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Capsaicin-Induced β-Adrenergic Action on Energy Metabolism in Rats: Influence of Capsaicin on Oxygen Consumption, the Respiratory Quotient, and Substrate Utilization[1] (42414)

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Abstract. The mode of action of capsaicin on energy metabolism was investigated in rats. The oxygen consumption was higher when capsaicin (6.0 mg/kg) was intraperitoneally injected than when it was not injected. The respiratory quotient (R.Q.) increased and then decreased after the administration of capsaicin. The levels of serum glucose and immunoreactive insulin rapidly increased after the administration of capsaicin. Also, liver glycogen rapidly decreased, in contrast to the serum glucose concentration which rapidly increased. The serum-free fatty acid level gradually increased after the administration of capsaicin. These alterations in energy metabolism on the administration of capsaicin were similar to those in the metabolism of epinephrine, and were specifically inhibited by various β-adrenergic blockers. On the other hand, the alterations were not affected by pretreatment with α-adrenergic or ganglion blockers. These results suggest that the mode of action of capsaicin on the enhancement of energy metabolism in rats comprises a direct (as an agonist) and/or an indirect (via catecholamine) β-adrenergic action. Therefore, it was speculated that the adrenergic action of capsaicin resulted, at least in part, in a decrease in the perirenal adipose tissue weight and serum triglyceride concentration in rats fed a high fat diet supplemented with capsaicin (T. Kawada *et al.*, J Nutr **116**:1272–1278, 1986).

Capsaicin (CAP) is a pungent principle of the hot red pepper, which is used as an important spice for enhancing the palatability of food and medicinally as a counterirritant. The chemistry, biochemistry, and pharmacology of capsaicin has been reviewed (1–4). Recent interest in capsaicin has been focused on its action as a relatively selective substance for small-diameter sensory neurons, many of which contain neuroactive peptides such as substance P, cholecystokinin, and somatostatin (5, 6).

Recent studies in our laboratory have shown that this pungent principle is readily transported through the wall of the gastrointestinal tract by means of nonactive transport into the portal vein (7), and it is mostly excreted into the urine as metabolites in rats (8). Furthermore, we have recently demonstrated that capsaicin enhances lipid metabolism in rats (9). These results suggest that capsaicin induces energy consumption in the animal body. However, the available information on the effect of capsaicin on energy metabolism in animals is limited (20). The present study was carried out to investigate the mode of action of capsaicin on the energy metabolism in rats by measuring oxygen consumption, the respiratory quotient (R.Q. = $\dot{V}_{CO2}/\dot{V}_{O2}$), serum glucose, liver glycogen, serum-free fatty acid, and serum immunoreactive insulin.

Materials and Methods. *Materials.* Capsaicin, of which the purity was determined to be over 99% by a HPTLC method as described elsewhere (10), was purchased from E. Merck (Darmstadt, GFR). Propranolol hydrochloride (Inderal injection), pindolol (Carvisken injection) and alprenolol hydrochloride (Apllobal injection) were obtained from ICI Pharmaceuticals, Ltd. (Osaka, Japan), Sankyo, Ltd. (Tokyo, Japan), and Fujisawa Pharmaceuticals Co., Ltd. (Osaka), respectively. Phentolamine mesylate (Regitin injection) and hexamethonium bromide (Methobromin injection) were purchased from Takeda Chemical Industries,

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Ltd. (Osaka) and Yamanouchi Pharmaceuticals Ltd. (Tokyo), respectively. Epinephrine (Bosmin injection) and norepinephrine (Nor-Adrenalin injection) were purchased from Daiichi Seiyaku, Ltd. (Tokyo) and Sankyo, Ltd., respectively. All other chemicals used were of guaranteed reagent grade.

Animals. Male Wistar rats, 6–7 weeks old, were used for experiments after at least 7 days feeding on a purified high fat diet (casein, 10%; starch, 40%; sucrose, 10%; lard, 30%; soybean oil, 5%; mineral mixture, 2%; vitamin mixture, 1%; cellulose, 2%) (9). The rats were injected intraperitoneally with capsaicin (3.0 or 6.0 mg/kg body wt), epinephrine (0.1 mg/kg), and/or blockers (propranolol, 3.0 mg/kg; pindolol, 2.0 mg/kg; alprenolol, 2.0 mg/kg; phentolamine, 10.0 mg/kg; or hexamethonium, 5.0 mg/kg). The dose of capsaicin used was related to that usually ingested by Thai people (11). Each blocker was injected 1 hr before the capsaicin administration. Capsaicin was suspended in a 0.9% saline solution containing 2% ethanol and 10% Tween 80. Controls were injected with a vehicle (a 0.9% saline solution containing 2% ethanol and 10% Tween 80).

Analysis of respiratory gas. A respiration apparatus was used according to the open-circuit method (open air-current system) (12, 13). The air-flow rate was 500 ml/min. Gas analysis was carried out with a respiratory metabolism measurement apparatus, a Beckman Model MAS-1, consisting of an oxygen analyzer (Beckman Model 755) and a carbon dioxide analyzer (Beckman Model 864) at 22°C. Since oxygen consumption of each rat used in our experiment was between 700 and 1000 ml/hr/$kg^{3/4}$ at rest, the mean changes are expressed as percentages of the mean value.

Collection of liver and serum. Rats injected with capsaicin and/or propranolol were lightly anesthetized with diethyl ether just before the measurement time, and their livers were quickly excised. The liver tissue was immediately subjected to glycogen analysis. Blood was collected by means of a heart puncture. Serum obtained after centrifugation was stored at −30°C before glucose, free fatty acid (FFA), and immunoreactive insulin (IRI) analyses.

Chemical analysis. Liver glycogen was determined by the method of Seifter and Dayton (14). Serum glucose and FFA were measured by the glucose oxidase–peroxidase method of Dahlqvist (15) and the method of Itaya and Ui (16), respectively. Serum IRI was determined with a "Rat Insulin Kit" obtained from Immuno Nuclear Corporation (Stillwater, Minn.).

Statistical analysis. Student's t test was used for statistical evaluation of the two means. Populations were subjected to analysis of the variance. When the F test was significant at $P < 0.05$, a comparison was made using the Aspin–Welch test (17). Group means were tested by means of standard one-way ANOVA techniques and Duncan's new multiple-range test (18). These calculations were performed with a NEC PC-8801 mkII microcomputer (NEC Co., Ltd., Tokyo) using the N_{88}-BASIC program (19).

Results. *R.Q. and O_2 consumption.* An increase in O_2 consumption began almost immediately after the rats had been injected with capsaicin (6.0 mg/kg) (Fig. 1). Oxygen consumption reached the maximal level within 20–30 min, and then gradually decreased and reached the initial level at 60 min. As shown in Fig. 1, the R.Q. was about 0.80 at rest when the rats were fed a high fat diet. The R.Q. of rats also began to increase almost immediately after the administration of capsaicin, and reached the maximal level (0.86–0.92) within 20–30 min, gradually decreasing to the initial level (about 0.80) at 60 min. Thereafter, this constant level was maintained for at least for 60 min, and then the R.Q. gradually decreased and reached the minimum level (about 0.75) at about 150 min after the capsaicin injection. The R.Q. level had returned to the initial level (about 0.80) by about 180 min. These alterations in O_2 consumption and R.Q. caused by capsaicin changed with the injection amount of capsaicin (Fig. 1). Rats fed a commercial diet containing a normal fat level showed about the same alterations in O_2 consumption and R.Q. with the capsaicin injection as rats fed a high fat diet (data not shown). Increases in O_2 consumption and R.Q. began rapidly after rats had been injected with epinephrine (0.1 mg/kg, ip) (Fig. 2). O_2 consumption reached the maximal level within 20–50 min, and then gradually decreased. The R.Q. of the rats also reached the maximal level within 20–50 min, and then rapidly decreased to about 0.74, which was lower than the initial level

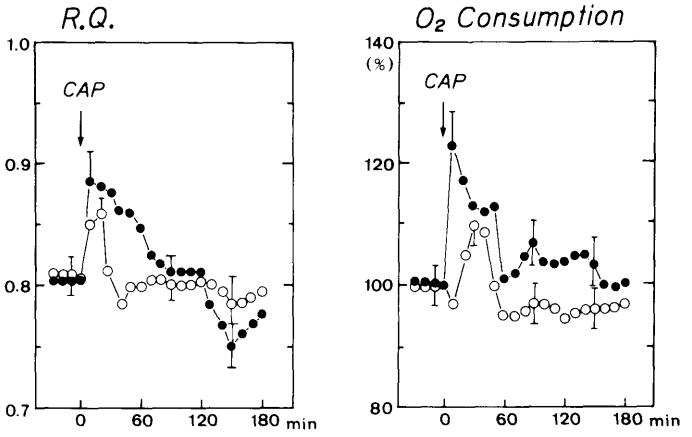

FIG. 1. Effects of capsaicin on the R.Q. and oxygen consumption. R.Q. and O_2 consumption determinations were performed at each time after ip injection of capsaicin (3.0 mg/kg, ○; 6.0 mg/kg, ●) into rats at 0 min. For O_2 consumption, the mean changes are expressed as percentages of the mean basal value, which was taken as 100. Values are means ± SEM for four rats.

(about 0.83). On the other hand, as shown in Fig. 2, the R.Q. of rats injected with norepinephrine (0.1 mg/kg, ip) hardly changed, although O_2 consumption rapidly increased.

Effects of capsaicin on metabolic parameters. Rats treated with vehicle alone showed no changes in any metabolic parameters. However, the levels of serum glucose, liver glycogen, serum FFA, and serum IRI were affected by the capsaicin injection (Table I). An increase in the serum glucose concentration began almost immediately after rats had been injected with capsaicin. The concentration reached the maximum level within 120 min, and then rapidly decreased. The liver glycogen level gradually decreased and then remained at constant level after 60 min in contrast to that with serum glucose. The serum FFA concentration gradually increased up to 180 min. The IRI level rapidly increased as the serum glucose concentration did with the capsaicin injection up to 60 min.

Effects of α- and β-adrenergic and ganglion blockers on the alterations in energy metabo-

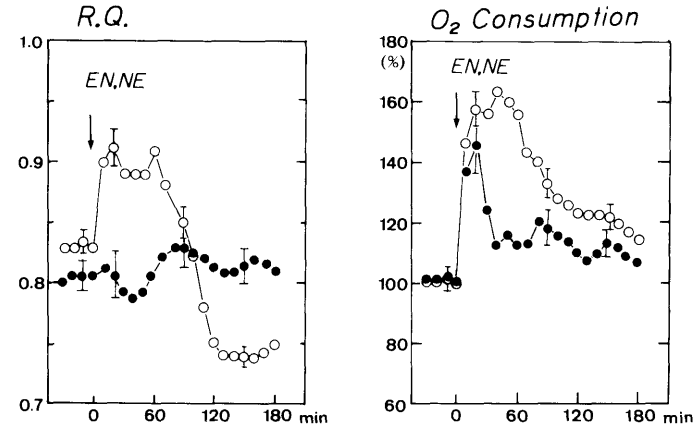

FIG. 2. Effects of epinephrine and norepinephrine on the R.Q. and oxygen consumption. R.Q. and O_2 consumption determinations were performed at each time after ip injection (0.1 mg/kg) of epinephrine (○) or norepinephrine (●) into rats at 0 min. O_2 consumption is expressed as in Fig. 1. Values are means ± SEM of the data for three or four rats.

TABLE I. EFFECTS OF CAPSAICIN ON SERUM GLUCOSE, LIVER GLYCOGEN, SERUM-FREE FATTY ACIDS, AND SERUM INSULIN

		Time after the administration of vehicle or CAP (min)				
		0	30	60	120	180
Glucose (mg/dl)	vehicle	170 ± 13 (4)	182 ± 8 (4)	160 ± 3 (4)	167 ± 9 (4)	154 ± 11 (4)
	CAP	170 ± 13 (4)	306 ± 51 (4)	393 ± 106 (4)	476 ± 46 (4)	226 ± 23 (4)
	P	n.s.	<0.05	<0.05	<0.02	<0.005
Glycogen (mg/g liver)	vehicle	51.3 ± 6.8 (4)	48.5 ± 6.4 (4)	44.6 ± 5.5 (4)	44.4 ± 8.0 (4)	34.8 ± 8.1 (4)
	CAP	48.2 ± 6.0 (4)	39.5 ± 3.8 (4)	25.6 ± 7.1 (4)	43.0 ± 0.7 (4)	33.9 ± 3.5 (4)
	P	n.s.	<0.05	<0.005	n.s.	n.s.
FFA (mg/dl)	vehicle	12.8 ± 0.8 (4)	12.5 ± 1.0 (4)	13.4 ± 0.4 (4)	12.4 ± 1.2 (4)	12.0 ± 0.8 (4)
	CAP	12.8 ± 0.8 (4)	13.6 ± 1.1 (4)	15.3 ± 2.2 (4)	16.1 ± 1.6 (4)	16.3 ± 1.7 (4)
	P	n.s.	n.s.	n.s.	<0.01	<0.005
IRI (ng/ml)	vehicle	2.04 ± 0.70 (6)	2.12 ± 0.45 (5)	1.86 ± 0.46 (5)	1.64 ± 0.36 (5)	n.d.
	CAP	2.04 ± 0.70 (6)	3.60 ± 1.05 (4)	5.25 ± 0.34 (4)	2.83 ± 0.35 (4)	n.d.
	P	n.s.	<0.05	<0.001	<0.005	

Note. Serum and liver parameter determinations were performed at each time after ip injection of the capsaicin vehicle (a 0.9% saline solution containing 2% ethanol and 10% Tween 80) or capsaicin (6.0 mg/kg body wt). Values are means ± SD, for the number of rats given in parentheses. Student's *t* test was used for statistical evaluation on the two means. Populations were subjected to analysis of the variance. When the *F* test was significant at $P < 0.05$, a comparison was made using the Aspin–Welch test. n.s., not significant; n.d., not determined.

lism due to capsaicin. Pretreatments in rats with an α-adrenergic blocker (phentolamine mesylate, 10.0 mg/kg) or a ganglion blocker (hexamethonium, 5.0 mg/kg) did not inhibit the alterations of O_2 consumption and R.Q. induced by the capsaicin injection (Fig. 3). On the other hand, rats previously injected with a β-adrenergic blocker (propranolol, 3.0 mg/kg) showed inhibition of the alterations in O_2 consumption and R.Q. when they were injected with capsaicin (6.0 mg/kg) (Fig. 4). Injections of other β-adrenergic blockers (pindolol, 2.0 mg/kg; alprenolol, 2.0 mg/kg) also completely inhibited the alterations in O_2

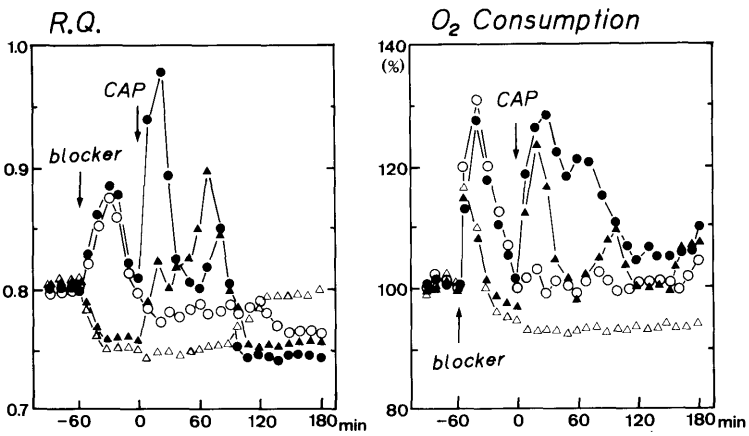

FIG. 3. Effects of an α-adrenergic blocker, a ganglion blocker, and capsaicin on the R.Q. and oxygen consumption. R.Q. and O_2 consumption determinations were performed at each time after ip injection of only an α-blocker, phentolamine mesylate (10.0 mg/kg, ○), into rats at −60 min, both phentolamine mesylate (10.0 mg/kg) at −60 min and capsaicin (6.0 mg/kg) at 0 min (●), only a ganglion blocker, hexamethonium (5.0 mg/kg, △ — △) at −60 min, and both hexamethonium (5.0 mg/kg) at −60 min and capsaicin (6.0 mg/kg) at 0 min (▲ — ▲). O_2 consumption is expressed as in Fig. 1. Values are means of the data for three or four rats.

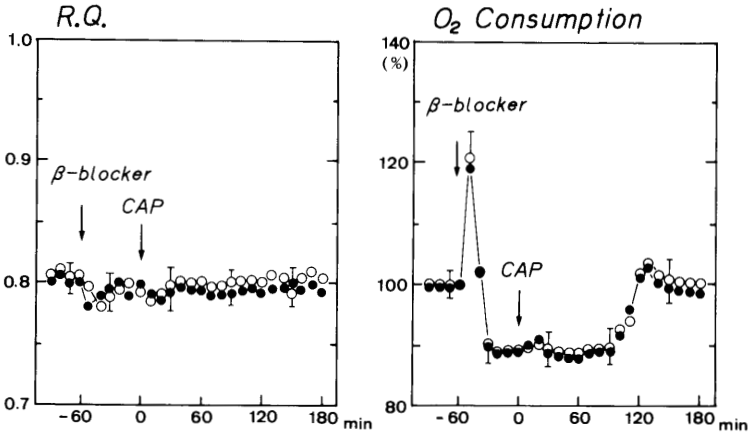

FIG. 4. Effects of a β-adrenergic blocker and capsaicin on the R.Q. and oxygen consumption. R.Q. and O_2 consumption determinations were performed at each time after ip injection of only a β-blocker, propranolol (3.0 mg/kg, ○) into rats at −60 min, and both propranolol (3.0 mg/kg) and capsaicin (6.0 mg/kg) at 0 min (●). O_2 consumption is expressed as in Fig. 1. Values are means ± SEM of the data for four rats.

consumption and R.Q. induced by the capsaicin injection. Furthermore, increases in the serum glucose and FFA concentrations with the capsaicin injection were completely inhibited by pretreatment with a β-adrenergic blocker (propranolol, 3.0 mg/kg) (Table II).

Discussion. Whether or not a nutrient is catabolized in animals, 1 liter of oxygen molecules to be catabolized is converted to about 4.7 kcal of heat under the standard temperature (0°C) and pressure (760 mm Hg) conditions (13). Therefore, it is suggested that the increase in O_2 consumption with the capsaicin injection leads to further heat production, or thermogenesis, in rats (Fig. 1). However, Issekutze *et al.* reported that the subcutaneous dose of capsaicin decreases the metabolic rate of mouse (20). The mechanism of action of capsaicin on energy metabolism might be therefore different from spices.

The R.Q. provides information as to the composition on the nutrient catabolized: carbohydrate only, 1.0; fat only, 0.708; protein only, 0.83. Therefore, we are able to determine by measuring the N-free R.Q. what kind of nutrients, especially carbohydrates or fats, are being catabolized in animals *in situ* (13). In this experiment, as a rapid increase in the R.Q. value was observed at an early stage after the capsaicin injection; endogenous carbohydrate (glucose derived probably from liver glycogen) may be catabolized as the main substrate in rats. On the other hand, since a decrease in the R.Q. value was observed at a later stage

TABLE II. EFFECT OF A β-ADRENERGIC BLOCKER ON SERUM GLUCOSE AND FREE FATTY ACIDS IN RATS INJECTED WITH CAPSAICIN

	Control	β-blocker	CAP	β-blocker + CAP
Glucose (mg/dl)	167 ± 9^a (4)	153 ± 8^a (4)	476 ± 46^b (4)	189 ± 24^a (5)
FFA (mg/dl)	12.4 ± 1.5^a (4)	12.8 ± 0.3^a (4)	16.1 ± 1.6^b (4)	13.7 ± 1.6^a (4)

Note. Serum parameter determinations were performed 2 hr after ip injection of the capsaicin vehicle into rats (Control group), 3 hr after injection of a β-blocker (propranolol, 3.0 mg/kg, ip) into rats (β-Blocker group), 2 hr after injection of capsaicin (6.0 mg/kg, ip) into rats (CAP group), and 2 hr after injection of capsaicin (6.0 mg/kg, ip) into rats preinjected with propranolol (3.0 mg/kg, ip) 1 hr before (β-Blocker + CAP group). Values are means ± SD, for the number of rats given in parentheses.

^{a,b} Means not sharing a common superscript letter are significantly different at $P < 0.05$ level as to one-way ANOVA techniques and Duncan's multiple-range test.

after the capsaicin injection, endogenous fat may then be catabolized as the main substrate in rats.

When rats were injected with capsaicin, the serum glucose level rapidly increased, but, on the other hand, the liver glycogen content rapidly decreased. The serum FFA concentration gradually increased after the capsaicin injection. Such changes in the levels of metabolic parameters well resemble the effects of epinephrine on energy metabolism (21–24), and do not conflict with the R.Q. results mentioned above. The results in Table I suggest that the serum glucose increase with the capsaicin injection was derived from liver glycogen. Serum IRI secretion is inhibited on the binding of epinephrine to the α-adrenergic receptor on the pancreatic β-cell and is enhanced by that to the β-receptor on the cell (25, 26). Whether the stimulatory effect of capsaicin on insulin secretion is due to the direct action of capsaicin on the pancreatic β-cell or whether it is indirect, e.g., via a glucose-stimulated release of insulin, cannot be concluded from the present results.

Previously, Tanaka (12) reported that epinephrine characteristically increases O_2 consumption, and raises or lowers the R.Q. in rats. The alteration of the image of energy metabolism by an epinephrine injection shown in Fig. 2 is very similar to that in the case of capsaicin shown in Fig. 1. Consequently, to elucidate the mechanism of action of capsaicin on energy metabolism, an experiment using various blockers of neuroreceptors was performed. When rats had been previously injected with a β-adrenergic blocker (propranolol), their O_2 consumption and R.Q. were not affected by a capsaicin injection (Fig. 4). However, pretreatment of rats with an α-adrenergic blocker (phentolamine, 10 mg/kg) or a ganglion blocker (hexamethonium, 5.0 mg/kg) seemed to enhance rather than to inhibit the alterations in O_2 consumption and the R.Q. induced by a capsaicin injection (Fig. 3).

These results suggest that the increase in heat production (thermogenesis) induced by capsaicin is based on a β-adrenergic action, which may coincide with the following two models for the possible mode and site of action of capsaicin: (i) the capsaicin molecule is a β-adrenergic agonist, and/or (ii) capsaicin releases catecholamine from sympathetic nerve systems. However, whether the enhancing effect of capsaicin on thermogenesis is due to either of the models cannot be concluded from the present results.

Recent studies in our laboratory demonstrated that capsaicin enhances lipid metabolism in rats; it lowers the perirenal adipose tissue weight and the serum triglyceride concentration in lard-fed rats (9). Therefore, it was speculated that the adrenergic action of capsaicin results in the enhancement of lipid metabolism in rats fed a high fat diet. Further biological and neurophysiological investigations are necessary to completely elucidate the mechanism of the action of capsaicin on energy metabolism.

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1. Szolcsanyi J. Capsaicin type pungent agents producing pyrexia. In: Milton AS, ed. Handbook of Experimental Pharmacology. Berlin, West Germany, Springer-Verlag, Vol **60**:pp437–478, 1982.
2. Nagy JI. Capsaicin: A chemical probe for sensory neuron mechanisms. In: Iverson LL, Iverson SD, Snyder SH, eds. Handbook of Psychopharmacology. New York, Plenum, Vol **15**:pp185–235, 1982.
3. Russell LC, Burchiel KJ. Neurophysiological effects of capsaicin. Brain Res Rev **8**:165–176, 1984.
4. Suzuki T, Iwai K. Constituents of red pepper spices: Chemistry, biochemistry, pharmacology, and food science of the pungent principle of *Capsicum* species. In: Brossi A, ed. The Alkaloids. New York, Academic Press, Vol **23**:pp227–299, 1984.
5. Gamse RS, Leeman SE, Holzer P, Lembeck F. Differential effects of capsaicin on the content of somatostatin, substance P and neurotensin in the nervous system of the rat. NS Arch Pharmacol **317**:140–148, 1981.
6. Jancso GT, Hokfelt T, Lundberg JM, Kiraly E, Halasz N, Nilsson, G, Terenius L, Rehfelt J, Steinbusch H, Verhofstad A, Elde R, Said S, Brown M. Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptides and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin, and 5-hydroxytryptamine. J Neurocytol **10**:963–980, 1981.
7. Kawada T, Suzuki T, Takahashi M, Iwai K. Gastrointestinal absorption and metabolism of capsaicin and dihydrocapsaicin in rats. Toxicol Appl Pharmacol **72**:449–456, 1984.
8. Kawada T, Iwai K. *In vivo* and *in vitro* metabolism

of dihydrocapsaicin, a pungent principle of hot pepper, in rats. Agric Biol Chem **49**:441–448, 1985.
9. Kawada T, Hagihara K-I, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr **116**:1272–1278, 1986.
10. Suzuki T, Kawada T, Iwai K. Effective separation of capsaicin and its analogues by reversed-phase high-performance thin-layer chromatography. J Chromatogr **198**:217–223, 1980.
11. Interdepartment Committee on Nutrition for National Defense. *Nutrition survey—The Kingdom of Thailand.* Washington, DC: US Government Printing Office, 1962.
12. Tanaka T. The mechanism of specific dynamic effect of ingested protein. J Japan Soc Nutr (Japanese) **31**: 1–7, 1978.
13. Kleiber M. The Fire of Life, An Introduction to Animal Energitics. Huntington, N.Y., Krieger, revised ed., 1975.
14. Seifter S, Dayton S. The estimation of glycogen in liver and muscle. Fed Pro **8**:249–250, 1949.
15. Dahlqvist A. Method for assay of intestinal disaccharidases. Anal Bochem **7**:18–25, 1964.
16. Itaya K, Ui M. Colorimetric determination of fatty acids in biological fluids. J Lipid Res **6**:16–20, 1965.
17. Snedecor GW, Cochram WG. Statistical Methods. Ames, The Iowa State Univ Press, 7th ed., 1980.
18. Duncan DB. Multiple range and multiple F-tests. Biometrics **11**:1–42, 1955.
19. Ishii S. Programs of statistical methods for biologists by N_{88}-BASIC, Tokyo, Baifukan, 1983.
20. Issekutz B Jr, Lichtneckert I, Nagy H. Effect of capsaicin and histamine on heat regulation. Arch Int Pharmacodyn Ther **81**:35–40, 1950.
21. Ahlquist RT. A study of the adrenotropic receptors. Amer J Physiol **153**:586–600, 1948.
22. Robert SS, Sacca L. Effect of epinephrine on glucose metabolism in humans: Contribution of the liver. Amer J Physiol **247**:E157–165, 1984.
23. Kawai Y, Arinze IJ. Beta-adrenergic receptors in rabbit liver plasma membranes. J Biol Chem **258**:4364–4371, 1983.
24. Fain JN, Garcia-Scinz JA. Adrenergic regulation of adipocyte metabolism. J Lipid Res **24**:945–966, 1983.
25. Katada T, Ui M. Islet-activating protein: Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores. J Biol Chem **254**:469–479, 1979.
26. Yamazaki S, Katada T, Ui M. Alpha$_2$-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. Mol Pharmacol **21**:648–653, 1982.

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