ELSEVIER

Alliin and volatile sulfur-containing compounds in garlic enhance the thermogenesis by increasing norepinephrine secretion in rats

Yuriko Oi, Miki Okamoto, Mina Nitta, Yutaka Kominato,* Syoji Nishimura,* Toyohiko Ariga,† and Kazuo Iwai

*Laboratory of Nutrition Chemistry, Faculty of Home Economics, Kobe Women's University, Suma-ku, Kobe 654, Japan; *Riken Chemical Industry Limited Company, Fushimi-ku, Kyoto 612, Japan; †Department of Agricultural and Biological Chemistry, College of Bioresource Sciences, Nihon University, 3-34-1, Shimouma, Setagaya, Tokyo 154, Japan*

The effects of alliin and volatile sulfur-containing compounds in garlic on thermogenesis were investigated in rats. Rats were fed high-fat diet with or without a ethanol extracted-garlic powder (EE-garlic) supplemented at 0.8% for 28 days. After feeding, mitochondrial protein content in interscapular brown adipose tissue (IBAT) and plasma norepinephrine secretion were significantly greater in rats given EE-garlic as compared with control rats. The effects of the administrations of EE-garlic and alliin on norepinephrine secretions were evaluated in anesthetized rats. The norepinephrine concentrations of arterial blood were significantly increased by EE-garlic and alliin administration, and these increases were dose dependent. The administration of allylsulfides having a different number of sulfur atoms (diallyldisulfide and diallyltrisulfide) also significantly increased the norepinephrine secretion. The effects of the administration of EE-garlic, alliin, and diallyldisulfide on thermogenesis were examined by the direct measurement of IBAT and rectal temperatures in anesthetized rats. The temperatures were significantly increased by the administration of these compounds, and the effect of diallyldisulfide disappeared in the presence of β-adrenergic blocker. These results suggest that the administration of alliin and volatile sulfur-containing compounds in garlic enhance the thermogenesis by increasing norepinephrine secretion via β-adrenergic action. (J. Nutr. Biochem. 9:60–66, 1998) © *Elsevier Science Inc. 1998*

Keywords: garlic; thermogenesis; norepinephrine secretion; interscapular brown adipose tissue; rats

Introduction

For many centuries, the medicinal properties of garlic have been well recognized, and it produces the sulfur-containing compounds including volatile compounds such as diallyldisulfide and diallyltrisulfide.[1] Stoll and Seebeck[2] reported that garlic contains odorless sulfur-containing compound known as alliin (S-allylcysteine sulfoxide) and an enzyme, alliin lyase (alliinase, EC. 4.4.1.4), which catalyzes alliin transformation into a volatile compound, allicin. The effects of these volatile or nonvolatile compounds in garlic on thermogenesis have not been investigated. We have recently reported that the supplementation (0.8%) of heat dried-garlic powder (contained 5.05 mg of total diallylsulfides/g) to the diet and the administration of diallyldisulfide, a major volatile sulfur compound of garlic, enhance triglyceride catabolism and growth of interscapular brown adipose tissues (IBAT) by increasing norepinephrine secretion in rats.[3] Norepinephrine secretion from the sympathetic nervous system controls thermogenesis of brown adipose tissue (BAT) by regulating uncoupling protein,[4–7] via β-adrenergic recepter, i.e., β-adrenergic pathway.[8–11] The present study was performed to investigate the effects of garlic administration on thermogenesis in rats, and especially to determine the effects of alliin and volatile sulfur-containing compounds in garlic on thermogenic activity of IBAT and

Address correspondence to Dr. Kazuo Iwai at Laboratory of Nutrition Chemistry, Faculty of Home Economics, Kobe Women's University, 2-1, Aoyama, Higashisuma, Suma-ku, Kobe 654, Japan.
Received January 21, 1997; accepted October 8, 1997.

Nutritional Biochemistry 9:60–66, 1998

655 Avenue of the Americas, New York, NY 10010

0955-2863/98/$19.00
PII S0955-2863(97)00167-8

norepinephrine secretion. To examine the IBAT growth and the norepinephrine secretion, the effects of ethanol extracted-garlic powder (EE-garlic; contained 1.81 mg of total diallylsulfides/g) on thermogenesis were investigated in high-fat diet fed rats by supplementation at 0.8% to the diet. In addition, the effects of EE-garlic, alliin, and volatile sulfur-containing compounds in garlic on thermogenesis were evaluated by the direct measurements of IBAT and rectal temperatures and the norepinephrine secretion in anesthetized rats. To determine the thermogenic pathway of the administration of major volatile sulfur-containing compound, the effect of administration of diallyldisulfide were also investigated by the direct measurement of IBAT and rectal temperatures in the presence of α- and β-adrenergic blocking agents.

Methods and materials

Animal care

Male Sprague-Dawley rats (Japan SLC., Shizuoka, Japan) were individually housed in stainless steel wire-bottom cages in a room maintained at 22 to 24°C and approximately 50% relative humidity. The room was lighted from 7:00 to 19:00 hr. The rats were given a commercial diet (CE-2: Japan Clea Inc., Tokyo, Japan). Tap water was freely available. The protocol of this study was approved by the Institutional Animal Care and Use Committee of Kobe Women's University, Faculty of Home Economics.

Chemicals

EE-garlic was practically odorless, and was a powdered ethanol extract of garlic bulb (Riken Chemical Industry Limited Co., Kyoto, Japan). The total volatile sulfur-containing compounds in EE-garlic were determined after the treatment of enzymatic reaction by alliin lyase, and they were analyzed by gas-chromatography using diallyldisulfide as a standard. The volatile sulfur-containing compounds were determined as diallyldisulfide equivalent. The data indicated that the EE-garlic used in this study contained 1.81 mg of total diallylsulfides/g; containing trace of diallylmonosulfide, 0.11 mg/g of diallyldisulfide, 1.4 mg/g of diallyltrisulfide, and 0.3 mg/g of diallyltetrasulfide. The total amounts of the volatile sulfur-containing compound in EE-garlic (1.81 mg/g) was approximately one third, as compared with the heat-dried garlic powder[3] (5.05 mg/g), and the EE-garlic had less odor than the heat-dried garlic powder. The commercial kits for assays of plasma triglycerides (Triglyceride G-Test) and plasma free fatty acids (NEFA C-Test) were obtained from Wako Chemical Ind., Osaka Japan. Alliin (purity, 99.9%) was synthesized by the method of Iberl et al.[12] followed by purification. S-Allyl-L-cysteine (99.9%) was synthesized by the method of Lancaster and Kelly.[13] Diallylsulfide (purity, 99.0%) and diallyldisulfide (88.9%, the remaining components were diallylmonosulfide 5.4% and diallyltrisulfide 5.3%) was purchased from Tokyo Chemical Ind. (Tokyo, Japan). Diallyltrisulfide was synthesized by the method of Kirner and Richter,[14] and was purified using chromatography.[15] Its purity was 73.4% and the remaining component was identified as diallyldisulfide (24.3%). Phentolamine and propranolol were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Experiment 1

The experimental diets were the high-fat diet (energy density, 21.21 MJ/kg) and were synthetic diets and contained the following ingredients, in grams per kilogram; casein 250, shortening 300, vitamins 17, minerals 50, cellulose 40, sucrose 300, and α-cornstarch 43.[3] One half of the animals received the high-fat diet alone, whereas the others received the same diet containing 0.8% of EE-garlic. Rats weighing 80 to 90 g were separated into two groups and were given the diet with or without EE-garlic for 28 days. Both groups of rats consumed the equal energy during the experimental period. At the end of the experimental period, the rats were individually transferred to a metabolic cage, where urine and feces were separately collected for 1 day. During the collection, urine samples were collected on 1 mL of 50% HCl. After the collection, urinary creatinine was measured by the method of Clark and Thompson.[16] Fecal samples were dried, weighed, and pulverized. The fecal samples were extracted by the method of Folch et al.[17] After the collection of urine and feces, in the fed state, rats were anesthetized by intraperitoneal injection of α-chrolarose and urethan (75 mg/kg and 750 mg/kg, respectively). Blood samples were collected from the abdominal aorta, and plasma was separated after centrifugation (3,000g for 15 min) and stored at −40°C until assayed. After blood sampling, the liver, kidney, perirenal fat pad, epididymal fat pad, and IBAT were immediately excised, washed in chilled saline, blotted, weighed, and stored at −40°C for further analyses. The plasma was treated with aluminum oxide for the determination of epinephrine and norepinephrine concentrations using HPLC with electrochemical detection.[18,19] Plasma triglycerides and free fatty acid concentrations were analyzed enzymatically using commercial kits. Plasma total and HDL cholesterol concentrations were measured according to the method of Pearson et al.[20] IBAT mitochondrial proteins were isolated by the method of Cannon and Lindberg,[21] and the protein contents were measured by the method of Lowry et al.[22]

Experiment 2

The effects of EE-garlic, alliin (allylcysteine sulfoxide), S-allyl-L-cysteine, and allylsulfides having a different number of sulfur atoms (volatile sulfur-containing compounds in garlic) on norepinephrine secretion were investigated by using blood samples obtained from the abdominal aorta. Rats were anesthetized as described above (in Experiment 1), and their rectal temperatures were maintained between 36.5°C and 37.5°C using a direct-current-heating pad. The rats were infused through the right femoral vein with 1 mL of either a vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or the same vehicle containing each of 0.12 g, 0.24 g or 0.36 g of EE-garlic, or 10, 20, or 30 mmol/L of alliin (1.77, 3.54, or 5.31 mg, respectively) or of S-allyl-L-cysteine (1.93, 3.86, or 5.79 mg, respectively). Blood samples were collected from the abdominal aorta 10 min after the infusion of each compound.[23] Blood samples were transferred to heparinized tubes, and plasma obtained by centrifugation at 3,000 g for 15 min, and stored at −40°C until assayed. Plasma norepinephrine was purified with aluminum oxide, assayed by HPLC with electrochemical detection as described previously.[18–19] Similarly, the rats were administered each 10 mmol/L of allylsulfides having a different number of sulfur atoms (diallylmonosulfide, 1.14 mg; diallyldisulfide, 1.46 mg; or diallyltrisulfide, 1.78 mg), and plasma norepinephrine secretions were measured by the same method as mentioned above.

Experiment 3

The effects of EE-garlic, alliin, and diallyldisulfide administration on thermogenesis were investigated by the direct measurement of IBAT and rectal temperatures. Rats were anesthetized as described above (in Experiment 1). A small incision was made above the scapulae, the IBAT was partially separated from the muscle below, and a thermister (Model BAT-12, Sensortek, NJ, USA) was placed under the IBAT pad. Another thermister was inserted in the rectum (C510, Technol Seven Limited Co., Yokohama, Japan). After the

Table 1 Effect of EE-Garlic supplementation on energy intake, body weight, liver weight, fecal lipid, urinary creatinine, perirenal adipose tissue weight, and epididymal fat pad weight in rats fed a high-fat diet for 28 days (Experiment 1)

	High-fat diet	High-fat EE-garlic-supplemented diet
Energy intake (MJ)[1]	6.321	6.321
Body weight (g)	261.6 ± 1.6	258.0 ± 3.8
Liver weight (g)	9.9 ± 0.1	9.3 ± 0.2
Liver lipid (g)	0.57 ± 0.03	0.55 ± 0.04
Fecal lipid (mg/day)	85.3 ± 15.3	97.4 ± 12.9
Urinary creatinine (μmol/day)	138.8 ± 34.5	105.2 ± 41.5
Perirenal fat pad weight (g)	1.78 ± 0.14	1.45 ± 0.04
Epididymal fat pad weight (g)	5.24 ± 0.31	3.76 ± 0.36*

[1]Rats were individually given the high-fat diet or the high-fat EE-garlic diet, and were received equal energy intake (6.321 MJ of total energy for 28 days) for each of the both group. Values are means ± SEM for seven or eight rats.
*Significantly different from high-fat diet group at $P < 0.01$.

IBAT and rectal temperatures reached a steady state, rats were given individually an intramuscular injection of 0.12 g of EE-garlic, or 10 mmol/L each of alliin (containing 1.77 mg/mL) or diallyldisulfide (containing 1.46 mg), which were dissolved in 1 mL of 0.9% NaCl solution containing of 2% ethanol and 0.5% Tween 80 as the vehicle or the vehicle alone. IBAT and rectal temperatures were recorded every min for 120 min. The effects were compared with the injection of vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80), and IBAT and rectal temperatures were monitored. The effects of diallyldisulfide administration in the presence or absence of α-blocker or β-blocker on thermogenesis were also investigated by the direct measurements of IBAT and rectal temperatures as mentioned above, after rats were given an intramuscular injection of α-blocker, phentolamine (3 mg/kg) or β-blocker, propranolol (10 mg/kg) before anesthesia. After IBAT and rectal temperatures reached a steady level, 10 mmol of diallyldisulfide (containing 1.46 mg/mL) was injected intramuscularly, the changes in temperatures were compared with those after the injection of 1 mL of the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80).

Statistical analysis

All data are presented as means ± SEM. Statistical analyses were performed using analysis of variance[24] and Duncan's multiple-range test.[25]

Results

Experiment 1

The mean values of perirenal fat pad weight and epididymal fat pad weight were significantly lowered by EE-garlic supplementation, although there were no differences in body weight, liver weight, urinary creatinine and perirenal adipose tissue weight (*Table 1*). Plasma concentrations of triglycerides and free fatty acids were significantly decreased by EE-garlic supplementation. In contrast, there were no significant differences in plasma total cholesterol or HDL cholesterol concentrations (*Table 2*). IBAT mitochondrial protein were significantly greater in rats given EE-garlic powder, although there were no significant differences in IBAT weight (*Figure 1*). Plasma norepinephrine concentrations were significantly increased by EE-garlic supplementation, while epinephrine levels were not significantly affected (*Figure 2*).

Table 2 Effect of EE-garlic supplementation on plasma concentration of triglycerides, free fatty acids, total cholesterol, and HDL-cholesterol in rats fed a high-fat diet (Experiment 1)

	High-fat diet	High-fat EE-garlic-supplemented diet
Triglyceride (mmol/L)	11.23 ± 1.04	5.18 ± 0.58*
Free fatty acids (μmol/L)	19.29 ± 1.52	13.40 ± 0.67*
Total cholesterol (mmol/L)	1.565 ± 0.070	1.490 ± 0.074
HDL cholesterol (mmol/L)	0.819 ± 0.135	0.549 ± 0.033

Values are means ± SEM for six or seven rats.
*Significantly different from high-fat diet group at $P < 0.01$.

Experiment 2

The effects of the administration of EE-garlic, alliin, and allyl-L-cysteine on norepinephrine secretions are shown in *Figures 3–5*. Plasma norepinephrine concentrations were significantly increased by administration of 0.24 and 0.36 mg of EE-garlic as compared with the infusion of a vehicle or of 0.12 mg of EE-garlic (Figure 3), and there was a significant positive correlation between the norepinephrine secretion and the EE-garlic dose ($P < 0.01$, $r = 0.65$). Plasma norepinephrine concentrations were significantly increased by administrations of 1.77, 3.54, and 5.31 mg of alliin as compared with the vehicle (*Figure 4*). The effect of the administration of alliin on plasma norepinephrine concentrations was also dose dependent ($P < 0.001$, $r = 0.934$). On the other hand, the effect of S-allyl-L-cysteine was not dose dependent within the dose used in this study (1.93 to 5.79 mg), although the effect of this compound was significant (*Figure 5*). The effects of the administration of allylsulfides having different numbers of sulfur atoms on plasma norepinephrine secretions are shown in *Figure 6*. Plasma norepinephrine concentrations were significantly

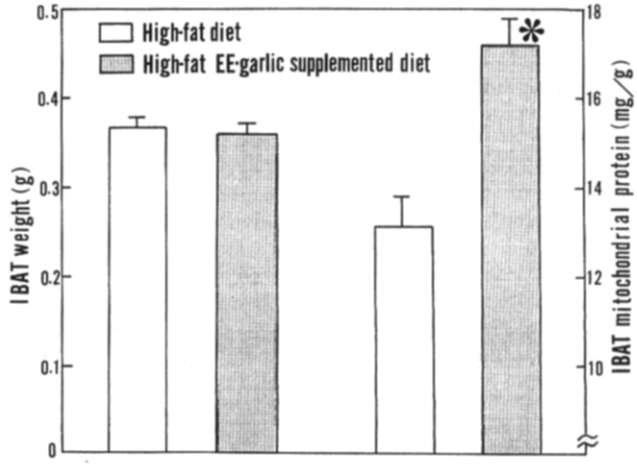

Figure 1 Effect of EE-garlic supplementation on IBAT weight and on IBAT mitochondrial protein contents in rats fed a high-fat diet for 28 days (Experiment 1). Values are means ± SEM of five or six rats. *Significantly different from high-fat diet fed group at $P < 0.01$.

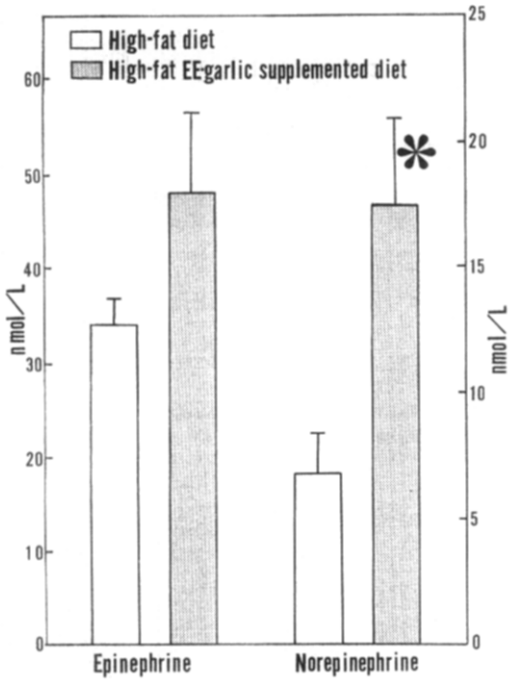

Figure 2 Effect of EE-garlic supplementation on epinephrine and norepinephrine secretions in plasma (Experiment 1). Values are means ± SEM of six or seven rats. *Significantly different from the high-fat diet fed group at $P < 0.01$.

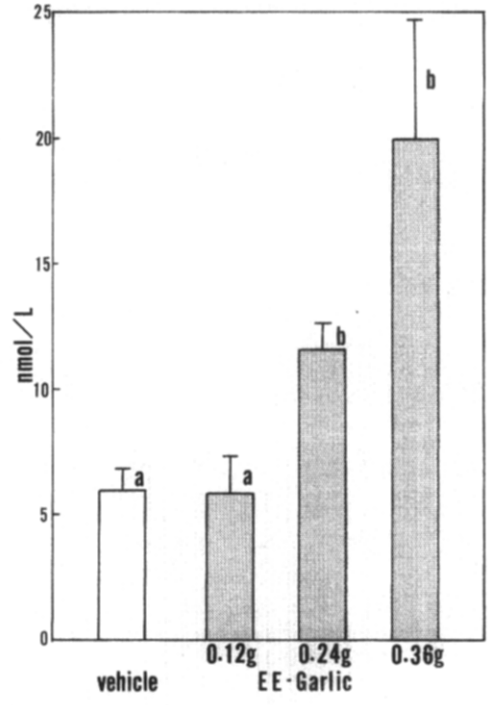

Figure 3 Effects of EE-garlic administration on plasma norepinephrine concentrations (Experiment 2) Rats were received an infusion of 1 mL of the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of 0.12, 0.24, or 0.36 g of EE-garlic into the right femoral vein. Ten min after the infusion, the abdominal aortic blood was collected. Values were means ± SEM for five or six rats. Means not sharing a common superscript letter are not significantly different at $P < 0.05$.

increased by the administration of diallyldisulfide and diallyltrisulfide (containing two and three sulfur atoms, respectively), whereas the effect of diallylmonosulfide was similar to that of the vehicle used for this experiment.

Experiment 3

The effects of the administration of EE-garlic, alliin, and diallyldisulfide on the thermogenesis are shown in *Table 3*. The increases in temperature of the IBAT and rectum in the administration of EE-garlic, alliin, and diallyldisulfide were significantly different from the vehicle alone. The effects of diallyldisulfide administration on temperature increases in the IBAT and rectum after the pretreatment with α-blocker or β-blocker are shown in *Table 4*. The effect of diallyldisulfide was not affected by the pretreatment of α-blocker, it disappeared when rats were pretreated by β-blocker. This finding suggests that the mechanism of the increased thermogenesis by diallyldisulfide administration was by β-adrenergic action.

Discussion

The present study has investigated the effects of alliin and volatile sulfur-containing compounds in garlic on thermogenesis in rats. From the measurement of IBAT mitochondrial protein and plasma norepinephrine secretion, it was suggested that growth of IBAT is enhanced more by EE-garlic supplementation in high-fat diet fed rats. Our previous paper has reported that supplementation of heat-dried garlic powder to the high-fat diet and diallyldisulfide enhances growth of IBAT by increasing norepinephrine secretion in rats.[3] It was suggested that effects of these two garlic preparations on thermogenesis were similar in the enhancement of IBAT growth and norepinephrine secretion, although the total amounts of the volatile sulfur-containing compounds in the EE-garlic was lowered to approximately one third, as compared with the heat-dried garlic powder.[3] Furthermore, it could be thought that there were very little of the volatile sulfur-containing compounds in EE-garlic unless these compounds were determined after the treatment of enzymatic reaction by alliin lyase. The present study indicated that the administrations of alliin (a nonvolatile sulfur-containing compound in garlic) and EE-garlic enhanced the norepinephrine secretion in situ and these increases were dose dependent. Therefore, we speculate that EE-garlic contains significant amounts of alliin, and that the alliin directly or indirectly stimulates the enhancement of norepinephrine secretion. In contrast, S-allyl-L-cysteine administration increased the norepinephrine secretion as compared to the administration of the vehicle, but this increase was not dose dependent. Accordingly, it was indicated that the mechanism of action for increasing norepinephrine secretion by S-allyl-L-cysteine was different from that of alliin. Sundaram and Milner documented the effects of organosulfur compounds in garlic on the growth of canine mammary tumor cells in culture.[26] They noted that water-soluble organosulfur compounds (S-allyl-cysteine) did not significantly alter the growth, but oil-soluble organosulfur compounds (diallylmonosulfide, diallyldisulfide, and diallyltrisulfide) markedly inhibited the growth of these cells.

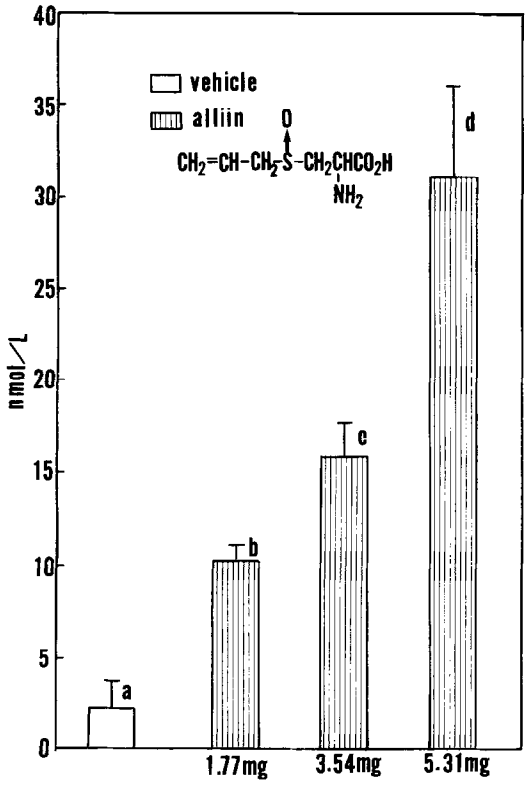

Figure 4 Effects of alliin administration on plasma norepinephrine concentrations (Experiment 2). Rats were received an infusion of 1 mL of the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of the vehicle containing alliin (S-allyl-L-cysteine sulfoxide) 1.77 mg (10 mmol/L), 3.54 mg (20 mmol/L) or 5.31 (30 mmol/L) into the right femoral vein. Ten minutes after the infusion, the abdominal aortic blood was collected. Values are means ± SEM of four or five rats. Means not sharing a common superscript are significantly different at $P < 0.05$.

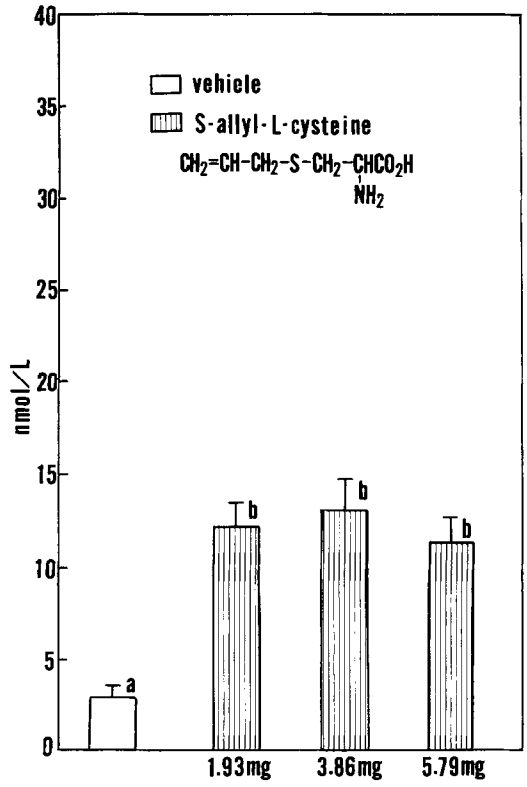

Figure 5 Effects of S-allyl-L-cysteine administration on plasma norepinephrine concentrations (Experiment 2). Rats were received an infusion of 1 mL of vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of the vehicle containing S-allyl-L-cysteine 1.93 mg (10 mmol/L), 3.86 mg (20 mmol/L), or 5.79 mg (30 mmol/L) into the femoral vein. Ten minutes after the infusion, the abdominal aortic blood collected. Values were means ± SEM of four or five rats. Means not sharing a common superscript letter are significantly different at $P < 0.05$.

The previous paper reported that diallyldisulfide enhances norepinephrine secretion in a dose-dependent manner.[3] In the present study, we found that norepinephrine secretion was increased by the administrations of diallyldisulfide and related sulfur-containing compounds in garlic, including alliin. This observation suggests that volatile and nonvolatile sulfur-containing compounds in garlic enhance norepinephrine secretion in rats. It may be that various tissues in the rat have alliin lyase-like enzyme activity (alliinase, EC. 4.4.1.4) that catalyzes the reaction of alliin to a volatile compound, diallylthiosulfinate (allicin); however, further experiments are necessary to clarify this point. It is possible that polysulfides in garlic collaborate with allyl portion and sulfur atoms, and play a role in the stimulation of norepinephrine secretion in rats.

BAT has long been recognized as a major site of metabolic heat generation (non-shivering thermogenesis) in mammalian neonates and in cold-exposed adult animals.[4,7,27] The major effector of BAT thermogenesis is believed to be norepinephrine released from the sympathetic nerve terminals densely innervating this tissue via β-adrenergic receptors.[28] In the present study, thermogenic effects of volatile and nonvolatile sulfur-containing compounds in garlic (diallyldisulfide and alliin, respectively), and EE-garlic were evaluated by direct measurements of temperatures of the IBAT and rectum temperatures. It was found that the nonvolatile sulfur-containing compound of garlic and alliin, enhanced thermogenesis similar to diallyldisulfide.

Furthermore, to determine the mechanism of thermogenesis by garlic constituents, we examined the effects of diallyldisulfide on IBAT and rectal temperatures in the presence or absence of α-adrenergic blocker, phentolamine, and β-adrenergic blocker, propranolol. Flaim et al.[8] reported the effects of α- and β-adrenergic antagonists (phentolamine and propranolol, respectively) on the neurally induced temperature changes of IBAT. These authors suggested that the temperature increase appears to be mediated via both α- and β-adrenergic receptors, although the β-pathway is quantitatively more significant. Mohel et al.[10] reported that oxidative metabolism of isolated brown adipocytes is directly increased in vitro by adding norepinephrine and is suppressed by the addition of β-blockers such as propranolol. Although norepinephrine, which is the natural mediator of thermogenesis in the BAT, interacts with both α-adrenergic and β-adrenergic receptors, most heat produced by brown adipocytes is caused by the interaction with β-receptors.[10] In the present study, it was found that the

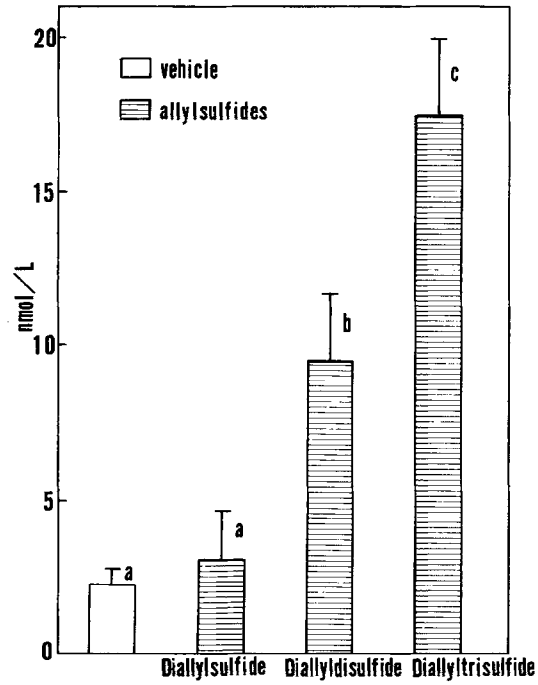

Figure 6 Effects of allylsulfides containing a different number of sulfur atoms on plasma norepinephrine concentrations (Experiment 2). Rats were received an infusion of 1 mL of the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of each 10 mmol/L containing 1.14 mg of diallylmonosulfide, 1.46 mg of diallyldisulfide, or 1.78 mg of diallyltrisulfide into the right femoral vein. Ten minutes after the infusion, the abdominal aortic blood was collected. Values are means ± SEM of four or five rats. Means not sharing a common superscript letter are significantly different at $P < 0.05$.

administration of diallyldisulfide enhanced thermogenesis by increasing temperatures caused by heat productions via β-adrenergic action. This observation is similar to the mechanism of thermogenesis regulated by capsaicin.[29,30] Furthermore, from the lowering effect of plasma triglyceride concentration, the increase of IBAT growth and norepinephrine secretion in rats fed a high-fat diet, we speculated that thermogenesis via β-adrenergic action by garlic supplementation resulted in the enhancement of triglyceride catabolism and energy expenditure.

We have also confirmed in the same method that the thermogenic effects of diallyldisulfide was demonstrated by comparison to the pungent compounds in some spices (allylisothiocyanate, piperine and capsaicin). The results

Table 3 Effects of EE-garlic, alliin and diallyldisulfide administration on the increase in temperatures of the IBAT and rectum (Experiment 3)[1]

	Increasing temperature (°C)[2]	
	IBAT	Rectum
Vehicle[3]	0.31 ± 0.06—a	0.32 ± 0.10—a
EE-garlic[3]	1.36 ± 0.18—b	1.37 ± 0.14—b
Alliin[3]	0.88 ± 0.18—b	0.92 ± 0.19—b
Diallyldisulfide[3]	1.18 ± 0.14—b	1.25 ± 0.17—b

[1]Rats were given individually the vehicle, alliin, or diallyldisulfide, and IBAT and rectal temperatures were directly measured by thermisters for 120 min. See the text in details.
[2]Increasing temperature denotes the differences between temperatures before the administration and at the maximum temperature after the administration.
[3]Rats were given an intramuscular injection of 0.12 g of EE-garlic and of 10 mmol/L each of alliin (1.77 mg) or diallyldisulfide (1.46 mg) that was dissolved in 1 mL of the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80).
Values were means ± SEM. Means not sharing a common letter (a, b) are significantly different at $P < 0.05$.

Table 4 Effects of diallyldisulfide on increasing temperatures of the IBAT and rectum in the presence or absence of the α-adrenergic blocker, phentolamine, or β-adrenergic blocker, propranolol (Experiment 3)[1]

	Increasing temperature (°C)[2]	
	IBAT	Rectum
Vehicle[3]	0.30 ± 0.04	0.32 ± 0.10
Diallyldisulfide[3]	0.70 ± 0.11*	0.71 ± 0.08*
⟨α-blocker⟩		
Vehicle	0.56 ± 0.09	0.56 ± 0.09
Diallyldisulfide	1.26 ± 0.14*	1.20 ± 0.23*
⟨β-blocker⟩		
Vehicle	0.24 ± 0.10	0.26 ± 0.10
Diallyldisulfide	0.33 ± 0.18	0.34 ± 0.17

[1]Rats were given an intramuscular injection of α-blocker (phentolamine) or β-blocker (propranolol) followed by the injection of the vehicle or diallyldisulfide solution. IBAT and rectal temperatures were directly measured by thermisters for 120 min. See the text in details.
[2]Increasing temperature denotes the differences between temperatures before administration and at the maximum temperature after the administration of diallyldisulfide.
[3]Rats were given an intramuscular injection of 10 mmol/L of diallyldisulfide (1.46 mg) dissolved in the vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80). Values were means ± SEM.
*Asterisks indicate significant differences between the vehicle and the diallyldisulfide at $P < 0.01$.

Table 5 Effects of diallyldisulfide and the pungent compounds in some spices on the thermogenesis of IBAT and rectum[1]

	Increasing temperature (°C)[2]	
	IBAT	Rectum
Vehicle[3]	0.00 ± 0.17—a	0.00 ± 0.11—a
Diallyldisulfide[3]	0.88 ± 0.21—b	1.10 ± 0.25—c
Allylisothiocyanate[3]	0.51 ± 0.12—a	0.48 ± 0.02—b
Piperine[3]	0.75 ± 0.08—b	0.78 ± 0.09—bc
Capsaicin[3]	1.79 ± 0.26—c	1.75 ± 0.35—c

[1]Rats were given individually the vehicle, diallyldisulfide, or some pungent compounds. IBAT and rectal temperatures were directly measured by thermisters for 120 min. See the text in details.
[2]Increasing temperature denotes the differences between temperatures before the administration and at the maximum temperature after the administration.
[3]Rats were given intramuscular injection of 10 mmol of each allylisothiocyanate (0.99 mg), piperine (2.85 mg), diallyldisulfide (1.46 mg), or capsaicin (3.05 mg) that was dissolved in 100 μL of the vehicle (dimethylsulfoxide). Diallyl disulfide and allylisothiocyanate were obtained from Tokyo Chemical Ind., Tokyo, Japan. Piperine and capsaicin were obtained from Sigma Chemical Co., St. Louis, MO, USA.
Values were means ± SEM. Means not sharing a common letter are significantly different at $P < 0.05$.

were shown in *Table 5*. It was found that thermogenesis was enhanced by the administration of these pungent compounds and that the potential for inducing thermogenesis was capsaicin > diallyldisulfide > piperine > allylisothiocyanate (*Table 5*). Therefore, the present study indicate that alliin and volatile sulfur-containing compounds in garlic enhance thermogenesis via β-adrenergic action of the sympathetic nervous system by increasing norepinephrine secretion in rats.

Acknowledgments

The authors wish to thank Dr. T. Tamura, the University of Alabama at Birmingham, Medical Center, Birmingham, Alabama, for reading this manuscript and for his kind advice.

References

1 Semmler, F.W. (1892). Über das atherische Ol des Knoblauchs (*Allium sativum*). *Arch. Pharm.* **230,** 434–448
2 Stoll, V.A., Seebeck, E. (1948). Über Alliin, die genuine Muttersubstanz des Knoblauchöls. *Helv. Chim. Acta.* **31,** 189–210
3 Oi, Y., Kawada, T., Kitamura, K., Oyama, F., Nitta, M., Kominato, Y., Nishimura, S., and Iwai, K. (1995). *J. Nutr. Biochem.* **6,** 250–255
4 Deseautels, M. and Himms-Hagen, J. (1979). Roles of noradrenaline and protein synthesis in the cold-induced increase in purine nucleotide binding by rat brown adipose tissue mitochondria. *Can. J. Biochem.* **57,** 968–976
5 Klingenberg, M. (1990). Mechanism and evolution of the uncoupling protein of brown adipose tissue. *Trends Biochem. Sci.* **15,** 108–112
6 Mory, G., Bouillaud, F., Combes-George, M., and Ricquire, D. (1984). Noradrenaline controls the concentration of the uncoupling protein in brown adipose tissues. *FEBS Letters* **166,** 393–397
7 Ricquier, D. and Mory G. (1984). Factors affecting brown adipose tissue activity in animal and man. *Clin. Endocrinol. Metab.* **13,** 501–520
8 Flaim, K.E., Horwitz, B.A., and Horwitz, J.M. (1977). Coupling of signals to brown fat: α- and β-adrenergic responses in intact rats. *Am. J. Physiol.* **232,** (Regulatory Integrative Comp. Physiol., 1) R101–R109
9 Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB J.* **4,** 2890–2898
10 Mohell, N., Nedergaard, J., and Cannon B. (1983). Quantitative differentiation of α- and β-adrenergic respiratory responses in isolated hamster brown fat cells: evidence for the presence of an β_1 adrenergic component. *Europ. J. Pharmacol.* **93,** 183–193
11 Rothwell, N.J. and Stock, M.J. (1981). Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis. *Pflüegers Arch.* **389,** 237–242
12 Iberl, B., Winkler, G., Muller, B., and Knobloch, K. (1990). Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Med.* **56,** 320–326
13 Lancaster, J.E. and Kelly, K.E. (1983). Quantitative analysis of the S-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa* L.) *J. Sci. Food Agric.* **34,** 1229–1235
14 Kirner, W.R. and Richter, G.H. (1929). α-Furfuryl chloride and its derivatives. 2. *J. Am. Chem. Soc.* **51,** 3131–3135
15 Nishimura, H., Wijaya, H., and Mizutani, J. (1988). Volatile flavor compounds and antithrombotic agents: vinyldithiins from *Allium victorialis* L. *J. Agric. Food Chem.* **36,** 563–566
16 Clark, L.C. and Thompson, I.L. (1949). Determination of creatinine and creatine in urine. *Anal. Chem.* **21,** 1218–1221
17 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–507
18 Watanabe, T. Kawada, T., and Iwai, K. (1987). Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. *Agric. Biol. Chem.* **51,** 75–79
19 Watanabe, T., Kawada, T., Yamamoto, M., and Iwai, K. (1987). Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochem. Biophys. Res. Comm.* **142,** 259–264
20 Pearson, S., Stern, S., and McGavack, T.H. (1953). A rapid, accurate method for the determination of total cholesterol in serum. *Anal. Chem.* **25,** 813–814
21 Cannon, B. and Lindberg, O. (1979). Mitochondria from brown adipose tissue: Isolation and properties. *Meth. Enzymol.* **55,** 65–78
22 Lowry, O.H., Rosebrough, N.J. Farr, A.L., and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
23 Watanabe, T., Kawada, T., and Iwai, K. (1988). Effect of capsaicin pretreatment on capsaicin-induced catecholamine secretion caused by capsaicin in rats. *Proc. Soc. Exp. Biol. Med.* **187,** 370–374
24 Snedecor, G.W. and Cochran W.G. (1980). *Statistical Methods*, 7th ed. The Iowa State University Press, Ames, IA USA
25 Duncan, D.B. (1955). Multiple range and multiple F-tests. *Biometrics* **11,** 1–42
26 Sundaram, S.G. and Milner, J.A. (1993). Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. *Cancer Let.* **74,** 85–90
27 Rothwell, N.J. and Stock, M.J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281,** 31–36
28 Nicholls, D.G. and Locke, R.M. (1984). Thermogenic mechanism in brown fat. *Physiol. Rev.* **64,** 1–64
29 Kawada, T., Watanabe, T., Takaishi, T., Tanaka, T., and Iwai, K. (1986). Capsaicin-induced β-adrenergic action on energy metabolism in rats: Influence of capsaicin on oxidative consumption, the respiratory quotient, and substrate utillization. *Proc. Soc. Exp. Biol. Med.* **183,** 250–256
30 Watanabe, T., Kawada, T., Kurosawa, M., Sato, A., and Iwai, K. (1991). Thermogenic action of capsaicin and its analogs. In: *Obesity: Dietary Factors and Control.* Japan Sci. Soc. Press, Tokyo/S. Karger, Basel