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Effect of Ginseng Saponins on Enhanced Dopaminergic Transmission and Locomotor Hyperactivity Induced by Nicotine

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Several studies have shown that behavioral hyperactivity induced by psychomotor stimulants is prevented by ginseng saponins. In an attempt to investigate whether the effect of ginseng saponins is through their inhibitory action on the enhanced dopaminergic transmission by psychomotor stimulants, we examined the effects of ginseng total saponin (GTS) presynaptically on nicotine-induced dopamine (DA) release in the striatum of freely moving rats using *in vivo* microdialysis technique and postsynaptically on the *in vitro* and *in vivo* binding of [^{3}H]raclopride to DA D_2 receptors. Also, we examined the effects of GTS on nicotine-induced locomotor hyperactivity and on nicotine-induced Fos protein expression in the nucleus accumbens and striatum. Systemic pretreatment with GTS (100 and 400 mg/kg, intraperitoneally (i.p.)) resulted in a dose-dependent inhibition of locomotor hyperactivity induced by nicotine. GTS decreased nicotine-induced DA release in the striatum in a dose-dependent manner. However, GTS had no effects on resting levels of locomotor activity and extracellular DA in the striatum. GTS inhibited the *in vitro* binding of [^{3}H]raclopride to rat striatal membranes with an IC_{50} of 5.14 ± 1.09 μM. High doses of GTS (400 and 800 mg/kg, i.p.) resulted in decreases in the *in vivo* binding of [^{3}H]raclopride in the striatum. GTS decreased nicotine-induced Fos protein expression in the nucleus accumbens and striatum, reflecting the inhibition by GTS of nicotine-induced enhancement of dopaminergic transmission. The results of the present study suggest that GTS acts not only on dopaminergic neurons directly or indirectly to prevent nicotine-induced DA release but also postsynaptically by binding to DA D_2 receptors. This may explain the blocking effect of GTS on behavioral activation induced by nicotine and conceivably by other psychostimulants. Our data raise the possibility that GTS, by attenuating nicotine-induced enhancement of dopaminergic transmission, may prove to be a useful therapeutic agent for nicotine addiction and warrant further investigation on its effect on nicotine's rewarding property.

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Keywords: ginseng total saponin; nicotine; dopamine release; dopamine D_2 receptor; Fos protein; locomotor activity

INTRODUCTION

Evidence suggests that nicotine, the neuroactive compound of tobacco, has common neurochemical as well as behavioral properties with other addictive drugs such as cocaine and amphetamine (Henningfield and Heishman, 1995; Pontieri *et al*, 1996). It is well documented that nicotine stimulates dopamine (DA) release in the nucleus accumbens and striatum (Imperato *et al*, 1986; Damsma *et al*, 1989; Toth *et al*, 1992; Benwell and Balfour, 1994; Nisell *et al*, 1994a; Pontieri *et al*, 1996; Mirza *et al*, 1996; Marshall *et al*, 1997). This property has been related to the addictive and behavioral properties of nicotine (Benwell and Balfour, 1992; Corrigall *et al*, 1992; Di Chiara and Imperato, 1988).

Ginseng, the root of *Panax ginseng* CA Meyer, has been used widely as a herbal medicine across the world. Many investigators have reported its chemical and pharmacological properties and saponin appears to be responsible for most of the pharmacological effects of ginseng. Behavioral studies have suggested that ginseng saponins or ginsenosides may act on the central dopaminergic system. Pretreatment with ginseng saponins or ginseng extract was able to block the development of behavioral sensitization induced by cocaine (Kim *et al*, 1995a), methamphetamine (Tokuyama *et al*, 1992), and morphine (Kim *et al*, 1995b). The conditioned place preference induced by these drugs was also antagonized by ginseng saponins (Kim *et al*,

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1996a, b). Also, it has been shown that ginseng saponins inhibit nicotine-induced hyperlocomotion, behavioral sensitization, and conditioned place preference (Kim and Kim, 1999). However, the neurochemical mechanisms underlying the behavioral effects of ginseng saponins are not well understood.

Convergent evidence suggests that drugs of abuse exert their locomotor stimulating and rewarding effects by increasing dopaminergic transmission. Therefore, it seems possible that the behavioral effects of ginseng saponins reflect their blockade of enhanced dopaminergic transmission by those drugs. In an attempt to examine this hypothesis, we investigated the following: (1) the effect of ginseng total saponin (GTS) on nicotine-induced locomotor hyperactivity; (2) the effect of GTS presynaptically on nicotine-induced DA release in the striatum of freely moving rats using *in vivo* microdialysis technique; (3) the effect of GTS postsynaptically on the *in vitro* and *in vivo* binding of [^{3}H]raclopride to DA D_2 receptors; and (4) the effect of GTS on nicotine-induced Fos protein expression in the nucleus accumbens and striatum.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, weighing 280–320 g, were used for *in vivo* microdialysis, behavioral, *in vitro* receptor binding, and immunohistochemical studies. Male ICR mice (25–30 g) were used for *in vivo* binding studies. The animals were group-housed in light-, temperature-, and humidity-controlled animal quarters with food and water available *ad libitum*. All procedures involving animal use were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Seoul National University Hospital Animal Care and Use Committee.

Drugs and Chemicals

(−)-Nicotine hydrogen tartrate was purchased from Sigma Chemical Co. (St Louis, MO). GTS was a gift from Korea Ginseng and Tobacco Research Institute (Daejeon, Korea). GTS (a characterized saponin mixture quantitatively containing nine major glycosides known as ginsenosides (Rb_1 20.14%, Rb_2 10.19%, Rc 11.34%, Rd 4.63%, Re 12.27%, Rf 3.01%, Rg_1 16.44%, Rg_2 2.01%, and Rg_3 2.64%) and other minor ginsenosides and components (17.33%), according to an HPLC method of Ko *et al* (1992), from *P. ginseng*) was extracted and purified using the method of Ando *et al* (1971). [^{3}H]raclopride (79.3 Ci/mmol) was purchased from New England Nuclear (Boston, MA).

Surgery and Microdialysis Procedures

Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally (i.p.)). Using aseptic techniques, a guide cannula (CMA/12, CMA Microdialysis, Solna, Sweden) aimed to terminate in the dorsal striatum (AP 1.0, L 3.2 from bregma; H 3.0 from dura) (Paxinos and Watson, 1986) was stereotaxically implanted and attached to the skull using skull screws and dental cement. The cannula was then closed with a tight-fitting stainless-steel obturator. The microdialysis was performed in freely moving rats. Following 3-day recovery, a 4 mm microdialysis probe (CMA/12, CMA Microdialysis) connected via a dual liquid swivel to a syringe pump was inserted into the guide cannula and perfused with an artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4; pH 7.4) at a constant rate of 1.5 μl/min. Dialysate samples were collected during 20 min sampling intervals via outlet tubing connected to a microfraction collector (CMA Microdialysis). The position of the probes was verified by histological examination at the end of experiments.

Analytical Procedures

The dialysate (injection volume 30 μl) was assayed for DA using HPLC coupled to an ESA Coulochem II 5200A electrochemical detection system with an oxidation potential of +320 mV. The detector was equipped with a high-performance analytical cell (ESA model 5014), which is tailored for use in microdialysis applications. The mobile phase was composed of 75 mM monobasic sodium phosphate, 0.1 mM EDTA, 1.4 mM octanesulfonic acid and 10% acetonitrile, and adjusted to pH 3.2 with HPLC grade phosphoric acid. The separation of monoamine metabolites was performed on a Waters Nova-Pak C-18 column (4 μm, 150 × 3.9 mm). The flow rate of the system was 1.0 ml/min. DA in dialysates was expressed as a percentage of three baseline samples collected immediately before nicotine treatment.

Drug Treatments

Nicotine was dissolved in the artificial cerebrospinal fluid to the concentration of 10 mM (expressed as free base) and infused for 60 min locally into the striatum through the dialysis probe. GTS (100 and 400 mg/kg, dissolved in saline) was injected i.p. 60 min before nicotine was administered.

Measurement of Locomotor Activity

The effect of GTS on nicotine-induced behavioral hyperactivity was evaluated in rats by measuring locomotor activity using a stabilimeter interfaced to a personal computer system. The stabilimeter, in which a loudspeaker is used both as platform and transducer, was constructed based on the design described by Parreno *et al* (1985). In our preliminary studies, this equipment provided a good reproducibility and a high sensitivity for measuring locomotor activity of small animals. Each animal was placed on the stabilimeter and after a habituation period of 60 min followed by the measurement of baseline activity for 60 min, nicotine (10 mM) was infused for 60 min locally into the striatum through the dialysis probe. Control animals were implanted with the dialysis probe and infused with the artificial cerebrospinal fluid. Locomotor activity was measured during 20 min intervals for a total of 180 min after the administration of nicotine. GTS (100 and 400 mg/kg) was injected i.p. 60 min prior to nicotine.

In Vitro Inhibition Experiments

Inhibition by GTS of the *in vitro* binding of [^{3}H]raclopride to striatal DA D_2 receptors was investigated. Rats were killed by rapid decapitation. The striata were quickly dissected, frozen on a block of dry ice, and stored at -70°C until needed. The binding of [^{3}H]raclopride to rat striatal membranes was conducted as described by Lidow *et al* (1989). Briefly, tissues were homogenized in ice-cold 50 mM Tris · HCl buffer (pH 7.4) and centrifuged at 45 000 *g* for 10 min. The resulting pellet was resuspended in 20 volumes of buffer, recentrifuged, and resuspended at 10 mg wet weight/ml of incubation buffer (50 mM Tris · HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$). Inhibition assays of [^{3}H]raclopride were performed using a fixed concentration of [^{3}H]raclopride (4.3 nM) and increasing concentrations of GTS (10 pM to 10 mM). The incubations were conducted in a final volume of 250 µl for 30 min at 20–22°C. The incubation was terminated by rapid filtration through Whatman GF/B filters previously soaked in 0.05% polyethylenimine using a Brandel MP-48LT filtering manifold (Gaithersburg, MD). The radioactivity trapped in the filters was measured in a liquid scintillation counter (Tri-Carb 2500 TR, Packard Instrument Co., Meriden, CT) after addition of 5 ml Aquassure scintillator (Packard). Nonspecific binding was defined using 1 µM (+)-butaclamol.

In Vivo Binding Experiments

The effect of GTS on the *in vivo* binding of [^{3}H]raclopride was investigated by pretreating mice with different doses of GTS (50–800 mg/kg, i.p.) 60 min before injection of [^{3}H]raclopride. [^{3}H]raclopride (1 µCi in 0.2 ml saline) was injected intravenously into a tail vein, and 15 min later, the animals were killed by cervical dislocation. Different brain regions were immediately dissected on ice, weighed and placed into glass vials. After digestion of the tissues with Soluene-350 (Packard), 10 ml of Formula 989 scintillator (Packard) was added and the samples counted in a liquid scintillation counter.

Fos Immunohistochemistry

The effect of GTS on nicotine-induced Fos protein expression in dopaminergic target areas was examined. Rats received subcutaneous (s.c.) injection of nicotine (0.4 mg/kg; expressed as free base) 2 h before anesthesia (ketamine 80 mg/kg, i.p. plus xylazine 10 mg/kg, i.p.). GTS (100 mg/kg) was administered i.p. 60 min before nicotine injection. Rats were transcardially perfused with ice-cold 0.1 M phosphate buffered saline (PBS), pH 7.4 followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed and post-fixed with the same fixative for 2 h at room temperature and immersed in a 20% sucrose solution overnight at 4°C. The frozen tissue was cut into 30 µm thick sections on a cryostat. Coronal sections were rinsed in PBS (2×5 min) and placed in 0.3% H_2O_2 in methanol for 15 min and then washed in PBS (3×5 min). The sections were preincubated in a 2% normal goat serum (DAKO, Glostrup, Denmark) in PBS-T (0.1% Triton X-100 in PBS) for 40 min to block nonspecific staining and then incubated with the primary antibody (rabbit polyclonal Fos antibody, Oncogene Research, Cambridge, MA) diluted in 1 : 1000 for 48 h at 4°C. The sections were washed in PBS-T (3×5 min) and incubated for 1 h with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA). After washing with PBS (3×5 min), the sections were incubated for 1 h with PBS-T containing avidin-biotinylated horseradish peroxidase complex (1 : 100, Vector Laboratories). The sections were washed in PBS (3×5 min) and then rinsed for 10 min in 0.175 M sodium acetate buffer (pH 7.5). The immunoreactive complex was revealed using diaminobenzidine (DAB) and nickel sulfate ($NiSO_4$) (0.2 mg/ml DAB, 25 mg/ml $NiSO_4$ and 0.83 µl/ml 3% H_2O_2 in 0.175 M sodium acetate) for 4 min. Sections were washed with PBS twice and mounted on silane-coated slides, air-dried, dehydrated through graded ethanols into xylene, and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA). No Fos-like immunoreactivity has been observed when the primary antibody was omitted. The number of Fos-positive nuclei were counted by using the IBAS image analysis system (Zeiss, Germany) within a squared field area of 210×210 µm for the nucleus accumbens and 420×420 µm for the striatum using $\times 200$ magnifications. The mean value of the three to six sections was calculated from each selected region.

Statistical Analysis

Unless otherwise stated, values are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) or two-way ANOVA (drug treatment × time) with repeated measures on the time factor. The origin of significant effects was further examined by *post hoc* comparisons using the Bonferroni technique.

RESULTS

Effect of GTS on Nicotine-Induced Locomotor Hyperactivity

Figure 1 shows the effect of GTS on nicotine-induced hyperlocomotion. Local infusion of nicotine (10 mM) into the striatum via the dialysis probe increased the locomotor activity (maximal response = 589% of controls). This effect was attenuated by systemic pretreatment with GTS. An ANOVA indicated a significant effect of drug treatments ($F_{3,12} = 8.7$, $p < 0.005$). *Post hoc* comparisons revealed that GTS attenuated the nicotine-induced hyperlocomotion in a dose-dependent manner. GTS (100 and 400 mg/kg, i.p.) decreased the nicotine-induced maximal locomotor activity by 37% ($p < 0.01$) and 58% ($p < 0.005$), respectively. However, GTS (100 and 400 mg/kg, i.p.) had no effect on resting levels of locomotor activity until 180 min after injection ($F_{2,9} = 0.7$, $p =$ NS) (Figure 3).

Effect of GTS on Nicotine-Induced DA Release

Figure 2 shows the effect of GTS on nicotine-induced changes in extracellular DA in the striatum. Local infusion of nicotine (10 mM) into the striatum produced an increase in extracellular DA in the striatum (maximal response = 500 ± 78% of basal levels). This effect was

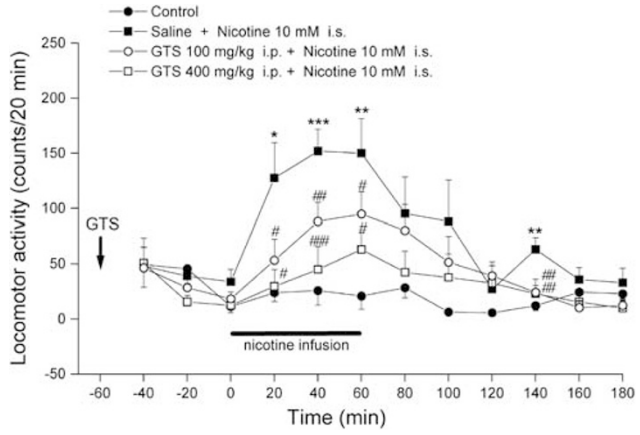

Figure 1 Effect of GTS on nicotine-induced hyperlocomotion. Nicotine (10 mM) was infused intrastriatally (i.s.) for 60 min beginning at time 0. GTS (100 and 400 mg/kg) was administered i.p. 60 min before nicotine infusion. Results are means ± SEM of four independent experiments. $^{*}p<0.05$, $^{**}p<0.005$, $^{***}p<0.001$ *vs* the control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.005$ *vs* the saline plus nicotine group.

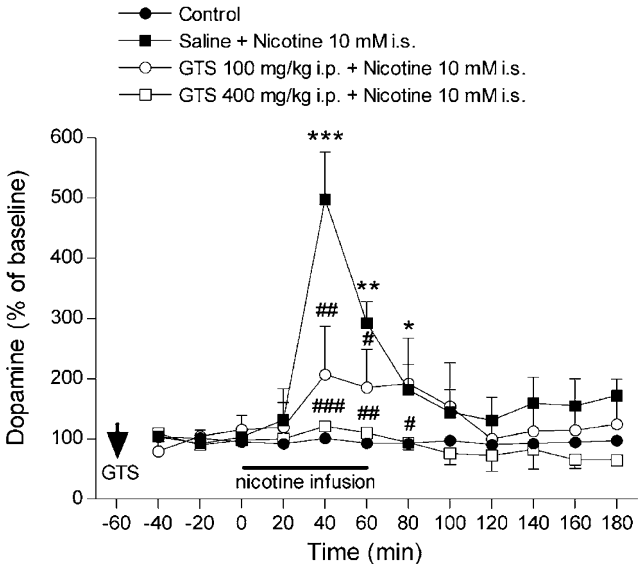

Figure 2 Effect of GTS on nicotine-induced changes in extracellular DA in the striatum. Nicotine (10 mM) was infused intrastriatally (i.s.) for 60 min beginning at time 0. GTS (100 and 400 mg/kg) was administered i.p. 60 min before nicotine infusion. Results are expressed as a percentage of three baseline samples and are means ± SEM of 4–6 independent experiments. $^{*}p<0.05$, $^{**}p<0.005$, $^{***}p<0.001$ *vs* the control group; $^{\#}p<0.05$, $^{\#\#}p<0.005$, $^{\#\#\#}p<0.001$ *vs* the saline plus nicotine group.

attenuated by GTS. An ANOVA indicated a significant effect of drug treatments ($F_{3,16}=9.2$, $p<0.001$). *Post hoc* comparisons revealed that GTS attenuated the nicotine-induced DA release in a dose-dependent manner. GTS (100 and 400 mg/kg, i.p.) inhibited the nicotine-induced maximal DA response by 58% ($p<0.005$) and 76% ($p<0.001$), respectively. However, GTS (100 and 400 mg/kg, i.p.) had no effect on resting levels of extracellular DA in the striatum until 180 min after injection ($F_{2,11}=0.9$, $p=$ NS) (Figure 3).

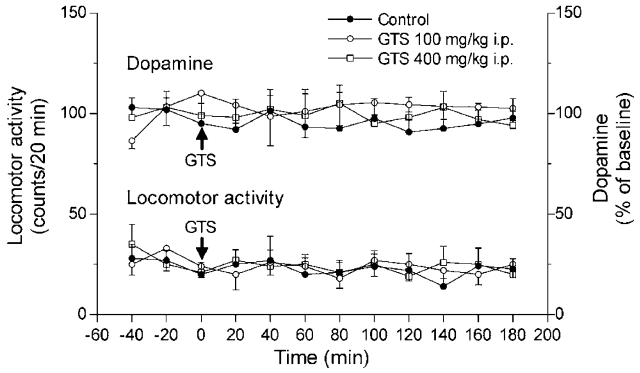

Figure 3 Effect of GTS on resting levels of locomotor activity and extracellular DA in the striatum. GTS (100 and 400 mg/kg) was administered i.p. at time 0. There were no significant differences in resting levels of locomotor activity and extracellular DA between control and GTS-treated animals. Results are means ± SEM of 4–5 independent experiments.

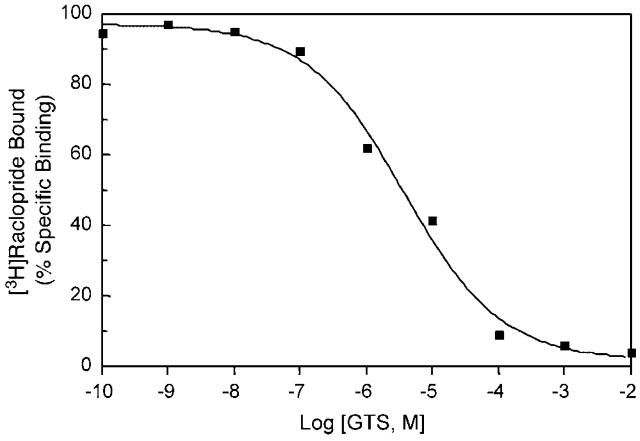

Figure 4 Inhibition of specific [^{3}H]raclopride binding by GTS in rat striatal membranes. Presented are the results of a representative experiment; points are the means of triplicate determinations.

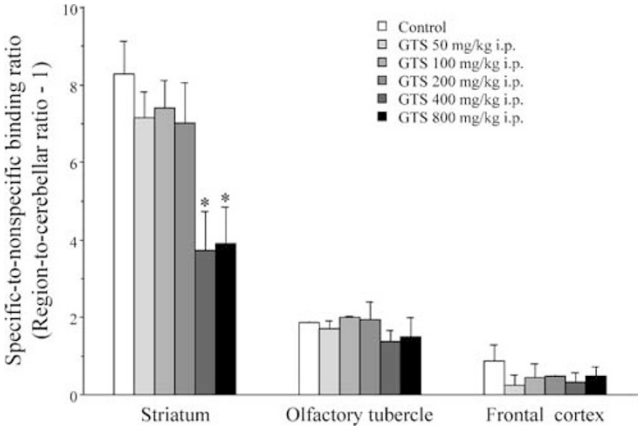

Figure 5 Effect of GTS on the *in vivo* binding of [^{3}H]raclopride in brain regions. GTS (50–800 mg/kg) was administered i.p. 90 min before intravenous injection of [^{3}H]raclopride. *In vivo* [^{3}H]raclopride binding was measured 15 min after radiotracer injection. Results are expressed as ratios of specific-to-nonspecific binding (region-to-cerebellar ratio—1) and are means ± SD; $n=4$ for each group. $^{*}p<0.0001$.

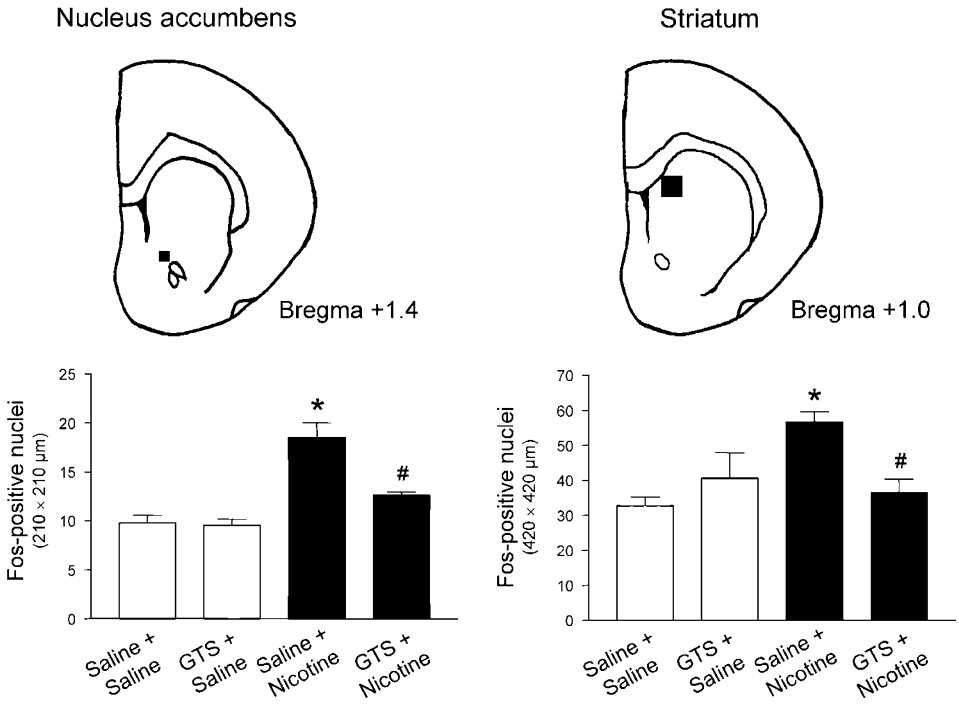

Figure 6 Effect of GTS on nicotine-induced Fos protein expression in the nucleus accumbens and striatum, with schematic drawings indicating areas in which Fos-positive nuclei were counted. GTS (100 mg/kg) was administered i.p. 60 min before nicotine injection (0.4 mg/kg, s.c.). Results are means ± SEM of four independent experiments. $^{*}p<0.05$ *vs* the saline plus saline group, $^{\#}p<0.05$ *vs* the saline plus nicotine group.

Effect of GTS on the *In Vitro* Binding of [^{3}H]raclopride

GTS inhibited the binding of [^{3}H]raclopride to striatal membranes with an IC_{50} of 5.14 ± 1.09 μM (mean ± SEM of five independent experiments) (Figure 4).

Effect of GTS on the *In Vivo* Binding of [^{3}H]raclopride

Figure 5 shows the effect of GTS on the *in vivo* binding of [^{3}H]raclopride in different brain regions. Doses of 50–200 mg/kg GTS resulted in 11–15%, but not significant decreases in the *in vivo* binding of [^{3}H]raclopride in the striatum. Increasing the dose of GTS to 400 and 800 mg/kg led to abrupt decreases in striatal [^{3}H]raclopride binding by 55% ($p<0.0001$) and 53% ($p<0.0001$), respectively. Regional analysis of the binding data indicated that the maximal effect of GTS on the *in vivo* binding of [^{3}H]raclopride occurred in the striatum, a region rich in DA D_2 receptors.

Effect of GTS on Nicotine-Induced Fos Protein Expression

Figure 6 shows the effect of GTS on nicotine-induced Fos protein expression in dopaminergic target areas. Nicotine (0.4 mg/kg, s.c.) increased the number of Fos-positive nuclei in the nucleus accumbens and striatum by 89% ($p<0.05$) and 73% ($p<0.05$), respectively. GTS (100 mg/kg, i.p.) decreased the nicotine-induced Fos protein expression in the nucleus accumbens and striatum by 32% ($p<0.05$) and 36% ($p<0.05$), respectively. GTS did not influence resting levels of Fos-positive nuclei in the nucleus accumbens and striatum (see Figure 6).

DISCUSSION

This study demonstrated that systemic pretreatment with GTS inhibits nicotine-induced hyperlocomotion and striatal DA release in a dose-dependent manner. As behavioral hyperactivity produced by nicotine is associated with an increase in DA release in the striatum as well as in the nucleus accumbens (Shim *et al*, 2001), the inhibitory action of GTS on nicotine-induced DA release in the striatum explains, at least in part, its blocking effect on behavioral activation produced by nicotine. We also found that GTS binds, with a modest affinity, to DA D_2 receptors both *in vitro* and *in vivo*. Because GTS had no effect on resting levels of extracellular DA, the inhibition by GTS of *in vivo* binding of [^{3}H]raclopride is not due to changes in synaptic DA levels. We do not know the function of GTS at DA D_2 receptor sites. In the present study, the dose-dependent effect of GTS against intrastriatal infusion of nicotine was greater for the reduction of locomotor activity (57% greater reduction with GTS 400 mg/kg compared with GTS 100 mg/kg) than for the reduction of striatal DA release (31% greater reduction with GTS 400 mg/kg compared with GTS 100 mg/kg). Although it is not certain whether the magnitude of locomotor activity changes proportionally to that of DA release, this finding suggests that mechanisms other than DA release inhibition may be involved in the behavioral effect of high-dose GTS. This idea is supported by our finding that high doses of GTS (400 and 800 mg/kg) inhibited the *in vivo* binding of [^{3}H]raclopride to DA D_2 receptors in the striatum. Also, it has been reported that GTS showed an antidopaminergic action at postsynaptic DA receptor sites by inhibiting apomorphine-induced climbing behavior (Kim *et al*, 1996a). In addition, high-dose ginseng

extract inhibited adenylate cyclase activity in rat brain tissues (Petkov, 1978). Taken together, it is likely that GTS may act on DA D_2 receptors as an antagonist. The results of the present study suggest that GTS acts not only on dopaminergic neurons directly or indirectly to prevent nicotine-induced DA release but also postsynaptically by binding to DA D_2 receptors. This may explain the blocking effect of GTS on behavioral activation induced by nicotine.

As nicotine increases DA release by stimulating nicotinic acetylcholine receptors (nAChRs) located in the terminal and somatodendritic regions of the dopaminergic neurons (Marshall *et al*, 1997; Mifsud *et al*, 1989; Wonnacott *et al*, 1990; Nisell *et al*, 1994a, b), it is possible that GTS inhibits DA release by acting on nAChRs in the dopaminergic neurons. Supporting this idea, it has been reported that ginseng saponins reduced acetylcholine-evoked Na^+ influx and catecholamine secretion in bovine adrenal chromaffin cells, but did not affect catecholamine secretion induced by high K^+ (an activator of voltage-sensitive Ca^{2+} channels) or veratridine (an activator of voltage-sensitive Na^+ channels) (Tachikawa *et al*, 1995). Also, it has been shown that GTS had no effect on DA release induced by intrastriatal infusion of high K^+ solution (Shim *et al*, 2000). These data suggest that GTS blocks the nAChRs or the receptor-operated Na^+ channels (but not voltage-sensitive Na^+ and Ca^{2+} channels), inhibit Na^+ influx through the channels and consequently reduce both Ca^{2+} influx and DA release. In our preliminary studies, GTS did not affect the binding of [^{3}H]nicotine to rat striatal membranes. This suggests that GTS may act on the receptor-operated ion channels rather than nAChRs or on the nAChR subtypes not labeled by [^{3}H]nicotine. However, there is possibility that GTS acts on GABAergic and glutamatergic neurons, receptors of the neurotransmitters, or nAChRs located in these neurons, because these neurons interact with dopaminergic neurons in the mesoaccumbens and nigrostriatal systems to modulate DA release (Mansvelder and McGehee, 2002; Smolders *et al*, 1995). Indeed, several studies provided evidence that ginsenosides may regulate the $GABA_A$ receptor. For example, ginsenosides differentially regulated [^{3}H]flunitrazepam and [^{3}H]muscimol binding to the $GABA_A$ receptor in a rat brain membrane fraction (Kimura *et al*, 1994). Prolonged infusion with ginsenoside Rc but not with ginsenoside Rg_1 into rat brain elevated [^{3}H]muscimol binding to the $GABA_A$ receptor in a region-specific manner (Kim *et al*, 2001). In addition, Tsang *et al* (1985) reported that ginseng saponins inhibited uptake of GABA in rat brain synaptosomes. Also, it has been shown that ginsenosides attenuate glutamate-induced injury of neuronal and non-neuronal cells in the brain (Abe *et al*, 1994; Seong *et al*, 1995; Kim *et al*, 2002).

Ginseng saponins were able to attenuate behavioral activation induced not only by nicotine but also by other psychostimulants, such as cocaine (Kim *et al*, 1995a), methamphetamine (Tokuyama *et al*, 1992), and morphine (Kim *et al*, 1995b). Interestingly, we found that GTS also inhibited cocaine-induced increase in extracellular DA without affecting [^{3}H]WIN 35 428 binding to the DA transporter in rat striatum (data not shown). In other words, GTS attenuated the behavioral and extracellular DA increasing effects of psychostimulants that behave with different mechanisms (ie, increasing DA release (nicotine) *vs* blocking DA transporters (cocaine)). This suggests that GTS may act on the final common pathways of the negative feedback loop for the regulation of dopaminergic neurotransmission.

Drug treatments affecting dopaminergic transmission modulate the expression of Fos protein encoded by *c-fos* proto-oncogene in the striatum (Graybiel *et al*, 1990; Robertson *et al*, 1990; Herrera and Robertson, 1996; Moratalla *et al*, 1996). Acute nicotine elevates Fos expression in various brain regions, including dopaminergic target areas (Ren and Sagar, 1992; Matta *et al*, 1993; Pang *et al*, 1993; Kiba and Jayaraman, 1994; Panagis *et al*, 1996; Salminen *et al*, 1996; Valentine *et al*, 1996). In the present study, GTS decreased nicotine-induced Fos protein expression in the nucleus accumbens and striatum. This reflects the inhibition of nicotine-induced enhancement of dopaminergic transmission by GTS. From our results, the effect of GTS may be due in large measure to its inhibitory effect on DA release because DA D_1 receptor, but not D_2 receptor, mediates nicotine-induced Fos expression in the striatum and nucleus accumbens (Kiba and Jayaraman, 1994; Nisell *et al*, 1997; Svenningsson and Le Moine, 2002). In addition, GTS may exert an inhibitory effect on DA D_1 receptors, which should be investigated by further studies. We found that GTS did not affect resting levels of Fos expression. This is in line with the finding that GTS had no effect on resting levels of extracellular DA.

In this study, for the microdialysis and locomotor activity experiments, nicotine was perfused via the microdialysis probe in a concentration of 10 mM at a rate of 1.5 μl/min (ie, in a dose of 15 nmol/min). Previous microdialysis studies have shown that local perfusion of 1–10 mM (1.5–40 nmol/min) nicotine elicited a reliable and consistent increase in DA content of the striatal dialysate samples (Marshall *et al*, 1997; Lecca *et al*, 2000; Shim *et al*, 2001). Lecca *et al* (2000) reported that perfusion of 0.2 nmol/min nicotine failed to increase DA content, while perfusion of 2 or 10 nmol/min nicotine significantly enhanced DA content in striatal dialysate samples. Also, in our pilot studies, 5 or 10 mM nicotine produced a stable and reliable increase in both DA release and locomotor activity. Thus, we used 10 mM nicotine in our experiments.

A major limitation of this study is that more direct measures of nicotine reinforcement, such as nicotine self-administration and nicotine-induced conditioned place preference, were not employed to assess the effects of GTS. The effects of GTS on those measures of nicotine reinforcement should be examined before drawing definitive conclusions on the potential effects of GTS on nicotine intake. To our knowledge, there is no clinical trial evidence or anecdotal report on the relationship between ginseng use and decreased tobacco consumption. Obviously, clinical studies for the elucidation of the efficacy of ginseng in smoking cessation treatment should be performed in the future.

In conclusion, the results of the present study suggest that GTS acts not only on dopaminergic neurons directly or indirectly to prevent nicotine-induced DA release, but also postsynaptically by binding to DA D_2 receptors. This may explain the blocking effect of GTS on behavioral activation induced by nicotine and conceivably by other psychostimulants. Our data raise the possibility that GTS, by

attenuating nicotine-induced enhancement of dopaminergic transmission, may prove to be a useful therapeutic agent for nicotine addiction and warrant further investigation on its effect on nicotine's rewarding property.

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