Metabolism of Theanine, γ-Glutamylethylamide, in Rats

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The metabolism of theanine, one of the major amino acid components in tea (*Camellia sinensis*), was studied in rats. High-performance liquid chromatography (HPLC) with fluorometric detection was used to evaluate the nature of theanine's metabolites in plasma, urine, and tissues. In the urine samples collected after administration of 100, 200, and 400 mg each of theanine, intact theanine, L-glutamic acid, and ethylamine, these compounds were detected in a dose-dependent manner. When 200 mg of theanine was orally administered to rats, the plasma concentrations of theanine and ethylamine reached their highest levels about 0.5 and 2 h after administration, respectively. It seems most likely that the enzymatic hydrolysis of theanine to glutamic acid and ethylamine was accomplished in the kidney. These results indicate that orally administered theanine is absorbed through the intestinal tract and hydrolyzed to glutamic acid and ethylamine in the rat kidney.

Keywords: *Theanine; metabolism; glutamic acid; ethylamine*

INTRODUCTION

Theanine, the chemical structure of which is γ-glutamylethylamide, is by far the major amino acid components in Japanese green tea leaves. It was first isolated and identified in the late 1940s by Sakato (1949), and several investigators later reported it as an intensely ninhydrin-reactive substance in other forms of tea (Cartwright et al., 1954; Roberts and Wood, 1951; Feldheim et al., 1986). The other natural source of theanine so far discovered is the mushroom *Xerocomus badius* (Casimir et al., 1960). Although most amino acids are found at lower levels, theanine accounts for >50% of total free amino acids in tea. The content of theanine in tea is between 1 and 2% (on average) of the total dry weight (Goto et al., 1994, 1996). In a recent study, Ekborg-Ott et al. (1997) found that D-theanine was present at significant level in tea samples.

Regarding the pharmacological effects of theanine, previous papers indicated a reduction in blood pressure in spontaneously hypertensive rats (Yokogoshi et al., 1995), a relief from convulsions induced by caffeine (Kimura and Murata, 1971a,b, 1980; Kimura et al., 1975), and an influence on the brain levels of norepinephrine, serotonin, 5-hydroxyindoleacetic acid, and dopamine (Kimura and Murata, 1986; Yokogoshi et al., 1998a,b). The structural similarity of theanine to glutamic acid, a neurotransmitter in the brain, also prompted researchers to investigate its possible action on the central nervous system as a glutamate antagonist (Shinozaki and Ishida, 1978; Maruyama and Takeda, 1994). Moreover, oral intake of theanine results in the generation of α-electric waves in the occipital and parietal regions of the human brain (Kobayashi et al., 1998).

Theanine was shown to be absorbed by a common Na^+-coupled cotransporter in the intestinal brush-border membrane (Kitaoka et al., 1996), but information on its metabolic fate is limited. A previous study demonstrated that ethylamine was detected in human urine collected after ingestion of a tea extract, presumably as the result of the hydrolysis of theanine (Asatoor, 1966). The question to be considered next is whether theanine truly acts as the source of urinary ethylamine, because green tea leaves contain ethylamine itself (Tsushida and Takeo, 1984). The aim of this study was, therefore, to measure the plasma and urinary levels of theanine and ethylamine by HPLC with fluorometric detection for the purpose of evaluating the metabolic fate of theanine after oral administration to rats.

MATERIALS AND METHODS

Chemicals and Reagents. Theanine used in the present study was a commercial product from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Ethylamine hydrochloride was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Standard amino acids, *o*-phthalaldehyde (OPA), *N*-acetyl-L-cysteine (AcCys), HPLC grade acetonitrile, and other reagents were purchased from Wako Pure Chemical Industry, Ltd. (Osaka, Japan).

Animals. Six-week-old male Wistar rats, weighing 110−140 g, were purchased from Japan SLC, Inc. (Hamamatsu, Japan), and were acclimatized for 2 days in a room with controlled temperature (23 ± 2 °C) and a 12-h cycle of light and dark (7:00 a.m.−7:00 p.m.). All of the rats were fed a commercial diet (type CE-2, CLEA Japan, Tokyo, Japan) ad libitum, with free access to tap water during the experimental period, and then fasted for 16 h before being orally administered theanine. Various amounts of theanine (0, 100, 200, and 400 mg) dissolved in 2 mL of water were orally administered to fasted rats ($n = 3$ in each dosage group).

Sample Collection. *Urine.* Urine was collected for 24 h after administration and kept at −20 °C until use.

Blood. Blood was drawn from the heart with a heparinized syringe under pentobarbital anesthesia (Nembutal, Dainabot Co., Osaka, Japan) 2 h after administration of theanine.

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10.1021/jf981113t CCC: $18.00
Published on Web 03/26/1999

Plasma samples were obtained by centrifugation (2000*g*, 10 min, 4 °C) immediately after blood collection and then stored at −20 °C.

To observe the effect of the time-dependent appearance of theanine and its metabolites after oral administration of 200 mg of theanine dissolved in distilled water, blood was drawn from the heart before (0 min) and at 15, 30, 60, 120 and 240 min after administration.

Metabolic Studies. Rat tissues (brain, heart, lung, liver, kidney, and spleen) were removed and flushed with saline. These tissues were then homogenized with 9 volumes of 0.1 M sodium phosphate buffer (pH 7.4). In the standard assay, 0.5 mL of tissue homogenate was incubated with 0.5 mL of 2 μmol/mL theanine dissolved in 0.1 M sodium phosphate buffer. The reaction was stopped by the addition of 4 mL of acetonitrile. After centrifugation at 2000*g*, 4 °C, for 10 min, the levels of theanine and ethylamine in the supernatant were measured by the HPLC method.

HPLC Analysis. Fifty microliters of thawed plasma and 200 μL of acetonitrile were mixed vigorously for deproteinization. After centrifugation at 2000*g*, 4 °C, for 10 min, 20 μL of the supernatant was diluted with 180 μL of water in a microtube. This sample was added to 400 μL of the fluorescence derivative reagent, which consisted of 40 mM OPA and 40 mM AcCys in 50 mM borate buffer (pH 10)/methanol (10:1, v/v). The resultant mixture was passed through a 0.45-μm membrane filter, and 20 μL of the filtrate was injected into the HPLC system. For treatment of the 24-h urine collected, thawed urine was deproteinized by adding 4 volumes of acetonitrile and prepared in the same manner as above.

Chromatographic System. A Shimadzu LC-6B HPLC system (Kyoto, Japan) was used. This system consisted of an SCL-6B system-controlled dual pump, an SIL-6B automatic injector, a CTO-6A column oven, an RF-530 fluorescence detector, and a Chromatopack C-R3A data processor. A Wakopak 3C18 column (Wako Pure Chemical Industries Ltd., 4.6 mm i.d. × 150 mm) with a guard column (4.6 mm i.d. × 10 mm), all of which operated at 40 °C, was used. Mobile phase A was 20 mM sodium phosphate buffer (pH 5.8) with 5% acetonitrile; mobile phase B was acetonitrile, and the proportions were controlled by a gradient programmer. The column was eluted with a linear gradient from 100% A and 0% B to 95% A and 5% B in 15 min. The elution was then maintained at 95% A and 5% B until 25 min. The gradient was changed linearly to 87% A and 13% B from 25 to 40 min and maintained at 87% A and 13% B until 55 min. The mobile phase was rechanged linearly to 70% A and 30% B from 55 to 65 min and maintained at 70% A and 30% B until 80 min. The gradient was then changed back to 100% A and 0% B for the analysis of the next sample. The mobile phase was delivered at a constant flow rate of 0.8 mL/min. The derivatives were monitored by fluorescence detection with excitation at 340 nm and emission at 445 nm.

RESULTS

Chromatograms. Figure 1A shows a typical chromatogram obtained with a standard mixture of theanine and other amino acids (20 kinds in total). These compounds could be separated by gradient elution within 80 min. The peaks of amino acid derivatives were identified from the retention times. The retention times of glutamic acid, theanine, and ethylamine were 7, 24, and 67 min, respectively. Thus, the resolutions using the above-described method are satisfactory. This method was applied to the analysis of biological samples from rats.

Urinary Excretion of Theanine and Its Metabolites. As shown in Figure 1B, the HPLC chromatogram of urine indicated some distinct peaks due to the metabolites of theanine, and these were identified by the retention time of each compound as glutamic acid, theanine, and ethylamine, in that order. The peaks of other amino acids could not be seen in this chromatogram because the setting sensitivity of the urine analysis was much lower than that of the plasma. When distilled water was administered, urinary theanine, glutamic acid, and ethylamine were not detected. The urinary excretion profiles of theanine, glutamic acid, and ethylamine after administration of 0, 100, 200, or 400 mg theanine are summarized in Table 1. At doses of 100, 200, or 400 mg of theanine, the molecular percentages of theanine excreted into urine during 24 h were 2, 14, and 21, respectively, and those of ethylamine were 26, 22, and 21, respectively.

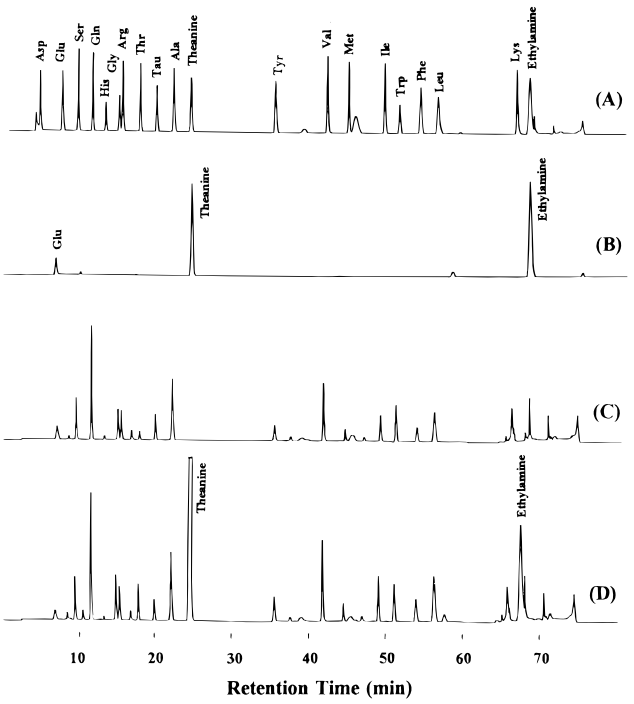

Figure 1. Chromatograms of authentic amino acids and the urinary and plasma extract analyzed by HPLC with fluorometric detection: (A) 10 nmol/mL of authentic amino acids; (B) urine collected 24 h after oral administration of 400 mg of theanine; (C) plasma 2 h after administration of distilled water; (D) plasma 2 h after administration of 200 mg of theanine. The separating conditions are described in the text.

Table 1. Urinary Excretion of Theanine, L-Glutamic Acid, and Ethylamine after Theanine Administration[a]

	theanine dose[b]			
	0 mg	100 mg	200 mg	400 mg
theanine, μmol	ND[c]	12 ± 6	165 ± 16	487 ± 207
glutamic acid, μmol	ND	5 ± 3	70 ± 6	132 ± 18
ethylamine, μmol	ND	151 ± 2	255 ± 27	481 ± 66

[a] Urine was collected 24 h after a single administration. Values are mean ± SD from three rats. [b] One hundred, 200, and 400 mg of theanine denote 575, 1150, and 2300 μmol, respectively. [c] ND, not detected.

Dose-Dependent Incorporation of Theanine into Plasma. Parts C and D of Figure 1 show chromatograms of rat plasma extracts. The plasma samples were prepared from distilled water (control sample, Figure 1C) and 2 h after a single oral administration of 200 mg of theanine (Figure 1D) and analyzed by HPLC. Although a theanine peak was not detected in the control sample, the plasma from the rats that received 200 mg of theanine had a distinct theanine peak. The peak of ethylamine was shown in the chromatogram of rat plasma extract obtained after theanine administra-

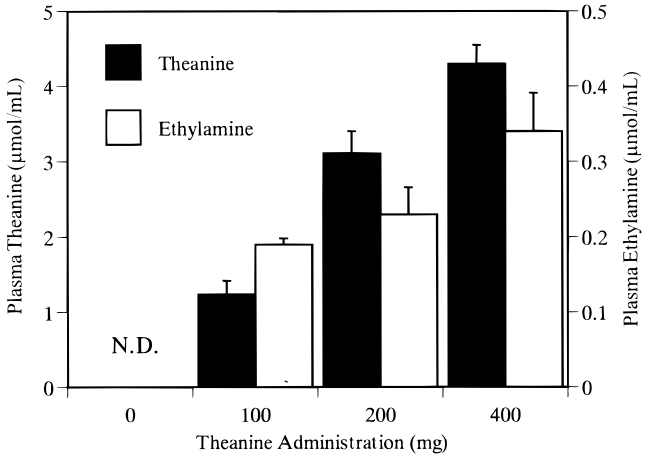

Figure 2. Dose-dependent incorporation of theanine into rat plasma after its oral administration. Plasma was taken 2 h after a single oral administration of 0, 100, 200, and 400 mg of theanine. Solid and open bars indicate plasma levels of theanine and ethylamine, respectively. Each value represents the mean ± SD from three rats.

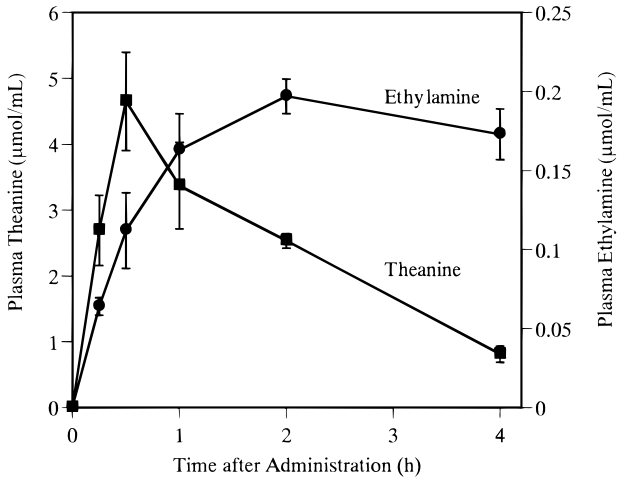

Figure 3. Time course plots of the concentrations of theanine and ethylamine in rat plasma after theanine administration. Plasma was taken after a single oral administration of 200 mg of theanine. Each point represents the mean ± SD from three rats.

tion but was not detected in the control plasma. Figure 2 shows the concentrations of theanine and ethylamine in plasma after an oral administration at doses of 0, 100, 200, and 400 mg per rat. At 2 h after a single oral dose, plasma theanine was significantly increased, and the levels reached 1.24, 3.11, and 4.30 μmol/mL when the rats received 100, 200, and 400 mg of theanine, respectively.

Time Course Changes of Plasma Levels of Theanine and Ethylamine. As shown in Figure 3, the concentrations of theanine in rat plasma obtained after oral administration of 200 mg of theanine began to increase rapidly and reached the highest level after 30 min of dosing. The maximum level of theanine found in the plasma ranged from 4.21 to 5.51 μmol/mL at the 200-mg dosing of three rats. On the other hand, the ethylamine level in plasma was gradually increased after theanine administration, and the time difference between theanine and ethylamine in reaching the maximum concentration was obvious.

Metabolism of Theanine in Tissues. Using the rat tissues of brain, heart, lung, liver, kidney, and spleen,

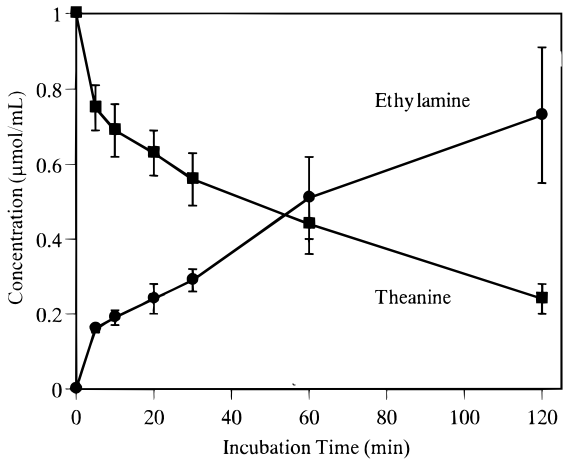

Figure 4. Time course of theanine decomposition and ethylamine formation in renal homogenate of rats. Theanine was added to the homogenate at a concentration of 1 μmol/mL, and the reaction was stopped by the addition of acetonitrile.

the enzymatic hydrolysis of theanine was examined. Among the tissue homogenates tested, the occurrence of ethylamine in the reaction mixture was virtually restricted to kidney. The time course formation of ethylamine in the reaction mixture with kidney homogenate was associated with the decomposition of theanine, as indicated in Figure 4.

DISCUSSION

The metabolic fate of theanine after its oral administration has not been sufficiently verified. To confirm whether the hydrolysis of theanine could take place in the body, plasma and urinary theanine and ethylamine after a single oral administration of theanine to rats were measured using HPLC with fluorometric detection. OPA-AcCys was used as the derivative reagent in precolumn amino acid analyses, and their derivatives were efficiently resolved on reversed-phase columns (Buck and Krummen, 1984; Nimura and Kinoshita, 1986). The method used in this study allowed the simultaneous analysis of theanine, ethylamine, and other amino acids by gradient elution within 80 min, which is suitable for the determination of theanine and ethylamine in plasma obtained after a single oral administration.

The literature to date has suggested that the formation of ethylamine is not due to bacterial action in the intestine (Asatoor, 1966). Therefore, it seems reasonable to conclude that theanine may be taken up into the blood circulation through the intestinal tract and then distributed to tissues. The kidney would seem to be the most effective site for the enzymatic hydrolysis of theanine. If we assume that the breakdown of theanine into ethylamine of the kidney in the in vitro study could be extended into the in vivo stage, the appearance of ethylamine in plasma can be explained by the metabolism of theanine after a single oral administration. The maximum concentration of ethylamine in plasma after oral administration was much lower than that of theanine. This result led us to presume that most of the ethylamine generated was immediately excreted into urine, with only a part being circulated in plasma.

As mentioned above, studies have been performed on the effects of theanine on the central nervous system. Osborn et al. (1986) reported that trace amines such as ethylamine, octopamine, and tryptamine stimulated

inositol phospholipid hydrolysis in a dose-dependent manner in rat cortical slices. Ethylamine is a constituent of many plant roots (Sasaoka, 1965; Tunmann and Linde, 1958) and is produced by the enzymatic decarboxylation of alanine (Crocomo and Fowden, 1970; Takeo, 1974). The ethylamine content in tea leaves is ~1.5−3.0% of the total free amino acids (Tsushida and Takeo, 1984). Although it would be of some interest to know whether ethylamine, as either the metabolite of theanine or foodstuff, might play an important role in the central nervous system, there is still not enough evidence to clarify this matter.

Because tea is widely used as a daily beverage, many people ingest theanine when drinking it. For a better evaluation of the effects of ingesting theanine, it is necessary to observe the effects of not only a single dose but also of its long-term ingestion. Further investigation is necessary to elucidate the in vivo effects of theanine and its metabolites.

ABBREVIATIONS USED

OPA, *o*-phthalaldehyde; AcCys, *N*-acetyl-L-cysteine; HPLC, high-performance liquid chromatography.

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Received for review October 2, 1998. Revised manuscript received February 12, 1999. Accepted February 19, 1999.

JF981113T