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Influence of Alkylamides of Glutamic Acid and Related Compounds on the Central Nervous System. IV.[1] Effect of Theanine on Adenosine 3′,5′-Monophosphate Formation in Rat Cerebral Cortex

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Theanine (L-N-ethylglutamine, a constituent of Japanese green tea), L-glutamine or caffeine did not affect adenosine 3′,5′-monophosphate (cAMP) formation in cerebral cortex slices from rats. In addition, theanine and L-glutamine did not show any effect on cAMP formation in the presence of caffeine. Theanine did show an inhibitory effect on norepinephrine-stimulated cAMP formation in the presence or absence of caffeine. Theanine also showed an inhibitory effect on histamine-stimulated cAMP formation in the absence of caffeine, but not in the presence of caffeine. It appears that theanine does not antagonize caffeine in cAMP metabolism, but does inhibit the stimulation of cAMP formation by norepinephrine.

Keywords——cAMP; theanine; L-glutamine; caffeine; norepinephrine; histamine

The greatest capacity to form adenosine 3′,5′-monophosphate (cAMP) among mammalian tissues is found in the central nervous system.[3] A considerable amount of evidence that cAMP plays an important role in nervous system function has accumulated.[4]

The authors have reported that theanine (L-N-ethylglutamine), a constituent of Japanese green tea, protected mice from convulsions induced by a toxic dose of caffeine[5] and depressed the spontaneous motor activity of mice enhanced by a lower dose of caffeine.[1] Methylxanthines, including caffeine, are known to have inhibitory action on the phosphodiesterase which hydrolyzes cAMP.[6]

This paper describes an investigation of the interaction of theanine and caffeine on cAMP formation in the brain tissue, in comparison with the effect of L-glutamine, which showed a depressive action similar to that of theanine.[1,5]

Experimental

Animals——Wistar male rats (weighing about 180 g) were used.

Materials——Theanine was synthesized according to the method of Furuyama *et al.*[7] Adenine-8-^{14}C (Daiichi Pure Chem.), norepinephrine (Fluka), cAMP (Boehringer), histamine, L-glutamine (Wako Pure Chem. Ind.), and other reagent-grade chemicals were purchased.

Procedures——Twelve cerebral cortex slices (0.25 mm in thickness) from 3 rats were prepared by the usual method and the slices were chopped to a size of 0.35×0.35 mm with a McIlwain mechanical chopper (Hotta Rikagakukikai Seisakusho, Tokyo) at room temperature. The chopped slices were preincubated in 5 ml of Krebs–Ringer bicarbonate buffer containing 10 mM glucose and 1 μM EDTA at 37.5° for 20 min under

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an O_2–CO_2 (95: 5) atmosphere, then the slices were "pulse-labelled" with 1 μCi (63.5 nmol) adenine-8-^{14}C at 37.5° for 40 min by the method of Shimizu *et al.*[8] A portion (4—7 mg of protein) of the labelled slices was incubated with the drugs listed in Table I in the Krebs–Ringer bicarbonate buffer at 37.5° for 10 min. The slices were assayed for cAMP-^{14}C and total radioactivity according to the method of Shimizu *et al.*[8] and for protein contents by the method of Lowry *et al.*[9]

Results

Figure 1 shows an example of the formation of cAMP in slices of rat brain cortex incubated with norepinephrine, histamine+caffeine, and theanine. The stimulation of cAMP formation by norepinephrine or histamine+caffeine was maximal at 10 min, while the formation of cAMP was not affected by theanine for 15 min.

Table I shows the effect of combinations of theanine or L-glutamine with caffeine, norepinephrine and histamine. Theanine and L-glutamine did not affect respiration by rat brain cortex slices in the presence of glucose as a substrate at a dose of 0.1 mM,[5b] but did affect the respiration at a dose of 10 mM (not published). Thus, the effect of theanine or L-glutamine on cAMP formation was investigated at 10 mM. In this experiment, no effect of 1 mM caffeine on the formation of cAMP was observed. Theanine, like L-glutamine, did not affect cAMP formation in the presence or absence of caffeine. The stimulation of cAMP formation by norepinephrine was significantly depressed by theanine. Both theanine and L-glutamine depressed the cAMP formation stimulated by norepinephrine in the presence of caffeine. Theanine also depressed the formation of cAMP stimulated by histamine, though this effect was not observed in the presence of caffeine.

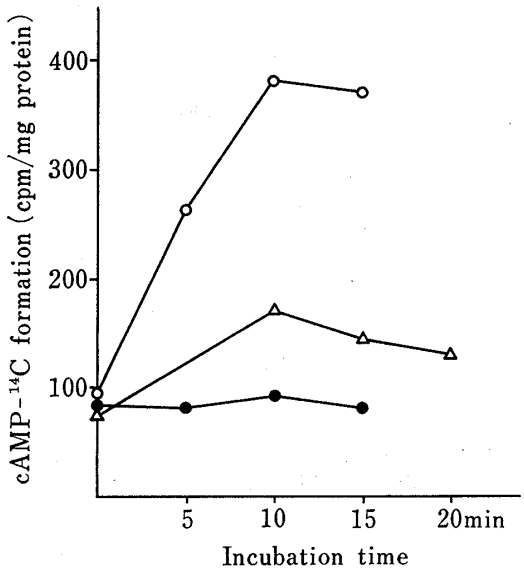

Fig. 1. Time Course of the Accumulation of cAMP-^{14}C in Slices of Rat Cerebral Cortex in Response to Norepinephrine, Histamine+caffeine, or Theanine

—○— : norepinephrine, 0.5 mM.
—△— : histamine, 0.5 mM+caffeine, 1 mM.
—●— : theanine, 10 mM.

Table I. Effects of Theanine and L-Glutamine on the Formation of cAMP in Rat Cerebral Cortex Slices under Various Conditions

Compounds	Formation of cAMP-^{14}C (% conversion[a])							
	Control		Theanine (10 mM)		Effect %	L-Glutamine (10 mM)		Effect %
Nil	0.19	±0.030(3)	0.20	±0.045(3)	+5.3	0.23	±0.047(3)	+15.5
Caffeine (1 mM)	0.17	±0.015(4)	0.18	±0.006(4)	+5.9	0.21	±0.020(4)	+23.5
Norepinephrine (0.5 mM)	0.81	±0.090(5)	0.52	±0.050(4)[b]	−35.8	0.57	±0.033(4)	−29.6
Norepinephrine (0.5 mM) +caffeine (1 mM)	0.98	±0.072(5)	0.71	±0.088(4)[b]	−27.6	0.61	±0.082(4)[c]	−37.8
Histamine (0.5 mM)	0.33	±0.029(4)	0.25	±0.006(4)[b]	−24.2	0.31	±0.022(4)	−6.1
Histamine (0.5 mM) +caffeine (1 mM)	0.34	±0.026(5)	0.38	±0.019(4)	+11.8	0.40	±0.042(5)	+17.7

Labelled slices were incubated for 10 min.
Numbers in parentheses are the numbers of experiments.

a) mean±s. e., % conversion $= \frac{\text{cpm of cAMP-}^{14}\text{C in the slices}}{\text{cpm of total }^{14}\text{C present in the slices}} \times 100$

Significant difference from control value : *b*) $p < 0.05$, *c*) $p < 0.02$

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Discussion

Vernikos-Danellis and Harris[10] reported that 10 mM caffeine or theophyline inhibited the phosphodiesterase of rat brain cortex homogenates *in vitro*. Other reports showed that 0.5 mM theophylline depressed the accumulation of cAMP by adenine[11] or by electrical stimulation[12] in brain cortex slices of the guinea pig. No alteration in cAMP formation was observed in the presence of 1 mM caffeine in this experiment.

L-Glutamine (10 mM), like theanine (10 mM), did not influence cAMP formation in the rat cerebral cortex slices. However, Shimizu *et al.*[13] reported that L-glutamine (10 mM) did enhance cAMP formation in guinea pig cerebral cortex slices. The discrepancy may be due to the species difference in the experimental animals used. No interaction between caffeine and theanine or L-glutamine was observed with regard to cAMP formation.

Theanine showed a significant depressing effect on norepinephrine-stimulated cAMP formation in the presence or absence of caffeine. These results suggest that theanine does not antagonize the action of caffeine on cAMP formation, but antagonizes the stimulating action of norepinephrine.

Histamine-stimulated cAMP formation was also depressed by theanine, but this inhibition was not observed in the presence of caffeine. This phenomenon suggests that the inhibiting effect of theanine on the action of histamine is different from its effect on the action of norepinephrine.

Studies on the influence of theanine on the disposition of brain monoamines are in progress.

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