Metabolic fate of theanine after its oral administration to rats.

Tomonori Unno[1], Takami Kakuda[1], Takashi Hayakawa[2] and Haruhito Tsuge[2]

1: Ito en, Ltd., 21 Mekami, Sagara, Shizuoka 421-0516, Japan
2: Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Summary

Theanine, γ-glutamylethylamide, is the major amino acid components in green tea. Although the pharmacological studies have been performed on effects of the central nervous system, the investigation on the intestinal absorption and metabolism of theanine has been superficial. In order to evaluate the metabolic fate of theanine after oral administration to rats, we measured the metabolites in plasma, urine and tissues by high-performance liquid chromatography.

In the urine sample collected after administration of 100, 200 and 400 mg each of theanine, intact theanine, L-glutamic acid and ethylamine were detected in a dose-dependent manner. The plasma concentrations of theanine and ethylamine reached the highest levels about 0.5 and 2 h after oral administration of 200 mg theanine, respectively. In vitro study using rat tissues of brain, heart, lung, liver, kidney and spleen resulted in the fact that this enzymatic hydrolysis of theanine to L-glutamic acid and ethylamine was virtually accomplished in the kidney.

These results indicate that orally administered theanine in absorbed through the intestinal tract and hydrolyzed to L-glutamic acid and ethylamine in the rat kidney.

Keywords
Theanine, Intestinal absorption, Metabolism, Rat

Introduction

Theanine is by far the major amino acid components in green tea leaves. Theanine was shown to be absorbed by a common Na^+-coupled cotransporter in the intestinal brush-border membrane, but information on its *in vivo* metabolic fate is limited. A previous paper demonstrated that ethylamine was detected in human urine after ingestion of a tea extract. Therefore, we considered that theanine could act as the source of urinary ethylamine after theanine administration to rats.

Materials and Methods

Animal Study. Six-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan) were used in this study. Various amounts of theanine (0, 100, 200, and 400 mg) dissolved in 2 ml of water were orally administered to rats under being fasted for 16 h. Urine was collected for 24 h after administration. Blood was drawn from the heart with a heparinized syringe under pentobarbital anesthesia 2 h after theanine administration, and plasma was immediately obtained by centrifugation.

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

Metabolic Study. Rat tissues (brain, heart, lung, liver, kidney, and spleen) were removed and homogenized with 9 volumes of 0.1 M sodium phosphate buffer (pH7.4). Theanine solution

was added to tissue homogenates at the final concentration of 1 μmol/ml. The reaction was incubated at 37℃ and stopped by the addition of acetonitrile. After centrifugation, the levels of theanine and ethylamine in the supernatant were measured.

HPLC Analysis. The levels of theanine and ethylamine in plasma and urine of rats were measured by HPLC with fluorometric detection after deriving with fluorescence reagent of *o*-phthalaldehyde and *N*-acetyl-L-cysteine.

Results

Urinary Theanine and Its Metabolites. HPLC chromatogram of urine collected after theanine administration indicated some distinct peaks due to the metabolites of theanine, identifying with L-glutamic acid and ethylamine (chromatogram does not shown). The urinary excretion of theanine, L-glutamic acid and ethylamine are summarized in Table 1.

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
L-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.

Plasma Profiles of Theanine and Ethylamine. Figure 1 shows the plasma concentrations of theanine and ethylamine after its oral administration. Plasma theanine levels were 1.24, 3.11 and 4.30 μmol/ml when the rats received 100, 200 and 400 mg of theanine.

As shown in Fig. 2, the concentrations of theanine in rat plasma obtained after oral administration of 200 mg of theanine began to increase rapidly and reached the maximum level after 0.5 h of dosing. On the other hand, the plasma ethylamine level was gradually increased after theanine administration. There was time difference between theanine and ethylamine in reaching their maximum plasma level.

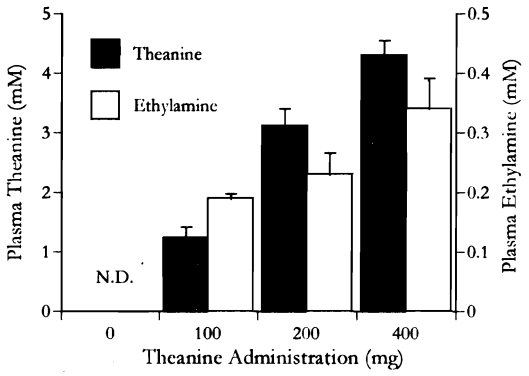

Fig.1. Dose-dependent incorporation of theanine into rat plasma after its oral administration. Plasma was taken 2 h after a single administration of 0, 100, 200 and 400 mg of theanine. Each value represents the mean ± SD from 3 rats.

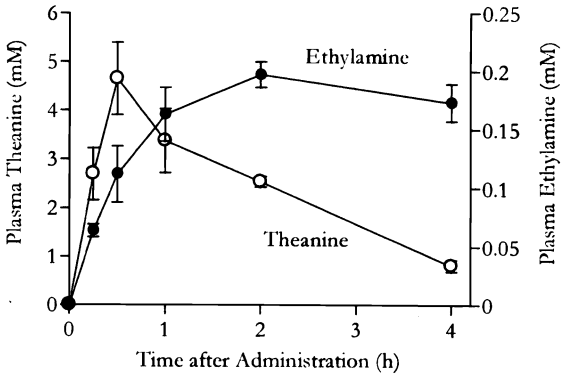

Fig. 2. Time-course of plasma level of theanine and ethylamine after theanine administration. Plasma was taken after a single administration of 200 mg of theanine. Each point represents the mean ± SD from 3 rats.

Metabolism of Theanine in Rat Tissues. *Using* the rat tissues of brain, heart, lung, kidney and spleen, 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 was associated with the decomposition of theanine (Fig. 3).

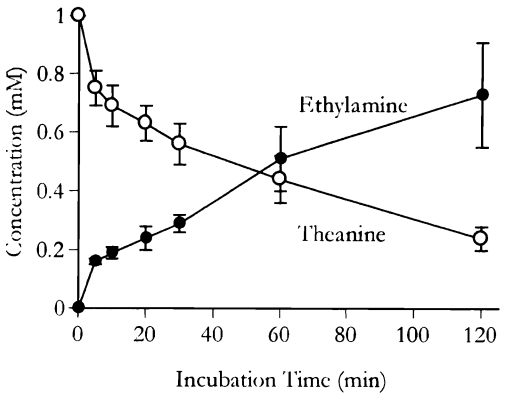

Fig. 3. Time course of theanine decomposition and ethylamine formation in renal homogenate of rats. Theanine was added to the homogenate at a concentration of 1 μmol/ml, and the reaction was stopped by the addition of acetonitrile.

Discussion

The literature to date has suggested that ethylamine could be produced after ingesting a tea extract, presuming that it owe to the hydrolysis of theanine. However, the *in vivo* metabolism of theanine has been sufficiently verified. Therefore this study was carried out to confirm whether the hydrolysis of theanine could take place in the body system.

When the theanine was orally administered to rats, we could detect the plasma and urinary theanine and ethylamine in a dose-dependent fashion. Previous report indicated that theanine could be absorbed by a Na^+-coupled cotransporter. 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. Additionally, it would seem that the most effective site for the enzymatic hydrolysis of theanine is kidney. Considering that the highest level of ethylamine was much lower than that of theanine, most of resulting ethylamine may be immediately excreted into urine, with only a part being circulated in plasma.

The question then arises about how theanine was hydrolyzed to L-glutamic acid and ethylamine. Although the purification and identification for theanine hydrolyzing enzyme are under being investigated, we now interpret that *glutaminase* could participate with decomposing theanine to L-glutamic acid and ethylamine in the rat kidney. It is known that rat kidney contains two distinct *glutaminase* activities; one is the mitochondrial phosphate-dependent and the other is maleate-activated and phosphate-independent. Our preliminary study suggests that theanine can be hydrolyzed by a maleate-activated and phosphate-independent *glutaminase*. Further investingation is necessary to elucidate the principle of hydrolyzing theanine by *glutaminase*.

Part of this work has already been presented in *Journal of Agricultural and Food Chemistry*, **47**, 1593-1596 (1999).