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RAPID COMMUNICATION

SOME PUNGENT PRINCIPLES OF SPICES CAUSE THE ADRENAL MEDULLA TO SECRETE CATECHOLAMINE IN ANESTHETIZED RATS[1]

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Abstract. We recently reported that capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the rat adrenal medulla. In this study, the effects of some pungent principles of spices on adrenal catecholamine secretion were investigated as compared with that of capsaicin. An increase in catecholamine, especially epinephrine, secretion was observed not only on capsaicin infusion but also on piperine (a pungent principle of pepper) and zingerone (ginger) infusion. Even on infusion of the same amount (650 nmol/kg, i.v.), the order of potency as to catecholamine secretion was capsaicin >> piperine $\geq$ zingerone. While, sulfur-containing and volatile pungent principles, allylisothiocyanate (mustard, etc.) and diallyldisulfide (garlic, etc.), did not even cause slight catecholamine secretion. Furthermore, these adrenergic secretagogues were readily transported via the gut into the body. These results indicate that some pungent principles of dietary spices can induce a warming action via adrenal catecholamine secretion. © 1988 Society for Experimental Biology and Medicine

Introduction Spices with a hot taste have long been used as important seasonings to enhance the palatability of food and also been used as medicinal drugs all over the world (1). They have specific plant origins, as shown in Table 1, and can be chemically classified into two groups, namely, volatile sulfur-containing compounds and nonvolatile ones. In particular, capsaicin (CAP), a pungent principle of hot red pepper, has been extensively examined as a relatively selective substance for peptidergic sensory neurons (2-5).

When CAP-containing food is eaten by humans, the pungent principle is supposed empirically induce a warming action although no biochemical or physiological studies on the energy metabolism involved have been reported except for those of Henry and Emery (6) and ours. The former reported that the intake of spiced food (meals with chilli and mustard sauces) results in a marked increase in the energy metabolic rate (6). In our very recent papers, we postulated that CAP enhances the energy metabolism in rats through activation of the sympathetic nervous system (7-9), and then stimulates the lipid metabolism (10). Furthermore, we have demonstrated directly that CAP evokes catecholamine

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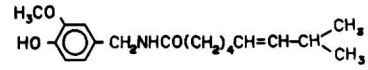
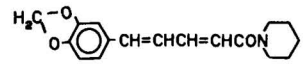
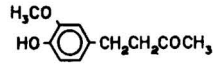
Table 1. The common names, plant origins and chemical structures of typical pungent principles of spices.

pungent principle	abbreviation	origin	chemical structure
capsaicin	CAP	hot pepper	
piperine	PIP	pepper	
zingerone	ZIN	ginger	
allylisothiocyanate	AITC	mustard *etc*	$CH_2=CHCH_2NCS$
diallyldisulfide	DADS	garlic *etc*	$CH_2=CHCH_2SSCH_2CH=CH_2$

secretion from the adrenal medulla of anesthetized rats mainly through the action of the central nervous system(11). The present study was carried out to investigate whether pungent principles other than CAP cause the adrenal medulla to secrete catecholamine or not in anesthetized rats. Furthermore, it was performed to clarify the gastrointestinal absorption of some adrenergic secretagogue principles in rats in situ.

Materials and Methods

Materials. CAP (grade I) and piperine (PIP) were purchased from Sigma Chemical Co. (Saint Louis, MO). Zingerone (ZIN) was obtained from K & K Laboratories (Plainview, NY). Allylisothiocyanate (AITC) and diallyldisulfide (DADS) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other chemicals were of guaranteed reagent grade.

Preparation of animals and sampling of plasma. Male Wistar rats weighing 220-260 g were used after anesthetization with urethane (750 mg/kg, i.p.) and chloralose (75 mg/kg, i.p.). The rats were infused with each pungent principle solution (650 nmol/kg), in 0.9% saline with 0.5% Tween 80 and 0.1% ethanol, into the right femoral vein by means of an infusion pump for 1 min. The dose of capsaicin used were 650 nmol = 200 μg/kg rat. The amount was equivalent to a 60 kg man's ingestion of 4 g of dry hot pepper containing 0.3% of capsaicin, the normal amounts usually taken by Asian people (up to 5 g dry hot pepper/kg man per meal) (12). Hexamethonium bromide and atropine sulfate (1 mg/kg and 5 mg/kg, respectively) were injected 5 min before the pungent principle administration. The operation procedure was described in detail elsewhere (13).

Gastrointestinal absorption of pungent principles in situ. Male Wistar rats weighing 220-260 g were used after overnight fasting. The rats were anesthetized with urethane-chloralose as described above. Emulsions (500 μl), composed of 2.6 mM PIP or ZIN, 2% Tween 80 and 0.4% ethanol in saline, were injected into the jejunal loop (4 cm) and then left in situ for 60 min. The operation and extraction procedure were described elsewhere (14).

Measurement of plasma catecholamine Catecholamine(CA) in the adrenal venous plasma was determined by the HPLC-EC method described in detail elsewhere (8).

Measurement of PIP and ZIN. The HPLC system consisted of a Shimadzu Model LC-3A (Kyoto, Japan), a Cosmosil 5C_8 separation column (150 x 4.6 mm I.D.; Nakarai Chemicals, Kyoto), a Shimadzu

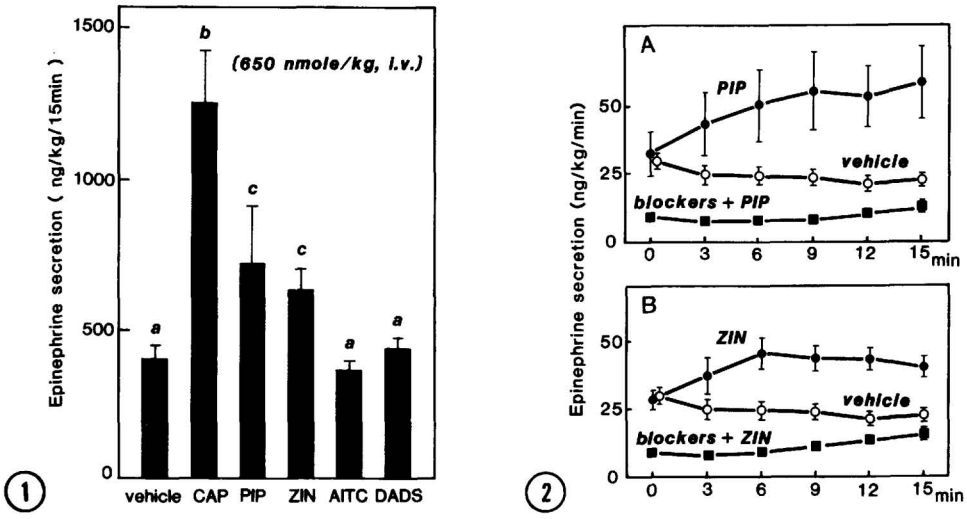


Fig. 1. Effects of capsaicin (CAP), piperine (PIP), zingerone (ZIN), allylisothiocyanate (AITS) and diallyldisulfide (DADS) on adrenal epinephrine secretion. Rats were intravenously infused with each pungent principle solution (650 nmol/kg) or the vehicle (a 0.9% NaCl solution containing 0.1% ethanol and 0.5% Tween 80) for 1 min. Adrenal venous blood was collected for 15 min after the solution administration. The values are means ± SEM for 4-9 rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$.

Fig. 2. Effects of cholinergic blockers on the adrenal epinephrine secretion caused by piperine and zingerone. **A**, piperine (PIP) administration; **B**, zingerone (ZIN) administration; closed circles, PIP or ZIN (650 nmol/kg, i.v.) was administered for 1 min at time 0 without cholinergic blockers; closed squares, hexamethonium bromide and atropine sulfate (1 mg/kg and 5 mg/kg, i.v., respectively) injected 5 min before 650 nmol/kg of PIP or ZIN administration; open circles, the vehicle, a 0.9% NaCl solution containing 0.1% ethanol and 0.5% Tween 80. The values are means ± SEM for 6-9 rats.

Model SPD-2A uv detector (280 nm) and an E1A microcomputer. The mobile phases for PIP and ZIN analysis were 50% and 30% CH_3CN, respectively. The flow rates for PIP and ZIN analysis were 0.7 and 0.6 ml/min, respectively. The retention times of PIP and ZIN were 10 and 6 min, respectively. The detection limits for PIP and ZIN were 1 and 3 ng at a signal-to-noise ratio of 5:1, respectively.

Statistical analysis The data are presented as means ± SEM, and were statistically analyzed by means of analysis of variance (15) and Duncan's Multiple-Range test (16).

Results and discussion

CA secretion with pungent principles of spices The results of our previous in situ experiments suggested that CAP evokes CA secretion from the adrenal medulla of anesthetized rats (9). In the present study, an increase in CA secretion was observed not only on CAP infusion but also on PIP and ZIN infusion (Fig. 1). Even on infusion of the same amount (650 nmol/kg, i.v.), CAP evoked greater CA secretion than PIP or ZIN. The total amount of epinephrine (E) secreted over 15 min with infusions of the vehicle, CAP, PIP and ZIN were 339.2 ± 44.6 (n=7), 1253.5 ± 170.7

(n=4), 718.8 ± 194.0 (n=7) and 632.8 ± 65.4 ng/kg (n=9), respectively. On the other hand, norepinephrine(NE) secretion was also observed when CAP, PIP and ZIN were infused into rats. The value being fairly low compared with that for E secretion in each case (data not shown). While, sulfur-containing volatile pungent principles, AITC and DADS, did not cause even slight CA secretion from the adrenal medulla of anesthetized rats (Fig. 1).

Time-course of the response to PIP and ZIN

Figure 2 shows the time-courses of E secretion from the adrenal medulla. A moderate increase in E secretion was seen in rats infused with a PIP or ZIN solution (650 nmol/kg, i.v.), in contrast to a significant increase on CAP infusion (650 nmol/kg = 200 μg/kg, i.v.), as previously reported (9). However, E secretion began without a detectable lag after the infusion of both PIP and ZIN, in the same manner as in the case of CAP.

Effects of cholinergic blockers on PIP and ZIN-induced adrenal CA secretion

Cholinergic blocking by hexamethonium bromide (nicotinic receptor-blockade) and atropine sulfate (muscarinic receptor-blokade) decreased the CAP-induced adrenal E secretion, as previously reported (11). As shown in Fig. 2, the pretreatment of rats simultaneously with both cholinergic blockers significantly attenuated the PIP and ZIN-induced E secretion, as in the case of CAP. These results suggest that the PIP and ZIN-induced adrenal CA secretion is elicited through activation of the adrenal sympathetic nerves in the same manner as in the case of CAP, as reported previously (11).

Gastrointestinal absorption of CAP, PIP and ZIN in situ

Since the above pungent principles that activate sympathetically-mediated adrenal secretion are daily taken in as food components, knowledge of their gastrointestinal absorption is very important. We have demonstrated that CAP is readily transported via the gastrointestinal tract and then absorbed through non-active transport into the portal vein (12). However, it is not known whether PIP and ZIN can be absorbed or not. So the rates of absorption of CAP, PIP and ZIN from the gastrointestinal tract of rats were investigated, in ligated jejunal loops. A significant amount of these pungent principles disappeared from the loops in 1 hour. Since no metabolite derived from these pungent principles was detected in the gut contents, the rates of absorption of CAP, PIP and ZIN could be determined to be 85.0 ± 2.5 (n=4), 86.7 ± 5.0 (n=4) and 96.5 ± 2.5 % of the dose per hour per 4 cm-length of loop (n=4), respectively.

The present results suggest that only non-volatile pungent principles, namely, PIP and ZIN, like CAP, can evoke CA secretion from the adrenal medulla of rats, and that the stimulatory sympathetic CA release is mediated by cholinergic receptors. As a possible mechanism for all the sympathetically-mediated effects of the principles, pungent principle-induced hypotension might be considered. Detailed study on the mechanism is now in progress. When CAP, PIP, and/or ZIN-containing spiced food is eaten and these materials are absorbed in the gastrointestinal tract, increased secretion of CA from the adrenal medulla will be induced. Therefore, it is considered that the secretion of CA evoked by dietary pungent principles is a daily phenomenon. Furthermore, it is noteworthy that the sympathetic nervous system, via CA, plays a critical role in the regulation of mammalian thermogenic responses to dietary intake and cold exposure (15-18). So the present findings are strong biochemical and physiological evidence for the possible warming action, thermogenesis, due to pungent principle-containing spiced foods.

References

1. Pruthi JS. Spices and Condiments: Chemistry, Microbiology, Technology. In: Chichester CO, Mrak EM, Stewart GF, eds. Advances in Food Research. New York, Academic Press, suppl. 4, p1, 1980.

2. Games R, Leeman E, Holzer P, Lembeck F. Differential effects of capsaicin on the contents of somatostatin, substance P and neurotensin in the nervous system of the rat. Naunyn-

Schmiedeberg's Arch Pharmacol **317:** 140-148, 1981.

3. 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.

4. Buck SH, Burks TF. The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Rev **38:** 179-226, 1986.

5. Franco-Cereceda A, Henke H, Lundberg JM, Petermann JB, Hokfelt T, Fisher JA. Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. Peptides **8:** 399-410, 1987.

6. Henry CK, Emery B. Effect of spiced food on metabolic rate. Hum Nutr Clin Nutr **40C:** 165-168, 1986.

7. Kawada T, Watanabe T, Takaishi T, Tanaka T, Iwai K. Capsaicin-induced beta-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, respiratory quotient, and substrate utilization. Proc Soc Exp Biol Med **183:** 250-256, 1986.

8. Watanabe T, Kawada T, Iwai K. Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. Agric Biol Chem **51:** 75-79, 1987.

9. Watanabe T, Kawada T, Yamamoto M, Iwai K. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. Biochem Biophys Res Commun **142:**259-264, 1987.

10. Kawada T, Hagihara K-I, Iwai K. Effect of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr **116:** 1272-1278, 1986.

11. Watanabe T, Kawada T, Kurosawa M, Sato A, Iwai K. Excitation of adrenal sympathetic efferent nerve and catecholamine secretion caused by capsaicin in rats. Amer J Physiol in press.

12. Limlomwongse L, Chaitauchawong C, Tongyai S. Effect of capsaicin on gastric acid secretion and mucosal blood flow in the rat. J Nutr **109:** 773-777, 1979.

13. Watanabe T, Kawada T, Iwai K. Effect of capsaicin pretreatment on capsaicin-induced catecholamine secretion from the adrenal medulla in rats. Proc Soc Exp Biol Med **187:** 370-374, 1988.

14. 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.

15. Snedecor GW, Cochram WG. Statistical Methods. Iowa, The Iowa State Univ Press, 7th ed., 1980.

16. Duncan DB. Multiple range and multiple F-test. Biometrics **11:** 1-42, 1955.

17. Landsberg L, Young JB. Autonomic regulation of thermogenesis. In: Girardier L, Stock MJ, eds. Mammalian Thermogenesis. London, Chapman and Hall, p99, 1983.

18. Woo R, Daniels-Kush R, Horton ES. Regulation of energy balance. In: Olson R, Beutler E, Broquist H, eds. Annu Rev Nutr. California, Annual Reviews Inc., Vol. 5, p411, 1985.

19. Sims EA. Energy balance in human beings: The problems of plenitude. In: Aurbuch GD, McCormick DB, eds. Vitamins and Hormones. New York, Academic Press, Vol. **43,** p1, 1986.

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