Published in final edited form as:

Brain Res. 2009 January 12; 1248: 184–190. doi:10.1016/j.brainres.2008.10.064.

Effects of ginseng saponin on acute cocaine-induced alterations in evoked dopamine release and uptake in rat brain nucleus accumbens

Seong-Yeol Nah^a, Kamal S. Bhatia^b, Johnnie Lyles^b, Everett H. Ellinwood^{b,c}, and Tong H. Lee^{b,*}

^a Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul Korea 143-701

^b Department of Psychiatry, Duke University Medical Center, Durham, NC, 27710 U.S.A

^c Department of Pharmacology, Duke University Medical Center, Durham, NC, 27710 U.S.A

Abstract

In traditional medicine, *Panax* ginseng has been used to treat various behavioral effects of psychostimulants (e.g., cocaine) and other drugs of abuse and to ameliorate withdrawal symptoms. The neurochemical bases for this efficacy, however, remain to be elucidated. We previously used the real-time fast-scan cyclic voltammetry in rat nucleus accumbens slices to demonstrate that cocaine not only enhances DA release evoked by single-pulse electrical stimulation and inhibits DA uptake during application but also further increases the release upon washout (termed a “rebound” release enhancement). In the present study, we determined whether co-application and washout of ginseng total saponin (GTS), the active ingredient of *Panax* ginseng, with cocaine attenuate cocaine-induced enhancement of evoked DA release, DA uptake inhibition and/or withdrawal-associated rebound enhancement. Cocaine rapidly potentiated the DA release within the first 10 min of application, and acute cocaine withdrawal caused a rebound increase. Co-application of GTS with cocaine inhibited the release enhancement and subsequently prevented the rebound increase during acute withdrawal. The effect of GTS was concentration-dependent. In contrast, GTS had no significant effects on the cocaine-mediated DA uptake inhibition. These results suggest that the attenuation of the cocaine-induced enhancement of impulse-dependent DA release, rather than uptake inhibition, might be one of the pharmacological bases for attenuation of behavioral effects of cocaine and amelioration of acute withdrawal symptoms by ginseng.

Keywords

Panax ginseng; ginsenosides; dopamine; cocaine; nucleus accumbens; fast scan cyclic voltammetry

*Address Correspondence to: Tong Hyon Lee, M.D., Ph.D., 120 Sands, Box 3870, Duke University Medical Center, Durham, NC 27710, U.S.A., Telephone: (919) 684-4374, Fax: (919) 681-8369, email: tong.lee@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, is a traditional folk medicine that is reported to have many beneficial effects. Ginseng saponins, which are also called ginsenosides, are the main active components responsible for the actions of ginseng. Ginsenosides are well characterized and known to have a four-ring steroid-like structure with sugar moieties attached and exert their diverse effects in central and peripheral nervous systems (Fig. 1) (Nah et al., 2007). Ginseng alone or with other herbal medicines has been used in traditional folk medicine to block the actions of abused drugs and to ameliorate their side effects. Experimental studies have provided scientific rationale for this traditional use of ginseng. For example, systemic ginseng saponins inhibit the development of tolerance to the analgesic and hyperthermic effects of chronic morphine treatment in rodents (Bhargava and Ramarao, 1990; Bhargava and Ramarao, 1991). Moreover, ginsenosides attenuate the development of tolerance to the inhibitory effect of morphine on electrically-evoked contraction of guinea-pig ileum (Watanabe et al., 1988).

In addition to interactions with the opioid systems, recent reports have demonstrated that GTS also attenuates cocaine- or amphetamine-induced behavioral activity such as hyperactivity and conditioned place preference (Kim et al., 1995; Tokuyama et al., 1996; Halladay, et al., 1999; Lee et al., 2008). Enhancement of the central dopamine (DA) neurotransmission may play an important role in mediating behavioral effects of psychostimulants (Lee et al., 2001). However, mechanisms underlying the efficacy of GTS in inhibiting the psychostimulant-induced behaviors remain to be fully elucidated. We previously demonstrated that acute cocaine not only inhibits DA uptake but also enhances DA release evoked by single-pulse electrical stimulations; remarkably, this release enhancement becomes more pronounced upon cocaine washout (termed a “rebound” increase; Lee et al., 2001). In the present study, we determined, using real-time measurements of the extracellular DA concentrations in slice preparations, whether GTS can attenuate effects of cocaine on evoked DA release and uptake in the nucleus accumbens. The results show that, while exerting minimal effects on DA uptake inhibition, GTS attenuates both the release enhancement and the rebound increase during cocaine application and withdrawal, respectively. The efficacy of ginseng against behavioral effects of cocaine and associated withdrawal symptoms may be partly mediated by a selective inhibition of the DA release enhancing effects of the drug. Of note, the term “release” is used throughout this report to denote DA release evoked by a single-pulse electrical stimulation.

2. Results

During perfusion with ACSF, a single-pulse electrical stimulation evoked rapid DA release in the nucleus accumbens slice (“baseline release”); the peak concentrations were evident within 50–150 ms of the stimulus. The extracellular DA concentrations returned to baseline within 1–2 s. Control values for the DA release parameter (i.e., $[DA]_p$) ranged 0.45–3.2 μM , and those for the uptake parameters (i.e., K_m and V_{max}) ranged 0.17–0.33 μM and 1.7–6.5 $\mu\text{M/s}$, respectively. No changes in the background-subtracted cyclic voltammograms (i.e., no DA “signature” signal) were noted between electrical stimulations, indicating no detectable “spontaneous” DA release under our experimental conditions.

2.1. Effects of GTS on cocaine-induced enhancement of evoked DA release and withdrawal-associated rebound

As previously demonstrated (e.g., Lee et al., 2001), application of 10 μM cocaine alone rapidly increased evoked DA release (i.e., $[DA]_p$) to its plateau level within 10 min (Fig. 2A). Cocaine washout (acute withdrawal) was also associated with a distinctive rebound increase in DA release over the maximal amount measured during cocaine perfusion. As shown in Fig. 2A, $[DA]_p$ values progressively increased during the first 30 min of cocaine withdrawal before

beginning a gradual decline. This rebound increase is a unique phenomenon observed during acute withdrawal from 10 but not 1 or 3 μM cocaine (Lee et al., 2001). Such high concentrations may be achieved during intravenous or intrapulmonary (i.e., crack smoking) delivery of the drug (Volkow et al., 1997; Stuber et al., 2005). Continuous perfusion of GTS (300 $\mu\text{g/ml}$) alone for 130 min did not alter evoked DA release (Fig. 2A).

A mixed-design two-way ANOVA on the release area-under-the-curve (AUC, cumulative DA release) revealed significant effects of drug treatment ($[F(5,20)=26.505]$, $p < 0.001$) and time period ($[F(1,20)=35.800]$, $p < 0.001$); furthermore, there was a significant interaction between the two factors ($[F(5,20)=14.672]$; $p < 0.001$). Newman-Keul's *post-hoc* comparisons showed an increase in the cumulative amount of evoked DA release during the 30-min cocaine application compared to the ACSF group (Fig. 3A, left panel, $*p < 0.001$). GTS (100 or 300 $\mu\text{g/ml}$) co-applied with cocaine significantly attenuated this enhancement (Fig. 3A, left panel, $^{\#}p < 0.05$ and $^{+}p < 0.001$ versus cocaine alone group for 100 and 300 $\mu\text{g/ml}$ GTS, respectively). This inhibitory effect was dose-dependent as the two GTS groups were significantly different from each other ($^{\#\#}p < 0.05$).

Acute cocaine withdrawal led to a significant rebound enhancement of evoked DA release (AUC) compared to the cocaine perfusion period (Fig. 3A, left and right panels, $**p < 0.001$), and GTS attenuated this rebound in a dose-dependent manner (Fig. 3A, right panel, $^{+}p < 0.001$ versus cocaine alone group, $^{++}p < 0.05$ versus 100 $\mu\text{g/ml}$ GTS). In addition, no significant differences in the cumulative DA release were observed between the GTS + cocaine washout and continuous GTS + cocaine groups (Fig. 3A, right panel). To the extent acute cocaine withdrawal is the necessary and sufficient condition for the DA release rebound (Lee et al., 2001), the similar amounts of DA release in these two experimental groups further confirm that GTS attenuates the rebound increase associated with acute cocaine withdrawal.

2.2. Effects of GTS on cocaine-mediated DA uptake inhibition

As shown in previous reports (e.g., Lee et al., 2001), cocaine inhibited DA uptake (i.e., increased K_m) in the nucleus accumbens (Fig. 2B). Compared to the rapid enhancement of evoked DA release within the first 10 min of application, 10 μM cocaine inhibited DA uptake with a much slower time-course (Fig. 2B). Thus, this inhibition did not reach a plateau until 20–30 min of perfusion. Furthermore, DA uptake monotonically recovered back to the pre-cocaine levels during acute withdrawal. We have previously demonstrated that DA uptake inhibition can be maintained during continuous cocaine application (Lee et al., 2001). GTS alone (300 $\mu\text{g/ml}$) also did not affect DA uptake during 130 min of continuous application (Fig. 2B).

A mixed-design two-way ANOVA found significant differences in DA uptake (i.e., K_m) among treatment groups ($[F(5,20)=7.398]$, $p < 0.001$) and time period ($[F(1,20)=23.900]$, $p < 0.001$). Significant interactions between the two factors were also observed ($[F(5,20) = 9.204]$, $p < 0.001$). Cocaine significantly inhibited DA uptake after 30 min of perfusion (Fig. 3B, left panel, $*p < 0.05$ versus ACSF group) and this inhibition showed a complete abatement after 60 min of cocaine withdrawal (Fig. 3B right panel). In contrast to its efficacy against cocaine-induced DA release enhancements, GTS did not alter DA uptake inhibition by 10 μM cocaine or its recovery during cocaine withdrawal. Thus, the three GTS + cocaine groups exhibited significant inhibition of DA uptake after 30 min of perfusion (Fig. 3B, left panel, $^{\#}p < 0.007$ versus ACSF group), and they were not different from the group perfused with cocaine alone. After 60 min of cocaine washout, no uptake differences were observed among the cocaine alone, GTS (100 $\mu\text{g/ml}$) + cocaine and GTS (300 $\mu\text{g/ml}$) + cocaine groups. Finally, prolonged co-application of cocaine and GTS (300 $\mu\text{g/ml}$) for 130 min exerted minimal effects on cocaine-mediated inhibition of DA uptake (Fig. 3B, right panel, $^{+}p < 0.009$ versus all the other five groups).

3. Discussion

The present study demonstrates that GTS may selectively suppress enhancements of evoked DA release ($[DA]_p$) during cocaine application and acute withdrawal (washout) in a concentration-dependent manner. In contrast, the baseline DA release and cocaine-induced uptake inhibition are minimally affected by GTS. Overall, these results are consistent with previous microdialysis finding that GTS reduces cocaine-stimulated DA efflux in the rat nucleus accumbens (Lee et al., 2008). Importantly, the present study extends the microdialysis results by demonstrating that GTS inhibits cocaine-induced facilitation of the central DA neurotransmission by selectively attenuating the effects of cocaine on evoked (action potential-dependent) DA release but not on the uptake.

Additional studies are needed to elucidate precise neurobiological mechanism(s) underlying the efficacy of GTS against cocaine-mediated enhancement of DA release. However, several mechanisms could be considered. First, GTS might interact with presynaptic DA nerve terminals in the nucleus accumbens and inhibit the vesicular release and rebound enhancement caused by acute cocaine withdrawal. Consistent with this hypothesis, it has been shown that ginseng root extract and ginsenosides inhibit voltage-gated Ca^{2+} channels in the dorsal root ganglion nociceptive neurons and adrenal chromaffin cells (Nah and McCleskey, 1994; Nah et al., 1995; Lee et al., 2006). Ginsenosides also inhibit brain voltage-gated Na^{+} channels (Kang et al., 2005; Lee et al., 2005; Lee et al., 2006). However, it is unlikely that direct presynaptic inhibition of Ca^{2+} or Na^{+} channels is the main element for GTS-induced attenuations of cocaine-mediated changes in DA release. Thus, GTS alone exerts minimal effects on DA release evoked by single-pulse electrical stimulation (Fig. 2).

In addition to the voltage-gated ion channels, GTS might also affect presynaptic signal transduction pathways in DA systems (Elkins et al., 2003; Zhang et al., 2005; Wei et al., 2007), and consequently interfere with the pharmacological effects of cocaine on DA neurotransmission. We have previously demonstrated that treatment of GTS attenuates both intracellular Ca^{2+} release and extracellular Ca^{2+} entry in cultured hippocampal neurons (Jeong et al., 2004). For example, GTS might decrease the free cytosolic Ca^{2+} concentrations by blocking its release from intracellular and extracellular Ca^{2+} sources, might influence cocaine-mediated Ca^{2+} -calmodulin dependent kinase activities (Elkins et al., 2003; Zhang et al., 2005; Wei et al., 2007). This inhibition, in turn, might attenuate enhancements of evoked DA release during cocaine application and acute withdrawal. It is interesting to note that the “typical” antipsychotic haloperidol, which is a relatively potent calmodulin antagonist (Prozialeck and Weiss, 1982) exerts similar effects on DA release enhancements as GTS (Lee et al., 1998) in contrast to other DA antagonists such as sulpiride (D2) or SCH 39166 (D1), which lack the anti-calmodulin efficacy (Lee et al., 2001). Further investigations using selective inhibitors/antagonists may lead to precise elucidation of mechanisms underlying the GTS-mediated attenuation of acute cocaine effects on DA release.

In general, the effective concentration range of GTS against the cocaine-mediated enhancement of DA release (100 – 300 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$ exerting minimal effects) tends to be higher than those reported in previous reports (see above). For example, ED_{50} are in the range of 40–120 $\mu\text{g/ml}$ for Ca^{2+} channel regulation in neuronal cell cultures (Nah et al., 1995; Choi et al., 2001a), 4.4 $\mu\text{g/ml}$ for Ca^{2+} -activated Cl^{-} channel in *Xenopus* oocytes (Choi et al., 2001b) and approximately 30 $\mu\text{g/ml}$ for heterologously expressed Na^{+} channel (Lee et al., 2005). In addition to differences in the cell types, targeted mechanisms, the types of experimental preparations used in the present study (i.e., slices versus cell cultures in the others) may account for the apparent potency differences.

It is well known that one of the main pharmacological effects of cocaine is to enhance DA neurotransmission through inhibition of DA uptake (Kiyatkin et al., 2000; Stathis et al., 1995; Volkow et al., 1997). In the present study, we could not observe any effects of GTS on DA uptake inhibition by cocaine, during either drug application or washout; GTS alone also had minimal effects on DA uptake (Fig. 3). These results suggest that GTS might not interact with the DA transporter (DAT) or mechanisms modulating its activity. Combined with its positive effects on evoked DA release, the present results suggest that GTS exerts differential effects on cocaine-dependent enhancement of DA release and uptake inhibition. It is noted that GTS can also attenuate the increased striatal DA efflux induced by amphetamine (Halladay et al., 1999). However, in contrast to cocaine, amphetamine increases DA efflux primarily through promoting “reverse transport” of DA via the DA transporter (Fischer and Cho, 1979; Jones et al., 1998). Further studies are needed to elucidate the mode of action for GTS in inhibiting amphetamine-induced DA release reported in the above study. Interestingly, ginseng might attenuate behavioral effects of cocaine and amphetamine through two distinct DA release mechanisms (vesicular release and reverse transport, respectively, for cocaine and amphetamine).

Changes in DA uptake (e.g., cocaine-induced inhibition) can modulate DA release through alterations in autoreceptor stimulation or DAT-mediated “reverse” transport (e.g., Jones et al., 1998; Lee et al., 1996). However, these release/uptake interactions are unlikely to have confounded the analysis outcome or interpretation of GTS effects on cocaine-enhanced DA release. This conclusion is based on a number of considerations including that: (1) we used a well-established curve-fitting model (Wightman and Zimmerman, 1990) to analytically separate and estimate the DA release and uptake parameters ($[DA]_p$ and K_m/V_{max} , respectively); (2) the use of single-pulse electrical stimulations to evoke DA release eliminates potential autoinhibitory effect of DA molecules that accumulate during multiple-pulse electrical stimulation; (3) blockade of DA autoreceptors with sulpiride minimally affects DA release and uptake changes during cocaine application and washout (Lee et al., 2001); and (4) alterations in evoked DA release and uptake inhibition exhibit distinct time-courses during drug application and washout (Fig. 2), thus indicating distinct modulatory changes for these two processes.

How can these *in vitro* effects of GTS on DA release be translated into the positive efficacy of ginseng in blocking cocaine-induced behavioral changes? In previous studies, actions of cocaine to potentiate DA neurotransmission have been mainly attributed to DA uptake inhibitions; this cocaine-induced-blockade of DA uptake could be one of the mechanisms underlying its addictive property (Koob and Bloom, 1988; Carroll et al., 1992; Reith et al., 1997). In addition to enhancement of DA neurotransmission via uptake inhibition, however, cocaine also increases DA efflux (Scheel-Kruger et al., 1977; King et al., 1993). Real-time voltammetric studies, which can experimentally resolve DA release and uptake, have demonstrated that cocaine directly enhances DA release induced by electrical stimulations or spontaneous action potentials in the nucleus accumbens (Stamford et al., 1989; Jones et al., 1995; Williams et al., 1995; Lee et al., 1998; Lee et al., 2001; Aragona et al., 2008). Finally, we have demonstrated a rebound release enhancement during acute withdrawal. Thus, multiple mechanisms might underlie cocaine-mediated potentiation of DA neurotransmission and behaviors as well as the “therapeutic” efficacy of ginseng against cocaine abuse (Volkow et al., 1997; Kiyatkin et al., 2000; Kim et al., 1995; Tokuyama et al., 1996; Lee et al., 2008).

In summary, we have demonstrated for the first time that GTS inhibits pharmacological effects of cocaine by attenuating its enhancing effects on evoked DA release and the rebound increase associated with acute withdrawal. Thus, GTS-mediated attenuation on cocaine-induced behavioral alterations may be achieved through the inhibitions of these release-associated changes rather than interfering at the DA transporter level. Finally, these inhibitory effects of

GTS on cocaine-mediated changes in impulse-dependent DA release might be one of pharmacological bases for the efficacy of *Panax ginseng* in the treatment of cocaine abuse.

4. Experimental procedure

4.1. Experimental animals and materials

Male Sprague-Dawley rats (Charles Rivers, Raleigh, NC; weighing 250–300 g at the time of sacrifice) were housed two per cage under a 12/12 light/dark cycle; they were cared for in accordance with the “Principles of laboratory animal care” (NIH publication 85-23, revised 1985), and a protocol approved by the Duke Institutional Animal Use and Care Committee. Food and water were available *ad libitum*. GTS contained ginsenoside Rb₁ (17.1%), Rb₂ (9.07%), Rc (9.65%), Rd (8.26%), Re (9%), Rf (3%), Rg₁ (6.4%), Rg₂ (4.2%), Rg₃ (3.8%), Ro (3.8%), Ra (2.91%) and other minor ginsenosides (Jeong et al., 2004). Figure 1 shows the structures of the eight representative ginsenosides.

4.2. Preparation of brain slices

Animals were euthanized by decapitation and coronal slices (400 μ m thick) containing the anterior nucleus accumbens (+1.7–2.7 mm versus bregma; Paxinos and Watson, 1986) were prepared in ice-cold artificial cerebrospinal fluid (ACSF) using a vibratome (Technical Products International, St. Louis, MO). The ACSF composition was (in mM): 124 NaCl, 1.0 KCl, 1.24 KH₂PO₄, 1.3 MgSO₄, 26 NaHCO₃, 2.4 CaCl₂, and 10 glucose, saturated with 95/5 % O₂/CO₂. Following 1–2 hr for recovery in a holding chamber (room temperature), a single slice was transferred to a submersion-type recording chamber, and perfused with the control ACSF at ~1.8 ml/min (32.5 $\pm$ 0.5° C).

4.3. *In vitro* fast-scan cyclic voltammetry and measurement of DA

In vitro fast-scan cyclic voltammetry was performed using carbon-fiber $r = 5 \mu$ m, Thorne P-55, Amoco, Greenville, SC) microelectrodes prepared as described previously (Wightman and Zimmerman, 1990). The electrodes were beveled, and dip-coated with nafion (2.5% weight/volume) to exclude anions from the electrode surface. The electrodes were placed in the accumbens core, ~75 μ m below the slice surface and their potential linearly scanned from –400 mV to +1000 mV and back to –400 mV (held at –400 mV between scans) at 350 V/sec every 100 ms (EI-400 amplifier in the “two-electrode” mode, Ensman Instrumentation, Bloomington, IN). A Ag/AgCl electrode served as a combined reference/auxiliary electrode. To obtain DA concentration versus time plots, the current at the peak oxidation potential for DA in each voltammogram (typically ~600 mV, current averaged between 500 and 700 mV) was converted to concentration based on post-calibration of the electrode with DA (0.5–8 μ M). Background-subtracted cyclic voltammograms were constructed by subtracting the baseline current from voltammograms of interest to confirm the identity of the released substance with DA.

DA release was evoked by single-pulse electrical stimulation (biphasic, constant current, 350 μ A, 2 ms each phase) delivered, 50 ms after a voltammetric scan, via a bipolar stimulation electrode. This electrode was constructed by twisting insulated 150- μ m stainless steel wires (Plastic Products, Roanoke, VA). The stimulating electrode was placed on the slice surface with its center 100–200 μ m from the working electrode. The electrical stimuli were computer generated and delivered via a stimulus isolation unit (Isolator-10, Axon Instrument, Foster City, CA).

Following 3–4 consecutive stable baseline measurements (every 3 min), the slices were first exposed to 0, 30, 100 or 300 μ g/ml GTS for 40 min followed by GTS + 10 μ M cocaine for 30 min. This cocaine perfusion period was followed by additional 60-min perfusion with either GTS alone (0 – 300 μ g/ml; i.e., cocaine washout) or GTS + cocaine (continuous cocaine).

Direct comparison of the continuous and washout groups were to allow a determination of potential effects of GTS on the unique DA kinetic changes associated with cocaine washout *per se* (Lee et al., 2001). Voltammetric measurements during the drug perfusion periods were made every 5 min.

The endogenous DA kinetics was analyzed by fitting changes in extracellular DA concentrations to a nonlinear kinetic model developed by Wightman (see Wightman & Zimmerman, 1990 for review). The kinetic equation was given by

$$d[DA]/dt = ([DA]_p) - V_{\max}/\{(K_m)/[DA] + 1\}$$

where $[DA]_p$ was the concentration of DA released “instantaneously” by a single stimulus pulse, and (K_m) and $V_{\max}$ are Michaelis-Menten reuptake rate constants (Wightman & Zimmerman, 1990). A locally-written, non-linear, Simplex-based fitting routine was used to estimate the 3 parameters for baseline and pre-cocaine GTS data. Since cocaine behaves as a competitive inhibitor under our experimental conditions (Lee et al., 1998), the values of the $V_{\max}$ were fixed at the baseline level, and the K_m and $[DA]_p$ determined. Changes in the endogenous DA kinetics were determined by first normalizing the $[DA]_p$ and K_m values to the respective baseline values recorded immediately before the start of drug perfusion. The normalized DA release data were converted to respective AUC for the 30-min drug application period and the first 30 min of drug. For the time-course results in Fig. 2, every other ratio data points (i.e., every 10 min) are plotted against time for clearer presentation. All data are presented as mean $\pm$ S.E.M.

4.4. Statistical analysis

The AUC for DA release and individual K_m values were analyzed with mixed-design two-way analyses of variance (ANOVA) with the drug treatment group and time period (perfusion and withdrawal) as the main factors. Newman Keul’s test was used for *post-hoc* individual mean comparisons. Statistical significance levels for all the tests were set at the $p < 0.05$ level.

Acknowledgments

This work was supported by grants of BK21 project, KRF (KRF-2005-015-E00222), KOSEF funded by the MEST (R01-2008-000-10448-0) and Konkuk University Sabbatical Leave to SYN and R01DA12768 to THL.

References

- Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM. Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. *J Neurosci* 2008;28:8821–8831. [PubMed: 18753384]
- Bhargava HN, Ramarao P. Antagonism of the acute pharmacological actions of morphine by panax ginseng extract. *Gen Pharmacol* 1990;21:877–80. [PubMed: 2279687]
- Bhargava HN, Ramarao P. The effect of Panax ginseng on the development of tolerance to the pharmacological actions of morphine in the rat. *Gen Pharmacol* 1991;22:521–525. [PubMed: 1869026]
- Carroll FI, Gao Y, Abraham P, Lewin AH, Lew R, Patel A, Boja JW, Kuhar MJ. Probes for the cocaine receptor. Potentially irreversible ligands for the dopamine transporter. *J Med Chem* 1992;35:1813–1817. [PubMed: 1588560]
- Choi S, Jung SY, Kim CH, Kim HS, Rhim H, Kim SC, Nah SY. Effect of ginsenosides on voltage-dependent Ca^{2+} channel subtypes in bovine chromaffin cells. *J of Ethnopharmacol* 2001a;74:75–81. [PubMed: 11137351]

- Choi S, Rho SH, Jung SY, Kim CS, Park CS, Nah SY. A novel activation of Ca^{2+} -activated Cl^- Channel in *Xenopus* oocytes by ginseng saponins: evidence for the involvement of phospholipase C and intracellular Ca^{2+} mobilization. *British J Pharmacol* 2001b;132:641–648.
- Elkins RL, Orr TE, Rausch JL, Fei YJ, Carl GF, Hobbs SH, Buccafusco JJ, Edwards GL. Cocaine-induced expression differences in PSD-95/SAP-90-associated protein 4 and in Ca^{2+} /calmodulin-dependent protein kinase subunits in amygdalae of taste aversion-prone and taste aversion-resistant rats. *Ann N Y Acad Sci* 2003;1003:386–390. [PubMed: 14684468]
- Fischer JF, Cho AK. Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. *J Pharmacol Exp Ther* 1979;208:203–209. [PubMed: 762652]
- Halladay AE, Lu YL, Palmer J, Oh KW, Wagner GC. Acute and chronic effects of ginseng total saponin and amphetamine on fixed-interval performance of rats. *Planta Med* 1999;65:162–164.
- Jeong SM, Lee JH, Kim S, Rhim H, Lee BH, Kim JH, Oh JW, Lee SM, Nah SY. Ginseng saponins induce store-operated calcium entry in *Xenopus* oocytes. *Br J Pharmacol* 2004;142:585–593. [PubMed: 15148256]
- Jones SR, Gainetdinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J Neurosci* 1998;18:1979–1986. [PubMed: 9482784]
- Jones SR, Garri PA, Wightman RM. Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *J Pharmacol Exp Ther* 1995;274:396–403. [PubMed: 7616424]
- Kang DI, Lee JY, Yang JY, Jeong SM, Lee JH, Nah SY, Kim Y. Evidence that the tertiary structure of 20(S)-ginsenoside Rg_3 with tight hydrophobic packing near the chiral center is important for Na^+ channel regulation. *Biochem Biophys Res Commun* 2005;333:1194–201. [PubMed: 15979567]
- Kim HS, Kang JG, Seong YH, Nam KY, Oh KW. Blockade by ginseng total saponin of the development of cocaine induced reverse tolerance and dopamine receptor supersensitivity in mice. *Pharmacol Biochem Behav* 1995;50:23–27. [PubMed: 7700950]
- King GR, Kuhn C, Ellinwood EH. Dopamine efflux during withdrawal from continuous or intermittent cocaine. *Psychopharmacology* 1993;111:179–184. [PubMed: 7870949]
- Kiyatkin EA, Kiyatkin DE, Rebec GV. Phasic inhibition of dopamine uptake in nucleus accumbens induced by intravenous cocaine in freely behaving rats. *Neuroscience* 2000;98:729–741. [PubMed: 10891616]
- Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. *Science* 1988;242:715–723. [PubMed: 2903550]
- Lee TH, Gee KR, Ellinwood EH, Seidler FJ. Combining “caged-dopamine” photolysis with fast-scan cyclic voltammetry to assess dopamine clearance and release autoinhibition in vitro. *J Neurosci Meth* 1996;67:221–231.
- Lee TH, Gee KR, Ellinwood EH, Seidler FJ. Altered cocaine potency in the nucleus accumbens following 7-day withdrawal from intermittent but not continuous treatment: voltammetric assessment of dopamine uptake in the rat. *Psychopharmacology* 1998;137:303–310. [PubMed: 9683008]
- Lee TH, Balu R, Davidson C, Ellinwood EH. Differential time-course profiles of dopamine release and uptake changes induced by three dopamine uptake inhibitors. *Synapse* 2001;41:301–310. [PubMed: 11494401]
- Lee JH, Jeong SM, Kim JH, Lee BH, Yoon IS, Lee JH, Choi SH, Kim DH, Rhim H, Kim SS, Kim JI, Jang CG, Song JH, Nah SY. Characteristics of ginsenoside Rg_3 -mediated brain Na^+ current inhibition. *Mol Pharmacol* 2005;68:1114–1126. [PubMed: 16014805]
- Lee JH, Jeong SM, Kim JH, Lee BH, Yoon IS, Lee JH, Choi SH, Lee SM, Park YS, Kim SS, Kim HC, Lee BY, Nah SY. Effects of ginsenosides and their metabolites on voltage-dependent Ca^{2+} channel subtypes. *Mol Cells* 2006;21:52–62. [PubMed: 16511347]
- Lee B, Yang CH, Hahm DH, Lee HJ, Han SM, Kim KS, Shim I. Inhibitory effects of ginseng total saponins on behavioral sensitization and dopamine release induced by cocaine. *Biol Pharm Bull* 2008;31:436–441. [PubMed: 18310906]
- Nah SY, McCleskey EW. Ginseng root extract inhibits calcium channels in rat sensory neurons through a similar path, but different receptor, as mu-type opioids. *J Ethnopharmacol* 1994;42:45–51. [PubMed: 8046943]

- Nah SY, Park HJ, McCleskey EW. A trace component of ginseng that inhibits Ca²⁺ channels through a pertussis toxin-sensitive G protein. *Proc Natl Acad Sci USA* 1995;92:8739–8743. [PubMed: 7568008]
- Nah SY, Kim DH, Rhim H. Ginsenosides: are any of them candidates for drugs acting on the central nervous system? *CNS Drug Rev* 2007;13:381–404. [PubMed: 18078425]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; San Diego: 1986.
- Prozialeck WC, Weiss B. Inhibition of calmodulin by phenothiazines and related drugs: structure activity relationships. *J Pharmacol Exp Ther* 1982;222:509–516. [PubMed: 6286920]
- Reith MEA, Xu C, Chen NH. Pharmacology and regulation of the neuronal dopamine transporter. *Eur J Pharmacol* 1997;324:1–10. [PubMed: 9137907]
- Scheel-Kruger, J.; Braestrup, C.; Nielson, M.; Golembiowska, K.; Mogilnicka, E. Cocaine: discussion on the role of dopamine in the biochemical mechanisms of action. In: Ellinwood, EH.; Kilbey, MM., editors. *Cocaine and other stimulants, advances in behavioral biology*. New York: Plenum Press; 1977. p. 373–408.
- Stamford JA, Kruk ZL, Millar J. Dissociation of the actions of uptake blockers upon dopamine overflow and uptake in the rat nucleus accumbens: in vivo voltammetric data. *Neuropharmacology* 1989;28:1383–1388. [PubMed: 2533329]
- Stathis M, Scheffel U, Lever SZ, Boja JW, Carroll FI, Kuhar MJ. Rate of binding of various inhibitors at the dopamine transporter in vivo. *Psychopharmacology (Berl)* 1995;119:376–384. [PubMed: 7480516]
- Stuber GD, Wightman RM, Carelli RM. Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. *Neuron* 2005;46:661–669. [PubMed: 15944133]
- Tokuyama S, Takahashi M, Kaneto H. The effect of ginseng extract on locomotor sensitization and conditioned place preference induced by methamphetamine and cocaine in mice. *Pharmacol Biochem Behav* 1996;54:671–676. [PubMed: 8853188]
- Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, Vitkun S, Logan J, Gatley SJ, Pappas N, Hitzeman R, Shea CE. Relationship between subjective effects of cocaine and dopamine transporter occupancy. *Nature* 1997;386:827–830. [PubMed: 9126740]
- Wei Y, Williams JM, Dipace C, Sung U, Javitch JA, Galli A, Saunders C. Dopamine transporter activity mediates amphetamine-induced inhibition of Akt through a Ca²⁺/calmodulin-dependent kinase II-dependent mechanism. *Mol Pharmacol* 2007;71:835–842. [PubMed: 17164407]
- Watanabe J, Oh KW, Jin HS, Takahashi M, Kaneto H. A non-opioid mechanism in the inhibitory effect of ginseng saponins on electrically evoked contractions of guinea-pig ileum and mouse vas deferens. *J Pharmacobiodyn* 1988;11:453–458. [PubMed: 3171886]
- Wightman RM, Zimmerman JB. Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Res Rev* 1990;15:135–144. [PubMed: 2282449]
- Williams JE, Wiczorek W, Willner P, Kruk ZL. Parametric analysis of the effects of cocaine and cocaine pretreatment on dopamine release in the nucleus accumbens measured by fast cyclic voltammetry. *Brain Res* 1995;678:225–232. [PubMed: 7620892]
- Zhang D, Zhang L, Tang Y, Zhang Q, Lou D, Sharp FR, Zhang J, Xu M. Repeated cocaine administration induces gene expression changes through the dopamine D1 receptors. *Neuropsychopharmacology* 2005;30:1443–1454. [PubMed: 15770241]

Ginsenosides	R1	R2	R3
Rb ₁	-Glc ₂ -Glc	-H	-Glc ₆ -Glc
Rb ₂	-Glc ₂ -Glc	-H	-Glc ₆ -Ara(pyr)
Rc	-Glc ₂ -Glc	-H	-Glc ₆ -Ara(fur)
Rd	-Glc ₂ -Glc	-H	-Glc
Re	-H	-O-Glc ₂ -Rha	-Gc
Rh ₁	-Glc ₂ -Glc	-H	-Glc
Rh ₂	-H	-O-Glc ₂ -Rha	-Glc
Rg ₁	-H	-O-Glc	-Glc
Rg ₃	-Glc ₂ -Glc	-H	-H

Fig. 1. Chemical structure of the major types of ginsenosides. Numbers indicate the carbon in the glucose ring that links the two carbohydrates. Abbreviations for carbohydrates are as follows: Glc = glucopyranoside; Ara(fur) = arabinofuranose; Ara(pyr) = arabinopyranoside; and Rha = rhamnopyranoside.

Fig. 2.

Time-course of GTS effects on cocaine-induced enhancement of evoked DA release and uptake inhibition. (A) Cocaine (10 μM) rapidly increased evoked DA release ($[DA]_p$) during perfusion (black bar), followed by a “rebound” increase during washout (gray bar). GTS attenuated both cocaine-induced enhancement of evoked DA release and the rebound. These inhibitory effects of GTS were concentration-dependent; 30 $\mu\text{g}/\text{ml}$ GTS was without significant effects (data not shown). (B) Cocaine (10 μM) progressively inhibited DA uptake as indicated by increased K_m during perfusion. However, this uptake inhibition, in contrast to the release enhancement, abated toward baseline during acute withdrawal. Co-application of GTS with cocaine had no significant effects on either cocaine-induced DA uptake inhibition or its

abatement. Continuous (Cont) co-application of cocaine and GTS (100 $\mu\text{g/ml}$) also had no effects on cocaine-induced DA uptake inhibition. The respective black and gray bars indicate the time periods of drug application and washout. The inset shows representative single DA release and uptake events at selected time points for the ACSF control group. Traces 1 – 3 correspond to the time points for baseline (immediately before cocaine application), 30-min cocaine perfusion and 30-min washout. Scale bars: vertical, 1 μM ; horizontal, 2 sec.

Fig. 3.

Effects of GTS on the DA release AUC enhancement and uptake inhibition. (A) Cocaine treatment significantly increased cumulative DA release (left panel; $*p < 0.001$ versus ACSF group) and led to a rebound increase in DA release during washout ($**p < 0.001$, “Acute withdrawal” versus “Perfusion” periods). GTS significantly inhibited cocaine-induced DA release enhancement in a concentration-dependent manner (left panel; $\#p < 0.05$ and $+p < 0.001$ versus cocaine alone group for 100 and 300 $\mu\text{g/ml}$, respectively; $\#\#p < 0.05$ versus 100 $\mu\text{g/ml}$). The rebound increase was also inhibited in the same manner (right panel; $+p < 0.001$ versus cocaine alone group; $++p < 0.05$ versus 100 $\mu\text{g/ml}$). (B) Cocaine significantly inhibited DA uptake (left panel; $*p < 0.05$ versus ACSF controls); 60-min cocaine washout rapidly led to a

recovery of DA uptake to the pre-cocaine level (** $p < 0.001$, “Acute withdrawal” versus “Perfusion” periods).