Roles as metabolic regulators of the non-nutrients, capsaicin and capsiate, supplemented to diets

By Kazuo IWAI,^{*)}^{†)} Ayaka YAZAWA,^{**)} and Tatsuo WATANABE^{**)†)}

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Abstract: Capsaicin is a pungent principle of hot red pepper and a non-nutrient. Capsaicin supplementation to high fat diet prevents fat deposit in the adipose tissue through secretion of adrenaline from the adrenal gland via activation of adrenal sympathetic nerve in rats. The chemical structure of a capsaicin analog found in non-pungent red pepper which was selected and fixed from a pungent variety was recently determined by our group to be a novel compound, capsiate. The gastrointestinal absorption and the metabolism of capsiate after oral administration in rats was studied. Capsiate rapidly disappeared from upper region of the gastrointestinal tract. It was cleaved to vanillyl alcohol and vanillic acid. The cleaved products were excreted in the urine as a conjugated complex such as glucuronide. The effect of capsiate on adrenaline secretion from the adrenal medulla of anesthetized rats was investigated. Capsiate evoked rapid and transient adrenaline secretion as dose-dependent manner. Capsazepine, a specific inhibitor of vanilloid (capsaicin) receptor subtype 1 (VR1), blocked capsiate-induced adrenaline secretion to show the participation of VR1 for this response. These results support that non-nutrients such as capsaicin and capsiate are well absorbed into blood and play important roles in regulating energy and lipid metabolism.

Key words: Capsaicin; capsiate; gastrointestinal absorption; metabolism; adrenaline secretion; vanilloid receptor.

Introduction. Capsaicin, (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide, is a major pungent principle of hot red pepper fruits of *Capsicum* species. Hot red pepper are now one of the most heavily and widely consumed spices in the world because of their pungency and color. In red pepper fruits, the pungent principle was found to be composed of at least 14 analogs which have been termed collectively as capsaicinoid. Capsaicin is recognized as the major component comprising approximately 70% of the total pungent acid-amide, whereas dihydrocapsaicin accounts for 30% or less. Other remaining compounds were found only in trace amounts.¹⁾

Capsaicin has been studied intensively for the various pharmacological actions including long-lasting or permanent desensitization to chemical noxious stimuli by destroying nerve ending of primary afferents or depleting

putative neurotransmitters, i.e., substance P, cholecystokinin, vasoactive intestinal polypeptide or calcitonin gene-related peptide.¹⁾⁻⁴⁾ Despite the extensive use as a pungent principle in food, information on physiological and nutritional effects of capsaicin is obscure, and both beneficial and adverse effects have been reported.¹⁾

We first investigated the absorption and metabolism of capsaicin and its analogs in rats *in vivo* and *in situ*, because Monsereenusorn⁵⁾ reported in 1980 that a mixture of capsaicin and dihydrocapsaicin (Sigma's capsaicin) was absorbed in the everted sac of the jejunum, whereas Kim and Park⁶⁾ postulated in 1981 that capsaicin and its analogs were poorly absorbed in the gut. Our results indicated that capsaicin and its analogs were well absorbed (more than 80% of the dose) at the stomach and the superior portion of the small intestine by a non-active process, and that they were absorbed approximately 50% of dose in stomach, 80% in jejunum, and 70% in ileum, respectively, within 1 h after the administration.⁷⁾ Also, the appearance of [³H]dihydrocapsaicin administered was found in portal blood and the activity increased in proportion to incubation time. The [³H]dihydrocapsaicin in blood was associated with

^{*)} Laboratory of Nutrition Chemistry, Faculty of Home Economics, Kobe Women's University, 2-1, Aoyama, Higashisuma, Suma-ku, Kobe, Hyogo 654-8585, Japan.

^{**)†)} School of Food and Nutritional Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422-8526, Japan.

^{†)} Correspondence to: K. Iwai.

Fig. 1. Proposed action mechanism of energy metabolism enhancement by capsaicin (CAP).

serum albumin.⁷⁾

Further, we investigated details of the metabolism of dihydrocapsaicin in rats by thin-layer chromatography, HPLC and GC-MS. The results indicated that within 48 h of oral administration of dihydrocapsaicin (20 mg/kg body weight) to male adult rats, unchanged dihydrocapsaicin and eight of its metabolites were identified in urine, i.e., dihydrocapsaicin (8.7% of the total dose), vanillylamine (4.7%), vanillin (9.6%), vanillyl alcohol (37.6%) and vanillic acid (19.2%) as free forms and/or their glucuronides. The proportion of free and glucuronide metabolites in urine were 14.5% and 60.5% of the total dose. These results strongly support that capsaicin and its analogs supplemented to diets are well absorbed into blood.

We investigated the physiological and nutritional effects of capsaicin-supplemented diets. In proportion to human intake of red pepper in Southeast Asia, 0.014% of capsaicin was fed to rats with high-fat diet. Capsaicin feeding for 10 days lowered perirenal and epididymal adipose tissue weights and serum triglyceride concentration dose-dependently.⁸⁾ By the respiratory gas analysis in

rats, capsaicin was shown to cause enhancement of energy metabolism similar to the effect of adrenaline administration and this increase was inhibited by the β -adrenergic receptor blocker.⁹⁾ Capsaicin's adrenaline-like effect was fully inhibited by the adrenomedullation.¹⁰⁾ In anesthetized rats, capsaicin caused adrenal sympathetic nerve excitation¹¹⁾ and adrenaline secretion^{11,12)} in a dose-dependent manner. In extirpated adrenal gland, no direct action of capsaicin to stimulate adrenaline secretion was found.¹¹⁾ These data clearly show that capsaicin inhibits fat deposition in the adipose tissue in high-fat diet-fed rats through the enhancement of energy metabolism after adrenaline secretion from the adrenal gland via β -adrenergic receptor stimulation.

In late 1980s, resiniferatoxin, a phorbol ester-like compound in *Euphorbia resinifera*, was found to be an ultrapotent agonist for capsaicin receptor. Thereafter, capsaicin receptor was termed as vanilloid receptor because of the common structure of capsaicin and resiniferatoxin containing vanillyl moiety.¹³⁾ Recently, a vanilloid receptor was cloned from rat dorsal root ganglion and named vanilloid receptor subtype 1 (VR1).¹⁴⁾ It

Fig. 2. Structure of pungent compound capsaicin (upper) and non-pungent compound capsiate (lower).

is also highly expressed in the hypothalamus.¹⁵⁾ Many of capsaicin's action is known mediated by VR1 activation. We further evaluated the participation of VR1 in capsaicin-induced adrenaline secretion by two methods. At first, pharmacological dose of capsaicin was used¹⁶⁾ to produce impairment or destruction of capsaicin-sensitive neuron, which shows dense distribution of VR1. The other was to use capsazepine,¹⁷⁾ a specific antagonist of VR1. Adrenaline secretion after capsaicin injection was almost completely inhibited by both treatments, indicating apparent involvement of VR1 for this action.

Brown adipose tissue (BAT) activation was known to be important for energy expenditure.¹⁸⁾ Effect of capsaicin supplementation on BAT was investigated. It was found to increase uncoupling protein 1 indicating the activation of BAT. This activation of BAT is thought to be another factor for the energy metabolism enhancement by capsaicin.¹⁹⁾

Figure 1 illustrates the summary of the action mechanism of acute capsaicin action in preventing fat deposition following oral administration in rats.

The relationship between pungency and adrenaline secretion-inducing effect was studied by the use of capsaicin analogs and related compounds.^{17),20)} Interestingly, there was no clear relationship between pungency and the activity for adrenaline secretion.²⁰⁾ Some non-pungent analogs as myristoyl vanillylamide or oleoyl vanillylamide (olvanil) showed strong adrenaline secretion, while pungent analogs such as heptanoyl vanillylamide did not evoke the response.

'CH-19 Sweet' is a non-pungent variety of red pepper selected from the pungent cultivar 'CH-19' (*Capsicum annuum* L.) and fixed by Yazawa *et al.*²¹⁾ They found that 'CH-19 Sweet' accumulates capsaicin-like substances instead of capsaicin.²¹⁾ Recently, we isolated and determined the chemical structure of

a novel non-pungent capsaicin-like compound as 4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonen-3-yl 4-methoxy-3-hydroxybenzoate named capsiate by spectroscopic methods and comparison with the spectral data of an authentic capsaicin.²²⁾ The center linkage is the only structurally different part between capsaicin and capsiate, i.e., an amide bond in capsaicin and an ester linkage in capsiate (Fig. 2). Interestingly, 'CH-19 Sweet' contains capsiate analogs such as dihydrocapsiate and nordihydrocapsiate in the same ratio as found in capsaicinoid in hot red peppers.²³⁾ In this work, we first investigated the absorption and metabolism of capsiate, then the ability of capsiate to secrete adrenaline in rats.

Materials and methods. *Materials.* Capsiate was prepared enzymatically.²⁴⁾ Salt mixture (AIN-93) and vitamin mixture (AIN-93M) were purchased from Oriental Yeast Co. (Chiba, Japan). Activated alumina for catecholamine purification was obtained from Wako Pure Chem. Ind. (Osaka, Japan). All other chemicals were of guaranteed reagent grade or higher.

Gastrointestinal absorption of capsiate. Male Sprague-Dawley rats weighing 220-230 g were fasted overnight with free access to water. Three mg of capsiate suspended in a basal diet (casein, 200 mg; soluble starch, 350 mg; sucrose, 300 mg; salt mixture, 40 mg; soybean oil, 100 μ L; and saturated phenol red solution, 2 mL) were administered to the rats using a stomach tube. After 1 or 3 h following the administration, rats were anesthetized with ether and the whole blood was collected from the heart and kept on ice. Serum was obtained by centrifugation of the blood. The gastrointestinal tract was taken out and was divided into six segments (stomach, duodenum, jejunum, ileum, cecum, and large intestine) by pinching both side of the each segment with ligatures. The content of the each organ was extracted three times with 5 mL of 2 mM sodium deoxycholate. The amount of phenol red in each extract was determined as a volume marker of the basal diet by optical absorbancy (560 nm) at pH 11.0 and 7.2. The residual organs were extracted with 5 mL of chloroform-methanol solution (2:1, v/v). The amount of capsiate in all extracts and the serum sample was determined by HPLC.

Metabolism of capsiate. Male Sprague-Dawley rats weighing 220-230 g were used after 3-day feeding with a purified diet containing 20% casein, 50% soluble starch, 15% sucrose, 8% soybean oil, 4% salt mixture, 1% vitamin mixture and 2% cellulose, and with water *ad lib*. Capsiate suspension in 200 μ L olive oil (20 mg/kg) was administered to the rats with a stomach tube. Urine and

Fig. 3. Remaining capsiate in digestive tracts after oral administration in rats. Three mg of capsiate with purified diet was administered *per os* to overnight-fasted rats. Values are means $\pm$ S.E. of 3 rats. N.D., not detected.

feces were separately collected using a metabolic chamber every 24 h for 2 day. Half of the urine was extracted with ethyl acetate after acidification to measure the free form of metabolites. The other portion of urine was adjusted to pH 5.0 with saturated NaHCO_3 solution and then treated with β -glucuronidase to investigate the glucuronides of metabolites. Metabolite contents were analyzed by HPLC. The dried feces were pulverized and then extracted with chloroform/methanol (2:1,v/v). The extracts were purified by solid phase extraction with a Mega bond elute C18 cartridge (VARIAN, Harbor City, CA, U. S. A.) using methanol as the elution solvent, then submitted to HPLC analysis.

Measurement of adrenal catecholamine secretion. Male Sprague-Dawley rats weighing 250-270 g were anesthetized with α -chloralose and urethane at 50 and 500 mg/kg, respectively. The rectal temperature was maintained constant by means of a heating pad. After rats were heparinized, capsiate solution was administered through a right femoral vein cannula for 1 min. Adrenal venous blood was continuously collected through a cannula in the left adrenal vein. An equivalent volume of heparinized saline solution to the collected blood was simultaneously infused from the femoral vein as a transfusion. After centrifugation of the collected blood, 10 μL of 10% sodium pyrosulfite was added to the plasma. The plasma was kept at -20°C until assay. 3,4-Dihydroxybenzylamine was added to the plasma as an

Fig. 4. Capsiate-induced adrenaline secretion from the adrenal medulla in rats. Capsiate or vehicle was intravenously administered at 0 min in anesthetized rats. Values are means $\pm$ S.E.M. of 5-7 rats. * $P < 0.05$ (significantly different from control).

internal standard. Catecholamine was adsorbed onto activated alumina at pH 8.6, washed with methanol and water, then eluted with 0.4 *N* perchloric acid. The amount of catecholamine was analyzed by HPLC.

Results and discussion. *Absorption of capsiate.* The remaining capsiate in digestive tracts after oral administration of 3 mg of capsiate with purified diet in overnight-fasted male rats was evaluated (Fig. 3). Phenol red was used as a non-absorptive marker. Upper figure is the data for 1 h after administration. Almost all capsiate detected was in the stomach, but only 18% of administered capsiate. After 3 h, the capsiate content was decreased to half in the stomach and slight capsiate was detected in large intestine. Little putative metabolites of capsiate detected in all alimentary canal. These results indicate that capsiate can be absorbed quickly in the upper gut like capsaicin.

Metabolism of capsiate. By analyzing urine and feces after oral administration of capsiate (20 mg/kg) in purified diet-fed rats metabolism of capsiate was investigated. Although no capsiate was detected in the urine within 48 h, vanillyl alcohol and vanillic acid, the possible metabolites of capsiate, existed in large quantities. About 70% of the initial dose of capsiate was excreted as its metabolites in the urine within 48 h. The total amounts, sum of free forms and glucuronide conjugates, of vanillyl alcohol and vanillic acid in the urine were 11% and 41% at 0-24 h and 3 and 6% during 24-48 h, respectively. Major form of metabolites was the glucuronide conjugates (80% of total metabolites). On the other hand, any intact capsiate, vanillyl alcohol and

Fig. 5. Effect of capsazepine on capsiate-induced adrenaline secretion from the adrenal medulla in rats. Capsazepine was administered 5 min before capsiate injection at time 0 in anesthetized rats. Values are means $\pm$ S.E.M. of 5-10 rats. * $P < 0.05$ (significantly different from control).

vanillic acid were not detected in the feces. On metabolism of capsiate, it is likely that capsiate is decomposed or biotransformed to vanillyl alcohol, vanillic acid, and their glucuronide conjugates.

Effect of capsiate on adrenaline secretion.

Figure 4 shows the change of adrenaline level in anesthetized rats after capsiate injection. At 0.2 mg/kg, capsiate facilitated adrenaline secretion immediately. And the maximum secretion observed at 0-3 min after administration, while the total adrenaline secretion level was one tenth or less than that of the same dosage of capsaicin. After that, the secretion quickly decreased. At 0.5 mg/kg, the response increased 3-fold. When more capsiate of 1.0 mg/kg was injected the maximal response was the same as at 0.5 mg/kg. These results clearly show that capsiate can evoke dose-dependent adrenaline secretion. Interestingly, the time course of adrenaline secretion pattern evoked by capsiate was somewhat different from that of capsaicin or other stimulants. Capsaicin induces biphasic adrenaline secretion, i.e., quick and intense response and slow and moderate one.¹⁷⁾ Both olvanil, one of the non-pungent capsaicin analog, and resiniferatoxin, pungent and ultrapotent capsaicin analog, shows slow-onset response only.¹⁷⁾ The reason for such difference in adrenaline response are now under investigation.

The participation of the vanilloid receptor was tested at 0.5 mg/kg of capsiate injection. Capsazepine pretreatment (20 mg/kg) effectively inhibited capsiate-induced adrenaline secretion (Fig. 5). Thus, it is apparent that the capsiate-induced adrenaline secretion is

caused through vanilloid receptor similar to the capsaicin-induced secretion. Recently, the direct action of capsaicin or capsiate to vanilloid receptor was investigated and the affinity and the maximum response of both compounds found to be similar.²⁵⁾ And the reason why the pungency of capsaicin and capsiate is dramatically different was suspected to be due to the difference of the hydrophobicity of the two compounds.²⁵⁾ The vanilloid receptor in human tongue is thought to distribute in deep position. The hydrophobicity of capsaicin is moderate enough to penetrate into the vanilloid receptor in tongue but that of capsiate is too high to penetrate and capsiate seems to be easily decomposed by esterases in tongue because it has an ester bond in its structure.

Ohnuki *et al.* showed that intragastric administration of capsiate to mice promoted energy metabolism and reduced the accumulation of body fats *in vivo* and the effectiveness of capsiate was similar to that of capsaicin.²⁶⁾ But the intensity of adrenaline secretion in rats is not so strong as that evoked by capsaicin as shown above. Further study will be necessary.

These results described above strongly support that non-nutrients such as capsaicin and capsiate are well absorbed into blood and influence lipid catabolism through secretion of adrenaline from the adrenal gland via activation of adrenal sympathetic nerve. Thus, it is important and necessary in food and nutritional sciences to identify and define metabolic regulatory effects of non-nutrients including spices and herbs supplemented to diets.

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