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Effect of Theanine on Norepinephrine and Serotonin Levels in Rat Brain[1)]

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The effects of i.p. administered theanine (L-*N*-ethylglutamine), a constituent of Japanese green tea, on the levels of norepinephrine (NE) and serotonin (5-HT) in the brain of rats with or without coadministration of caffeine were investigated, and compared with those of glutamine.

Theanine decreased the NE level, whereas no change was observed with glutamine or caffeine. The decrease of NE induced by theanine was reversed by caffeine. In rats pretreated with pargyline, a monoamine oxidase inhibitor, theanine significantly increased the NE level compared with the control. However, it did not enhance the NE levels increased by caffeine. Thus, theanine may decrease the NE levels by releasing this neurotransmitter.

Theanine did not alter the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in rats pretreated with or without pargyline, indicating that this amide affects neither 5-HT synthesis nor its degradation. Caffeine increased the levels of 5-HT and 5-HIAA in normal rats to similar extents. This effect was depressed by theanine. In rats pretreated with pargyline, the levels of 5-HT and 5-HIAA were not altered by caffeine, and theanine did not modify the outcome. It may be concluded that the action of theanine is related to the possible inhibition of 5-HT release by caffeine. The effect of glutamine on the levels of 5-HT was somewhat different from that of theanine.

Keywords——theanine; glutamine; caffeine; norepinephrine; serotonin; rat brain

Caffeine is widely used as a behavioral stimulant, and a number of neurochemical effects of caffeine have been reported.[2)] However, the molecular mechanisms involved are still unclear. Methylxanthines can inhibit phosphodiesterase and thus prevent inactivation of adenosine 3′, 5′-monophosphate (cAMP),[3)] increase the turnover of catecholamines,[4)] and increase the brain levels of tryptophan, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA).[5)]

In a series of experiments, we have shown that theanine (L-*N*-ethylglutamine), a constituent of Japanese green tea,[6)] protected mice from convulsion induced by a toxic dose of caffeine,[7)] depressed the spontaneous locomotor activity of mice enhanced by a lower dose of caffeine,[8)] and inhibited the stimulation of cAMP formation by norepinephrine (NE) in the rat brain cortex *in vitro*.[1)]

In this report, we describe the effects of theanine on the levels of NE and 5-HT in the brain of rats with or without coadministration of caffeine, and compare them with the action of glutamine, which has similar pharmacological effects.[7,8)]

Experimental

Animals——Male Wistar rats weighing about 180 g were used. Rats were given commercial chow (Oriental, MF) and tap water *ad libitum*.

Chemicals——Theanine was synthesized by the method of Furuyama *et al.*[9)] Pargyline hydrochloride was synthesized according to the method of Martin.[10)] Caffeine (Japanese Pharmacopeia grade) was recrystallized from water. L-Glutamine (Wako Pure Chem. Ind.), norepinephrine tartrate (Nakarai Chem. Co.), serotonin creatine sulfate (E. Merck A. G.), 5-hydroxyindoleacetic acid (Nakarai Chem. Co.), and other reagent-grade chemicals were purchased.

Determination of NE and 5-HT Levels——Rats were administered i.p. theanine or caffeine dissolved in water, or glutamine suspended in 0.5% tragacanth solution. The doses of theanine and glutamine were 10 mmol/kg, which is enough to decrease the convulsive effect of caffeine.[7a] Control rats were administered saline or 0.5% tragacanth solution. The rats were killed by decapitation at an appropriate time after the administration of drugs, and the whole brain minus the cerebellum was removed and immediately used for the determination of NE, 5-HT and 5-HIAA by the method of Cox and Perhach.[11] This is a fluorometric method for the determination of catecholamines, 5-HT and 5-HIAA, in a single brain sample utilizing the method of Chang[12] for the adsorption of catecholamines onto alumina, the method of Ansell and Beeson[13] for the extraction of 5-HT, and the method of Maickel *et al.*[14] for the determination of 5-HT and 5-HIAA. Excision of the brain was performed at 2.00—3.00 p.m. A Hitachi 204 spectrofluorophotometer was used.

Statistics——Data were analyzed by the use of Student's *t* test.

Results

Table I shows the NE concentrations in the brain in groups of rats which were administered theanine, glutamine and the combination of each amide with caffeine.

On administration of theanine, the NE concentration was decreased about 16% at 30 min and 9% at 75 min, whereas no change was observed after the administration of glutamine. Caffeine did not have any significant effect on the NE concentration. However, the decrease of NE induced by theanine was reversed by caffeine.

TABLE I. Effects of Theanine, Glutamine and Caffeine on the Concentration of NE in Rat Brain

Compound[a]	Time[b] (min)	NE[c] (μg/g wet wt.)
Control		0.555±0.0169(9)
Theanine	30	0.464±0.0097(5)[d]
	75	0.504±0.0082(5)[e]
Caffeine[f]	75	0.590±0.0235(5)
Theanine+caffeine[f]	75	0.594±0.0074(5)
Control		0.546±0.0118(9)
Glutamine	30	0.544±0.0239(5)
	75	0.558±0.0159(5)
Caffeine[f]	75	0.596±0.0225(5)
Glutamine+caffeine[f]	75	0.589±0.0188(5)

a) Doses of theanine and glutamine were each 10 mmol/kg i.p. *b*) Time after the administration of theanine, glutamine or vehicle. *c*) Mean±S.E. Significance of difference from the control group: *d*) $p<0.01$, *e*) $p<0.05$. *f*) Caffeine (100 mg/kg i.p.) was administered 15 min after the administration of the amides or vehicle.

TABLE II. Effects of Theanine and Caffeine on the Concentration of NE in Rat Brain after Pargyline Treatment

Compound	NE[a] (μg/g wet wt.)
Control	0.739±0.0183(6)
Theanine	0.816±0.0294(5)[b]
Caffeine	0.842±0.0369(6)[b]
Theanine+caffeine	0.847±0.0236(6)[c]

Rats were given a solution of pargyline hydrochloride (75 mg/kg i.p.) 90 min before the administration of theanine or vehicle, and caffeine was given 15 min after. Doses of theanine and caffeine were the same as indicated in the footnote of Table I. *a*) Mean±S.E. Significance of difference from the control group: *b*) $p<0.05$, *c*) $p<0.01$.

Table III. Effects of Theanine, Glutamine and Caffeine on the Concentrations of 5-HT and 5-HIAA in Rat Brain

Compound	5-HT[a] (μg/g wet wt.)	5-HIAA[a] (μg/g wet wt.)
Control	0.621±0.0472(5)	0.528±0.0379(5)
Theanine	0.653±0.0654(5)	0.499±0.0258(5)
Caffeine	0.968±0.0491(6)[b]	0.838±0.0235(6)[b]
Theanine+caffeine	0.763±0.0351(5)[c]	0.708±0.0367(6)[d]
Control	0.694±0.0489(6)	0.517±0.0198(5)
Glutamine	0.724±0.0361(6)	0.529±0.0179(5)
Caffeine	1.13 ±0.066 (5)[b]	0.790±0.0521(5)[e]
Glutamine+caffeine	1.09 ±0.064 (5)	0.713±0.0459(5)

The concentrations of 5-HT and 5-HIAA were determined 75 min after the administration of the amides or vehicle. Other experimental conditions were as indicated in the footnote of Table I. *a*) Mean± S.E. Significance of difference from the control group: *b*) $p<0.001$, *e*) $p<0.05$; from the group administered caffeine alone: *c*) $p<0.01$, *d*) $p<0.02$.

Table IV. Effects of Theanine, Glutamine and Caffeine on the Concentrations of 5-HT and 5-HIAA in Rat Brain after Pargyline Treatment

Compound	5-HT[a] (μg/g wet wt.)	5-HIAA[a] (μg/g wet wt.)
Control	1.62±0.142(7)	0.332±0.0415(8)
Theanine	1.94±0.093(8)	0.375±0.0454(8)
Caffeine	1.51±0.146(7)	0.428±0.0361(5)
Theanine+caffeine	1.46±0.140(6)	0.424±0.0251(6)
Control	1.36±0.076(9)	0.315±0.0307(7)
Glutamine	2.00±0.128(9)[b]	0.337±0.0241(9)
Caffeine	1.19±0.101(7)	0.375±0.0186(8)
Glutamine+caffeine	1.58±0.122(7)[c]	0.373±0.0266(7)

Rats were given a solution of pargyline hydrochloride (75 mg/kg i.p.) 90 min before the administration of theanine, glutamine or vehicle. The concentrations of 5-HT and 5-HIAA were determined 75 min after the administration of the amides or vehicle. Other experimental conditions were as indicated in the footnote of Table I. *a*) Mean±S.E. Significance of difference from the control group: *b*) $p<0.001$; from the group administered caffeine alone: *c*) $p<0.05$.

The effects of theanine and caffeine on the elevated NE concentration produced by pretreatment with pargyline, a monoamine oxidase (MAO) inhibitor, are shown in Table II. Theanine and caffeine increased the NE concentration significantly. However, the NE concentration after the administration of theanine with caffeine was equal to that after caffeine alone.

The concentrations of 5-HT and 5-HIAA after the administration of theanine, glutamine and caffeine are shown in Table III. In agreement with the result of Berkowitz and Spector,[5a] caffeine increased the concentrations of 5-HT and 5-HIAA, significantly. Neither theanine nor glutamine altered the concentrations of 5-HT and 5-HIAA. However, the increases induced by caffeine were significantly reversed by theanine administration, while they were not altered by glutamine administration.

Table IV shows the effects of theanine, glutamine and caffeine on the concentrations of 5-HT and 5-HIAA in the brain of rats pretreated with pargyline. The 5-HT concentration was increased and the 5-HIAA concentration was reduced by the pretreatment with pargyline, as a

result of MAO inhibition. Theanine and caffeine did not alter these concentrations, and the concentrations of 5-HT and 5-HIAA after the administration of the combination of theanine and caffeine were similar to those after caffeine alone. On the other hand, glutamine significantly increased the 5-HT concentration in rats with or without caffeine.

Discussion

After i.p. administration, theanine decreased the NE level of rat brain. This effect may not be due to the inhibition of NE synthesis, because it was not observed when rats were preadministered pargyline, an MAO inhibitor. On the other hand, the decrease of NE level by theanine was not observed after the coadministration of caffeine. Yessaian *et al.*[15] showed that the NE levels in midbrain, medulla plus pons and cerebral hemisphere were decreased by the i.p. administration of γ-aminobutyric acid (GABA), and they suggested that GABA had the effect of releasing NE in the brain. Theanine was shown to increase the GABA level in the brain,[7a] and this phenomenon may be partially responsible for the decrease of NE level. Caffeine did not alter the NE level in rat brain, in agreement with other reports.[5d,16] However, the NE levels were increased by caffeine in the brain of rats pretreated with pargyline. Berkowitz *et al.*[16] also observed these phenomena and suggested that caffeine is able to increase the turnover of NE in the brain. An increase of NE turnover induced by caffeine has also been proposed by other investigators.[4] These and our results suggest that theanine may decrease the NE levels by releasing this neurotransmitter.

The present finding that caffeine produced an increase of 5-HT level is consistent with the reports of many investigators,[5] and this effect was maintained for more than 5 h (data not shown). Since the 5-HIAA level was increased by the administration of caffeine to a similar extent to that of 5-HT, it is considered that the increase of 5-HT level is not a result of MAO inhibition. The 5-HT level was increased by pretreatment with pargyline as expected, but caffeine did not have any additive effect. The rate of 5-HT turnover was reported not to be increased.[4c,5f,17] Thus, the increase of the 5-HT level by caffeine administration is not considered to be due to an increase of 5-HT synthesis or to MAO inhibition, but to the inhibition of 5-HT release.[4c,5a,f]

Theanine did not show any apparent effect on the brain levels of 5-HT and 5-HIAA of normal and pargyline-pretreated rats. These results indicate that this amide affects neither the synthesis nor the degradation of 5-HT. On the other hand, theanine inhibited the increase in the level of 5-HT caused by caffeine administration, and also that of 5-HIAA to a similar extent. Thus, it is considered that the action of theanine is related to the possible inhibition of 5-HT release by caffeine.

Glutamine did not change the levels of 5-HT and 5-HIAA in the brain of normal rats. However, the 5-HT level in the brain of pargyline-pretreated rats was increased by the administration of glutamine alone or in combination with caffeine. These results indicate that glutamine increased the turnover of 5-HT in the brain of rats with or without caffeine. Glutamine, like theanine, prevented the convulsion induced by caffeine[7] and depressed the increase of spontaneous locomotor activity by caffeine.[8] However, the effect of glutamine on 5-HT metabolism was somewhat different from that of theanine.

References and Notes

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