J. Food Sci. Nutr.
Vol. 1, No. 1, p. 69~74(1996)

Effect of Capsaicin on L-Ascorbic Acid Level in Various Tissues and Its Urinary Excretion in Rats

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Abstract

Capsaicin(CAP) is a pungent ingredient of hot pepper that has been used as a spicy food additive, preservative, and medicine. In this study, the effect of CAP on L-ascorbic acid(AsA) level in various tissues as well as its urinary excretion, and drug-metabolizing enzyme activity in rats were investigated. Rats fed AsA-deficient diets for 17 days were injected intraperitoneally with 1mg of CAP in 0.5ml of ethanol-Tween 80-saline(20 : 10 : 70, v/v/v). Control rats received the equal volume of the same solution without CAP. Urine was collected for 3 days after the CAP injection, and after 5 days tissues were removed ; their AsA contents were measured by high performance liquid chromatography combined with an electrochemical detector. In addition, hepatic microsomal ethoxyresorfin O-deethylase(EROD) activity was measured. Urinary AsA excretion changed significantly following CAP injection. One and two days after CAP injection, the urinary AsA increased 2- and 3-fold in the CAP injected group, compared to the control. AsA contents of liver and kidneys were higher in the CAP injected group than those of control, but the contents of adrenal glands and brain were lower than those of the control. Dehydroascorbic acid contents in adrenal glands of the CAP injected group were higher than those of control. In addition, hepatic microsomal EROD activities in the CAP injected group were higher than that of the control. These results suggested that a single large dose of CAP could temporarily cause the redistribution of AsA in tissues accompanying by its urinary excretion, by affecting probably a biological system including mixed function oxygenase system(MFOS).

Key words: capsaicin, L-ascorbic acid, tissue level, urinary excretion

INTRODUCTION

Some xenobiotics increase the requirements for micronutrients such as vitamin C or vitamin A, and they alter lipid and drug metabolism(1,2). For example, the intake of polychlorinated biphenyl(PCB) altered the urinary ascorbic acid(vitamin C, AsA) excretion and its tissue content(3,4). In addition, AsA deficiency decreased mixed function oxygenase system(MFOS) activity as well as P-450 content in microsomal drug-metabolism system(5-9), which is important in metabolizing xenobiotics.

Capsaicin(trans-8-methyl-N-vanillyl-6-nonenamide, CAP) is an active pungent ingredient in various species of *capsicum* fruits such as green or red hot peppers (10). It is extensively used as a spicy food additive in Korea, as well as in some other countries(11). Besides enhancing the taste and flavour of food, CAP exhibits a wide range of neurophysiological and neuropharmacological effects(12-17), as well as biochemical effects such as enhancing lipid metabolism(18). CAP is readily metabolized by CAP hydrolyzing enzyme (19), but MFOS is also involved in its metabolism(20). CAP may alter metabolism of micronutrients, particularly AsA, because it causes not only the chemical stimuli as xenobiotics stimulating drug metabolism, but also physiological stimuli, such as burning sensation, which would in turn alter neuropeptide secretion accompanied by neuronal stimulation.

Since Korean people consume the hot ingredient CAP in their daily food, investigating metabolic change of micronutrient is important. In order to get more basic information on whether CAP can alter AsA metabolism, it would be worthwhile to determine a short term effect of CAP before studying its long term effect. In this study, we investigated the effect of a large single dose of CAP on AsA metabolism by determining AsA level in various tissues as well as its urinary ex-

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cretion, and hepatic drug-metabolizing enzyme activity in rats.

MATERIALS AND METHODS

Reagents

CAP, AsA, dithioerythritol(DTE), $Ba(OH)_2$-$8H_2O$, Tween-80, tetra n-butylammonium bromide and methanol were purchased from Wako Chemical. Co. Ltd. (Japan). 7-Ethoxyresorufin, resorufin and NADPH were purchased from Sigma Chemical. Co.(USA). AsA-deficient diet was purchased from Oriental Yeast Co. Ltd.(Japan).

Experimental procedures

Sprague-Dawley male rats weighing 230~250g were provided with the AsA-deficient diet(Table 1). Water and diets were offered *ad libitum* through the experimental period. After 17 days of feeding the AsA-deficient diets, animals were divided into two groups, control and CAP supplemented, and then intraperitoneally injected with CAP solution or the vehicle control. CAP was dissolved in ethanol : Tween-80 : saline(20 : 10 : 70, v/v/v)(21), and 1mg of CAP in 0.5ml of the solution were injected intraperitoneally. Control rats received the equal volume of the vehicle without CAP. Every 24 hour urine for 5 days, following the CAP injection, was collected in 10ml of 20% HPO_3.

Sample preparation for AsA

Liver, spleen, adrenal glands, kidney and brain were removed, and stored at -70°C until AsA analysis. To determine the amounts of AsA, samples were extracted with 90% methanol/1mM EDTA.

Table 1. Composition of AsA-deficient diet

Composition	%
Corn	20
Sucrose	15
Soybean oil	15
Wheat bran	20
Casein	20
Alfalfa	20
Vitamin[1)]	1
Soybean meal	5
McCollum salt	4

[1)]Vitamin mixture per 100g diet : vitamin A 1,000IU, vitamin D 200IU, vitamin B_1 0.2mg, B_2 0.5mg, B_6 0.1mg, nicotinic acid 0.2mg, pantothenic acid 0.2mg, p-aminobenzoic acid 0.1mg, inositol 0.1mg, folic acid 0.2mg, phylloquinone 0.2mg

Determination of AsA and dehydroascorbic acid

The concentration of AsA and dehydroascorbic acid (DHA) were measured with high performance liquid chromatography(HPLC), combined with electrochemical detector at 750mV. An Inertsil-ODS column(40×150 mm, i.d., Gasukurokogyo Ltd., Japan) with a mobile phase of methanol/0.05M phosphate buffer containing 0.005M tetra-n-butylammonium bromide(20/70,v/v) was used. In order to determine DHA levels, the sample was reduced to AsA with DTE. Constitutive and produced AsA were determined as total AsA. DHA amounts were obtained by subtraction of constitutive AsA from total AsA. For that, DHA in the sample was reduced to AsA by adding an aliquot of aqueous 10mM DTE solution at 4°C(22). After 10min at 25°C, the samples was filtered with a Chromatodisk(0.45um, Kurabo Co.Ltd., Japan), and analyzed by HPLC.

Microsome preparation

The liver of each animal was perfused through the portal vein with ice-cold physiological saline. A sample of the liver was homogenized in 3 volumes of 0.05M Tris-HCl buffer, pH 7.4 with 1.15% KCl using a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 14,000×g for 15min ; the supernatant collected and centrifuged at 100,000×g for 1 hr(23). The microsomal pellet was resuspended in Tris-HCl, pH 7.4 with 1.15% KCl. All steps were carried out at 4°C. Protein concentration was determined by the Lowry method(24).

Ethoxyresorufin O-deethylase(EROD) activity

EROD activity was measured by a modification of the previously described method(25). The assay buffer used to determine EROD activity consisted of 0.1M potassium phosphate(pH 7.4), 2mg/ml bovine serum albumin, 10μM dicumarol, 5mM of glucose-6-phosphate, 5units/ml of glucose-6-phosphate dehydrogenase, and 2.5μM 7-ethoxyresorufin. After the addition of 5μM NADPH, the mixture was incubated at 37°C for 30min. Formation of resorufin by the microsomal fraction was

monitored fluorimetrically(Perkin Elmer LS-50 Fluorescence spectrophotometer) at an excitation wavelength of 550nm and emission wavelength of 585nm. The amount of formed resorufin was calculated by comparing the relative fluorescence to the fluorescence of known amount of resorufin. Results are expressed as ρ mole resorufin generated/min/mg/protein.

Statistical tests

The difference between the means of two groups was statistically analyzed by Student's t-test.

RESULTS

To determine whether CAP could affect organ weight, selected organs including brain, adrenal glands, spleen, kidney and liver were assessed. CAP had no effect on the various organ weights(Table 2).

Since AsA metabolism can be affected by various chemicals as well as physical stimuli(1,8,9), it was thought that CAP, a pungent ingredient, might modulate AsA metabolism by affecting biological system. As shown in Table 3, AsA contents in liver and kidney of CAP injected animals were significantly higher than those of control. AsA contents in adrenal glands and brain were lower in the CAP injected group compared to the control. Conversely, there was an increase of dehydroascorbic acid level, which is an oxidized form of AsA in adrenal glands in the CAP injected group compared to control(Table 4).

Fig. 1 shows change of urinary excretion of AsA for the experimental period. All animals fed the AsA-deficient diet maintained a consistant low level of AsA at days 14~17 after the initiation of AsA-deficient feeding. This indicates that the excretion was at an equilibrium as previously reported(26). However, the urinary excretion significantly increased in the CAP injected group. One and two days after CAP injection, the AsA excretion increased 2- and 3-fold respectively compared to the control. However, four days after CAP injection, the excretion of AsA returned to a level which was close to that of control.

Table 2. Effect of capsaicin on organ weights

Tissues(g/100g B.W.)	Control	CAP injected group
Brain	1.43±0.10	1.46±0.16[1)]
Adrenals	0.05±0.11	0.08±0.05
Spleen	0.57±0.11	0.51±0.13
Kidney	2.93±0.36	2.84±0.45
Liver	13.02±0.38	10.82±2.19

[1)]Values are means±SE(n=7)

Table 3. Effect of capsaicin on ascorbic acid level in various tissues

Tissues	Control	CAP injected group
	(mg/100g wet tissue)	
Liver	16.32± 4.04[1)a]	41.84± 1.13[a]
Kidney	5.26± 0.78[b]	7.05± 0.96[b]
Adrenals	461.60±46.61[c]	218.50±82.17[c]
Brain	46.09± 5.25[d]	36.53± 4.18[d]
Spleen	26.14± 4.82	22.49± 6.84

[1)]Values are means±SE(n=7)
Means with common superscript are significantly different (p<0.01)

Table 4. Effect of CAP on dehydroascorbic acid level in adrenal glands and brain

Tissues	Control	CAP injected group
	(mg/100g wet tissue)	
Adrenal glands	27.00±3.26[1)a]	47.65±2.35[a]
Brain	10.30±6.66	9.44±3.16

[1)]Values are means±SE
Means with common superscript are significantly different (p<0.01)

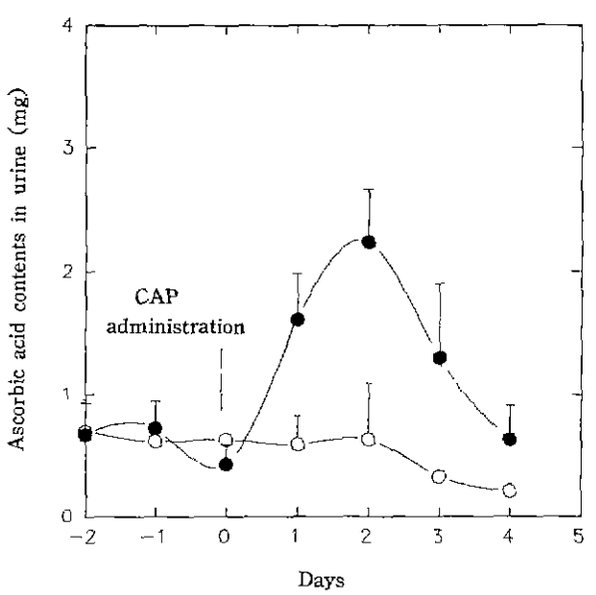

Fig. 1. Effect of capsaicin on urinary excretion of ascorbic acid.
Animals were injected intraperitoneally with 1mg CAP or the vehicle, and urine was collected every 24 hours. Concentrations of AsA and DHA in urine were determined by HPLC.
○: Control group, ●: CAP-supplemented group.

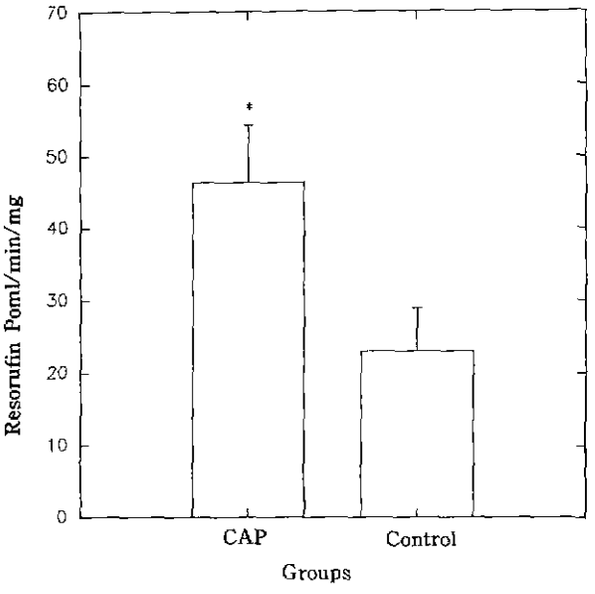

Fig. 2. Effect of capsaicin on hepatic microsomal ethoxyresorufin O-deethylase activity.
*Microsomal EROD activity was measured fluorimetrically and expressed as pmole resorufin generated /min/mg/protein. Means±SE ; *significantly different ($p<0.01$).*

Since phase I drug-metabolizing enzymes in MFOS are involved in CAP metabolism *in vivo*(19,20,23,27), CAP might be associated with AsA metabolism by acting xenobiotics stimulating the drug-metabolizing enzyme system. We studied the effect of CAP on EROD activity in liver, which is one of the cytochrome p-450 dependent enzymes in phase I, and found that the EROD activity increased in the CAP injected group compared to the control(Fig. 2).

DISCUSSION

The administration of xenobiotics generally induces the MFOS accompanied by an enlargement of various organs, especially liver(6-9). In this study, there were no changes of organ weights after CAP administration. Since CAP is readily metabolized by the CAP hydrolyzing enzyme as well as MFOS(19), as described below, its single injection might not be sufficient to cause a change of organ weight. It may also be possible that CAP acts differentially from other xenobiotics which increase organ weight even with a single injection.

AsA metabolism can be affected by various chemical stimuli. For example, xenobiotics such as PCB which activate hepatic drug-metabolizing enzyme also enhance AsA synthesis in the rat(1,8,9). Alternatively, in guinea pig, which is not capable of AsA synthesis, PCB cause a reduction of AsA in tissues(8,9). In our study, CAP injection resulted in an increased urinary AsA excretion one and two days after CAP injection by 2- and 3-fold higher levels compared to the control. The level of urinary AsA excretion returned to its initial level within 4 days. Besides the chemical stimuli by xenobiotics, physical stimuli, like immobilized stress alter temporarily urinary AsA excretion(28,29). The change in urinary AsA excretion with the various stresses was accompanied with physiological adaptation against the stress, resulting in a recovery to the initial excretion level(28-30). This may partially explain our observation that urinary excretion of AsA returned to its initial level four days after the CAP injection. This observation suggests that the adaptation could occur with the chemical stimuli. In addition, since the half-life of CAP *in vivo* has also been shown to be very short(19), CAP would be metabolized after 3~4 days, and thus might not affect change in urinary AsA excretion.

AsA contents in liver and kidney of the CAP injected group were higher, but its concentration in adrenal glands and brain were lower than those of the control (Table 3). Since AsA biosynthesis in most species of animals synthesizing AsA occurs mainly liver(31), the increased AsA content in liver might result from AsA synthesis. Alternatively, AsA in adrenal glands and brain decreased in the CAP injected group compared to control, but DHA content increased in the adrenal glands. The results suggested that CAP injection caused AsA oxidation in the tissues. The AsA oxidation, which would in turn lead to its consumption, could result in alteration of AsA metabolism. This may be associated with the decreased level of AsA in the CAP injected group compared to the control. Since some tissues level of AsA decreased but others did not, therefore, the change of AsA contents would result from AsA redistribution among the tissues rather than from AsA synthesis. This redistribution in tissues may occur through recirculation by the blood, which could be accompanied by the change of urinary AsA excretion. In addition, CAP can enhance adrenal medullary catecholamine secretion, which is associated with a stress response through the central nervous system(21,32). AsA is required *in vitro* to maximize the hydroxylation of dopamine to form norephinepirine in catecholamine biosynthesis(33), and the dopamine hydroxylation is

accompanied with AsA oxidation(33). Therefore, we are tempted to speculate that the enhanced catecholamine secretion with the stressful condition following CAP injection could result in AsA oxidation, as well as its consumption in some organs such as adrenal glands.

Most foreign chemicals, known xenobiotics, are biotransformed and detoxified by the drug metabolizing system(7). The initial step of CAP metabolism is the hydrolysis reaction of the acid-amide bond in the CAP molecule(19). However, it has been also shown that CAP and its analogues are hydroxylated to N-(4,5-dihydroxy-3-methoxybenzyl)-acrylamide by a microsomal mixed function oxidase *in vitro*(20). We have shown that CAP injection resulted in an increased microsomal EROD activity(Fig. 2). CAP has been shown to interact with hepatic drug metabolizing system *in vitro* by inhibiting ethylmorphine demethylation, such as SKF-525A(23). Contrary to its inhibition effect on drug metabolizing system *in vitro*, the effect of CAP *in vivo* is controversial. Some investigators reported that CAP inhibited aminopyrene and hexobarbital metabolism at a high dose(100mg/kg,i.p.)(34), while others reported that dietary CAP stimulated liver microsomal cytochrome p-450 content and enzyme activities of aryl hydroxylase in phase I drug-metabolizing enzymes(27). Recently, it was shown that CAP administration with ethanol synergistically increased p-450 content and enzyme activities of phase I drug metabolizing enzymes(35). Our result in this study supports that CAP metabolism *in vivo* was involved in increased phase I drug metabolizing enzyme activity such as EROD, and presumably this might also be associated with alteration of AsA metabolism.

Based on these results, we suggest that a single large dose of CAP can temporarily causes the redistribution of AsA in tissues accompanied by its urinary excretion in rats, probably by affecting other biological systems including MFOS. Hot ingredient CAP in daily food might alter micronutrients metabolism including AsA. The effect of dietary CAP on AsA metabolism is under study.

ACKNOWLEDGEMENTS

This work was supported by Kamibayashi Scholarship Foundation and Yamazaki Foundation for the Promotion of Spice Science. We are grateful to Hitomi Hashita and Mi-Ok Kim for their technical assistance.

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(Received April 15, 1996)