Intragastric administration of capsiate, a transient receptor potential channel agonist, triggers thermogenic sympathetic responses

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The sympathetic thermoregulatory system controls the magnitude of adaptive thermogenesis in correspondence with the environmental temperature or the state of energy intake and plays a key role in determining the resultant energy storage. However, the nature of the trigger initiating this reflex arcs remains to be determined. Here, using capsiate, a digestion-vulnerable capsaicin analog, we examined the involvement of specific activation of transient receptor potential (TRP) channels within the gastrointestinal tract in the thermogenic sympathetic system by measuring the efferent activity of the postganglionic sympathetic nerve innervating brown adipose tissue (BAT) in anesthetized rats. Intragastric administration of capsiate resulted in a time- and dose-dependent increase in integrated BAT sympathetic nerve activity (SNA) over 180 min, which was characterized by an emergence of sporadic high-activity phases composed of low-frequency bursts. This increase in BAT SNA was abolished by blockade of TRP channels as well as of sympathetic ganglionic transmission and was inhibited by ablation of the gastrointestinal vagus nerve. The activation of SNA was delimited to BAT and did not occur in the heart or pancreas. These results point to a neural pathway enabling the selective activation of the central network regulating the BAT SNA in response to a specific stimulation of gastrointestinal TRP channels and offer important implications for understanding the dietary-dependent regulation of energy metabolism and control of obesity.

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chili pepper cultivar, CH-19 sweet (22), and has an ester bond in its structure in place of the amide bond in capsaicin, making it highly vulnerable in the digestive system. Use of this TRP channel ligand, therefore, enables us to evaluate the effect of selective stimulation of TRP channels in the GI tract without affecting those in other sensory systems (5, 6, 54). Here, we show that selective activation of TRP channels, presumably the TRPV1 type, located in the upper GI tract is sufficient to trigger the thermogenic reflex through the intermediary step of activating vagal afferents and sympathetic efferents innervating the BAT. We demonstrated this by analyzing the effects of capsiate administration into the GI tract on the discharge patterns of the sympathetic nerves innervating BAT and on blood pressure, body temperature, heart rate, and norepinephrine (NE) turnover in anesthetized rats. This finding might have important implications in understanding the dietary-dependent regulation of energy metabolism and control of obesity.

MATERIALS AND METHODS

Animals and preparations. All animal procedures in the present study were approved by the Committee for Animal Experiments at Ajinomoto and were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Male Wistar rats weighing 300–400 g (Charles River) were housed individually and maintained on a 12:12-h light-dark cycle at 23°C. Diet chow (CRF-1, Charles River) and tap water were provided ad libitum. In a subgroup of rats, subdiaphragmatic bilateral vagotomy was performed 7–14 days before the recording of sympathetic nerve activity (SNA) or measurement of circulating 3-methoxy-4-hydroxyphenylglycol (MHPG; see below). Under pentobarbital anesthesia, a small incision was made along the midline of the abdomen, and 1- to 2-cm portions of the anterior and posterior vagal trunks were resected at the central part of the divergence of the hepatic branch or just below the diaphragm, respectively. In a sham-operated group, vagal trunks were just exposed under partial laparotomy. The food intake and body weight gain recovered to almost similar levels as those of naïve animals within 7–14 days after the vagotomy.

Recording of SNA. Efferent discharge of the unilateral sympathetic nerve innervating BAT was recorded according to previously described methods (37). In brief, after 80% feeding restriction for 24 h, rats were anesthetized with urethane (1 g/kg ip), and the right femoral artery and right femoral vein were cannulated with a heparinized polyethylene catheter (30 U/ml heparin in saline) for blood pressure monitoring and drug administration, respectively. Animals were positioned prone on a heating pad, the surface of which was maintained at 37°C. Rectal temperature (Trec) was continuously monitored and recorded. Blood pressure was continuously recorded using a conventional pressure transducer (BDT-100, BRC). A silicon-made catheter (0.96-mm outer diameter, ~80-mm length) was inserted orally so that the tip of the catheter remained in the gastric cavity for the administration of drugs. A postexperiment examination made on randomly selected animals indicated that the catheter tip was consistently located in the forestomach near the level of fundus. For BAT temperature (TBAT) measurements, the tip of a thermosensor connected to a thermotransducer (BDT-100, BRC) was inserted into the right interscapular BAT pad, and the incision was sutured with silk thread. For the measurement of BAT SNA, a small midline incision ~4 cm in length was made on the back around the level of the scapula.

After visual identification of the intercostal nerve, postganglionic SNA to BAT was recorded from a small nerve bundle of the nerve trunk dissected from the ventral surface of the right interscapular BAT pad after dividing the pad along the midline and reflecting it laterally. The central cut end of the nerve bundle was placed on a bipolar platinum hook recording electrode and immersed in liquid paraffin.

Efferent nerve activity was amplified (bandpass filtered at 150–1,000 Hz) with a MEG-2100 amplifier (Nihon-Koden) and, along with blood pressure, Trec, and TBAT in the same cases (10 kHz, 16-bit), was digitized using Chart software and a PowerLab interface (AD Instruments). In some experiments, we cooled the bottom back of the rats with an ice-cold bag while turning off the heating pad system and confirmed a robust increase in the nerve discharge in a similar manner as reported in previous reports (37, 41). In a subgroup of rats, the pylorus was ligated with silk thread under partial laparotomy immediately before the SNA measurement to delimit the distribution of the drug administered via the intragastric cannula within the gastric cavity. In most of the experiments, we confirmed that the stomach lumen contained fluid and that no remaining dietary chow was discernible.

Experimental procedures. Vehicle, capsaicin, or capsiate was administered via the intragastric cannula at a volume of 5 ml/kg and a rate of 1 ml/min. Ruthenium red (1%) was administered 30 min before the administration of capsiate solution, which also contained ruthenium red. Only vehicle was preadministered in the control experiments for ruthenium red. At the end of each recording, hexamethonium chloride (C6, 20 mg/kg) was injected via the femoral vein to measure SNA in the absence of the preganglionic influence.

Quantitative analyses of SNA. Nerve signals were analyzed with Chart (AD Instruments) and Igor Pro (WaveMetrics) software packages. To generate “integrated SNA” (int-BAT SNA), raw digitized SNA signals at 10-kHz sampling frequency were leaky integrated with a 1-s time constant by a function implemented in Chart software, and average integrated activity within each 1-min period was calculated. Changes in int-BAT SNA were evaluated as follows. The int-BAT SNA value at 15 min after the administration of C6 was subtracted from the raw values of the int-BAT SNA calculated for each minute to obtain int-BAT SNA free of postganglionic and noise components, which was then used for the evaluation of percent changes in int-BAT SNA relative to the mean int-BAT SNA over a 10-min period before intragastric administration.

We estimated the power spectral density function for int-BAT SNA as follows. First, baseline drift was cancelled by subtracting a moving averaged version (slow trend component) of the raw digitized SNA signals (sampled at 10 kHz) from the raw SNA signal. This wave was then smoothed by moving average over 100-ms windows to eliminate high-frequency components so as to avoid aliasing and was resampled at 200 Hz. On the 60-s period of these data, a fast Fourier transform was performed to obtain a raw estimate of the power spectral density function from 0–100 Hz at 0.03 Hz/bin. Weighted mean frequency was calculated from the power spectral density from 0.2–5 Hz at 0.5 Hz/bin, and a 5-min mean value was obtained. These analyses were made using IgorPro 5 software (WaveMetrics) with procedures written by one of the authors (F. Kato).

Measurement of circulating levels of MHPG. Rats weighing 250–300 g were anaesthetized with pentobarbital (40 mg/kg ip), and an indwelling catheter was inserted into the right jugular vein 1 wk before the blood sampling experiments. Blood (800 µl) was sampled via the indwelling catheter 180 min after the intragastric administration of vehicle or capsiate. MHPG was extracted with activated alumina and assayed by HPLC-electrochemical detection (HPLC-EC).

Measurement of the rate of NE turnover. The effect of capsiate on the NE turnover rate was assessed according to previously described methods (17, 68). Briefly, a total of 18 rats with the indwelling catheter in the right jugular vein was divided into three groups: pre, capsiate, and vehicle groups. Ten minutes after the injection of a NE synthesis inhibitor (methyl ester of α-methyl-p-tyrosine, 250 mg/kg ip), capsiate or vehicle was administered intragastrically via cannula, rats were left unanesthetized for 4 h, and the interscapular BAT, heart, and pancreas were then rapidly dissected out immediately after euthanasia by an overdose intravenous injection of pentobarbital and frozen at ~80°C. In the pre group, rats were killed 10 min after the
injection of a NE synthesis inhibitor. Tissues were homogenized in 0.2 N perchloric acid containing 1% sodium bisulfate and 1 mM EDTA. The homogenate was centrifuged, and catecholamines were extracted with activated alumina from the resulting supernatants. NE levels in the eluted samples were assayed by HPLC-EC.

**Statistics.** Differences between values were assessed by the appropriate statistical methods specified in the text below, and statistical significance was set at \( P < 0.05 \). Values shown in the text are means ± SE.

**Chemicals.** Capsiate used in this study was synthesized from side-chain fatty acid substances in capsaicinoids extracted from chili pepper (28) and therefore was composed of capsiate (53.6%), dihydrocapsiate (27.5%), and nortiodihydrocapsiate (4.6%) (30). Dihydrocapsiate and nortiodihydrocapsiate are almost equally vulnerable in aqueous conditions as well as in the digestive tract, with a similar bioavailability by oral ingestion (5, 54) and with a similar potency to TRPV1 channels to pure capsiate (52). C6 (Sigma) was stored as a stock solution and diluted immediately before the experiment. Capsaicin (Wako) and capsiate were prepared by dissolving in vehicle solution [ethanol-Tween 80-saline at 3:10:87 (vol/vol/vol), 5 ml/kg body wt] immediately before the intragastric administration. Ruthenium red (Sigma) was dissolved in saline when administered alone or in capsiate solution when coadministered.

**RESULTS**

**Augmentation of BAT SNA by the intragastric administration of capsiate.** We intragastrically administered capsiate at doses of 30, 100, and 300 mg/kg and observed changes in int-BAT SNA. Capsiate gradually increased the discharge activity of the BAT-innervating sympathetic nerve and, accordingly, int-BAT SNA. This effect developed over the course of a 3-h observation (Fig. 1A, right, representative example with 100 mg/kg administration). In contrast, there was no apparent change in BAT SNA or int-BAT SNA after the administration of vehicle (Fig. 1A, left). Such augmentation of int-BAT SNA was observed with 30–300 mg/kg capsiate in all six animals. The administration of 30, 100, and 300 mg/kg capsiate significantly increased int-BAT SNA compared with that after vehicle administration, as confirmed by two-way repeated ANOVA with Holm-Sidak method post hoc correction (\( P < 0.05 \)) using int-BAT SNA values sampled every minute for a 0- to 180-min period (Fig. 1B). Collectively, mean int-BAT SNA values over the 0- to 180-min period after 100 and 300 mg/kg capsiate administration were significantly larger than those after vehicle administration (Fig. 1C). Administration of 100 and 300 mg/kg capsiate resulted in a similar increase in int-BAT SNA, which was not significantly different between them (\( P = 0.124 \), ANOVA with Tukey correction). Such increase in int-BAT SNA was more manifest when values sampled at a later time were compared (Fig. 1D), primarily due to a gradual increase in background BAT SNA and partly due to a gradual increase in the incidence of burst pattern discharge, which was observed in a subset of rats receiving either 30, 100, or 300 mg/kg capsiate (e.g., Fig. 1B, right; described more in detail below). The mean int-BAT SNA during each 1-h period exhibited a gradual increase after capsiate administration, which was not the case for vehicle administration (Fig. 1D).

In a subset of animals, the increased int-BAT SNA was accompanied by the sporadic appearance of phases characterized by burst-like discharges of high amplitude lasting for 10–35 min (e.g., see “180 min” in Fig. 1A, right; such activity commenced at 162 min in this animal). Such burst-like activity was observed in three of six rats with 30 and 100 mg/kg administration and in two of six rats with 300 mg/kg administration. In rats receiving 30 mg/kg capsiate, the earliest burst appeared at 76–93 min (\( n = 3 \)) after the administration and eventually reappeared for the full observation period. In rats receiving 100 and 300 mg/kg capsiate, the earliest burst activity appeared at 16–162 (\( n = 3 \)) and 30–67 (\( n = 2 \)) min, respectively. As we failed to find any other differences in other biological signals, such as arterial blood pressure and \( T_{rec} \), between those showing such burst activity and those that did not, we pooled the data obtained from these rats. Even without such burst-like discharges, either in the rats showing no such bursts or in the periods without such burst in the rats showing such burst-like activities after capsiate administration, there was a steady and clear increase in int-BAT SNA, as shown in Fig. 1A, right (int-BAT SNA). The appearance of such sporadic burst phases resulted in a large variability of the mean int-BAT SNA among animals because the timing and duration of these activities varied substantially among preparations, especially in cases in which a larger dose was administered (error bars in Fig. 1B).

It is unlikely that this augmentation of int-BAT SNA after capsiate administration resulted from changes in the global sympathetic tone because, despite the large increase in int-BAT SNA, there were no significant differences between the presence and absence of capsiate in mean arterial blood pressure over the 3-h postadministration period (change from the mean preadministration value over a 10-min period: vehicle, \( -3.7 \pm 2.2 \) mmHg; and 100 mg/kg capsiate, \( -2.4 \pm 1.5 \) mmHg; \( n = 6 \) for each group) or in heart rate (vehicle, \( -6.9 \pm 7.8 \) beats/min; and 100 mg/kg capsiate, \( -11.4 \pm 4.1 \) beats/min).

Bilateral subdiaphragmatic vagotomy and pre- and coadministration of the non-competitive TRPV1 antagonist ruthenium red (1%) almost completely abolished the promotive effect of capsiate (Fig. 1, E and F), suggesting that the activation of TRP channels and the vagal afferent pathway is involved in these effects of capsiate. In addition, the significant promotive effect of intragastrically administered capsiate (100 mg/kg) was almost unchanged even after the pylorus ligation (Fig. 1G).

To confirm that these increases in SNA BAT after capsiate administration give rise to thermogenesis in BAT, we recorded \( T_{BAT} \) in a separate set of animals in which the midline incision on the back was sutured after placing the thermosensor so as to avoid artifactual heat loss and measured \( T_{BAT} \) as precisely as possible. The results shown in Fig. 1, H and I, demonstrate that capsiate (300 mg/kg) significantly increased \( T_{BAT} \) in a ruthenium red-sensitive manner and that this increase in \( T_{BAT} \) resulted in an increase in \( T_{rec} \) (Fig. 1J), which is in a good accordance with a recent report by Kawabata et al. (25) showing a TRPV1 channel-dependent increase in \( T_{BAT} \) after the intrajejunal administration of capsiate. Taken together, these observations point to a specific thermoregulatory pathway initiating at TRP channels in the gastric region, involving vagal afferents and terminating in the sympathetic nerve innervating the BAT.

**Augmentation of BAT SNA by capsiate shows typical changes in frequency components.** The most straightforward interpretation of these effects of capsiate is that the activation of gastric TRPV1 channels by capsiate stimulates the central premotor
Fig. 1. Effects of intragastric administration of capsiate on sympathetic nerve activity (SNA) to interscapular brown adipose tissue (BAT). A: representative traces at four time points obtained from a rat administered with 100 mg/kg vehicle (left) or capsiate (right) showing raw SNA (BAT SNA), integrated BAT SNA (int-BAT SNA), arterial blood pressure (AP), heart rate (HR), and rectal temperature (Trec). Horizontal bar = 1 min. 
B: mean time courses of int-BAT SNA changes expressed as means ± SE of every 5 min of a 1-min average of int-BAT SNA changes calculated as a percentage of baseline (n = 6 rats/group). Compared with vehicle (v), each dosage of capsiate significantly enhanced int-BAT SNA (Holm-Sidak method after two-way repeated ANOVA). 
C: enhancement of mean int-BAT SNA over a 0- to 180-min period by intragastric administration of capsiate (30, 100, and 300 mg/kg) calculated from B. Values are means ± SE. *P < 0.05 (by Dunnett’s test). 
D: time dependence of the enhancement by capsiate of int-BAT SNA. Ordinate, average int-BAT SNA over periods of 1–0 to 0 (pre), 0–60, 60–120, and 120–180 min. Values are means ± SE of 6 rats as shown in B. *P < 0.05 vs. vehicle (by Tukey’s test after two-way repeated ANOVA). 
E: effects of 100 mg/kg capsiate on mean int-BAT SNA over a 0- to 180-min period in sham-operated or vagomomized rats (n = 5). Values are means ± SE. *P < 0.05 by t-test. NS, not significant. 
F: effects of pretreatment with the transient receptor potential (TRP) channel antagonist ruthenium red (RR; 1%, n = 6) or saline (n = 5) on the increase of mean int-BAT SNA over a 0- to 180-min period after the administration of 300 mg/kg capsiate. Values are means ± SE. *P < 0.05 (by t-test). 
G: capsiate-induced enhancement of mean int-BAT SNA over a 0- to 180-min period in rats that received pyloric ligation or sham operation (n = 5). Values are means ± SE. *P < 0.05 by t-test. H: effects of 300 mg/kg capsiate and pretreatment with 1% RR on the change in BAT temperature (ΔTBAT). Data are shown as means ± SE of a 1-min average of ΔTBAT changes from baseline. At 30 min, vehicle (n = 5), capsiate (n = 6), or capsiate containing RR (n = 5) was administered after pretreatment with saline or RR at 0 min. *P < 0.05 (by the Holm-Sidak method after two-way repeated ANOVA). 
I: effects of 300 mg/kg capsiate and 1% RR on mean ΔTBAT of 30–210 min. Values are means ± SE. *P < 0.05 (by Tukey’s test). 
J: effects of 300 mg/kg capsiate and 1% RR on mean ΔTrec of 30–210 min. Values are means ± SE. *P < 0.05 (by Tukey’s test).

generator for thermogenesis (38), which, in turn, increases the descending command to the preganglionic sympathetic neurons innervating BAT. To examine this interpretation, we analyzed the frequency composition of the increased int-BAT SNA and the effect of blocking autonomic ganglia with hexamethonium thereon. Morrison et al. (37, 39) reported that increased BAT SNA in response to a local microinjection of bicuculline into the rostral raphe pallidus nucleus (rRPa) showed a characteristic pattern of increase of the frequency distribution in which the peak was located at ~2–4 Hz. Before capsiate administration, the power spectral density presented peaks corresponding to the respiratory cycles and harmonics (R and 2R in Fig. 2B, bottom), cardiac cycles (C in Fig. 2B, bottom), and a broad peak in the range of 1–10 Hz and peaking at ~2 Hz (Fig. 2B, bottom left). At 2–3 h after the intragastric administration of capsiate (100 mg/kg), a large amplitude and repeated burst-like discharge appeared in the int-BAT SNA (Fig. 2B, top), which resulted in a broad and large peak,
especially in the range of 1–5 Hz, in the power spectra, which completely masked the respiratory and cardiac rhythm-related peaks (Fig. 2, A, bottom, and B, bottom). These changes in the activities and frequency components are reminiscent of the results of power spectral analysis of BAT SNA in anesthetized rats in which bicuculline was injected into the rRPa (39), suggesting the possibility that these typical SNA patterns are generated in the central mechanisms regulating the thermogenic premotor networks. This interpretation is further supported by the effect of hexamethonium, which completely eliminated the typical peak at \( \sim 1–5 \) Hz in the presence of capsiate (C6 in Fig. 2B) as well as the cardiac- and respiratory-related components, indicating that these typical activities are generated in the supraspinal mechanisms.

**Sympathoexcitation by capsiate is tissue specific.** We then examined whether the sympathoexcitation seen after capsiate administration is a specific result of the activation of the thermoregulatory system by comparing the NE content between BAT and the heart and pancreas over a 4-h period after the intragastric administration of 100 mg/kg capsiate in freely moving animals (Fig. 3A). Capsiate significantly enhanced the turnover of NE in BAT, in good accordance with the increase in BAT SNA, but not in the heart or pancreas, suggesting that the increase in sympathetic outflow by capsiate predominantly occurs in the nerve innervating BAT. In addition, the capsiate-induced dose-dependent increase in the levels of circulating MHPG (Fig. 3B), an index showing the extent of peripherally released NE in the whole body (15), was abolished by vagotomy but was unaffected in rats without the adrenal organ (Fig. 3, C and D). These results suggest that GI TRPV1 channels are selectively linked, via vagus afferents, to the central networks that underlie the regulation of BAT metabolism and that their link to the general circulatory, digestive, and adrenal systems is negligible, if any.

**Effect of capsaicin on BAT SNA.** The above results strongly argue for a pivotal role in the already reported thermogenic effect of capsiate of the specific neural pathway originating in TRPV1 channels in the gastric region, mediated by vagal afferents and terminating in the sympathetic nerve innervating BAT (25, 43). In contrast, the thermogenesis that occurs after the administration of capsiate, another potent TRPV1 agonist, has been mostly attributed to the enhanced epinephrine release from the adrenal organs and subsequent direct activation of BAT (26, 63). If capsiacin could activate gastrointestinal TRPV1 receptors after intragastric administration, it is expected that the thermoregulatory pathway demonstrated above would be activated, resulting in an increased BAT SNA with a similar time course as that with capsiate. To examine this, we analyzed the effect of capsiate on BAT SNA. As expected, the intragastric administration of capsiate increased int-BAT SNA (Fig. 4A) in a dose-dependent manner in the range of 1.5–15 mg/kg (Fig. 4B). This increase was characterized by a

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**Fig. 2. Power spectral analyses of the effect of intragastric administration of capsiate on int-BAT SNA.** A: int-BAT SNA recorded in a rat (top) and the power spectral density (0.2–5 Hz) for each 1-min int-BAT SNA displayed in arbitrary color units (bottom). Capsiate (100 mg/kg) was administered via intragastric cannula as indicated (first arrow on the top), and C6 (20 mg/kg) was intravenously administered as indicated (arrow on the right). The color scale for the power spectral density [in arbitrary units (AU)] is shown on the right of the power spectral density. B: time-expanded versions of int-BAT SNA at different time points after capsiate administration (top) and the power spectral density function [ordinate, power spectral density (in AU)] estimated based on a 1-min data period including the 2-s period shown at the top. Note that the calibration bars (top) and division (bottom) correspond to the same AUs throughout the panels for each row except for the power spectral density function at 180 min after administration, in which 1 division corresponds to a 100-fold value of the AU. R and 2R indicate peaks corresponding to the respiratory and second harmonics of the respiratory rhythm, respectively, and C indicates a peak for the cardiac rhythm. These respiratory and cardiac rhythms were abolished by the administration of C6, indicating that these components did not result from mechanical movement of the recording electrode or fluctuation in the nerve-electrode contact in response to cardiac and respiratory movement of the body trunk. C: relation between changes by 100 mg/kg capsiate in the total power in the 0.2- to 5-Hz range relative to the preadministration value (pre-CST) and weighted mean frequency of the power spectral density. Filled circles, results from the 6 rats in which capsiate was administered: open circles, results from the vehicle administration (6 rats). Red circles shows means ± SE for all 6 animals/group (the SE of the weighted mean frequency was smaller than the radius of the marker circle).
It is well established that TRPV1 channels mediate nociception (59), and, accordingly, TRPV1 channel proteins have been seen as a promising target of antinociceptive drugs, a view that has stimulated the synthesis and development of many agonist and antagonists in the past decade (18, 48). However, such attempts have unexpectedly revealed the complicated effects of TRPV1 channel systems on body temperature regulation. For example, administration of TRPV1 channel agonists results in hypothermia and administration of certain types of peripherally acting antagonists causes aberrant hyperthermia, a clearly undesirable side effect encountered in pain relief therapies (18). This complicity arises, at least in part, from the fact that the widely distributed TRPV1 channels, including in the central nervous system, viscera, and peripheral organs and nerves, could be activated by systemic administration of these drugs (3). To understand the functional roles played by a specific set of TRP channels expressed in a specific region of the body, it is indispensable to stimulate these channels selectively in the region of concern while minimally affecting those in other systems. To address this issue, we took advantage of the chemical vulnerability in the digestive tract of a recently found analog of capsaicin, namely, capsiate (22), to describe the effects of gastrointestinal delimited activation of TRP channels (presumably TRPV1 type). Due to its chemical vulnerability, capsiate, when administered into the upper digestive tract, is rapidly degraded to vanillyl alcohol and (E)-8-methyl-6-nonenolic acid, two metabolites that are inactive to TRP channels (50, 57), in the alimentary mucosa. This rapid metabolism makes capsiate undetectable in the circulation in the portal blood and brain tissue in rats (6, 54) and humans (5). The results indicate that selective activation of TRP channels (presumably TRPV1 type) in the upper digestive tract induces increased thermogenesis through a reflex pathway composed of TRP channels (presumably TRPV1 type) located within the gastric wall, vagus nerve afferents, central thermogenic pattern generator (such as the rRPa), and sympathetic outflow to BAT. Each step of this newly identified regulatory pathway is discussed below.

TRP channels are the primary target of capsiate in the BAT SNA response. The effect of capsiate in activating BAT SNA was almost completely abolished by ruthenium red. Ruthenium red has been shown to be a noncompetitive antagonist of TRP channels that can inhibit the effect of 200 nM capsaicin on TRPV1 channels at an IC50 of 50 nM in isolated neurons (66). It has been demonstrated that the EC50 of capsiate in activating heterologously expressed TRPV1 channels is 290 nM (22), a value comparable with that of capsaicin (99 nM) in the same system. An intravenous injection of capsazepine, a specific antagonist of TRPV1 channels, abolishes the abdominal temperature rise with orally administered capsiate in nonanesthesia-sized mice (43). The rise in BAT and colonic temperature induced by intrajejunal administration of capsiate is absent in mice lacking TRPV1 protein (25). These lines of evidence strongly suggest that the primary mediator of the capsiate effect on BAT SNA presented here is TRP channels. The ratio of the number of molecules of capsiate to that of ruthenium red administered in the gastric lumen in the present study was ~10-fold, a value comparable with the ratio estimated based on the EC50 of these agonists and the IC50 of ruthenium red as discussed above (66). Although ruthenium red has potent effects on other types of TRP channels as well as proteins involved in intracellular Ca2+ mobilization such as ryanodine receptors, the most plausible target of intragastrically administered capsiate is TRP channels in activating BAT SNA and

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**DISCUSSION**

gradual increase in the basal discharge, which became significant at 60–120 and 120–180 min after administration, and a burst-like increase in discharge (Fig. 4A, 120 and 180 min), which are essentially similar to that with capsiate (Fig. 1A; although the temporal pattern of bursting slightly differed from that observed after capsiate, as shown in Fig. 1A, right). These results suggest an involvement of sympathetic excitation in the effect of capsiate. As it has been suggested that the effect of intragastric administration of capsiate on temperature also involves components not mediated by extrinsic nerves around the jejunum, unlike that of capsiate (25), this change in inter-BAT SNA might also involve other mechanisms than the above-shown reflex pathway, as expected from the slight difference in the time course of BAT SNA increase; we did not analyze the mechanism of the capsiate effect further in detail.

**Fig. 3.** Effects of intragastric administration of capsiate on norepinephrine (NE) turnover. A: NE content in the BAT, heart, and pancreas (panc) before and 4 h after intragastric administration of capsiate (100 mg/kg) or vehicle after the injection of a NE synthesis blocker. Data are expressed as means ± SE; n = 6. **P < 0.01 and *P < 0.05 vs. the pre group (by Tukey-Kramer test). B: circulating 3-methoxy-4-hydroxyphenylglycol (MHPG) levels 180 min after the intragastric administration of vehicle or capsiate (n = 14 rats/group). Values are means ± SE. P < 0.05 for values marked with “a” compared with “b” (by Tukey’s test). C and D: effects of ablation of the gastrointestinal vagus nerve (vag; C, n = 6–7 rats/group) or adrenal gland (adx; D, n = 24 rats/group) on circulating MHPG levels 180 min after the administration of 100 mg/kg capsiate. Values are means ± SE. *P < 0.05 (by t-test).
increasing body temperature. Whether these effects involve the activation of other proteins that are also sensitive to ruthenium red remains to be determined, and their involvement cannot be totally ruled out at this time.

This interpretation, that the activation of TRPV1 channels results in thermogenesis, is apparently inconsistent with recent arguments that TRPV1 agonist rather decreases body temperature (48). It is unlikely that the thermogenesis and/or increase in BAT SNA induced by the intragastric administration of capsiate resulted from desensitization of constitutively activated TRPV1 channels in the viscera (3, 56) for the following reasons: first, we have recently found that the afferent discharge of the gastric branch of the vagus nerve remains continuously increased after the intragastric administration of capsiate (100 mg/kg) (27), and, secondly, the increase in BAT SNA with capsiate (100 mg/kg) was observed to be unchanged in rats that had been repeatedly receiving capsiate (100 mg·kg⁻¹·day⁻¹) for 2 wk before BAT SNA measurement (data not shown). These observations argue against the notion that capsiate inhibited TRPV1 channels through their desensitization.

Rather, a plausible interpretation of the present results is that the afferent nerves expressing TRPV1 channels innervating the limited regions of the upper digestive tract are specifically linked to the brain network underlying diet-induced thermogenesis, unlike TRPV1 channels in the general viscera and other peripheral organs (3, 48). The following lines of observations support this interpretation. First, capsiate, a TRP channel agonist highly vulnerable in the digestive tract, promoted the extrinsic nerves around the jejunum (25), suggesting that TRPV1 channels outside the GI tract, including those in the somatosensory and gustatory systems, play no essential role in BAT activation. In addition to the well-established role of TRPV1 channels in mucosal defense (20), the present study is the first, to our knowledge, to demonstrate the novel role of gastric TRPV1 channels on the vagal afferent terminals as the initial trigger of the central thermoregulatory network underlying energy homeostasis.

Effects of capsiate require vagus nerve afferents. The present vagotomy experiments (Fig. 1D) clearly demonstrate that the vagus nerve innervating the abdominal organs is required for the increase in BAT SNA by capsiate. Nearly half of the neurons innervating the stomach wall in the nodose ganglion, where the soma of the primary afferent neurons are localized, show TRPV1 immunoreactivity (47, 69). Indeed, these nodose neurons express functional TRPV1 channels on the peripheral terminals because, in isolated nodose-vagus-gastric preparations from mice, luminal acidification and mechanical stimulation in the stomach increase the firing rate of nodose neurons, a phenomenon that is significantly attenuated in mice lacking TRPV1 channels (7). It is unlikely that capsiate activated TRPV1 channels arising from the myenteric nervous system (MNS) because the majority of TRPV1-positive immunoreactive fibers in the muscle layer and lamina propria of the alimentary tract are abolished by chronic denervation of the nerves extrinsic to the myenteric plexus and because the neuronal soma in the MNS are TRPV1 negative (24, 47, 62). The almost complete absence of the capsiate effect on BAT SNA in vagotomized rats (Fig. 1D) also rules out the involvement of TRPV1-expressing afferents of dorsal root ganglion origin.

Intragastric administration of capsiate activates the central thermoregulatory system. Neurons in the nodose ganglion project to the nucleus of the solitary tract (NTS) and form their first intracerebral synapses. The NTS collects various types of visceral information and sends projections to widely distributed...
brain structures involved in cardiorespiratory, digestive, and endocochlear homeostasis. A recent anatomic study demonstrated that the injection of pseudorabies virus into BAT gives rise to infected NTS neurons (4, 9), strongly supporting the presence of neuronal connections initiating in the NTS and terminating in BAT. Nakamura and Morrison (41) proposed a model of the neural pathways mediating skin cooling-evoked BAT thermogenesis arising from spinal primary afferents and transmitted via the dorsal root ganglion, dorsal horn, preoptic area, dorsal-medial hypothalamic nucleus, and rPAs. Exactly how NTS neurons receiving abdominal afferents that express TRPV1 channels send their information and interfere with this thermoregulatory pathway remains to be clarified in future studies.

Effect of capsiate is selective to sympathetic efferents to BAT. As demonstrated in this study, intragastric administration of capsiate activates the sympathetic nerve innervating BAT, the final efferent also involved in skin cooling-evoked thermogenesis (41). It is likely that this increase in the BAT SNA is directly linked to energy consumption through thermogenesis (8) because the same administration protocol increased T_{BAT} (Fig. 1, G–I) in accordance with reports that capsiate significantly increases UCP-1 mRNA expression in the BAT of mice (25). However, because the time course of the changes in T_{BAT} was not necessarily linearly parallel to that in BAT SNA (Fig. 1, B and H), it remains to be an open question whether any other unreported mechanism underlies the increase in T_{BAT} and body temperature in response to intragastric administration of capsiate.

It has been proposed that the premotor neurons controlling BAT metabolism are localized in the rPAs (for a review, see Ref. 38). In contrast, those controlling the sympathetic nerves underlying cardiovascular regulation reside mostly in the rostral ventrolateral medulla (36). Lines of evidence have indicated that the sympathetic premotor systems of the rostral ventrolateral medulla are not involved in the generation of BAT SNA. The present results for the intragastric administration of capsiate are in good accordance with a specific link between the intragastric TRPV1 systems and the sympathetic regulation of BAT metabolic activity. First, the marked increase in BAT SNA was not accompanied by significant changes in NE release to the heart and pancreas (Fig. 3). Second, the increase in the circulating MHPG level by capsiate was not affected by adrenalectomy, suggesting that the capsiate effect does not involve the activation of the preganglionic nerve innervating the adrenal gland. Third, when BAT SNA was increased with capsiate, the frequency composition of the BAT SNA below 5 Hz was quite similar to that reported with the spectral responses to cooling. In particular, the fact that clear peaks corresponding to the respiratory and cardiac rhythms in the autospectral function of the BAT SNA before capsiate administration were not increased by capsiate, despite a marked increase in other frequency components, further suggests that this increase is specific to BAT SNA and is not due to an increase in sympathetic outflow to the heart and vascular beds (37). Finally, an ingestion of CH-19 sweet, a cultivar of chili pepper containing capsiate but not capsaicin, by human subjects significantly elevated the tympanic temperature for >60 min without any significant increase in blood pressure or heart rate (19, 45), whereas a similar elevation of tympanic temperature after the ingestion of hot red pepper containing capsaicin but not capsiate was accompanied by an increase in blood pressure and heart rate (19). In addition, selective activation of sympathetic outflow to BAT and not to the heart also occurs by physiological stimuli, such as refeeding after starvation (17), suggesting that thermogenesis and cardiorespiratory regulation are under the distinct influence of ingestion-related sensory signals.

Physiological relevance of the specific pathway involved in the effects of capsiate. An issue that remains to be addressed here is why the activation of intragastric TRPV1 channels potentiates, not attenuates, thermogenesis. The most plausible interpretation is that intragastric TRPV1 channels do not encode temperature but rather other types of information, including those involved in dietary-induced thermogenesis (65). Lines of evidence have indicated that dietary-induced thermogenesis occurs in response to ingredients contained in digested foods and their metabolites, intrinsic molecules secreted in response to food intake, and osmotic stimulation by luminal content. It has been shown that a large variety of food ingredients, their metabolites, and digestion-related intrinsic molecules (e.g., gastric acid, bicarbonate, fatty acids, and fatty acid conjugates like oleoylthanolamine) as well as a distension of the digestive tract activate or upmodulate TRPV1 channels (1, 2, 7, 13, 34, 49). These findings support the primary role of TRPV1 channels in the upper GI tract as sensors for dietary-induced thermogenesis.

The long-lasting (>180 min) and developing increase in BAT SNA after a single bolus administration of capsiate (Fig. 1) was impressive but not surprising because it was in good accordance with the long-lasting increase in O2 consumption after a single administration of capsiate in mice (25, 44) and a single intake of capsiate-containing chili pepper in human volunteers (19). This time course of the effect is also reminiscent of the long-lasting (>180 min) increase in metabolic rate and body temperature after a 10-min intraduodenum infusion of hypertonic NaCl in anesthetized rats (46). Interestingly, this effect was also vagus nerve dependent, β-blocker sensitive, and resistant to adrenal demedullation (46). Such a long time course is in marked contrast to the increase in BAT SNA in response to cooling, which immediately terminates after the recovery of temperature (41), suggesting partial involvement of a distinct pathway. We (27) have recently reported that anafferent activity of the gastric branch of the vagus nerve after intragastric capsiate administration shows a long-lasting elevation after a single injection. This observation favors the sustained activation of intragastric receptors during this period. The radioactivity content of [C14]dihydrocapsiate, a capsiate analog, in the stomach remains high 6 h after intragastric administration (6), but whether any active form of capsiate can stay and continuously activate TRP channels in the stomach is an open question. In addition, these data suggest, rather than a direct, simple pharmacological effect downstream to receptor activation, some sort of late onset, nongraded, bursting response in BAT SNA once a certain threshold dose of capsiate has been injected, which is also an important subject in understanding the sensory-evoked autonomous activity generation in the central thermoregulatory network. The exact mechanism underlying this long-lasting and developing effect remains to be demonstrated in future studies and might provide insights into a physiological basis for long-lasting thermogenesis known to be induced by diet (14, 17).
Possible applications of capsiate as antiobesity dietary supplement. Chronic administration of capsiate increases $O_2$ consumption, resting energy expenditure, and fat oxidation and decreases abdominal adiposity in experimental animals and human subjects (23, 33, 44, 55). Interestingly, the reduction in abdominal adiposity with capsiate was much weaker in subjects carrying a single-nucleotide mutation in TRPV1 channels (55). On the other hand, in addition to the established role of the BAT in human neonates, recent studies (12, 51, 60, 61) have indicated that BAT activity in adult humans is significantly correlated to the degree of fat accumulation and body mass index. It is therefore tempting to speculate that repeated stimulation of this reflex originating in gastric TRPV1 channels and targeting the BAT might enable therapeutic alimentation-based control of accumulated fat in human obese subjects. One gram of dried hot pepper contains $\sim 0.09$ mg and $4.6$ mg of capsinoids and capsaicinoids, respectively (31). It follows that the effective doses of 100 mg capsiate/rat and 5 mg capsaicin/rat used in this study are almost equivalent to oral intakes of $\sim 1$ kg/rat and 1.1 g/rat, respectively, of dried hot pepper. On the other hand, the chili pepper cultivar CH-19 sweet, which is much less pungent, contains 1.3 mg capsinoids in 1 g of dried fruit (31), making it necessary to use 75 g/rat to attain a dose of 100 mg/rat. Because the ingestion of such a large amount of fruit is not practicable, oral administration of capsiate would be efficiently made with more concentrated dosage form to expect the pharmacological effects as described in this study. Treatment with capsiate, which is more selective and less pungent than capsaicin, would provide a promising strategy in antiobesity programs, especially for subjects during a period of restricted energy intake.

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DISCLOSURES


REFERENCES
