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Note

Theanine-induced Reduction of Brain Serotonin Concentration in Rats

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Following the administration of theanine, the brain tryptophan content significantly increased or tended to increase, but the contents of serotonin and 5-hydroxyindole acetic acid (5HIAA) decreased. The use of inhibitors of serotonin metabolism enable us to speculate that theanine reduced serotonin synthesis and also increased serotonin degradation in the brain.

Key words: theanine; tryptophan; brain serotonin; neurotransmitter; rat

Theanine, r-glutamylethylamide, is one of the major components of amino acids in Japanese green tea, and is a derivative of glutamic acid which is one of the neurotransmitters in the brain. Theanine was absorbed by a common Na^+-coupled co-transporter in the intestinal brush-border membrane,[1] and also incorporated into the brain *via* the leucine-preferring transport system of the blood-brain barrier.[2] It has also been reported that a high dose of theanine to spontaneously hypertensive rats significantly decreased the blood pressure.[3] On the other hand, it has been reported that serotonin-containing neurons in the central nervous system participated in sympathetic control. L-tryptophan, administered at a dose of 100 mg/kg, decreased blood pressure by an average of 30 mm of Hg during hypertension by increasing brain serotonin synthesis.[4] This effect was blocked by pretreating with an inhibitor of tryptophan hydroxylase (PCPA; Sigma Chemical Co., St Louis, MO, U.S.A.) which therefore blocked serotonin synthesis.[5] In this paper, it is reported whether the administration of theanine can affect the synthesis or degradation of brain serotonin by using an inhibitor of serotonin metabolism.

Young adult male rats of the Wistar strain weighing about 100 g (Japan SLC, Hamamatsu, Japan) were used with six rats per group. The room temperature was maintained at 24°C with a 12-hr light (7:00–19:00 hr) and dark cycle. In experiment 1, rats were intragastrically administered with saline or theanine (200 mg per 100 g of body weight; Taiyo Kagaku Co., Yokkaichi, Japan), and decapitated 2 hr after the administration. In experiment 2, rats were intragastrically administered with saline or theanine (200, 400, or 800 mg per 100 g of body weight), and brain 5-hydroxyindoles were determined 2 hr after the administration. In experiment 3, the animals were divided into six groups. Saline or theanine was intragastrically administered to each of three groups. Pargyline (5 mg per 100 g of body weight; Sigma Chemical Co., St Louis, MO, U.S.A.) or PCPA (20 mg per 100 g body weight; Sigma Chemical Co.) was also given twice, intraperitoneally, 1 hr before and 1 hr after (half amount of the first dose) administering saline or theanine. Serum tryptophan,[6] brain tryptophan,[6] serotonin,[7] and 5-hydroxyindole acetic acid (5HIAA)[7] were measured fluorimetrically. The statistical significance of differences between values was calculated by Duncan's multiple-range test after an analysis of variance.[8] The experimental procedures used in this study met the guidelines of the Animal Care and Use Committee of the University of Shizuoka.

As shown in Table I, the administration of theanine significantly increased or tended to increase the brain tryptophan concentration, while it significantly decreased the serotonin and 5HIAA concentrations in the brain. In the next experiment, the dose-dependent effect of theanine on brain 5-hydroxyindoles was examined. The concentration of brain tryptophan was increased in a dose-dependent manner by the administration of theanine. In our previous experiments, it was very difficult to obtain the same level of significance for the effect of theanine on brain tryptophan concentrations. By contrast, brain serotonin was consistently decreased to a significant extent by the administration of theanine. It has been reported that the contents of brain serotonin were regulated by brain tryptophan by a mechanism called precursor-mediated control of the neurotransmitter,[9] so the discrepancy in the changes of brain tryptophan and serotonin induced by administering theanine was interesting. Therefore, in the next experiment, the effect of theanine on the serotonin metabolism in the brain was investigated. To examine whether the reduction of serotonin induced by theanine was due to a decrease of serotonin synthesis or to an increase in serotonin degradation, we investigated the brain serotonin concentration after an injection of pargyline (an inhibitor of monoamine oxidase, for serotonin degradation) or of PCPA (an inhibitor of tryptophan hydroxylase, for serotonin synthesis). Pargyline administration caused a significant accumulation of brain serotonin, and completely inhibited the production of 5HIAA from serotonin; the increases in the con-

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Abbreviations: 5HIAA, 5-hydroxyindole acetic acid; LNAA, large neutral amino acid; PCPA, *p*-chlorophenylalanine.

Table I. Effect of Theanine with or without Pargyline or *p*-Chlorophenylalanine on Brain Tryptophan and 5-Hydroxyindoles of Rats[1]

Experiment	Treatment	Tryptophan		Brain	
		Serum	Brain	Serotonin	5HIAA[2]
Experiment 1		nmol/ml	nmol/g	ng/g	ng/g
Saline		—	14.1 ± 1.0^{a}[3]	574 ± 13^{b}	403 ± 26^{b}
Theanine, 100 mg/100 g of body weight		—	17.7 ± 1.4^{b}	482 ± 13^{a}	269 ± 12^{a}
Experiment 2					
Saline		—	14.1 ± 0.5^{a}	481 ± 14^{c}	284 ± 7^{d}
Theanine, 200 mg/100 g of body weight		—	15.6 ± 0.5^{b}	447 ± 8^{b}	265 ± 6^{c}
Theanine, 400 mg/100 g of body weight		—	16.1 ± 0.5^{b}	443 ± 11^{ab}	244 ± 6^{b}
Theanine, 800 mg/100 g of body weight		—	17.0 ± 0.5^{b}	416 ± 6^{a}	222 ± 5^{a}
Experiment 3					
Saline	Saline[4]	93.5 ± 2.4^{b}	13.5 ± 0.6^{b}	542 ± 13^{d}	490 ± 28^{c}
Theanine	Saline	102.8 ± 2.9^{bc}	14.4 ± 0.2^{bc}	482 ± 15^{c}	446 ± 14^{c}
Saline	Pargyline	93.0 ± 3.9^{b}	14.1 ± 0.5^{b}	945 ± 24^{f}	133 ± 7^{a}
Theanine	Pargyline	107.7 ± 2.4^{c}	15.9 ± 0.2^{c}	850 ± 10^{e}	130 ± 6^{a}
Saline	PCPA[5]	72.5 ± 2.9^{a}	14.4 ± 0.5^{bc}	425 ± 17^{b}	469 ± 24^{c}
Theanine	PCPA	70.5 ± 5.9^{a}	10.6 ± 0.3^{a}	356 ± 7^{a}	348 ± 19^{b}

[1] The rats were killed 2 hr after the administration of saline or theanine. Initial average body weights are 98 ± 1 g (experiments 1 and 3) and 115 ± 5 g (experiment 2).
[2] 5-hydroxyindole acetic acid.
[3] Mean ± SEM for six rats per group. Means within a column not followed by the same letter in each experiment are significantly different, Duncan's test ($p < 0.05$).
[4] Saline, pargyline or *p*-chlorophenylalanine was given twice, i.p., 1 hr before and 1 hr after the dose of theanine.
[5] PCPA, *p*-chlorophenylalanine.

centration of serotonin in the brain were 403 ng/g (in the control group) and 368 ng/g (in the theanine group). On the other hand, the administration of PCPA inhibited the production of brain serotonin and 5HIAA; the decreases in the concentrations of serotonin and 5HIAA in the brain were 117, 21 ng/g (in the control group) and 126, 98 ng/g (in the theanine group), respectively. Thus, synthesized and accumulated serotonin decreased as compared with that in the control group. The mechanism whereby theanine affected the brain serotonin metabolism, as to whether theanine would directly inhibit the enzyme activity of serotonin synthesis or whether it would enhance the release of brain 5-hydroxyindoles, is still unclear. In conclusion, theanine may decrease the serotonin synthesis and also enhance the degradation or release of serotonin, leading to an alteration in the concentrations of brain 5-hydroxyindoles.

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