Assessment of the Biological Similarity of Three Capsaicin Analogs (Capsinoids) Found in Non-Pungent Chili Pepper (CH-19 Sweet) Fruits

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CH-19 Sweet is a newly found chili pepper breed bearing much less pungent fruits. Because CH-19 Sweet fruits were found to contain three analogs (capsinoids) of capsaicin, a major component of pungency of hot peppers (the analogs are capsiate or CST, dihydrocapsiate or DCT, and nordihydrocapsiate or NDCT), we assessed in this study the bio-potencies of these three capsinoids by comparing them with capsaicin. The three capsinoids bound to transient potential vanilloid 1 (TRPV1) receptors expressed in cultured cells and activated Ca2+ influx in a concentration-dependent manner with similar magnitudes. In contrast to capsaicin, capsinoids at the same concentration induced virtually no nociceptive responses when applied to the eyes or the oral cavities of mice. Intravenous administration of capsaicin or 20-fold increased doses of each capsinoid to rats induced significant increases in plasma catecholamine levels. Orally administered, each capsinoid enhanced oxygen consumption in mice. Based on the present results, capsaicin and these three capsinoids should have similar bio-potency, though capsinoids do not generate pungency or sensory irritation.

Key words: capsiate; transient potential vanilloid 1 (TRPV1); sympathetic nervous system; energy metabolism; capsinoid

Capsaicin (CAP, Fig. 1) is a pungent substance of capsicum fruits known to have diverse biological activities. It is thought to prevent positive energy balance as well as obesity1,2 because it has been reported to increase catecholamine secretion,3,4 energy expenditure,5 and body fat loss after long-term treatment in experimental animals.5 Breakfast with hot peppers was reported to increase diet-induced energy expenditure in Japanese males.6 The strong pungency of hot peppers, but, restricts common long-term usage. Thus, in this context, reports that CH-19 Sweet, a non-pungent cultivar of red pepper, has biological activities similar to those of red pepper despite its non-pungency7–9 are noteworthy. Research with CH-19 Sweet or its extract expressing HEK293 cells, although they induced virtually no nociceptive responses in mice, unlike capsaicin. Intravenous administration of any of these compounds to rats elevated circulating catecholamine levels, and oral administration resulted in elevated energy consumption at similar magnitudes.

Materials and Methods

Materials. CAP was purchased from Sigma-Aldrich Japan (Tokyo). CST, DCT, and NDCT were synthesized enzymatically, as described previously.10 The purities of all compounds were confirmed to be over 98% by reversed-phase high performance liquid chromatography (HPLC). All other chemicals were guaranteed reagent-grade.

Measurement of intracellular Ca2+ concentrations in TRPV1-expressing HEK293 cells. Intracellular Ca2+ concentrations after capsaicin or one of the three capsinoid treatments were measured in HEK293 cells that stably expressed TRPV1 (named HEK293VR11 cells), essentially following a method described previously.11 Briefly, HEK293VR11 cells that stably express rat TRPV1 were maintained in
Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 250 ng/ml of amphotericin B at 37 °C under 5% CO₂/air conditions. The cells were loaded with cytoplasmic calcium indicator fura-2 AM (5 μM; Molecular Probes, Eugene, OR) in a loading buffer (5.37 mM KCl, 0.441 mM KH₂PO₄, 0.406 mM MgSO₄·7H₂O, 137 mM NaCl, 0.336 mM NaHPO₄·7H₂O, 5.56 mM glucose, 20 mM HEPES, 0.492 mM MgCl₂·6H₂O, 2.26 mM CaCl₂, and 0.1% bovine serum albumin) at pH 7.4. Time-dependent changes in fluorescence (excitation wavelength, 340 and 380 nm; emission wavelength, 500 nm) were recorded with a CAF-110 fluorospectrophotometer (JASCO, Tokyo). Each datum was expressed as a percentage of the response to 10 μM CAP in each experiment. Curve fitting and parameter estimation were carried out using the Prism4 program (GraphPad Software, La Jolla, CA).

The irritancy assay. Male ddY mice (5–6 weeks old, Japan SLC, Shizuoka, Japan) were housed in a controlled-lighting environment (lights on from 0700 to 1900 h) with food and water available ad libitum. The animals were placed individually in a transparent cage (19.5 cm × 12.0 cm × 12.0 cm) that also served as an observation chamber. After 1 h for adaptation, each mouse was taken out from its cage temporarily and administered the test reagent, as described below. Each animal was used in only one test. All the animal experiments were approved by the Animal Committee of Ajinomoto Co., Inc. (Tokyo), and the animals received care under the guidelines for care and use of laboratory-animals of this committee.

Taste aversion assay was performed by the method described by Spector et al.,17 with necessary modifications. Each animal was placed in a different chamber bedded with chips from the heating cage. Twenty μl of one of three capsinoids (0.5 mM in distilled water containing 5% dimethyl sulfoxide, DMSO) or CAP (0.5 mM) was administered to the right femoral vein for 1 min, and the 3-min period of blood sampling was started after injection. Before blood collection, 10 μl of 10% sodium pyrosulfite and 10 μl of 10% ethylenediaminetetraacetic acid (EDTA) were added to the collection tubes. After centrifugation of the blood, 300 μl of plasma was kept at −20 °C until assay. 3,4-Dihydroxybenzylamine was added to the plasma as an internal standard. Catecholamines (adrenaline and noradrenaline) were adsorbed onto activated alumina at pH 8.6, washed with methanol and water, and then eluted with 0.4N perchloric acid. The amount of catecholamines was analyzed by HPLC with an electrochemical detector, as follows: Separation was done with a reversed-phase silica gel column (Cosmosil 5C18-AR-II, φ4.6 × 250 mm; Nacalai Tesque, Kyoto, Japan) with a guard column (Cosmosil Guard Column 5C18-AR-II, φ4.6 × 10 mm) maintained at 30 °C, and detection was done using an electrochemical detector with the potential value set at 300 mV (CoalArray Multi-Channel ECD System, ESA Laboratories, Chelmsford, MA). Methanol buffer (8:92, v/v) composed of 50 mM potassium phosphate buffer (pH 3.4). 10 mM EDTA-2Na, and 100 mg/l sodium 1-octanesulfonate was used as the mobile phase with a flow rate of 1.0 ml/min.

Respiratory gas analysis. Five-week-old male Std ddY mice (Japan SLC) were housed in standard cages (33 × 23 × 12 cm) under controlled temperature (24 °C ± 0.5), humidity (50%), and lighting (lights on from 0700 to 1900 h). Each animal was anesthetized with urethane (1.2 g/kg of BW) and placed in a metabolic chamber after oral administration of vehicle or one of the three capsinoids (10 mg/kg of BW).

Utilizing a respiratory gas analysis system consisting of an acrylic metabolic chamber, gas analyzers, and a switching system (ARCO2000-RAT/ANI System, Arco System, Chiba, Japan), sampled gas from each metabolic chamber was measured as described previously.11 Briefly, room air was constantly pumped through the chamber, and expired air was dried in a thin cotton column and then introduced into a gas analyzer. Oxygen consumption was calculated as the area under the curve (AUC) from 3 to 80 min after reagent administration.

Results

The three capsinoids activated TRPV1

TRPV1 is activated by CAP and is also known as the capsaicin receptor. It is a non-selective cation channel expressed on the plasma membrane and is found in heat-sensitive neurons.19 We examined whether the capsi-
TRPV1 Activation by CAP and Capsinoids (CST, DCT, and NDCT).

TRPV1 activation was assessed by the change in Ca\(^{2+}\)-induced fluorescence of HEK293VR11 cells loaded with fura-2 AM. Data are expressed as percentages of the response to CAP at 10\(\mu\)M. Each point represents the mean value ± SEM for three to four experiments.

Irritancy-related effects of capsinoids on the oral cavity and the eye

In humans, the lesser pungency of the capsinoids is apparent upon eating CH-19 Sweet fruits. To confirm this in rodents, CAP and capsinoids CST, DCT, and NDCT were applied to the oral cavity or to the eyes of mice. At a dose (0.5 mM) of capsaicin that induced almost relentless gaping episodes (142.02 ± 7.11 s/3 min), the capsinoids induced many fewer gaping episodes that were not significantly different from the vehicle control (Table 1).

The capsinoids, moreover, did not induce an eye irritation response. The somata of sensory neurons at the eye surface are known to be located in the trigeminal ganglion. As in the gaping test, at a dose (0.5 mM) of capsaicin, which induced repeated and almost constant eye wiping episodes (20.33 ± 3.17 times/2 min), the capsinoids induced many fewer eye wiping episodes than that of CAP. Thus the potency of the capsinoids was approximately 1/10 that of CAP. These calcium responses by capsinoids (10\(\mu\)M) were not observed in the parent HEK293 cell (data not shown).

Plasma catecholamine concentrations after intravenous administration of capsaicin and the capsinoids

We have carried out experiments to measure plasma catecholamine from adrenal venous blood, particularly to assess adrenaline secretion from the adrenal medulla.\(^3\)\(^2\)\(^0\)

Table 1. Aversive Responses to Oral and to Ocular Application of 0.5 mM CAP, CST, DCT, or NDCT

<table>
<thead>
<tr>
<th>Time of gaping (s/3 min)</th>
<th>Number of eye wipeings (times/2 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>1.57 ± 0.38</td>
</tr>
<tr>
<td>CAP</td>
<td>142.02 ± 7.11*</td>
</tr>
<tr>
<td>CST</td>
<td>4.08 ± 1.54</td>
</tr>
<tr>
<td>DCT</td>
<td>8.32 ± 2.72</td>
</tr>
<tr>
<td>NDCT</td>
<td>10.69 ± 2.76</td>
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Values are the mean ± SEM (n = 6).

* \(p < 0.05\) vs. all other treatments by Fisher’s PLSD.
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potency was virtually equal among the three capsinoids.
The EC\textsubscript{50} concentration was approximately 1/10 of that of
capsaicin. The EC\textsubscript{50} value for CST was 0.58 \mu M, which is consistent with our previous report.\textsuperscript{15} The EC\textsubscript{50} values and maximum responses for DCT and NDCT were similar to that for CST. Thus TRPV1 activation potency was virtually equal among the three capsinoids.

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breakdown of the ester bond by hydrolysis before digestive absorption. But, Hachiya et al.\textsuperscript{28} reported that oral ingestion of capsicum-containing capsinoids without capsaicinoids (capsaicin or capsaicin analogs with an amide bond) activated the sympathetic nervous system in humans but did not change the blood pressure, whereas capsicum-containing capsaicinoids did. This apparent contradiction raises the question why capsinoids activate the sympathetic nervous system even though they do not enter the bloodstream in an intact form. The discrepant findings might be reconciled by the fact that TRPV1 is also expressed on peripheral nerve endings distributed in the gastric mucous membrane.\textsuperscript{29} The orally ingested capsinoids in the study of Hachiya et al.\textsuperscript{28} bound to TRPV1 expressed on the gastric surface and triggered it to activate the sympathetic nervous system.

Since it was reported that orally administration of CAP and of CST enhanced oxygen consumption in mice at similar magnitudes,\textsuperscript{1} we evaluated the bio-potency of three capsinoids orally administrated in mice with by calorimetry. The orally ingested capsinoids were to enhance energy consumption almost equally via detection of elevated oxygen consumption after treatment. Although the adrenaline secretion induced by intravenous NDCT was less than that induced by the other two capsinoids, the ability of oral NDCT to enhance energy metabolism was comparable. Thus, effenter projections can be differentiated according to the origin and/or intensity of afferent stimuli.

In this study, we found that DCT and NDCT had much less pungency than CAP, are agonists of TRPV1, and enhance energy consumption through activation of the sympathetic nervous system as well as those of CST. These results suggest DCT and NDCT contribute to the physiological functions of CH-19 Sweet fruits, including enhancing energy consumption.

References