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Inhibitory Effects of Ginseng Total Saponin on Methamphetamine-Induced Striatal Dopamine Increase in Mice

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Amphetamine-like compounds facilitate the release of dopamine (DA) from the synaptosomes and inhibit the DA uptake by the presynaptic neurons (Butcher *et al.*, 1988; Heikkila *et al.*, 1975). These enhanced neurotransmitter activities, particularly at the DA receptors, have been implicated in the locomotor and stereotypy responses, as well as rewarding properties of amphetamine and amphetamine-like stimulants. A single dose of amphetamine (1.0~5.0 mg/kg) produces dose-dependent changes in striatal DA and its metabolite concentrations within 1 hour of the drug administration (Kuczenski, 1977; Schmidt *et al.*, 1986). Generally, investigators agree that amphetamines increase the striatal DA synthesis and inhibit monoamine oxidase, thereby causing a transient increase in striatal DA and homovanillic acid (HVA), and a decrease in dihydroxyphenylacetic acid (DOPAC) levels (Hartman and Halaris, 1980; Miller *et al.*, 1980; Schmidt *et al.*, 1991).

On the other hand, blocking of methamphetamine-induced DA release with the DA uptake inhibitor nomifesine also was shown to block the increase in tissue concentrations of striatal DA produced by methamphetamine administration (Nash and Brodkin, 1991). The preventive effects of DA uptake blockers could be explained by the ability of these compounds to prevent methamphetamine entry into dopaminergic neurons.

Interestingly, it was reported that ginsenosides were potent inhibitors of neurotransmitter (norepinephrine, DA and serotonin) uptake (Tsang *et al.*, 1983). Moreover, we previously reported that GTS inhibited the methamphetamine-induced hyperactivity, sensitization and DA receptor supersensitivity, suggesting that these inhibitions may be closely related to the antidopaminergic actions at the pre- and post-synaptic DA receptors (Kim *et al.*, 1995; Kim *et al.*, 1996). Therefore, this study was performed to investigate the effects of GTS on the methamphetamine-induced DA transient elevation and the changes in levels of its metabolites in the mouse striatum.

Male Swiss-Webster mice (Charles River, Wilmington, MA) weighing 22-30 g were housed individually with free access to food and water in a temperature- and humidity-regulated room with a 12/12-hour light/dark cycle (lights on at 8:00 AM). Methamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline to a concentration of 0.2 mg/ml. GTS characterized saponin mixture quantitatively containing at least 11 glycosides as known ginsenosides [Ra (2.91%), Rb1 (18.26%), Rb2 (9.07%), Rc (9.65%), Rd (8.24%), Re (9.28%), Rf (3.48%), Rg1 (6.42%), Rg2 (3.62%), Rg3 (4.70%), Ro (2.82), and other minor ginsenosides and components (22.55%), according to an HPLC-method for separation and quantitative determination of ginsenosides by Soldati and Sticher with minor modification] from roots of *Panax ginseng* C. A. Meyer extracted and purified by Namba et al.'s methods. GTS was supplied from Korea Ginseng & Tobacco Research Institute and dissolved in 0.9% saline to concentration of 5 or 10 mg/ml.

Mice were intraperitoneally pretreated twice with GTS (50 or 100 mg/kg) at 2 hour inter-injection intervals. Mice were sacrificed 45 min after a subcutaneous injection of methamphetamine (2 mg/kg), and brains were removed and striatal tissue was dissected out and stored in liquid nitrogen until the HPLC assay with electrochemical detection for DA, DOPAC and HVA (Jakubovic *et al.*, 1987). Data were expressed as mean % SE. The statistical significance was first analyzed by variance (ANOVA). In case of significant variation, the individual values were compared by the Student's t-test.

A single administration of methamphetamine (2 mg/kg) increased the concentration of DA with concomitant decreases in the concentration of DOPAC in the mouse striatum, which is in agreement with previous reports (Heikkila *et al.*, 1980, Nash *et al.*, 1991). The pretreatment with two injections of GTS (100 mg/kg) caused a significant reduction in the methamphetamine-induced DA increase. Moreover, GTS (100 mg/kg) significantly restored the methamphetamine-in-

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Table I. Effects of GTS on the striatal DA, DOPAC and HVA concentrations after a single methamphetamine (MA)

	n	DA	DOPAC	HVA
Sal+Sal	6	9.83±0.24	0.83±0.06	1.29±0.13
Sal+MA	8	13.32±0.98*	0.47±0.02**	1.31±0.11
GTS 50+MA	12	10.48±0.75	0.50±0.05	1.42±0.14
GTS 100+MA	10	9.86±0.78#	0.58±0.04#	1.39±0.12
GTS 100+Sal	7	9.49±0.54	0.85±0.05	1.12±0.12

Values reported are in μg of compound/g of tissue. Data represents mean±SE of animals. *P<0.05, **P<0.01, values are significantly different from saline (Sal), #P<0.05, values are significantly different from MA control.

duced DOPAC decrease (Table 1).

Most research aimed at elucidating neurobiological mechanisms underlying the altered responsiveness to amphetamine and related stimulants has focused on DA dynamics, since converging evidence suggests that DA systems play a critical role in acute and chronic stimulant response (Kuczenski and Segal, 1989). A single methamphetamine increases striatal DA synthesis and elevates the tissue DA concentration. Moreover, the inhibition of monoamine oxidase by methamphetamine augments the DA concentrations and concomitantly reduces DOPAC (Miller *et al.*, 1980). These changes were transient and disappeared by 12 hrs GTS inhibited the methamphetamine-induced DA increase and also methamphetamine-induced DOPAC increase. Therefore, it is suggested that GTS may modulate either the release of dopamine or its degradation by monoamine oxidase. It has been reported that such an acute rise in striatal DA concentration may increase the synthesis of DA in the presynaptic neurons (Butcher *et al.*, 1988; Kuczenski and Segal, 1989). Generally, it has been postulated that drugs that reduce the availability of catecholamines in the presynaptic neuron attenuate the behavioral effects of stimulants (such as hyperactivity and self administration) in rodents (Pickens *et al.*, 1968). In a separate serious studies, we previously reported that GTS inhibited methamphetamine-induced hyperactivity, conditioned place preference and sensitization (Kim *et al.*, 1995; Kim *et al.*, 1996). Likewise, we have demonstrated that pretreatment with GTS reduced the dopaminergic toxicity induced by methamphetamine (Kim *et al.*, 1995, Kim *et al.*, 1996; Oh *et al.*, 1997) an effect known to be dependent on DA release (Wagner *et al.*, 1980; Wagner *et al.*, 1983).

Alternatively, the methamphetamine-induced DA release can be prevented by the pretreatment with DA uptake inhibitors, thereby reducing the tissue elevation of striatal DA produced by the methamphetamine administration (Nash and Brodkin, 1991). Tsang *et al.* have reported that ginsenosides are the potent inhibitors of DA uptake. Therefore, the ability of GTS to block the effects by methamphetamine could be linked to its ability to block the DA uptake.

In conclusion, biochemical analysis revealed that GTS inhibited the methamphetamine-induced DA increase and the methamphetamine-induced DOPAC decrease in the mouse striatum. These results indicate that GTS could modulate the methamphetamine-induced striatal dopaminergic neuronal systems.

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