Induction of Capsaicin-Hydrolyzing Enzyme Activity in Rat Liver by Continuous Oral Administration of Capsaicin

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Effects of continuous oral administration of capsaicin (CAP) on the CAP-hydrolyzing enzyme activity in liver were investigated in rats. Rats were given a standard laboratory diet supplemented with 14 mg of CAP/100 g of diet, ad libitum, for 3 days and 1 and 3 weeks. It was found that CAP administration for 1 and 3 weeks significantly increased the CAP-hydrolyzing enzyme activity compared with that of each control group. Reaction products were chromatographically identified as vanillyl alcohol, vanillin, and vanillic acid. The CAP-hydrolyzing enzyme activity found in rat liver was also detected in chicken, hog, and bovine livers. It was suggested that the initial step of CAP metabolism was the hydrolysis reaction of the acid–amide bond in the CAP molecule and that this hydrolysis would be the rate-limiting step in CAP metabolism. These results suggest that the CAP-hydrolyzing enzyme activity reached maximum induction by continuous oral administration of CAP for 1 week.

INTRODUCTION

Capsaicin (CAP), a pungent principal of hot red pepper, has long been used as spices, food additives, and drugs (Iwai et al., 1979; Suzuki and Iwai, 1984). The consumption of CAP in Japan has progressively increased in recent years. The pharmacological effects and toxicity of CAP were reported by many investigators (Bucks and Burks, 1986; Gamse et al., 1986; Millar et al., 1983). It has been suggested that dietary supplementation of CAP in high-fat diets lowered the perirenal adipose tissue and serum triglyceride concentration in rats due to enhancement of energy metabolism (Kawada et al., 1986a,b). Watanabe et al. have recently demonstrated that the enhancement of energy metabolism by CAP occurs via catecholamine secretion from the adrenal medulla (Watanabe et al., 1987) and that catecholamine secretion is mainly through activation of the central nervous system (Watanabe et al., 1988). Kawada and Iwai (1985) reported in vivo and in vitro metabolism of CAP and its analogues in rats and that the CAP-hydrolyzing enzyme was distributed in various organs of rats, with the highest activity located in the liver (Kawada and Iwai, 1985). In this paper, we report the effect of continuous oral administration of CAP on the induction of CAP-hydrolyzing enzyme activity in rat liver. We have also investigated the occurrence of CAP-hydrolyzing enzyme activity in other animal livers and in some commercially available enzyme preparations.

MATERIALS AND METHODS

Materials. Capsaicin (CAP) was obtained from Sigma Chemical Co. (purity 93%, Lot 128F-7100, St. Louis, MO). Vanillin, vanillyl alcohol, and vanillic acid were purchased from Wako Pure Chemical Industry Co. (Osaka, Japan).

Animals and Diets. Male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka, Japan), weighing 70–80 g, were given a standard laboratory diet (powdery CE-2; Japan Clea Inc., Tokyo) for 3 days before the experiments were started and then were divided into six groups as follows: CAP-fed groups and corresponding control groups (without CAP) for 3 days and 1 and 3 weeks. Rats were fed individually. A standard laboratory diet (powdery CE-2) was used as control diet, and CAP diet was supplemented at 14 mg of CAP/100 g of the standard laboratory diet. Diets were provided ad libitum. It had been reported that the amount of CAP supplemented to this experimental diet given to rats by pair feeding was approximately the same as that consumed by the rural Thai people (Nopanitaya, 1974; Kawada et al., 1986a). Nutritional components of the standard laboratory diet are as follows: moisture, 8.7%; protein, 24.8%; fat, 4.4%; fiber, 3.5%; ash, 7.0%; soluble non-nitrogen, 51.6%; and energy, 345.2 kcal/100 g. After the experimental periods of 3 days and 1 and 3 weeks, the liver was extirpated under pentobarbital anesthesia.

CAP-Hydrolyzing Enzyme Activity in Liver. The excised liver was homogenized with 1.5 volumes of 0.1 M potassium phosphate buffer (pH 7.2) and centrifuged at 10000*g* for 30 min. Then the supernatant was used for the assay of CAP-hydrolyzing enzyme activity. The reaction mixture consisted of 0.5 mL of 2 mM CAP in 0.1 M potassium phosphate buffer (pH 7.2) as substrate and 0.5 mL of the supernatant as an enzyme solution. It was incubated at 37 °C for 1 h, and the reaction was terminated by addition of 0.1 mL of 6 N HCl and then centrifuged at 10000*g* for 10 min to remove denatured protein. As a blank, the reaction mixture with 0.1 mL of 6 N HCl added before the incubation was used.

The remaining amount of CAP in the reaction mixture was determined by HPLC system (Irica Instruments, Kyoto, Japan), with Irica data processor CD-12. The mobile phase used for separating CAP was methanol–water (75:25 v/v). An ODS column, Irica RP-18T (Irica Instruments), 25 cm × 4 mm i.d., was used at room temperature (20–25 °C). The flow rate was kept at 0.5 mL/min. The CAP-hydrolyzing enzyme activity was estimated from the reduced amounts of CAP in the reaction mixture. A linear relationship was observed between the enzyme activity and protein concentration and also between the enzyme activity and the reaction time under this assay condition used. The protein concentration was determined according to the method of Lowry et al. (1951).

Test for CAP-Hydrolyzing Activity of the Commercially Available Enzyme Preparations. The CAP-hydrolyzing activity of some commercially available enzyme preparations having acid–amide hydrolase activity was investigated. The enzyme preparations tested were pepsin, peptidase, trypsin, protease, and aminoacylase. These enzyme preparations were purchased from Sigma: pepsin (No. P-6887, 3900 units/mg of protein), from

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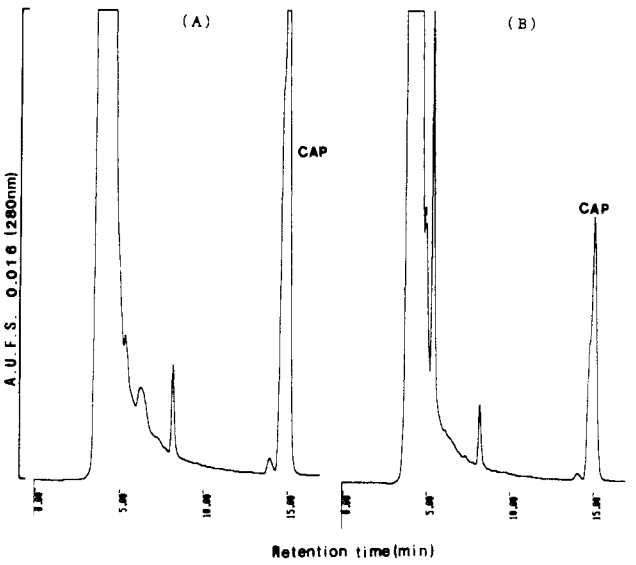

Figure 1. Chromatogram of the remaining amount of CAP analyzed by HPLC system after incubation under the assay condition: (A) blank; (B) reaction mixture. Analysis was carried out by HPLC system as follows: mobile phase, methanol–water (75:25 v/v); column, RP-18T (Irica Instruments), 25 cm × 4 mm i.d., at room temperature (20–25 °C); flow rate, 0.5 mL/min.

Table I. Effects of CAP-Hydrolyzing Enzyme Activity in Rat Liver after Continuous Oral Administration of Capsaicin

	enzyme act., munit		sp act., munit/mg of protein	
	control diet[b]	CAP diet[c]	control diet	CAP diet
3 days	230 ± 16	317 ± 78	2.34 ± 0.79	2.77 ± 0.57
1 week	204 ± 22	466 ± 12**	1.63 ± 0.19	4.23 ± 0.41**
3 weeks	224 ± 43	449 ± 92**	1.98 ± 0.79	3.96 ± 1.46*

[a] Unit definition: one unit will hydrolyze 1 μmol of CAP per gram of liver per hour under the assay condition. [b] Rats were fed ad libitum on a control diet (CE-2) for 3 days and 1 and 3 weeks. [c] Rats were fed ad libitum on a CAP diet (14 mg CAP/100 g of CE-2) for 3 days and 1 and 3 weeks. Values are means ± SE for five or six rats. Asterisks indicate significant difference between control and CAP groups: one asterisk, $p < 0.05$; two asterisks, $p < 0.01$. Other experimental details are under Materials and Methods.

porcine stomach mucosa; peptidase (No. P-7500, 0.115 unit/mg of solid), from porcine intestinal mucosa; trypsin (No. T-0134, 18 300 BAEE units/mg of protein), from porcine pancreas; proteases, from bacteria (No. P-0390, 12.9 units/mg of solid), from fungi (No. P-4789, 7.5 units/mg of solid), and from bovine pancreas (No. P-4630, 8 units/mg of solid); and amino acylase (No. A-8376, 900 units/mg of protein), from porcine kidney. The CAP-hydrolyzing activity was assayed according to the same method described above, using each 0.1% enzyme solution.

Statistical Analysis. The significance of differences between means with the same variance and with different variances by the *F*-test was evaluated by Student's *t*-test and Aspin Welch's test, respectively.

RESULTS

Figure 1 shows a chromatogram of a remaining amount of CAP in the reaction mixture and that in the blank assay after the incubation. The remaining amount of CAP in the reaction mixture markedly decreased compared with that of the blank. The diet intakes, body weight gain, and liver weight were unaffected by the continuous oral administration of CAP in each rat of all experimental periods. The CAP-hydrolyzing enzyme activity in rat liver after continuous oral administration of CAP for each experimental period is shown in Table I. One and 3 weeks of CAP administration resulted in a significant increase of the enzyme activity. The extents of increment were

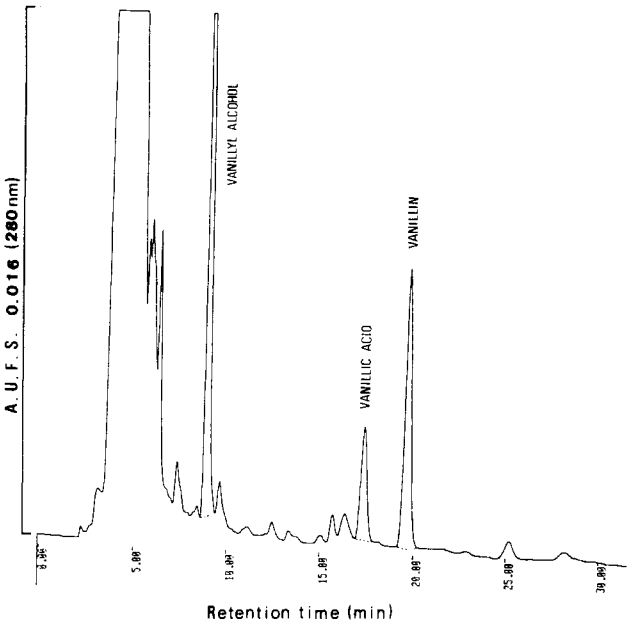

Figure 2. Chromatogram of the reaction products from CAP by crude CAP-hydrolyzing enzyme prepared from rat liver: (solid line) reaction mixture; (dotted line) blank. Analysis was carried out by HPLC system as follows: mobile phase, methanol–water–acetic acid (25:74:1, v/v) containing 0.01% sodium octanesulfonic acid; column, RP-18T (Irica Instruments), 25 cm × 4 mm i.d., at room temperature (20–25 °C); flow rate, 0.5 mL/min.

Table II. Reaction Products of Capsaicin with Crude CAP-Hydrolyzing Enzyme Prepared from Rat Liver

	nmol/g of liver	
	control diet[a]	CAP diet[b]
vanillyl alcohol	193.9 ± 23.1	161.4 ± 28.8
vanillin	16.8 ± 5.7	24.6 ± 4.9
vanillic acid	6.4 ± 1.7	8.1 ± 3.2
CAP hydrolyzed	223.8 ± 43.3	449.5 ± 92.5*

[a] Rats were fed ad libitum on a control diet (CE-2) for 3 weeks. [b] Rats were fed ad libitum on a CAP diet (14 mg of CAP/100 g of CE-2) for 3 weeks. Values are means ± SE for five or six rats. An asterisk indicates significant difference between control and CAP groups at $p < 0.05$. For other experimental details, see Figure 2.

Table III. CAP-Hydrolyzing Enzyme Activity in Liver of Some Animals

	enzyme act.,[a] munit	sp act., munit/mg of protein
chicken	546	5.97
hog	414	2.41
cow	410	2.57
rat[b]	204	1.63

[a] Unit definition: one unit will hydrolyze 1 μmol of CAP per gram of liver per hour under the assay condition. [b] Male Sprague-Dawley rat fed commercial diet (CE-2) ad libitum.

similar in both cases. CAP administration for 3 weeks showed enzyme activity similar to that after 1 week of administration, but there was no significant difference in the CAP administration for 3 days. The comparable increase in the specific activity of the enzyme in control and experimental rats (Table I) supported the idea that the observed induction was due to CAP specifically. These results suggest that CAP-hydrolyzing enzyme activity in rat liver is fully induced by the continuous oral administration of CAP for 1 week.

The chromatogram of reaction products of CAP with crude CAP-hydrolyzing enzyme from rat liver at 37 °C for 1 h is shown in Figure 2. Three peaks were identified as vanillyl alcohol, vanillic acid, and vanillin, respectively, by using reference compounds. The major reaction

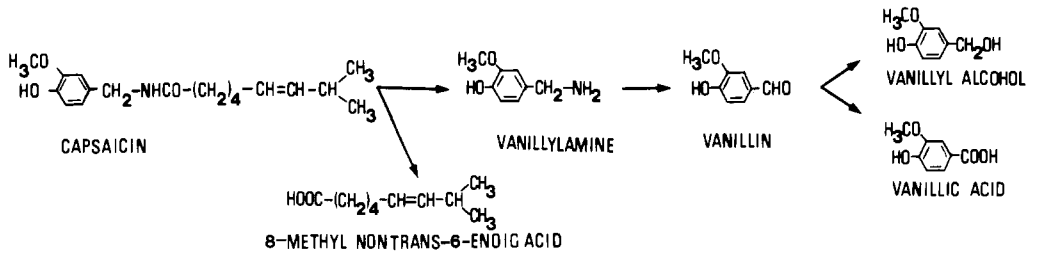

Figure 3. Metabolic pathway of capsaicin in rats.

product was vanillyl alcohol. Vanillin and vanillic acid were not so much found. Table II shows the reaction products of CAP by crude CAP-hydrolyzing enzyme prepared from rat liver after continuous oral administration of CAP for 3 weeks. There was a significant difference in the amounts of hydrolyzed CAP between control and CAP groups, although there were no significant differences in the amounts of vanillyl alcohol, vanillin, and vanillic acid.

CAP-hydrolyzing enzyme activity in the liver of some animals is shown in Table III. It was indicated that CAP-hydrolyzing enzyme activity found in rat liver was also detected in chicken, hog, and bovine livers. The highest enzyme activity was found in the chicken.

CAP-hydrolyzing enzyme activity was not found in any commercially available enzyme preparations tested, with a hydrolytic activity toward various acid–amid bonds, i.e., trypsin, proteases (bacterial and fungal proteases), peptidase, and aminoacylase.

DISCUSSION

The effects of CAP with various protein-level diets on the morphology of duodenal mucosa had been studied with young rats (Nopanitaya, 1974). CAP intensifies the damage to duodenal absorptive cells associated with a low-protein diet (Nopanitaya, 1974). Since, in the present study, rats were fed a sufficient protein level (24.8%), no alteration was observed in gastrointestinal tracts by the CAP administration during the course of the experiments. The results shown in Table I suggest that the CAP-hydrolyzing enzyme in the liver reached maximum induction by the continuous oral administration of CAP for 1 week and that the enzyme activity would be the maximum induction level. As shown in Figure 3, previous papers demonstrated the metabolic pathway of capsaicin and its analogues in rats in vivo (Kawada and Iwai, 1985); CAP was initially hydrolyzed to vanillylamine and 8-methylnon-*trans*-6-enoic acid, and the former compound was further transformed by oxidative deamination to vanillin, followed by oxidation to vanillic acid or reduction to vanillyl alcohol which were mainly excreted into urine as glucuronide conjugates (Kawada and Iwai, 1985). These metabolites were similarly detected as the reaction products with a ratio in the enzyme reaction mixture in vitro similar to that shown in Table II and Figure 2. Therefore, it was considered that the CAP metabolism in the reaction mixture might reveal the metabolic pathway observed in vivo in rats (Kawada and Iwai, 1985; refer to Figure 3). As seen in Table II, a significant difference was observed only in the amount of CAP hydrolyzed which corresponded to the amount of vanillylamine formed by the enzyme reaction, while vanillylamine could not be separated under the assay condition (refer to Figure 2). In the preliminary experiment (data not shown), when vanillylamine was used as a substrate for the enzyme reaction, there were no differences in the amounts of vanillyl alcohol and vanillic acid as the reaction products between CAP group and control group. These results suggest that the initial step of CAP metabolism in rats is the enzymic hydrolysis reaction of the acid–amide bond in the CAP molecule and that this hydrolysis would be the rate-limiting step in the CAP metabolism.

It has been reported that CAP was readily absorbed from the gastrointestinal tract by a nonactive process into the portal vein and that it was hydrolyzed partly during absorption (Kawada et al., 1984). The CAP-hydrolyzing enzyme had been shown to distribute in various organs of rats (Kawada and Iwai, 1985), and this enzyme was fully induced by continuous oral administration of 14 mg of CAP/100 g of diet for 1 week. These results suggest that the CAP absorbed would be rapidly hydrolyzed by the CAP-hydrolyzing enzyme in the liver and other tissues, and this enzyme activity would be concerned with the safety of daily intake of CAP as a spice constituent.

The CAP-hydrolyzing enzyme activity was also found in chicken, hog, and bovine livers (Table III). However, this enzyme activity could not be replaced by any commercially available enzyme preparations tested, which have a hydrolytic activity toward various acid–amide bonds. These results support the idea that the CAP-hydrolyzing enzyme may be a new type of enzyme with hydrolase activity. However, further investigations are needed.

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