Intake of Sweeteners and Pungent Ingredients Increases the Thermogenin Content in Brown Adipose Tissue of Rat

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The present work was undertaken to evaluate the potential effects of sweeteners (sucrose and saccharin) and pungent ingredients (capsaicin, a pungent principle of hot pepper; allyl isothyocyanate; and those of garlic and mustard) on the level of thermogenin, variously called uncoupling protein, 32-kDa protein, or GDP binding protein, in rat brown adipose tissue by the method of enzyme-linked immunosorbent assay. Fasting for over 24 h significantly decreased the interscapular brown adipose tissue weight and its thermogenin content. The intake of sweeteners at concentrations generally found in food without fasting condition significantly increased the thermogenin content. The intake of pungent ingredients slightly increased the thermogenin content. The results suggest that some seasonings cause thermogenin induction and result in enhancement of thermogenesis in animals during and after food intake.

INTRODUCTION

Brown adipose tissue (BAT) has attracted a great deal of interest because of its important role in energy regulation in rodents [for a review see Cannon and Nedergaard (1985); Trayhurn and Nicholls, 1986]. Energy regulation is performed through uncoupling of substrate oxidation from ATP production due to the presence of the mitchondrial membrane of a specific polypeptide, thermogenin, with a subunit molecular weight of 32 000 (Heaton et al., 1978). Seasonings, such as sweeteners and pungent ingredients, have long been used because they are refreshing stimuli to gustation and olfaction, they make insipid foods more appetizing, and so on, all over the world. We reported that some pungent ingredients enhance lipid metabolism (Kawada et al., 1986a) and energy metabolism (Kawada et al., 1986b) via catecholamine secretion from the adrenal medulla through sympathetic activation of the central nervous system (Watanabe et al., 1987a,b, 1988a,b; Kawada et al., 1988). Sympathetic nervous stimulation is the most important action in the regulation of thermogenesis and thermogenin synthesis in BAT (Hull and Segall, 1965; Nicholls and Locke, 1984; Rothwell and Stock, 1984; Minokoshi et al., 1986; Ricquier et al., 1986). The present study was carried out to determine the effects of sweeteners (sucrose and saccharin) and pungent ingredients (capsaicin, a pungent principle of hot pepper; allyl isothiocyanate; and those of garlic and mustard) on the level of thermogenin in rat BAT.

MATERIALS AND METHODS

Animals and Diets. Male Wistar rats (120–130-g body weight; Japan SLC Co., Hamamatsu, Japan) were individually housed in stainless steel wire-bottom cages in a room maintained at 22–24 °C and about 50% relative humidity. The room was lighted from 6:00 a.m. to 6:00 p.m. The rats were fed each diet for 10 days. The compositions of the experimental diets were as follows: control diet, 65.2% α-cornstarch, 20% casein, 5% cellulose powder, 5% corn oil, 3.5% mineral mixture (Kawada et al., 1986a), 1% vitamin mixture (Kawada et al., 1986a), and 0.3% D,L-methionine; sucrose diet, 20% sucrose, 45.2% α-cornstarch, and other ingredients as for the control diet; saccharin diet, 0.05% saccharin, 65.15% α-cornstarch, and other ingredients as for the control diet; capsaicin diet, 0.014% capsaicin, 65.186% α-cornstarch, and other ingredients as for the control diet; and allyl isothiocynate (AITC) diet, 0.182% AITC, 65.018% α-cornstarch, and other ingredients as for the control diet. The degrees of sweetness as pungency of the diets, which we adjusted to physiological levels, were the same for both the sucrose and saccharin diets (Inglett, 1981) and for the capsaicin and AITC diets (Kawada et al., 1986a). The same energy intake by the rats in each group was maintained by adjusting the feed intake (pair-fed). For starvation studies, rats were fed a commercial stock diet (MF; Oriental Yeast Co., Tokyo, Japan), and then the diet was removed for 1 or 3 days. Tap water was available ad libitum.

Isolation of Thermogenin from Rats. Purification of thermogenin was performed as described by Lin and Klingenberg (1980). Briefly, for cold adaptation, male Wistar rats (4 weeks old) were exposed to 4 °C for 2 weeks. Since the fasting period was a fairly important factor as to measurement of the thermogenin content, interscapular BAT (IBAT) was excised from the rats at 4–5 h after eating. For isolation of thermogenin, mitchondria in IBAT were extracted first with 3.2% Lubrol PX (a detergent) and then with Triton X-100. The resultant extract was applied directly to a hydroxyapatite column and eluted with 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 6.7. The breakthrough (nonabsorbable) fractions contained thermogenin. The purity of the thermogenin was checked by SDS–PAGE (Lin et al., 1980) and GDP binding (Cannon and Nedergaard, 1985). The isolated protein was stored at −20 °C and later used to prepare rabbit antibody and for the enzyme-linked immunosorbent assay (ELISA).

Preparation of Antithermogenin Antibody in a Rabbit. Rabbit anti-rat thermogenin serum was prepared according to the method of Cannon et al. (1982).

Enzyme-Linked Immunosorbent Assay. Standard procedures for indirect ELISA were performed as described by Cannon et al. (1982).

Chemicals. Sucrose and sodium saccharin were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Capsaicin and allyl isothiocyanate were obtained from Sigma Chemical Co. (St. Louis, MO) and Tokyo Kasei Kogyo Co. (Tokyo, Japan), respectively. Lubrol PX (a detergent) and hydroxyapatite (100–350 mesh; bovine serum albumin binding capacity, 17.1 mg/g) were purchased from Nakarai Tesuque Co. (Kyoto, Japan).

Statistical Analysis. The data are presented as means ± standard error from the mean (SEM) and were statistically analyzed by means of analysis of variance (Snedecor and Cochran, 1980) and Duncan's multiple-range test (Duncan, 1955).

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Table I. Effects of Fasting on the Body and IBAT Weights of Rats[a]

	fasting days		
	0	1	3
initial body wt, g	136.8 ± 3.7[#]	135.5 ± 1.2[#]	135.5 ± 1.0[#]
final body wt, g	136.8 ± 3.7[a]	109.8 ± 0.7[b]	75.9 ± 0.9[c]
IBAT wt, mg	216 ± 9[A]	156 ± 13[B]	96 ± 6[C]

[a] Rats were fasted for 0, 1, or 3 days. The values are means ± SEM for four rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$.

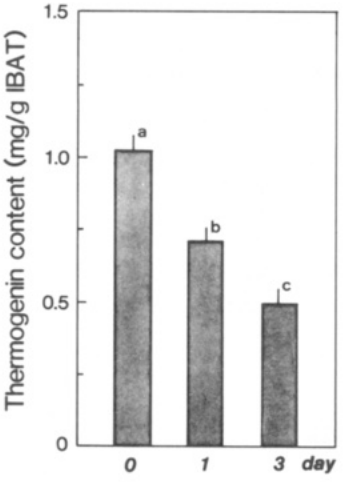

Figure 1. Effect of fasting on the thermogenin content of rat IBAT. Rats were fasted for 0, 1, or 3 days. The values are means ± SEM for four rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. Other experimental details are under Materials and Methods.

RESULTS AND DISCUSSION

Effects of Fasting on IBAT Weight and Thermogenin Content. BAT exhibits a unique property allowing a high heat production capacity. The prime activator of BAT thermogenesis is the β-adrenergic action of norepinephrine supplied through rich sympathetic innervation (Hull and Segall, 1965; Nicholls and Locke, 1984; Rothwell and Stock, 1984; Ricquier et al., 1986; Minokoshi et al., 1986). On the other hand, sympathetic nerve activity is suppressed during fasting and undereating, and the norepinephrine turnover rate and its metabolic clearance decrease (Young and Landsberg, 1977; Gross et al., 1979; O'Dea et al., 1982). Therefore, the nutrient status, primarily fasting, is considered an important factor in the regulation of BAT function.

In this study, the IBAT weight decreased with 1 day of fasting, the degree being greater than that in the case of body weight (Table I). Furthermore, the thermogenin content significantly decreased with 1 and 3 days of fasting (Figure 1). The extent of reduction was similar to that in the case of IBAT weight. These results are consistent with the observation of suppression of the sympathetic nervous system (Young and Landsberg, 1977), catecholamine metabolism (O'Dea et al., 1982), and specific binding of guanidine diphosphate to BAT mitochondria (Rothwell et al., 1984) and probably indicate that animals adapt to survive by reducing energy consumption during a fasting state. Therefore, the fasting period is a fairly important factor in the measurement of the thermogenin content.

Effects of Sweeteners on IBAT Weight and Thermogenin Content. The sucrose diet containing 20% sucrose, used as the standard diet in nutritional experiments, slightly decreased the IBAT weight compared to the control diet (Table II). On the other hand, the thermogenin content significantly increased in the sucrose diet

Table II. Effects of Sucrose and Saccharin on the Body and IBAT Weights of Rats[a]

	diet group		
	control	sucrose	saccharin
energy intake, kcal/day	43.7 ± 0.3[#]	44.0 ± 0.1[#]	43.9 ± 0.1[#]
body wt gain, g	38.8 ± 0.5[a]	37.3 ± 1.3[a]	39.2 ± 1.8[a]
IBAT wt, mg	267 ± 8[A]	250 ± 7[B]	238 ± 9[B]

[a] Rats were pair-fed each diet for 10 days. The values are means ± SEM for six rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$.

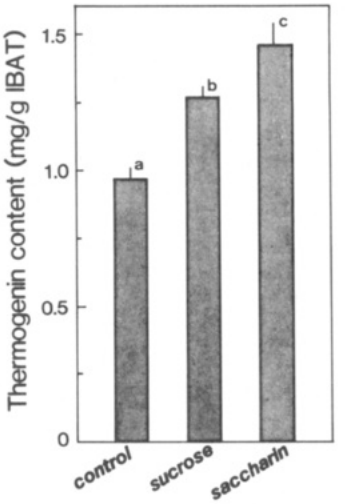

Figure 2. Effects of sucrose and saccharin on the thermogenin content of rat IBAT. Rats were pair-fed each diet for 10 days. The values are means ± SEM for six rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. Other experimental details are under Materials and Methods.

compared to that in the control diet (Figure 2). Also, glucoreceptors were detected in the liver, the portal vein, and the nucleus tractus solitarius, and the blood glucose level was monitored (Niijima, 1982; Mizuno and Oomura, 1984). Signals are transmitted via peripheral sympathetic nerves (Niijima, 1981). In this study, the amount of glucose produced from carbohydrate taken in was estimated the same in each diet group. Therefore, it was speculated that kinds of carbohydrate, especially their taste, and/or the timing of glucose production from carbohydrate during eating affected the BAT weight and thermogenin content fairly significantly. So we examined the effect of a nonnutritional sweetener, saccharin, on the IBAT weight and thermogenin content in rats. The saccharin diet, which was as sweet as the 20% sucrose one (Inglett, 1981), considerably decreased in IBAT weight compared to the control diet (Table II) but increased the thermogenin content fairly well (Figure 2). The degree of augmentation in the saccharin diet group was much greater than that in the case of the sucrose diet group (Figure 2). Rats preferentially take in saccharin as a "palatable" ingredient, but saccharin does not affect the blood glucose or insulin level, except for the activation of central nervous system via sensory stimulation by feeding, the so-called cephalic phase stimulation, due to palatability (Berthoud et al., 1980). These results suggest that the effects of sweeteners on the thermogenin content are due to palatability via cephalic phase stimulation. On the other hand, the IBAT weight reduction observed in the sweetener diet groups presumably resulted from the activation of BAT thermogenesis and the subsequent reduction in the number of white adipocytes in BAT, because white adipocytes sporadically appeared in IBAT in the control diet group, in contrast to in the sweetener diet groups.

Table III. Effects of Capsaicin and Allyl Isothiocyanate on the Body and IBAT Weights of Rats[a]

	diet group		
	control	capsaicin	AITC[b]
energy intake, kcal/day	38.9 ± 1.1[#]	37.5 ± 1.8[#]	36.8 ± 1.4[#]
body wt gain, g	37.6 ± 0.7[a]	35.4 ± 1.2[b]	33.4 ± 1.3[b]
IBAT wt, mg	256 ± 17[A]	233 ± 17[A]	250 ± 16[A]

[a] Rats were pair-fed each diet for 10 days. The values are means ± SEM for 12 rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. [b] AITC, allyl isothiocyanate.

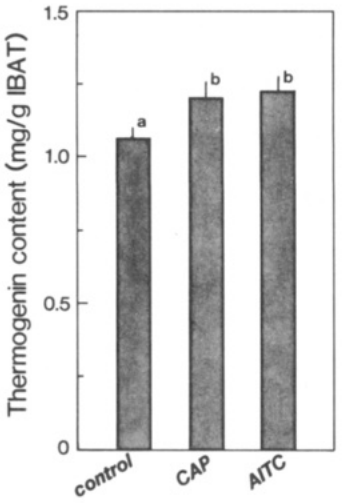

Figure 3. Effects of capsaicin and allyl isothiocyanate on the thermogenin content of rat IBAT. Rats were pair-fed each diet for 10 days. The values are means ± SEM for 12 rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. Other experimental details are under Materials and Methods. CAP, capsaicin: AITC, allyl isothiocyanate.

Effects of Pungent Ingredients on IBAT Weight and Thermogenin Content. We have reported that some pungent spices caused enhancement of energy metabolism (Kawada et al., 1986b) due to catecholamine secretion from the adrenal medulla through sympathetic activation of the central nervous system in rats (Watanabe et al., 1988a,b; Kawada et al., 1988). In humans, Henry and Emery (1986) reported that the ingestion of chili, containing capsaicin, and mustard, containing allyl isothiocyanate (AITC), sauces with meals results in a marked increase in energy metabolism. However, AITC and diallyl disulfide, "volatile" pungent ingredients of mustard, garlic, etc., did not cause even slight catecholamine secretion from the adrenal medulla of rats (Kawada et al., 1988). In this study, the degree of pungency of the AITC diet was approximately identical with that of the 0.014% capsaicin diet. The IBAT weight was not influenced by the intake of pungent diets (Table III). On the other hand, the thermogenin content increased moderately and to the same degree in both the pungent diet groups (Figure 3). Yoshida et al. (1988) reported that the intramuscular injection of capsaicin or isothiocyanate increased the IBAT temperature and increased GDP binding and mitochondrial oxygen consumption in IBAT. Capsaicin stimulates the heat and pain senses, as known well, and activates the sympathetic nerve system (Watanabe et al., 1988b), while sulfur-containing and volatile pungent ingredients, AITC and diallyl disulfide, predominantly stimulate the sense of smell during the intake of diet. In other words, all of these pungent ingredients activate the cephalic phase (Berthoud et al., 1980) via the senses at moderate concentrations. Therefore, it was suggested that the increase in the thermogenin content in both the pungent diet groups was mediated by the stimulation of the cephalic phase during the intake of each diet.

Recently, LeBlanc and colleagues revealed that palatability, through activation of the cephalic phase, evoked thermogenesis and pointed out that activation of the cephalic phase is the most important factor as to the appearance of diet-induced thermogenesis in rats fed a cafeteria diet (Diamond et al., 1985; LeBlanc and Brondel, 1985). Therefore, it was suggested that the intake of some seasonings, such as the sweeteners and pungent ingredients examined in this study, causes thermogenin induction via cephalic phase stimulation, which results in acceleration of thermogenesis during food intake.

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Registry No. Sucrose, 57-50-1; allyl isothiocyanate, 57-06-7; capsaicin, 404-86-4; saccharin, 81-07-2.