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Reduction Effect of Theanine on Blood Pressure and Brain 5-Hydroxyindoles in Spontaneously Hypertensive Rats

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The effect of theanine, one of the components of green tea, on the blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) was investigated by intraperitoneally administering theanine. The effect of glutamine, which is structurally similar to theanine, was also examined. When SHR were injected with various amounts of theanine (0, 500, 1000, 1500, and 2000 mg/kg), the change was dose-dependent, and a significant decrease in blood pressure was observed with the high doses (1500 and 2000 mg/kg). A dose of 2000 mg/kg of theanine did not alter the blood pressure of WKY, while the same dose to SHR decreased it significantly. On the other hand, glutamine administration to SHR did not change either the blood pressure or the heart rate. The brain 5-hydroxyindole level was significantly decreased by theanine administration to both WKY and SHR, the decrease being dose-dependent.

The physiological and pharmacological actions of various components of green tea such as catechins, caffeine, and γ-aminoisobutyric acid (GABA) have recently been investigated.[1–3] Blood pressure was especially affected by the administration of catechines,[1] caffeine,[2] and GABA.[3] It is known that the regulation of blood pressure is very dependent on catecholaminergic and serotonergic neurons in both the brain and periphery[4–7]; therefore, the ingestion of such neurotransmitter precursors as tyrosine and tryptophan affected the blood pressure.[4,7]

Theanine, L-*N*-ethylglutamine, is one of the major components of amino acids in Japanese green tea,[8] and is also one of the components which decides its taste (umami). Theanine is a derivative of glutamic acid, which is one of the neurotransmitters in the brain.

In this study, the effects of theanine on the blood pressure and the brain 5-hydroxyindole level were investigated.

Materials and Methods

Experimental design. Male Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) weighing 300 to 400 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in individual wire cages in a temperature- and humidity-controlled room (24°C and 50% relative humidity) with a 12-h cycle of light and dark. The animals were maintained on a commercial diet (CE-2; Nihon Clea, Japan). Theanine was purchased from Tokyo Chemical Industries (Tokyo, Japan). Various amounts of theanine (0, 500, 1000, 1500, and 2000 mg/kg body weight) were dissolved in a 0.9% NaCl solution (7 ml/kg body weight) and then administered intraperitoneally to SHR at 1:00 p.m. (experiment 1). WKY and SHR were injected with theanine (2000 mg/kg) intraperitoneally (experiment 2), and theanine (2000 mg/kg) or glutamine (2000 mg/kg) was injected intraperitoneally into SHR at 1:00 p.m. (experiment 3). Brain 5-hydroxyindoles were determined 2 h after the administration of theanine or glutamine to WKY and SHR (experiment 4).

Blood pressure measurement. The blood pressure and heart rate were measured before and 60 min after the administration of the substances by means of a monitor using a tail cuff (model MK-1000; Muromachi Kikai Co., Tokyo, Japan), and the differences in blood pressure were calculated. This measurement was performed during the period from noon to 6:00 p.m. The average normal values for systolic and diastolic blood pressure in SHR *vs.* WKY were 224.5 ± 5.5 *vs.* 150.8 ± 7.4 and 144.4 ± 6.7 *vs.* 100.9 ± 7.1 mmHg, respectively.

Brain 5-hydroxyindole analysis. After decapitation, the brain of each animal was immediately removed, frozen on dry ice and stored at −70°C until assayed. The concentration of tryptophan was determined by the method of Denckla and Dewey,[9] while serotonin[10] and 5-hydroxyindole acetic acid (5HIAA)[10] were assayed fluorimetrically.

Statistics. Statistical analyses were performed by using Student's *t*-test[11] or Duncan's multiple range test.[12] *P*-values of less than 0.05 are considered to be of statistical significance.

Results

Effects of various levels of theanine on the blood pressure and heart rate of SHR (experiment 1)

The dose-dependent changes in the blood pressure values (systolic, diastolic, and mean) resulting from the administration of theanine to SHR are shown in Fig. 1. Theanine tended to decrease the blood pressure, significant decreases being observed with high doses of theanine (1500 and/or 2000 mg/kg) in the systolic and/or mean blood pressure. The heart rate (beats/min) was not affected by the various doses of theanine.

Effect of theanine or glutamine on the blood pressure of WKY or SHR (experiments 2 and 3)

The effect of theanine on the blood pressure was investigated for both WKY and SHR (Fig. 2). With WKY, the blood pressure was not changed by the administration of theanine; however, with SHR, a significant decrease in blood pressure was observed by the administration of theanine. When glutamine was administered to SHR at the same dose as that of theanine (2000 mg/kg), there were no

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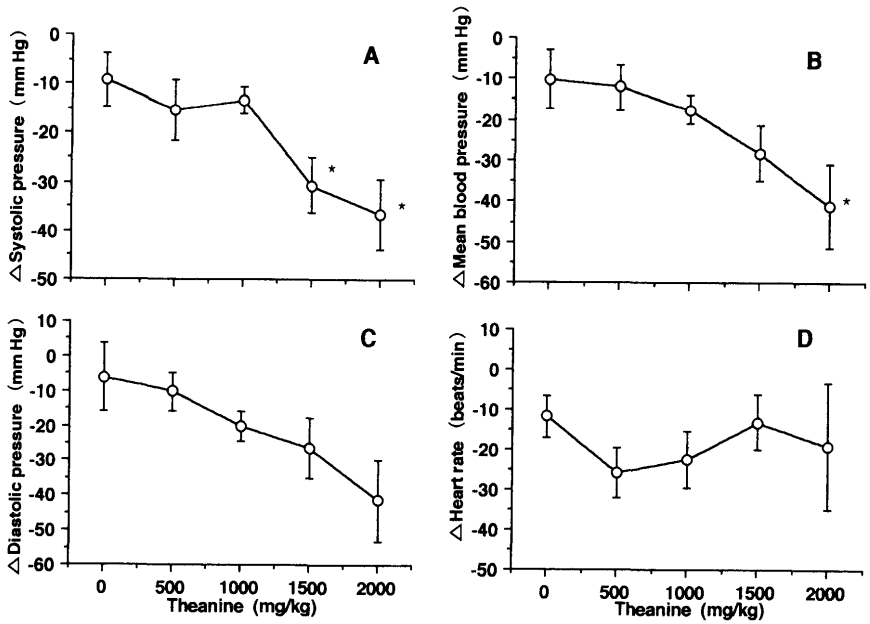

Fig. 1. Effect of Theanine Administration on the Systolic Blood Pressure (A), Mean Blood Pressure (B), Diastolic Blood Pressure (C) and Heart Rate (D) of Spontaneously Hypertensive Rats.

The blood pressure and heart rate were measured before and 60 min after the administration of various amounts of theanine. Each value represents the mean ± SEM for 5 rats and is expressed as the difference from the base value before administration. Asterisks indicate a significant difference from the value without theanine by Student's *t*-test ($p < 0.05$). The baseline for the systolic blood pressure of SHR is 222.7 ± 5.5 mmHg.

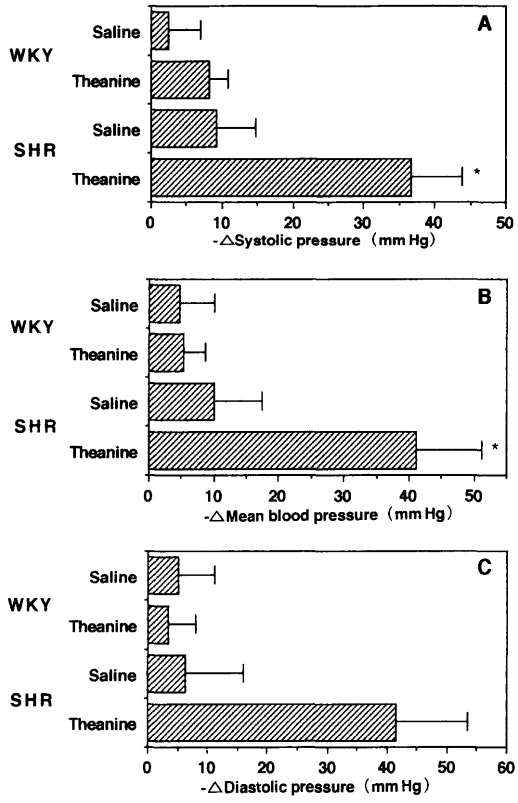

Fig. 2. Effect of Theanine Administration on the Systolic Blood Pressure (A), Mean Blood Pressure (B) and Diastolic Blood Pressure (C) of Conscious Wistar Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR).

Values represent the means ± SEM for 5 rats and are expressed as the difference from the base value before administration. Asterisks indicate a significant difference from the corresponding saline group by Student's *t*-test ($p < 0.05$). The dose of theanine administered was 2000 mg per kg of body weight. The baselines for the systolic blood pressure in SHR *vs.* WKY are 226.9 ± 4.6 *vs.* 150.8 ± 7.4 mmHg, respectively.

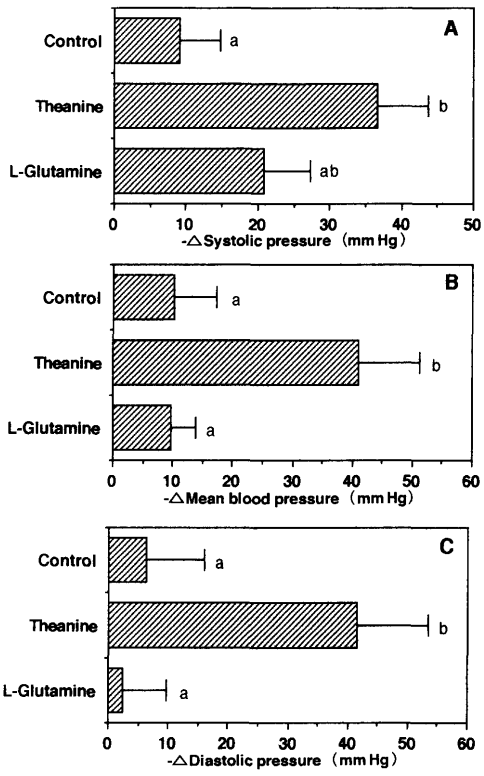

Fig. 3. Effect of Theanine or L-Glutamine Administration on the Systolic Blood Pressure (A), Mean Blood Pressure (B) and Diastolic Blood Pressure (C) of Spontaneously Hypertensive Rats.

Values represent the means ± SEM for 5 rats and are expressed as the difference from the base value before administration. Bars not followed by the same letter are significantly different by Duncan's multiple-range test ($p < 0.05$). The dose of theanine or glutamine administered was 2000 mg per kg of body weight. The baseline for the systolic blood pressure in SHR is 233.6 ± 5.6 mmHg.

Table Concentrations of Serotonin and 5-Hydroxyindole Acetic Acid (5-HIAA) in the Brain of Rats Injected with Theanine or Glutamine[1]

		Serotonin (ng/g of brain)	5-HIAA (ng/g of brain)
WKY[2]	Control (saline)	557 ± 18^{b}	405 ± 14^{ab}
WKY	Theanine (2000 mg/kg)	506 ± 10^{a}	390 ± 16^{ab}
SHR[3]	Control (saline)	592 ± 6^{c}	476 ± 13^{c}
SHR	Theanine (1000 mg/kg)	553 ± 8^{b}	406 ± 15^{ab}
SHR	Theanine (2000 mg/kg)	492 ± 15^{a}	354 ± 32^{a}
SHR	Glutamine (2000 mg/kg)	549 ± 8^{b}	425 ± 11^{bc}

[1] Means ± SEM ($n=5$ for WKY and $n=6$ for SHR). Means within a column not followed by the same letter are significantly different by Duncan's multiple-range test ($p<0.05$).
[2] WKY, Wistar Kyoto rats.
[3] SHR, Spontaneously hypertensive rats.

changes in either the blood pressure (Fig. 3) or the heart rate (data not shown).

Effects of theanine or glutamine on brain serotonin and 5-hydroxyindole acetic acid in WKY or SHR (experiment 4)

The changes in brain serotonin and 5HIAA resulting from the administration of theanine or glutamine to WKY and SHR are shown in Table I. Brain tryptophan was not changed by the administration of theanine to WKY and SHR (data not shown). The concentrations of brain serotonin and 5HIAA in SHR administered with saline were higher than those in WKY, while these concentrations were significantly decreased by the administration of theanine to WKY and SHR, the effect being dose-dependent.

Discussion

The effect of administering theanine on the blood pressure of SHR was studied. Increasing the dose of theanine resulted in a concomitant decrease in blood pressure, a significant decrease being observed with the high doses (1500 and 2000 mg/kg). The systolic blood pressure of SHR was especially affected by the administration of theanine. However, it is not known why such a high dose of theanine is required to suppress the blood pressure, this antihypertensive action of theanine being specific to SHR and not being observed with WKY. It is likewise not known why there is this specific effect on the blood pressure of SHR. Other antihypertensive substances such as tyrosine,[7] tryptophan,[4] GABA,[3] and catechins[1] have also been effective only on SHR, although the effective dose of these substances was not as high (100–200 mg/kg). Glutamine, which resembles theanine in structure, did not exhibit an antihypertensive action on SHR, while the heart rate was not affected by the administration of theanine to both SHR and WKY.

The reason why theanine administration decreases blood pressure is not known. Serotonin, a putative neurotransmitter in the mammalian central nervous system, is synthesized in the brain by 5-hydroxylation and decarboxylation of the essential amino acid, L-tryptophan. The continuing metabolism of serotonin is deamination by monoamine oxidase, and the product of this reaction, 5-hydroxyindole acetaldehyde, can be further oxidized to 5HIAA. In the case of the antihypertensive effect of tryptophan administration to SHR (*via* increased serotoninergic neurotransmission), several conditions for the inhibition of serotonin synthesis (*p*-chlorophenylalanine), a serotonin reuptake blocker (fluoxetine), or the coexistence of a large neutral amino acid that competes with tryptophan for transport into the brain have attenuated this antihypertensive action of tryptophan.[13] In the case of the antihypertensive effect of tyrosine administration to SHR (*via* increased catecholaminergic transmission), tyrosine injection has produced a marked decrease in blood pressure, this antihypertensive action being thought to be mediated by an increase in norepinephrine synthesis and release within the brain.[7] Therefore, the antihypertensive effect of some substances may be mediated by different mechanisms. In the present study, we investigated whether or not the brain 5-hydroxyindole levels in WKY and SHR would be affected by the administration of theanine or glutamine. Theanine caused significant decreases of serotonin and 5HIAA in the brain of SHR, and also of serotonin in WKY, but glutamine caused smaller decreases than these levels in SHR. It has been reported that theanine decreased the norepinephrine concentration in the brain, but did not change the concentrations of serotonin and 5HIAA.[14] This discrepancy in the effects of theanine on 5-hydroxyindoles may have been due to differences in the dosage or the sacrifice time after its administration. The mechanism underlying the decrease in 5-hydroxyindoles caused by theanine is not known, but it is considered that large amounts of theanine depress serotonin or 5HIAA synthesis directly or affect other factors which regulate serotonin turnover, such as insulin[15,16] and cAMP,[17] because theanine was significantly accumulated in the brain after its administration. The competitive inhibition by theanine of tryptophan incorporation into the brain *via* the blood-brain barrier is not plausible, because brain tryptophan was not changed by theanine administration, despite its large dose. We therefore presume that theanine is incorporated into the brain by different means from those for other amino acids. In our previous study, the concentration of serum cAMP in Wistar-strain rats administered with theanine (2000 mg/kg body weight) was significantly increased in comparison with that in the control group (saline): 5.07 ± 0.35 *vs.* 4.08 ± 0.11 pmol/ml, respectively. This increase in cAMP may have stimulated the secretion of insulin in the pancreas, which in turn stimulated the use of all amino acids for such purposes as protein synthesis in peripheral tissues, and caused the changes in brain 5-hydroxyindoles. Although the increase in brain serotonin caused by tryptophan administration has contributed to its antihypertensive effect,[13] in our study, the level of brain serotonin was inversely decreased, in spite of the occurrence of the antihypertensive effect, by the administration of theanine. Generally, the blood pressure is affected by the vascular resistance (caused by transmural pressure and blood flow), viscosity of the blood, peripheral resistance and other factors. In this study, an antihypertensive effect was only observed in SHR and also rapidly after theanine administration. Therefore, it is assumed that, through its antihypertensive action, theanine might affect such other pathways as the peripheral nervous system and peripheral blood vessels, and not the brain serotonin level. Further investigations on this mechanism as well as the reason for the requirement of such a high

dose of theanine to obtain an antihypertensive effect are needed.

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