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CAPSAICIN, A PUNGENT PRINCIPLE OF HOT RED PEPPER, EVOKES CATECHOLAMINE SECRETION FROM THE ADRENAL MEDULLA OF ANESTHETIZED RATS#

Tatsuo Watanabe, Teruo Kawada, Miyuki Yamamoto, and Kazuo Iwai*

Laboratory of Nutritional Chemistry,
Department of Food Science and Technology, Faculty of Agriculture,
Kyoto University, Sakyo-ku, Kyoto 606, Japan

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SUMMARY: Using a direct monitoring system for catecholamine (CA) secretion into the adrenal vein, we have demonstrated that capsaicin (CAP) evokes CA secretion from the adrenal medulla of pentobarbital-anesthetized rats. A significant increase in epinephrine (E) secretion was seen in rats infused with CAP (200 μg/kg, i.v.) without a detectable lag after the infusion. Norepinephrine (NE) secretion evoked by CAP was fairly weak compared with E secretion. The secretion of E evoked by CAP was dose-amount dependent. The stimulation of E release by CAP was barely detectable at 20 μg/kg, half-maximal at 100 μg/kg, and maximal at 600 μg/kg. When CAP (200 μg/kg) was infused into rats, the weight-ratio of E to NE was significantly higher (47.6) than when acetylcholine (12.5 μg/kg) was infused (13.0). These results indicate that CAP can evoke CA secretion from the adrenal medulla of rats.

Capsaicin (CAP), a pungent principle of hot red pepper, has long been used as an ingredient of spices and drugs all over the world (1). Recent interest in CAP has been focused on its action as a relatively selective substance for small-diameter sensory neurons (2-4), many of which contain neuroactive peptides such as substance P, gastrin/cholecystokinin, somatostatin and calcitonin gene-related peptide (5-8). We have demonstrated that CAP is readily transported via the gastrointestinal tract and then absorbed through nonactive transport into the portal vein (9). Most of the

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* To whom all correspondence should be addressed.

Abbreviations: CA, catecholamine; CAP, capsaicin; E, epinephrine; NE, norepinephrine; ACh, acetylcholine; i.v., intravenous; i.p., intraperitoneal.

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absorbed CAP is excreted as metabolites via the urine within 48 hr in rats (10). When CAP-containing food is eaten by humans, the pungent material is empirically supposed to induce a warming action although no biochemical or physiological studies on the energy metabolism involved have been reported except for ours. In our very recent studies, it was postulated that CAP enhances the energy metabolism of rats through the beta-adrenergic action of CAP in vivo (11-13). In this paper we will show directly that CAP evokes catecholamine secretion from the adrenal medulla of anesthetized rats.

MATERIALS AND METHODS

Materials. CAP was purchased from Sigma Chem. Co. (Saint Louis, MO; grade I, Lot No. 72F-06841). Sodium pentobarbital (Somnopentyl^{R}) was obtained from PITMAN-MOORE, INC. (Washington Crossing, N.J.). Acetylcholine chloride (ACh) and neostigmine bromide were purchased from Nakarai Chem. Co. (Kyoto, Japan) and Wako Pure Chem. Industry Co. (Osaka, Japan), respectively. All other chemicals were of reagent grade.

Animal experiments. The experiments were performed on sodium pentobarbital (60 mg/kg, i.p.) anesthetized male rats (Wistar, 210-230 g). Their rectal temperature was maintained between 36.5°C and 37.5°C through the use of a DC current heating pad (14). Each rat was infused with 1 ml of a heparinized (500 IU/ml) 0.9% saline solution into the right femoral vein by means of an infusion pump (TERUFUSION SYRINGE PUMP; TERUMO Co., Tokyo, Japan; model STC-521). Following midline laparotomy, the needle of a winged infusion set (G x 5/8" T.W.; TERUMO Co.) was inserted into the left adrenal vein via the left renal vein by a modification of the method of Yoshizaki (15). The rats were infused with a CAP solution (6-600 μg/kg) in 0.9% saline with 2% ethanol, 10% Tween 80, or acetylcholine chloride (12.5 μg/kg) in 0.9% saline into the right femoral vein by means of the infusion pump for 1 min. In the latter case, a neostigmine bromide solution (200 μg/kg) in 0.9% saline was pre-infused into the right femoral vein 5 min before the ACh infusion. Adrenal venous blood samples were collected for 15 min in glass culture tubes placed on ice. The adrenal venous blood flow rate in control and CAP-administered rats ranged between 131-250 μl/min and 131-265 μl/min, respectively. As blood was taken from the adrenal vein, an approximately equal volume of a heparinized saline solution was administered at a rate of 200 μl/min into the right femoral vein by means of the infusion pump. After centrifugation of the blood samples for 2 min at 10,800 x g, plasma samples were collected and 10% sodium pyrosulfite ($Na_2S_2O_5$) was added (50 μl/ml plasma). The samples were then frozen and stored at -20°C until assayed.

Measurement of CA in the adrenal medulla and venous plasma. CA in the adrenal medulla and venous plasma was determined by the HPLC-EC method described elsewhere (16).

Measurement of CAP in the adrenal venous plasma. CAP in the adrenal venous plasma was determined by the HPLC-EC method described previously (17).

Statistical analysis. The data are presented as means ± SEM and were statistically analyzed by means of analysis of variance (18) and Duncan's Multiple-Range test (19).

RESULTS AND DISCUSSION

Time-course response. Figure 1 illustrates the time course of E secretion from the adrenal medulla. A significant increase in E secretion was seen in rats infused not only with ACh but also with CAP. Secretion of E begins, however, without a detectable lag after an infusion of CAP, in contrast to in the case of ACh. Neostigmine was used as an ACh esterase inhibitor. When ACh (12.5 μg/kg) and CAP (200 μg/kg) were infused for 1 min, the maximal E secretion occurred between 6-9 min and 3-6 min, respectively, and then decreased in both cases. The total amount of E secretion over 15 min with infusions of ACh and CAP was 494.9 ± 159.5 (n=5) and 495.0 ± 102.8 ng/kg (n=10), respectively. The biological half-life of CAP in the adrenal venous plasma was 4.08 ± 0.25 min (n=5). Therefore, the rapid decrease in CA secretion evoked by CAP may be associated with the short biological half-life of CAP. On the other hand, NE was also secreted from the adrenal medulla when ACh or CAP was infused into anesthetized rats. However, NE secretion over 15 min with infusions of ACh and CAP was

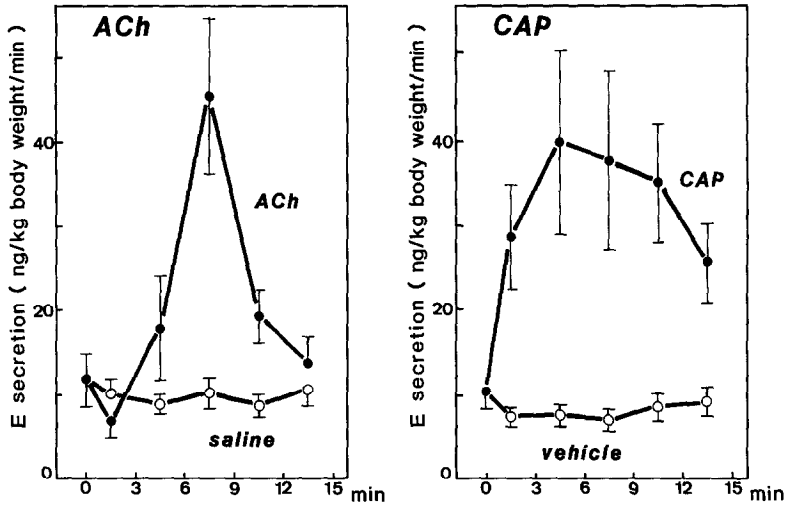

Figure 1. Time-course of epinephrine secretion from the rat adrenal medulla evoked by acetylcholine (left) and capsaicin (right). The rats were infused with acetylcholine chloride (12.5 μg/kg) with pre-treatment with neostigmine bromide (200 μg/kg) or capsaicin (200 μg/kg) for 1 min. The saline group was infused with a 0.9% saline solution 5 min after infusion of neostigmine bromide (200 μg/kg). The vehicle group was infused with a 0.9% saline solution with 2% ethanol and 10% Tween 80. Adrenal venous blood was collected at 3 min intervals to 15 min. The values are means ± SEM for 6-13 rats.

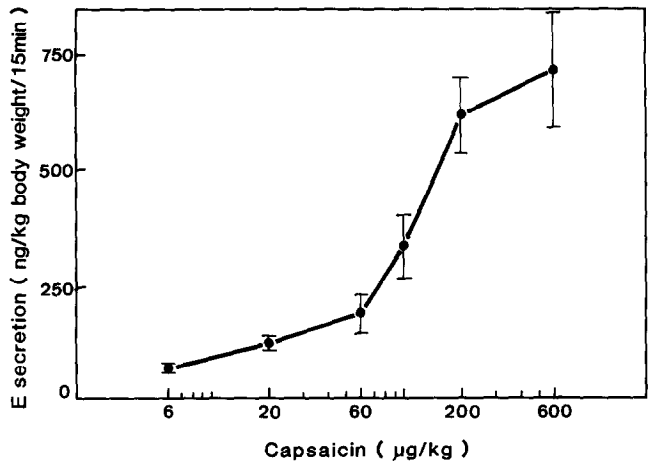

Figure 2. Dose-response curve for the effect of capsaicin on epinephrine secretion from the rat adrenal medulla. The rats were infused with capsaicin (6-600 μg/kg) for 1 min. Adrenal venous blood was collected for 15 min after the capsaicin infusion. The values are means ± SEM for 5-17 rats.

40.2 ± 10.5 (n=5) and 10.3 ± 1.9 ng/kg (n=10), respectively, these values being fairly low compared with those for E secretion in both cases.

Dependence of E secretion on the CAP dose-amount. It is well known that the stimulation of CA secretion from the adrenal medulla and isolated adrenal cells by ACh is dose amount-dependent (20-23). The secretion of E induced by CAP was also dose amount-dependent (Fig.2). The stimulation of E release by CAP was barely detectable at 20 μg/kg, half-maximal at 100 μg/kg, and maximal at 600 μg/kg. On the basis of the intake-amount of CAP from the diet (24) and the gastrointestinal absorption rate of CAP (9), CAP may be infused at 30-50 μg/kg/min into the mesenteric venous blood from the diet of rural Thai people. Therefore, it is considered that the secretion of CA from the adrenal medulla evoked by dietary CAP is a daily phenomenon.

The ratio of E to NE secreted due to ACh and CAP into the adrenal venous plasma. As shown in Fig. 3, although the amounts of E and NE in the adrenal venous plasma varied after stimulation of the adrenal gland with ACh, their ratio remained in the neighborhood of 13.0 as in the case of a saline infusion. However, in the case of CAP the ratio was significantly higher (47.6) than with other treatments ($P < 0.01$). This is in contrast to that in the case of perfusate of adrenal gland tissue stimulated with

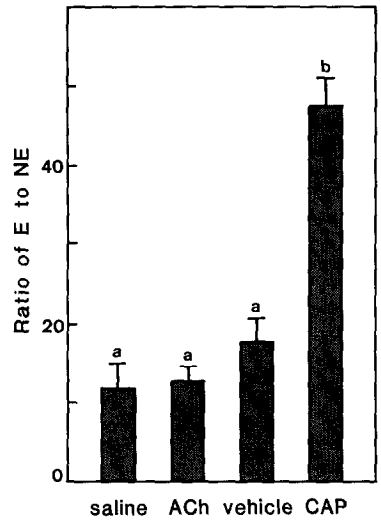

Figure 3. Weight-ratio of epinephrine to norepinephrine secreted from the rat adrenal medulla due to acetylcholine and capsaicin. The experimental conditions were as in Fig. 1. The weight-ratio of epinephrine to norepinephrine was determined on the basis of the cumulative secretion for 15 min after the infusion of both secretagogues. The values are means ± SEM for 6-13 rats. Means not sharing a common superscript letter are significantly different at $P < 0.01$.

nicotine, ACh and excess K, the ratio of E to NE being slightly higher than that in the case of the adrenal medulla of anesthetized rat (2.73) (22). In this study, the ratio of E to NE in the adrenal medulla was determined to be 4.12 ± 0.20 (n=5).

These results suggest that CAP can evoke CA secretion from the adrenal medulla of rats. When CAP-containing food is eaten and the CAP concentration reaches the physiological level, it will cause an increase in CA secretion. The present findings are strong biochemical and physiological evidence for the beta-adrenergic action of CAP on the energy metabolism previously suggested in our laboratory (13), and thus the possible warming action of CAP-containing food. Furthermore, pretreatment of rats with atropine sulfate (muscarinic receptor-blockade; 10 mg/kg, i.v.) and hexamethonium bromide (nicotinic receptor-blockade; 2 mg/kg, i.v.), which produced 80% inhibition of CA secretion from the adrenal medulla in response to the dose of 2-deoxy-D-glucose (300 mg/kg, i.v.), blocked hardly CA secretion from the adrenal medulla evoked by CAP (200 μg/kg, i.v.). Therefore, the different time courses and ratios of E to NE secreted in

response to the dose of CAP and ACh might be due to the difference of receptors and/or other factors. Detailed study on the mechanism of stimulation by CAP of CA secretion from the adrenal medulla is now in progress.

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