

Capsaicin-Like Anti-Obese Activities of Evodiamine from Fruits of *Evodia rutaecarpa*, a Vanilloid Receptor Agonist

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Abstract: Evodiamine, a major alkaloidal principle of *Evodia* fruits (*Evodia rutaecarpa*, Rutaceae), showed vanilloid receptor agonistic activities comparable to capsaicin. The Chinese literature refers to *Evodia* fruits as a “hot nature” herb. In spite of the similarities in the actions of evodiamine and capsaicin *in vitro*, evodiamine has no perceptible taste, including a peppery hot taste. Therefore, the effectiveness of evodiamine and the extract of *Evodia* fruits in preventing obesity on male C3H mice, or male SD rats were examined. When evodiamine was supplemented at 0.03% of the diet and fed to mice for 12 days, the perirenal fat weight became significantly lower than in the control group. The epididymal fat mass was also decreased in the evodiamine diet group. When evodiamine was supplemented at 0.02% in the form of ethanol extract of *Evodia* fruits to the high-fat diet and fed to rats for 21 days, the body weight, the perirenal fat weight, epididymal fat weight, the levels of serum free fatty acid, total lipids in the liver, triglyceride in the liver, and cholesterol level in the liver were significantly reduced as compared with the control diet group. Furthermore, both lipolytic activity in the perirenal fat tissue and specific GDP binding in brown adipose tissue mitochondria, as the biological index of enhanced heat production, were significantly increased in the evodiamine fed rats. Fasting mice subcutaneously administered 1–3 mg/kg evodiamine showed decreased core body temperature by 1–2 °C. This hypothermic effect was prevented by the pretreatment of intraperitoneally administered 10 mg/kg capsazepine, a vanilloid receptor antagonist. On the other hand, food-sated mice subcutaneously administered 1–3 mg/kg evodiamine showed unchanged core body temperature and increased tail skin temperature by more than 5 °C, suggesting the increased energy expenditure by enhanced heat dissipation. In conclusion, we have demonstrated that a novel non-pungent vanilloid receptor agonist, evodiamine, mimics the characteristic anti-obese effects induced by capsaicin. Evodiamine would induce heat loss and heat production at the same time and dissipate food energy, preventing the accumulation of perivisceral fat and the body weight increase.

Key words: Evodiamine, capsaicin, vanilloid receptor, *Evodia rutaecarpa*, Rutaceae, obesity treatment.

Introduction

The regional distribution of body fats is now recognized as a very important component of the obesity-related health hazards, including type II diabetes and coronary heart diseases (1). Dietary supplementation of 0.014% capsaicin, a pungent principle of hot red pepper, in the high-fat diets containing 30% lard lowers the visceral adipose tissue weight and serum triglyceride concentration in rats (2) with the enhancement of energy metabolism. The enhancement of energy metabolism could be due to the increase of thermogenesis in brown adipose tissue (3) through the stimulation of sympathetic nerve system (4). Energy expenditure for thermogenesis in brown adipose tissue (BAT) serves either to maintain body temperature in the cold environment or to waste food energy. We hypothesized that the anti-obese effects of capsaicin are evoked by the stimulation of sensory nerve activities by interacting with vanilloid receptors. Thus, the extracts of various herbs and edible plants were screened for potential vanilloid receptor agonists to find a novel antiobestic agent. As a result, we found that evodiamine is a potent vanilloid receptor agonist like capsaicin (5), (6).

Evodiamine is a major alkaloidal component of *Evodia* Fruits (dried fruits of *Evodia rutaecarpa* Bentham, Rutaceae). *Evodia* fruits known as “Wu-Chu-Yu”, have been prescribed for the treatment of headache, thoracoabdominal pain and vomiting that are caused by cold, or cold constitution, in both traditional Chinese and Japanese medicine. The Chinese literature refers to *Evodia* fruits as a “hot nature” herb, the same category to which chili pepper belongs. However, evodiamine does not have any perceptible taste, including peppery hot taste. The extremely hot taste is a major limitation to the use of capsaicin in the treatment of obesity. Therefore, evodiamine might be much more useful in obesity treatment.

In this report, we examined the effectiveness of evodiamine and the extract of *Evodia* fruits in preventing obesity.

Materials and Methods

Animals and diets

Male mice of the C3H strain (9-week-old, Japan SLC, Tokyo, Japan) or male rats of the SD strain (4-week-old, Japan SLC) were used in this experiment. Room temperature was main-

tained at $22 \pm 2^\circ\text{C}$ with a 12-h light-dark cycle (lights on 0700–1900 h). All the animals were housed individually in plastic cages (215 mm \times 320 mm \times 140 mm for a mouse, or 280 mm \times 440 mm \times 180 mm for a rat) and were allowed free access to diet and water during the acclimation period. Powdered diet was available in a stainless feeder positioned at the corner of each cage.

The basal diet was that recommended by the American Institute of Nutrition (AIN) and is given in Table 1. Casein (vitamin free), sodium cholate, and choline chloride were purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Lard, sucrose, and cornstarch were purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). Mineral mixture (AIN-76) and vitamin mixture (AIN-76) were purchased from CLEA Japan Inc. (Tokyo, Japan). Cellulose powder (type D) was purchased from Advantec Toyo, Inc. (Tokyo, Japan).

The evodiamine diet containing 0.03% evodiamine (purity > 99.0%, Kishida Chemical Co., Ltd., Osaka, Japan) was prepared according to the composition of Table 1. In the experiments using rats, the high-fat diets containing 30% fat were used in order to induce obesity much more effectively. The high-fat control diet and the high-fat *Evodia* diet were prepared according to the composition of Table 2. The extract of *Evodia* fruits were prepared as mentioned below. Such a high fat diet could not be used in the experiments of mice because mice avoided eating the diet containing 30% fat.

Table 1 Composition of experimental powdered diets for mice

	Basal Diet % (w/w)	Evodiamine Diet % (w/w)
Evodiamine	–	0.03
Casein	20	20
Lard	10	10
Sucrose	10	10
Mineral mixture*	4	4
Vitamin mixture*	1	1
Cellulose powder	2	2
Sodium cholate	0.125	0.125
Choline chloride	0.2	0.2
Corn starch	52.675	52.645

* AIN-76 diet.

Table 2 Composition of experimental powdered diets for rats

	Basal Diet %(w/w)	High-Fat Control Diet %(w/w)	High-Fat <i>Evodia</i> Diet %(w/w)
Extract of <i>Evodia rutaecarpa</i>	–	–	1.35
Casein	20	20	20
Lard	5	15	15
Corn oil	5	15	15
Sucrose	30	30	30
Mineral mixture*	4	4	4
Vitamin mixture*	1	1	1
Cellulose powder	2	2	2
Choline chloride	0.2	0.2	0.2
Corn starch	32.80	12.80	11.45
Evodiamine (w/w%)	0	0	0.02

* AIN-76 diet.

The body weight increase and the diet intake were measured every day during all the experimental period. The spilled feed was collected by the use of sieves. Both the feed remaining in the feeder and spilled in the cage were put together, dried in the oven at 80°C for 3 h, and weighed.

Process for preparing the extract of *Evodia* fruits

To 2.5 kg of *Evodia* fruits (*Evodia rutaecarpa*, Rutaceae, a bulk product for pharmaceutical use meeting the standards of the Japanese Pharmacopoeia, Lot. No. 07097, Takasago Yakugyo Co. Ltd., 1-1-2 Minami, Tennohji-cho, Abeno-ku, Osaka, Japan) was added 10 litres of ethanol, followed by maceration for 2 days at room temperature. After collection of the extract, the same treatment was repeated three times to obtain 30 litres of ethanol extract. The ethanol extract was filtered using a filter cloth (Miracloth, Calbiochem-Novabiochem Corp., CA, USA), and the filtrate was concentrated to dryness under reduced pressure to give ca. 100 g of extract. Ethanol solution of the extract was subjected to high performance liquid chromatography (column: YMC-Pack ODS-AM; 4.6 mm \times 25 cm, mobile phase: a 50% aqueous solution of acetonitrile, detection wavelength: 254 nm) to determine the evodiamine content. The content of evodiamine in the extract was 1.48%. The evodiamine content in the high-fat *Evodia* diet was adjusted to 0.02% by adding the 1.35% *Evodia* extract to the high-fat control diet as shown in Table 2.

Effect of evodiamine on visceral fat of a mouse

After acclimation to a basal diet for 7 days, mice (C3H, male, 9-week-old) were fasted for 24 h and then divided into two groups each consisting of 4 animals on the basis of body weight. One of the groups was fed with the basal diet and the other group was fed with the evodiamine diet. Both groups were allowed free access to the diet. After having been fed for 12 days, the mice of both groups were fasted for 1 day, anesthetized with 50 mg/kg pentobarbital, and killed. The perirenal fat pad and the epididymal fat pad of each mouse were excised immediately and weighed.

Effect of the extract of *Evodia* fruits on weight, visceral fat and lipolysis in a rat

The following experiment was carried out using a diet containing extract of *Evodia* fruits prepared as mentioned above. Although rats do not avert evodiamine itself, rats somewhat avoided eating the diet containing the extract of *Evodia* fruits. Therefore, 24 h-delayed pair-feeding was conducted so as to equalize the amount of diet taken by the rats in each group, the high-fat *Evodia* diet group and the high-fat control diet group. Four-week-old male SD rats were preliminarily fed with the basal diet for 7 days. The 9 pairs of rats were chosen on the basis of the diet intake and body weight increase rate. Each pair showed the same diet intake and body weight increase per day, but had the different body weights in such a way that one rat had the same body weight as the other but one day later. Thus, eighteen rats were divided into two groups: the high-fat *Evodia* diet group and the high-fat control diet group. One rat in each group was fed ad libitum with the high-fat *Evodia* diet. The other rat in each pair was given the high-fat control diet so as to feed equal amounts to each of the same pair. After the pair-feeding was continued for 21

days, the rats in the high fat control diet group were fasted for 24 h, anesthetized with 50 mg/kg pentobarbital and killed. The end of the experiment in the high-fat control diet group was delayed by 24 h. Blood was collected from the abdominal aorta for separation of serum. The perirenal fat pad, epididymal fat pad and interscapular brown adipose tissue (IBAT) of each rat were excised immediately and weighed.

Determination of the lipolytic activity

The lipolytic activity was determined according to the procedure described by Suzuki et al. (7). The amount of free fatty acids in the reaction mixture was determined using a commercially available kit (Determiner NEFA, Kyowa Medex Co., Ltd., Tokyo, Japan). The activity value was obtained by subtracting the value of the pre-reaction control sample from the value of the reaction sample.

Effects of extract of Evodia fruits on GDP binding in IBAT

IBAT samples were weighed and homogenized in ice-cold medium (pH 7.2) containing 250 mM sucrose and 5 mM potassium TES and 2 mM potassium EDTA. The major portion of the homogenate was used to prepare mitochondria. The mitochondria were isolated by differential centrifugation according to the procedure described by Cannon and Lindberg (8). Cytochrome c oxidase activity was determined spectrophotometrically according to the method described by Kamiryo (9) with a spectrophotometer (U-3210, Hitachi, Tokyo, Japan). Recovery of mitochondrial cytochrome c oxidase from the IBAT homogenate was determined and used for the calculation of total mitochondrial protein and guanosine-5'-diphosphate (GDP) binding per IBAT depot. Tissue protein content was measured by the Lowry method.

Mitochondrial GDP binding was determined according to the method described by Nicholls et al. (10) using [^3H]GDP (96.2 KBq/ml, and 9.7 μM ; Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, England) and [^{14}C]sucrose (4.55 KBq/ml; Amersham Pharmacia Biotech UK, Ltd.). The reaction mixture was filtered and the filter with mitochondrial tissues was counted for [^3H] and [^{14}C] by scintillation spectrometry in a Beckman LS5000TD liquid scintillation counter (Beckman Coulter Inc., Fullerton, CA, U.S) using scintillation counting fluid, Sintisol EX-H (Wako Pure Chemical Industries, Ltd., Osaka, Japan). [^{14}C]sucrose was included to calculate the volume of medium trapped on the filter.

Biochemical analysis of serum and liver

Various lipid concentrations were determined using commercially available kits as follows: with Triglyceride G-Test Wako (Wako Pure Chemical Industries Ltd., Tokyo, Japan); serum and liver cholesterol with Determiner TC555 (Kyowa Medex Co., Ltd., Tokyo, Japan); free fatty acids with Determiner NEFA (Kyowa Medex Co., Ltd., Tokyo, Japan). Liver lipids were extracted and determined gravimetrically by the method of Folch et al. (11). Other biochemical indexes were determined using commercially available kits (Wako Pure Chemical Industries Ltd., Osaka, Japan) as follows: glucose with Glucose C-II Test Wako (mutarotase-GOD-method); insulin with Glazyme Insulin-EIA Test; transaminase, GPT and GOT, with Transaminase C-II Test Wako.

Effects of evodiamine on the body temperature

The change of body temperature was measured under unanesthetized conditions, because heat production in BAT was suppressed under anesthesia as reported (12). A wireless temperature probe (IPTT-100 Implantable Programmable Temperature Transponder, Bio Medic Data Systems, Inc., NJ, U.S.A.) was implanted into the abdomen of each male C3H mouse (6-week-old, Japan SLC) using an implanting handle (IMI II, Bio Medic Data Systems, Inc., NJ, U.S.A.) under the anesthetization of 50 mg/kg pentobarbital. The mice were allowed to recover from surgery for a period of 7–10 days prior to testing. All the animals were housed individually in plastic cages and were allowed free access to water and diet, CE-2 pellet diet (CLEA Japan Inc., Tokyo, Japan). The resting core body temperature was measured between 0900–1000 h in the room ($22 \pm 2^\circ\text{C}$) everyday by a scanning/programming device (DAS-5001 Console System, Bio Medic Data Systems, Inc., NJ, U.S.A.).

Changes in tail skin temperature were measured by a thermographic image analyzing system, Thermo-Viewer (JTG-5200, JEOL Datum Ltd., Tokyo, Japan), for 15 min before and 60 min after injection at 30-sec interval. Temperature of tail skin is not uniform. Accordingly, the highest temperature at a fixed area on the tail skin was measured by the use of image analyzing software (TG-5000CNTA, JEOL Datum Ltd., Tokyo, Japan). All the measurements were conducted during 0900–1200 h. Compound solutions prepared in ethanol/Tween80/saline (1:1:8) were administered in volumes of less than 0.2 ml at the indicated doses. Capsazepine (Funakoshi Co., Ltd., Tokyo, Japan) or vehicle was administrated intraperitoneally 20 min prior to subcutaneous administration of evodiamine. Every solution injected was kept at $35-6^\circ\text{C}$ before injection.

Results

Effects of evodiamine on visceral fat of a mouse

Capsaicin is known to suppress food intake in rodents because of its pungency. On the other hand, evodiamine is not pungent and has no perceptible taste. Even though mice were fed ad libitum, there was no significant difference in diet intake between the evodiamine diet group and the basal diet group (3.21 ± 0.18 g/day and 3.30 ± 0.16 g/day, respectively. Mean \pm s.d. $N = 4$. Significant differences were determined by Student's t-test with 95% confidence limits.).

The amount of the perirenal fat in the evodiamine diet group was significantly smaller than that in the basal diet group (0.083 ± 0.016 g and 0.116 ± 0.020 g, respectively. $P < 0.05$). The amount of the epididymal fat was also somewhat smaller in the evodiamine diet group than the basal diet group (0.219 ± 0.020 g and 0.246 ± 0.038 g, respectively).

Effects of the extract of Evodia fruits on weight, visceral fat and lipolysis in a rat

The body weight increase was significantly reduced in the high-fat *Evodia* diet group compared with that in the high-fat control diet group, despite the amounts of calorie intake by rats in each group being equalized by applying the 24 h-delayed pair-feeding (Table 3). The high-fat *Evodia* diet group

Table 3 Effects of evodiamine on various biochemical parameters in rats fed diets with or without the extract of *Evodia* fruits for 3 weeks

	High-Fat Control Diet group		High-Fat <i>Evodia</i> Diet group	
Calorie intake (kJ/21days)	5950.8	± 272.1	5906.0	± 268.3
Final body weight (g)	252.7	± 10.5	226.8	± 12.8***
Body weight increase (g)	138.2	± 8.7	112.3	± 11.5***
Body weight increase per calorie intake (g/kj)	0.023	± 0.001	0.019	± 0.002***
Epididymal fat (g)	4.06	± 0.72	2.28	± 0.41***
Perirenal fat (g)	5.68	± 1.18	3.06	± 0.82***
Lipolytic activity (μmol/g/h)	3.19	± 1.02	6.35	± 2.78**
Serum				
Triglyceride (mg/dl)	42.8	± 22.9	35.2	± 12.2
Cholesterol (mg/dl)	48.0	± 13.9	64.8	± 7.7*
Free fatty acids (μEqv/l)	611.3	± 73.5	439.4	± 99.5*
Glucose (mg/dl)	125.4	± 22.1	126.9	± 8.6
Insulin (μU/ml)	15.2	± 16.7	4.1	± 6.3
GPT (IU/l)	6.3	± 1.3	6.2	± 1.3
GOT (IU/l)	29.4	± 2.7	30.5	± 5.8
Liver				
Total lipids (mg/g liver)	101.8	± 17.0	86.9	± 10.4*
Triglyceride (mg/g liver)	34.5	± 8.5	21.7	± 5.7*
Cholesterol (mg/g liver)	4.0	± 0.6	3.4	± 0.6*
BAT				
IBAT wt (g)	0.342	± 0.041	0.401	± 0.120
IBAT wt/body wt	0.0014	± 0.0002	0.0018	± 0.0005
Cytochrome c oxidase Activity in IBAT (μmol/IBAT/min)	8.30	± 3.24	12.11	± 5.35
Mitochondrial protein content in IBAT (mg/IBAT)	10.52	± 2.45	8.43	± 3.36
Specific GDP binding in IBAT mitochondria (pmol/mg-mitoprotein)	241.3	± 84.3	443.9	± 150.6**
Total GDP binding in IBAT (pmol/IBAT)	2503	± 1025	3464	± 1238

Values shown are mean ± s.d. (n = 9). Significant differences between the high-fat *Evodia* diet group and the high-fat control diet group are indicated by *P < 0.05, **P < 0.01, *** p < 0.001 (Student's t-test)

showed significantly less body weight increase per calorie intake in comparison with the high-fat control diet group. The amounts of the perirenal fat and the epididymal fat in the high-fat *Evodia* diet group were significantly smaller than those in the high-fat control diet group.

The levels of serum free fatty acid, total lipids in the liver, liver triglyceride, and liver cholesterol level in the high-fat *Evodia* diet group were significantly lower than those in the high-fat control diet group. The level of serum triglyceride in the high-fat *Evodia* diet group was also lower than that of the high-fat control diet group. The level of serum cholesterol in the high-fat *Evodia* diet group was significantly higher than those in the high-fat control diet group, but not significantly different from that of the rats fed basal diet (60.5 ± 8.2 mg/dl, N = 9). The level of liver cholesterol in the high-fat *Evodia* diet group was significantly smaller than that in the high-fat control diet group, and not significantly different from that of the rats fed basal diet (3.1 ± 0.4 mg/g liver, N = 9). These results suggest that the levels of cholesterol in serum and liver returned to normal level in the high-fat *Evodia* diet group. The level of serum insulin was much smaller than the high-fat control diet

group, but no significant difference in serum glucose level from the high-fat control diet was observed, suggesting the improved insulin resistance. Two liver damage indexes, GPT and GOT, remained at normal levels, suggesting no liver toxicity.

The difference of lipolytic activities among these groups was also examined (Table 3). In this experiment, the "lipolytic activity" expresses the sensitivity or the amount of the active hormone-sensitive lipase in fat tissue to norepinephrine, because an excessive amount of norepinephrine was added to the reaction medium. The high-fat *Evodia* diet group, with one half of visceral fat in comparison with the high-fat control group, showed a two-fold higher lipolytic activity per unit weight than the the high-fat control diet group.

Effects of extract of *Evodia* fruits on GDP binding in IBAT, "a thermogenic indicator"

To clarify whether evodiamine activates brown adipose tissue (BAT) function, mitochondrial guanosine diphosphate (GDP) binding in interscapular brown adipose tissue (IBAT) were compared between the high-fat *Evodia* diet and the high-fat control diet (Table 3). Whereas there was no significant difference in IBAT weight between two groups, IBAT weight per body weight increased significantly in the high-fat *Evodia* diet group compared with the high-fat control diet group.

Total mitochondrial protein content in IBAT and total GDP binding per IBAT pads were calculated from the recovery of cytochrome c oxidase. Specific GDP binding in IBAT mitochondria was increased significantly in the high-fat *Evodia* diet group compared with the high-fat control diet group. Total GDP binding in IBAT was also increased in the high-fat *Evodia* diet group compared with the high-fat control diet group, however, there were no significant differences.

Effects of evodiamine on the body temperature

Subcutaneously administrated evodiamine at the dose of 1–3 mg/kg caused drop in the core body temperature by more than 1 °C in the food-deprived (18 h) mice (Fig. 1). However, in the food-sated mice which had been allowed free access to food until the beginning of the experiments evodiamine at these doses (1–3 mg/kg) did not cause a drop in the core body temperature and only higher dose of evodiamine (10 mg/kg) produced hypothermic effects.

The drop in core body temperature caused by 10 mg/kg evodiamine treatment in food-sated mice was abolished after pretreatment with 10 mg/kg capsazepine, a potent vanilloid receptor antagonist (Fig. 2). Pretreatment with capsazepine or vehicle alone had no effect on core body temperature.

Body temperature is maintained at a constant level by the balance of heat production and heat dissipation. Therefore, it is possible that enhanced heat production prevented the drop of core body temperature in the food-sated mice. In order to examine whether evodiamine (1–3 mg/kg) induces heat loss in food-sated animals, the change in tail skin temperature after the subcutaneous injection of 1 mg/kg evodiamine was measured by the use of thermography. Immediately after the injection of evodiamine, a transient rise in tail skin tempera-

ture was observed (Fig. 3). Maximal elevation in tail skin temperature was more than 5 °C. These results indicated that heat dissipation occurs not only in the food-fasted animals but also in the food-sated animals.

Discussion

In this study, we demonstrated that evodiamine prevented perivisceral fat accumulation and body weight increase through the enhancement of lipolysis, BAT activation and heat dissipation, resulting in the waste of food energy. Evodiamine is already known to have vasodilatory effects (13), (14) and hypothermic effects (15), body temperature retaining effects (16), whereas its anti-obese effects had not been reported. These effects of evodiamine would be the result of the activation of the sensory nervous system by binding to the vanilloid

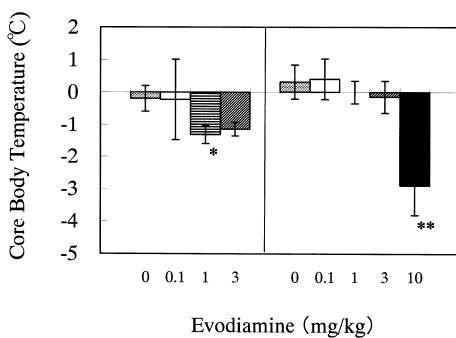


Fig. 1 Induction of hypothermia by evodiamine in food-fasted and food-sated mice. The changes in core body temperature were measured 1 h after the subcutaneous injection of evodiamine at the indicated doses. Changes in core body temperature are defined as the difference from the pre-injection levels (Food-fasted mice: $35.3 \pm 0.4^\circ\text{C}$; Food-sated mice: $36.3 \pm 0.4^\circ\text{C}$). Values shown are mean \pm s.d. ($n = 4-6$). Significant differences between vehicle treated group and evodiamine treated groups are indicated by * $P < 0.05$, ** $P < 0.01$ (Dunnett's multiple comparison test).

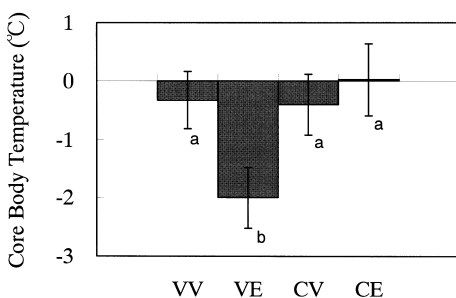


Fig. 2 The inhibitory effect of capsazepine on the hypothermic effect of evodiamine. Mean changes in core body temperature from initial pre-injection state ($35.9 \pm 0.6^\circ\text{C}$) in food-sated mice tested at normal ambient temperatures ($22 \pm 2^\circ\text{C}$) after various treatments are measured by the use of wireless thermo-sensors implanted in the abdominal cavity. Core body temperature was measured 1 hr after subcutaneous injection of evodiamine 10 mg/kg (E) or its vehicle (V). Capsazepine 10 mg/kg (C) or its vehicle (V) was intraperitoneally injected 20 min before evodiamine injection. VV: Vehicle + Vehicle VE: Vehicle + Evodiamine CV: Capsazepine + Vehicle CE: Capsazepine + Evodiamine. Values shown are mean \pm s.d. ($n = 4$). Means without a common letter (a,b) are significantly ($P < 0.01$) different by Tukey's multiple comparison test.

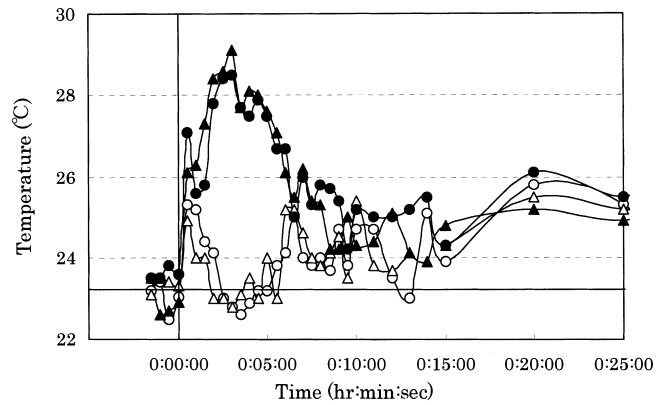


Fig. 3 Time course of the changes in tail skin temperature measured by a thermographic device after intraperitoneal injection of evodiamine or its vehicle in food-sated mice. Evodiamine was injected in two mice (\bullet, \blacktriangle), and its vehicle was also injected in two mice (\circ, \triangle) at the same time.

(capsaicin) receptor like other physiological responses we reported (5), (6). Capsaicin not only has anti-obese activities but also has hypothermic effects (17) as evodiamine does. Vascular dilatory effects of capsaicin-sensitive sensory-motor nerves have been reviewed by Rubino and Burnstock (18). As shown in this report, the hypothermic effect of evodiamine was inhibited by the pretreatment with a vanilloid receptor antagonist capsazepine. We supposed that during food deprivation evodiamine caused more heat loss to the environment by vasodilation that cannot be compensated by heat production and resulted in hypothermia, because of attenuated sympathetic drive to BAT. Attenuation of sympathetic drive to BAT and reduction of heat production by food-deprivation is well documented in the report (19).

References

- Lamarche B. Abdominal obesity and its metabolic complications: implications for the risk of ischaemic heart disease. *Coronary Artery Disease* 1998; 9: 473–81
- Kawada T, Hagiwara K, Iwai K. Effect of capsaicin on lipid metabolism in rats fed a high fat diet. *Journal of Nutrition* 1986; 116: 1272–8
- Yoshida T, Yoshikawa K, Wakabayashi Y, Nishioka H, Kondo M. Effects of capsaicin and isothiocyanate on thermogenesis of interscapular brown adipose tissue in rats. *Journal of Nutritional Science and Vitaminology* 1988; 34: 587–94
- Watanabe T, Kawada T, Kato T, Harada T, Iwai K. Effects of capsaicin analogs on adrenal catecholamine secretion in rats. *Life Science* 1994; 54: 369–74
- Kobayashi Y, Nakano Y, Hoshikuma K, Yokoo Y, Kamiya T. The bronchoconstrictive action of evodiamine, an indoloquinazoline alkaloid isolated from the fruits of *Evodia rutaecarpa*, on guinea-pig isolated bronchus: Possible involvement of vanilloid receptors. *Planta Medica* 2000; 66: 526–30
- Kobayashi Y, Hoshikuma K, Nakano Y, Yokoo Y, Kamiya T. The positive inotropic and chronotropic effects of evodiamine and rutaecarpine, indoloquinazoline alkaloids isolated from the fruits of *Evodia rutaecarpa*, on the guinea pig isolated right atria: Possible involvement of vanilloid receptors. *Planta Medica* 2001; 67: 244–8

- ⁷ Suzuki M, Saitoh S, Yashiro M, Hariu J. Dietary effects on liver and muscle glycogen repletion in exhaustively exercised rats: energy composition and type of complex carbohydrates. *Journal of Nutritional Science and Vitaminology* 1984; 30: 453–66
- ⁸ Cannon B, Lindberg O. Mitochondria from brown adipose tissue: isolation and properties. *Methods in Enzymology* 1978; 55: 65–78
- ⁹ Kamiryo T. Peroxisome. In: Mohri H, Kagawa Y (eds.). *Jikken Seibutsugaku Kohza*. Maruzen, Tokyo: 1984; 6 : 125–126
- ¹⁰ Nicholls DG. Hamster brown-adipose-tissue mitochondria. Purine nucleotide control of the ion conductance of the inner membrane, the nature of the nucleotide binding site. *European Journal of Biochemistry* 1976; 62: 223–8
- ¹¹ Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 1957; 226: 497–509
- ¹² Shimizu Y, Saito M. Activation of brown adipose tissue thermogenesis in recovery from anesthetic hypothermia in rats. *American Journal of Physiology* 1991; 261: R301–4
- ¹³ Chiou WF, Chou CJ, Shum AY, Chen CF. The vasorelaxant effect of evodiamine in rat isolated mesenteric arteries: mode of action. *European Journal of Pharmacology* 1992; 215: 277–83
- ¹⁴ Chiou WF, Liao JF, Chen CF. Comparative study of the vasodilatory effects of three quinazoline alkaloids isolated from *Evodia rutaecarpa*. *Journal of Natural Products* 1996; 59: 374–8
- ¹⁵ Tsai TH, Lee TF, Chen CF, Wang LCH. Thermoregulatory effects of alkaloids isolated from Wu-chu-yu in afebrile and febrile rats. *Pharmacology, Biochemistry and Behavior* 1995; 50: 293–8
- ¹⁶ Kano Y, Zong QN, Komatsu K. Pharmacological properties of galenical preparation. XIV. Body Temperature retaining effect of the Chinese traditional medicine, “goshuyu-to” and component crude drugs. *Chemical & Pharmaceutical Bulletin (Tokyo)* 1991; 39: 690–2
- ¹⁷ Hayes AG, Oxford A, Reynolds M, Shingler AH, Skingle M, Smith C, Tyres MB. The effects of series of capsaicin analogues on nociception and body temperature in the rat. *Life Science* 1984; 34: 1241–8
- ¹⁸ Rubino A, Burnstock G. Capsaicin-sensitive sensory-motor neurotransmission in the peripheral control of cardiovascular function. *Cardiovascular Research* 1996; 31: 467–9
- ¹⁹ Barton C, York DA, Bray GA. Bombesin-induced hypothermia in rats tested at normal ambient temperatures: contribution of the sympathetic nervous system. *Brain Research Bulletin* 1995; 37: 163–8

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