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Effects of Ginseng Total Saponin on Caffeine-induced Stimulation of Locomotor Activity and the Related Brain Catecholamine Contents in Mice

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Abstract□ This study was undertaken to investigate the effect of ginseng total sapoin (GTS) on locomotor activity that had been increased by caffeine. Catecholamines, noradrenaline and dopamine, possible mediators for the locomotor activity, were measured in the mouse whole brain, cortex and the remainder. The locomotor activity was measured in circular activity cages equipped with six light sources and photocells. The catecholamine contents in the mouse brain were determined by HPLC-fluorescence detection. GTS (50 and 100 mg/kg) reduced the increased locomotor activity by caffeine (25 mg/kg) dose-dependently. Caffeine increased the norepinephrine and dopamine in mouse whole brain and cortex dose-dependently. GTS reduced the norepinephrine in the remainder, and reduced the dopamine in the cortex which had been increased by caffeine.

Keywords□ Ginseng total saponin, caffeine, locomotor activity, brain catecholamine.

Introduction

Caffeine is widely consumed in beverages and use as a psychotropic stimulant agent with effects mood, cognitive performance and motor activity. Caffeine antagonizes potently the effects of adenosine and its analogs[1,2]. Adenosine regulates the action of central nervous system(CNS) excitability by the modulation of catecholamine biosynthesis[2]. Antagonists of adenosine such as caffeine and other methylxanthines increase spontaneous locomotor activity[3,4]. Caffeine has biphasic effects on locomotor activity, raising the activity at lower doses and reducing it at higher doses[5]. The fact that caffeine-induced stimulation of locomotor activity can be prevented by the treatment of catecholamine synthesis inhibitors suggests that catecholamine mediates some of the behavioral effects of caffeine[6]. The biosynthesis and turnover of norepinephrine(NE) in the brain is increased by caffeine[7]. Caffeine also increases plasma catecholamine level[8].

It was reported that ginseng total saponin(GTS) shows CNS-depressant action, in the inhibition of spontaneous and exploratory movement and in potentiation of CNS-depressants[9]. GTS also increases the locomotor activity at low doses but decreases it at high doses[10]. Chronic administration of ginseng butanol fraction (2.5 mg/kg) appears to decrease caffeine-induced spontaneous activity in mice[11].

The present study was undertaken to investigate the effects of GTS on caffeine-induced stimulation of locomotor activity. The brain levels of NE and dopamine(DA), possible mediators for the locomotor activity[6,12,13], were measured in mouse whole brain, cortex and the remainder to examine any correlation between the inhibition of GTS on caffeine-induced stimulation of locomotor activity and the related catecholamine content.

Materials and Methods

Experimental animal and test drug

ICR male mice weighing 20-23 g in a group of

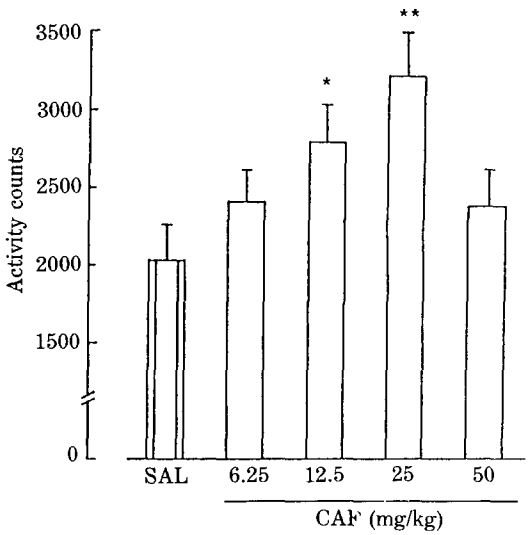

Fig. 1. Effect of caffeine (CAF) on locomotor activity in mice. CAF was administered intraperitoneally (i.p.) to mice. * $p<0.05$, ** $p<0.01$

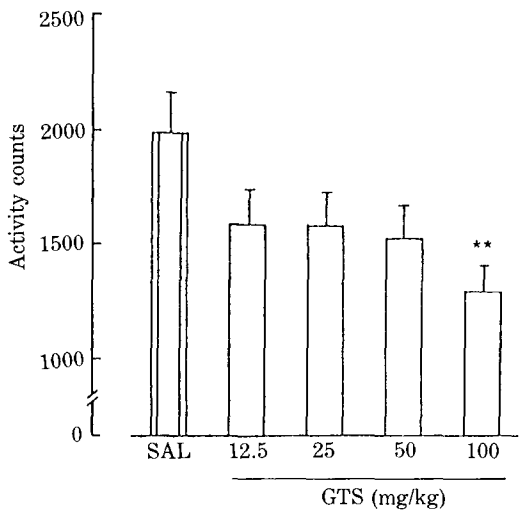

Fig. 2. Effect of ginseng saponin (GTS) on locomotor activity in mice. GTS was administered i.p. to mice. ** $p<0.01$

10-15. were used in all experiments. Animals were housed under controlled temperature, and food and water were provided *ad libitum*.

GST (gift from Korea Ginseng and Tobacco Research Institute) and caffeine-sodium benzoate [50:50(w/w), Sigma] were used in these experiments. Caffeine doses were expressed in terms of the free base of the drug. These drugs were dissolved in saline solution and administered intraperitoneally (i.p.).

Measurement of locomotor activity

The locomotor activity was measured with a circular activity cage[14]. Each cage was 35 cm in diameter and 20 cm in height, and equipped with six light sources and photocells placed just above the floor level.

To test the effects of GTS and caffeine on locomotor activity, each mouse was placed in the activity cage immediately after injection of these drugs for a 5 min preambulation period followed by a 45 min activity recording period. GTS was administered 4 hrs prior to the injection of caffeine 25 mg/kg to test the effects of GTS on caffeine-induced stimulation of locomotor activity. Saline solution was administered i.p. for the control group.

Measurement of NE and DA in mouse brain

The mouse was decapitated and the brain was immediately removed and chilled in an ice bath. All further procedures were conducted at 0-5 °C. The mouse whole brain, cortex and the remainder were homogenied with 4 times of 0.25 M sucrose solution. NE and DA in the sample preparations were determined by high-performance liquid chromatography with fluorescence detection[15].

Statistics

The data were expressed as mean ± S. E.. The difference in the means for different responses in different treatment groups were analyzed by the Student's t-test.

Results

Effects of GTS on caffeine-induced stimulation of locomoto activity

A dose-response relationship or caffeine-induced stimulation of locomotor activity was shown in Fig. 1. Caffeine in doses of 12.5 to 25 mg/kg increased locomotor activity significantly ($p<0.05$, $p<0.01$) but caffeine 50 mg/kg did not incraese the activity significantly. Thus, caffeine 25 mg/kg was used for

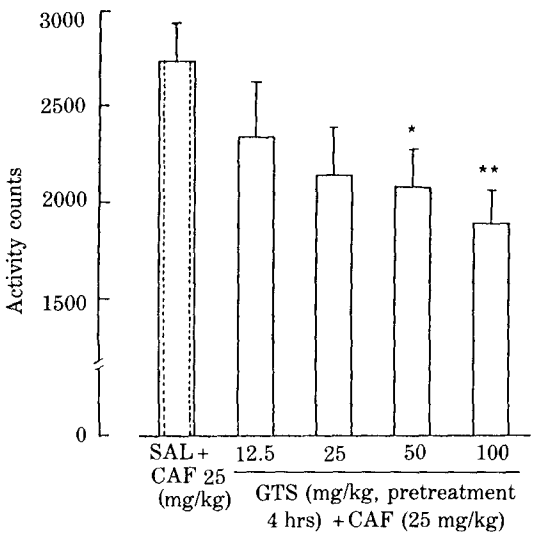

Fig. 3. Effect of GTS on locomotor activity increased by CAF. GTS was administered i.p. 4 hrs prior to the injection of CAF (25 mg/kg) in mice. * $p<0.05$, ** $p<0.01$

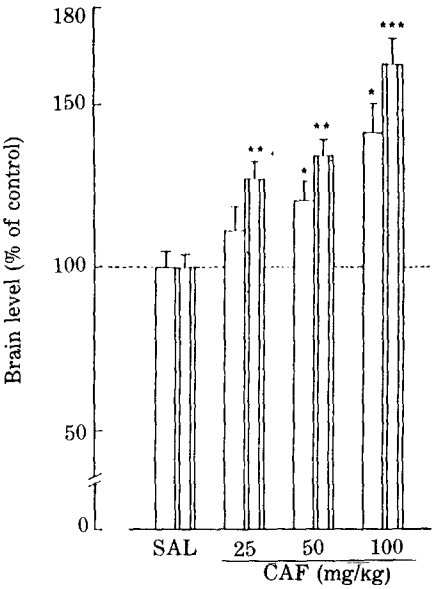

Fig. 4. Effect of CAF on the brain levels of norepinephrine (NE) and dopamine (DA) in mice. CAF was administered i.p. 40 min prior to the decapitation. Data were shown as a percent of the content in control mice (NE, 0.655 ± 0.039; DA, $1.289\pm0.065\mu g/g$ wet tissue). □, NE; ▥, DA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

the test dose of caffeine-induced stimulation in this experiment.

All doses of GTS (12.5-100 mg/kg) decreased locomtor activity. GTS 100 mg/kg especially showed a significant reduction ($p<0.01$) (Fig. 2).

To begin with, the effects of GTS on caffeine-induced locomotor activity at various pretreatment time intervals of GTS was examined. The most significant reduction of GTS was appeared in the group with 4 hrs pretreatment. Therefore, GTS was administered i.p. 4 hrs prior to the injection of caffeine 25 mg/kg. GTS reduced the caffeine-induced stimulation of locomotor activity dose-dependently (Fig. 3).

Effects of GTS on caffeine-induced brain cathcholamine contents

All doses of caffeine (25 to 100 mg/kg) increased the levels of NE and DA in the mouse whole brain dose-dependently. Especially, the DA content was increased most strongly than that of NE by caffeine (Fig. 4). Administration of GTS 50 and 100 mg/kg did not alter the endogenous levels of NE and DA in the mouse whole brain. However, GTS 100 mg/kg reduced the level of DA which had been increased

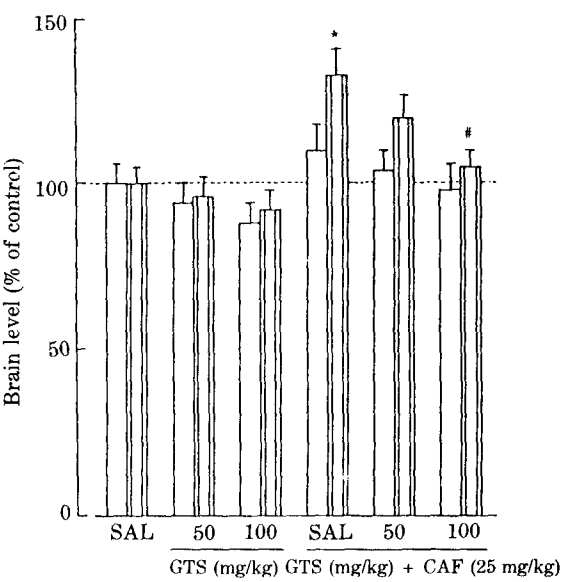

Fig. 5. Effect of GTS on the brain levels of NE and DA in mice. GTS was administered i.p. 4 hrs prior to the injection of CAF (25 mg/kg). For other details, refer to Fig. 4. * $p<0.05$, compared with that of saline (SAL); # $p<0.05$, compared with that of SAL+CAF (25 mg/kg).

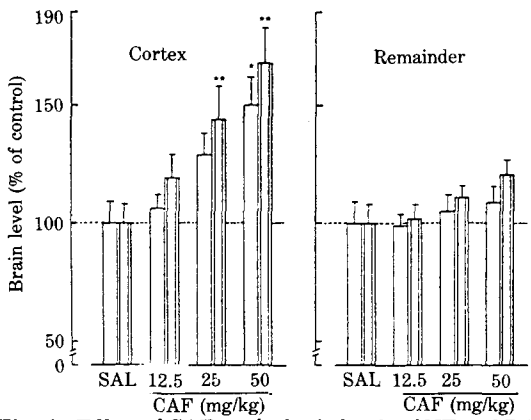

Fig. 6. Effect of CAF on the brain levels of NE and DA in mice. CAF was administered i.p. 40 min prior to the decapitation. For other details, refer to Fig. 4. * $p<0.05$, ** $p<0.01$.

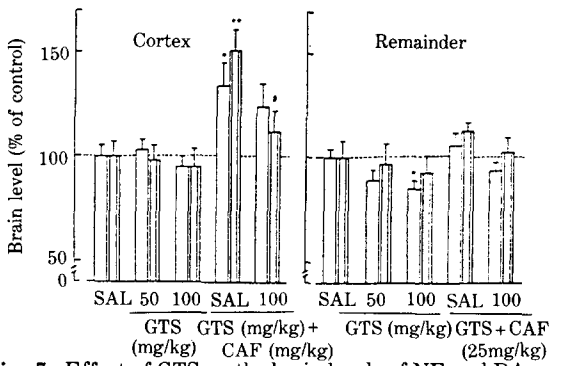

Fig. 7. Effect of GTS on the brain levels of NE and DA in mice. GTS was administered i.p. 4 hrs prior to the injection of CAF (25 mg/kg). For other details, refer to Fig. 4. * $p<0.05$, ** $p<0.01$, compared with that of SAL; #$p<0.05$, compared with that of SAL+CAF (25 mg/kg).

by caffeine 25 mg/kg, but did not reduce the level of NE (Fig. 5).

Administrations of caffeine and GTS modified the levels of NE and DA in the whole brain (Fig. 4 and 5). So the whole brain was dissected into the cortex and the remainder to investigate which part of the brain these drugs affected.

Caffeine (12.5 to 50 mg/kg) increased the levels of NE and DA in brain cortex dose-dependently. In the remainder, the levels of NE and DA were increased somewhat but not significantly. And the level of DA was increased more strongly than that of NE (Fig. 6).

Effects of GTS on the levels of NE and DA in cortex and the remainder was shown in Fig. 7. Administration of GTS in doses of 50 and 100 mg/kg did not alter the endogenous levels of NE and DA in brain cortex, but GTS 100 mg/kg reduced the revel of NE in the remainder. In the cortex, GTS 100 mg/kg reduced significantly the content of DA that had been increased by caffeine (25 mg/kg) but did not reduce the NE content.

Discussion

Caffeine is a potent stimulant of the CNS, acting particulary on the medulla oblongata and brain cortex. In this experiment, caffeine in dose upto 25 mg/kg increases the locomotor activity but 50 mg/kg of caffeine does not increase activity. All doses of caffeine (12.5-100 mg/kg) increase the levels of NE and DA in whole brain, especially in the cortex. It is probable that the depression of locomotor activity at higher doses of caffeine (50 mg/kg), while the levels of NE and DA are increased, is caused by convulsion.

Caffeine inhibits phosphodiesterase, the enzyme that degrades c-AMP[16], and elevates c-AMP contents at the cellular level[17]. The increase in c-AMP may, in turn, activate or sensitize catecholamine postsynaptic receptor[6]. Iversen[18] suggested that adenyl cyclase and c-AMP may be involved in the DA receptor mechanism. The mesolimbic DA system has an important role in the mediation of the behavioral actions of psychomotor stimulations[19]. It is suggested that at least part of the potentiating effect of caffeine on the L-DOPA-induced hypermotility is related to the increased cerebral levels of DA[20]. Caffeine may promote synthesis and/or release of the catecholamine[21]. White *et al.*[6] reported that synthesis of catecholamine is necessary for caffeine-induced stimulation of locomotor activity. Otherwise, it was reported that caffeine in high doses increases c-AMP levels in brain but at low doses decreases c-AMP levels in mouse cortex and subcortex while having no effect in the cerebellum[22].

Caffeine and other methylxanthines are competi-

tive inhibitors at adenosine binding sites in the brain, with relative affinities that correlate with relative potencies for stimulating locomotor activity of mice[1,22].

Some of the CNS effects of caffeine at high concentration may be due to an interaction with benzodiazepine receptors[23]. Low or moderate caffeine concentration may cause stimulatory behavior, whereas high concentration cause convulsion which appears as decreased activity. The decrease in activity may be mediated via the GABA/benzodiazepine receptor system[24].

Therefore, this experiment permits the assumption that caffeine-induced stimulation of locomotor activity was due to the increase of both NE and DA contents in brain cortex.

GTS, 12.5-100 mg/kg, depresses the locomotor activity in mice. It was reported that GTS appears to have a stimulating effect in small doses (2.5 and 5 mg/kg), while it has an inhibitory effect in large doses (50 and 100 mg/kg) on the CNS[10]. GTS does not alter the levels of NE and DA in the whole brain and cortex, but decreases the level of NE in the remainder. These data suggest that some of the CNS inhibitory effects of GTS are correlated with catecholamine levels in the remainder.

In conclusion, GTS reduced caffeine-induced stimulation of locomotor activity dose-dependently and also reduced the level of DA which had been increased by caffeine in the whole brain and the cortex. So we could hypothesize that at least part of the inhibitory action of GTS on the caffeine-induced stimulation of locomotor activity might be due to a reduction of the level of DA in the cortex which had been increased by caffeine.

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