant differences in IBAT weight, tissue protein content, or mitochondrial protein content in IBAT were observed among the 5 (ephedrine, capsaicin, isothiocyanate, saline control, and DMSO control) groups. However, GDP binding and oxygen consumption in IBAT increased significantly \((p < 0.05-0.01)\) in the ephedrine, capsaicin, and isothiocyanate groups compared with the control or DMSO groups (Table 1).

DISCUSSION
The hypothesis that functional abnormalities of brown adipose tissue (BAT), which is a main effector in diet-induced thermogenesis \((1-3)\) as well as non-shivering thermogenesis \((4)\), cause obesity has been proven using various obese animal models \((1-14)\). Therefore, the activity of reduced BAT function in obese animals may lead to the mitigation of obesity. In this study, we examined whether capsaicin and isothiocyanate, which are found in spices, activate BAT thermogenesis by measuring IBAT temperature, mitochondrial GDP binding, which is a thermogenic indicator, and mitochondrial oxygen consumption in IBAT in rats. The results were compared with those of ephedrine, a compound known to activate BAT function.

The results of the present study showed that the intramuscular injection of capsaicin or isothiocyanate activates BAT function, in a way similar to the intramuscular injection of ephedrine in rats. Therefore, to clarify whether capsaicin or isothiocyanate is effective for mitigating obesity, the effect of long-term administration of these substances to obese animals must also be examined.

Because BAT is controlled by the sympathetic nervous system (SNS) \((2, 4)\) and because many catecholamine receptors exist on BAT \((2, 4)\), intramuscular injection of ephedrine brought about an expected rapid increase in BAT temperature. However, the intramuscular injection of 0.6 mg/kg capsaicin produced hypothermia. This is the same finding as that reported by Jancso-Gabor et al. \((20)\) which shows that capsaicin administration at low doses stimulates the hypothalamic warmth detectors and produces hypothermia. However, intramuscular injection of more than 0.7 mg/kg capsaicin significantly increased IBAT temperature. Isothiocyanate, also, at a dose of more than 3.0 mg/kg increased IBAT temperature.
and about 70 to 80 min was required for the administration of capsaicin or isothiocyanate to increase IBAT temperature. This suggests that these substances do not work on BAT and the ventromedial hypothalamic nucleus which is the center of SNS directly. Our findings are in agreement with those by Watanabe et al. (21–23) which reported that capsaicin enhances energy metabolism through the secretion of catecholamines from the adrenal medulla. A detailed study of the mechanism by which BAT function is activated by capsaicin or isothiocyanate administration should be done using adrenomedullated rats and rats with denervated IBAT.

This study showed that the administration of capsaicin or isothiocyanate activates BAT thermogenesis, as ephedrine has been shown to do in rats.

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