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The Effect of Ginseng Extract on Locomotor Sensitization and Conditioned Place Preference Induced by Methamphetamine and Cocaine in Mice

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TOKUYAMA, S., M. TAKAHASHI AND H. KANETO. *The effect of ginseng extract on locomotor sensitization and conditioned place preference induced by methamphetamine and cocaine in mice.* PHARMACOL BIOCHEM BEHAV **54**(4) 671–676, 1996.—Repeated IP injections of 2 mg/kg methamphetamine (MA) or 20 mg/kg cocaine at 48-h intervals induced reverse tolerance to their ambulation-enhancing effects (behavioral sensitization). Furthermore, the reappearance of the sensitized state was observed at the time of readministration of MA or cocaine even after a 30-day discontinuation of drug administration. A concomitant injection of ginseng extract (GE), 200 mg/kg, IP, suppressed the development of reverse tolerance and the reappearance of sensitization to MA and cocaine. Conditioned place preference to MA (1, 2, and 4 mg/kg, IP) and cocaine (1, 4, 10, and 20 mg/kg, IP), was completely blocked by GE, 200 mg/kg, IP combined treatment with MA or cocaine. Meanwhile, spontaneous motor activity and place preference were not affected by GE alone. These results provide evidence that GE may be useful clinically for the prevention of adverse actions of MA and cocaine.

Ginseng extract Methamphetamine Cocaine Ambulometer Reverse tolerance
Behavioral sensitization Conditioned place preference Reinforcing effect

MUCH attention has been paid to ginseng saponins, which have long been used for medicinal purposes in Eastern countries because they possess multiple pharmacological actions. These actions are mediated by the central nervous system and include suppression of exploratory and spontaneous movements (28), prolongation of hexobarbital sleeping time (29), and inhibition of the conditioned avoidance response (23). Furthermore, ginseng extract (GE) has been reported to antagonize the antinociception produced by morphine and to inhibit the development of tolerance to and dependence on this narcotic (12,13).

It is well known that repeated exposure to opiate (1,9,24) and psychostimulant drugs (9,10) often results in behavioral sensitization (development of reverse tolerance to their ambulation-enhancing effects), which is regarded as one model for studying the psychotoxicity of these drugs. Recently, it has been reported that the development of reverse tolerance to the effect of morphine is blocked by GE (12). Methamphetamine (MA) sensitization has also been reported to be blocked by GE (30). However, the effect of GE on sensitization in conjunction with other psychostimulants is still unknown.

The conditioned place preference paradigm is considered a reliable measure of the reinforcing properties of drugs and is particularly useful for evaluating the reinforcing effect of psychostimulants (3,5,25). A variety of drugs including MA (8,15) and cocaine (17,18,26) have been shown to produce a place preference in rats. It is believed that this property of MA and cocaine is at least one of the critical factors leading to abuse of and dependence on these drugs. Accordingly, examination of the influence of GE on the reinforcing effect of these psychostimulants using the conditioned place preference paradigm may provide information of value for the development of therapy for the abuse of and dependence on psychostimulants.

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In the present study, we examined the effect of GE on the development of sensitization to the ambulation-enhancing effect of MA and cocaine. In addition, the influence of GE on the reinforcing effects of MA and cocaine was evaluated with the conditioned place preference paradigm.

METHOD

Animals

Male mice of the ddY strain weighing 18 to 20 g (Otsubo Exp. Animals, Nagasaki, Japan) were purchased and housed in groups of 20 animals per plastic cage. They were kept in a room maintained at 21 ± 2°C under a natural day/night regime with free access to a standard laboratory diet (MF; Oriental Yeast, Tokyo, Japan) and tap water. After reaching 23 to 25 g, they were used for experiments.

Drugs

Standardized Panax ginseng extract powder G115 containing 18% of ginseng total saponins (GE; gift from Dr. H.-S. Kim, Chungbuk National University, Korea), methamphetamine-HCl (MA; Dainippon Pharm. Co., Osaka, Japan), and cocaine-HCl (Takeda Pharm. Co. Osaka, Japan) were dissolved in saline in a volume of 0.1 ml/10 g body weight. GE (100 or 200 mg/kg, IP) or saline (0.1 ml/10 g, IP) was given 60 min prior to the MA or cocaine injection.

Measurement of Locomotor Activity

Using the Ambulometer (O'hara Co., Ltd., Tokyo, Japan), ambulatory activity was measured for 120 min after MA (1 or 2 mg/kg, IP) or cocaine (10 or 20 mg/kg, IP) injection. The principle by which the device operates and the measures obtained have been described in detail by Hirabayashi and Alam (8). Briefly, each mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm in height. A slight tilt of the activity cage caused by a horizontal movement of the animal was detected by microswitches. Total activity counts were automatically recorded.

Apparatus for Conditioned Place Preference Testing

The place preference conditioning procedure was conducted in a chamber consisting of three compartments made of acryl-resin board: two end compartments (15 × 15 × 15 cm: W × L × H) and a middle compartment (10 × 10 × 12 cm: W × L × H). The middle compartment was separated from the others by removable guillotine doors. One end compartment was white with a textured floor; the other was black with a smooth floor. One side of both the white and black compartments consisted of transparent Plexiglas to allow the observation of the movement of the animals placed inside. The middle compartment was gray and served as a small, neutral region between the black and white compartments.

Procedure for Place Conditioning

Animals were immediately confined for 30 min to one compartment after injection of MA (1, 2, and 4 mg/kg, IP) or cocaine (1, 4, 10, and 20 mg/kg, IP), and to the other compartment after injection of saline. Animals that had been injected with drugs were confined to one of the end compartments on one day, and to the other of the end compartments after the injection of saline (0.1 ml/10 g) on the following day. This conditioning cycle was performed three times (for a total of 6 days). The kind of injection (drug or saline) and the type of compartment (white or black) was counterbalanced across subjects. The control mice were injected with saline instead of drugs during each of the conditioning sessions. Tests of conditioning were conducted on day 7. On day 7, preference for a particular compartment was assessed in the drug-free state, after placing the animals in the neutral middle compartment and allowing them free access to each compartment. The time spent in each compartment during a 30-min session was measured. Conditioning scores represent the time spent in the drug-paired compartment minus the time spent in the saline-paired compartment.

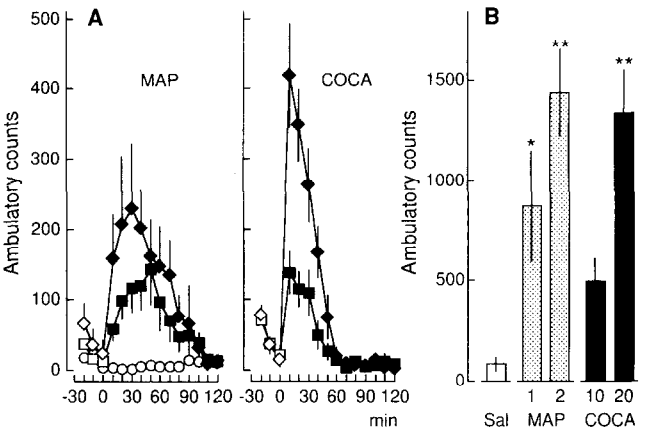

FIG. 1. Ambulation enhancing effect of MA and cocaine. Left: the ambulatory activity was measured in 10-min intervals for 120 min after 1 mg/kg (■) or 2 mg/kg (◆) of MA or 10 mg/kg (■) or 20 mg/kg (◆) of cocaine, IP injection. The control group (○) received saline. Each point indicates the mean ± SEM of the data obtained from five to six mice. Right: cumulative ambulatory activity for 120 min following drug injection. Each column indicates the mean ± SEM of the data obtained from five to six mice. $*p < 0.05$, $**p < 0.01$, compared with the control group (Dunnett's test).

Statistics

The results were expressed as the means ± SEM. Following one-way or two-way analyses of variance (ANOVA) for repeated measurements on the overall data to assess statistical significance, differences between the group means were analyzed with Dunnett's test for the locomotor activity experiments, and Wilcoxon's test for the conditioned place preference experiments. A difference was considered statistically significant at $p < 0.05$. Post hoc comparisons among means were conducted only following significance in the overall ANOVA.

RESULTS

As shown in Fig. 1, both MA (1 and 2 mg/kg) and cocaine (10 and 20 mg/kg) significantly and dose dependently enhanced ambulatory activity for 2 h relative to the saline treated group, $F(4, 21) = 9.99$, $p < 0.001$.

Repeated injections of MA, 2 mg/kg, or cocaine, 20 mg/kg, at 48-h intervals increased ambulatory activity; that is, reverse tolerance to the ambulation-enhancing effect of those psychostimulants was observed. For MA, ANOVA revealed an effect of group, $F(1, 84) = 46.03$, $p < 0.001$; an effect of time, $F(6, 84) = 2.96$, $p < 0.01$; but no group × time interaction,

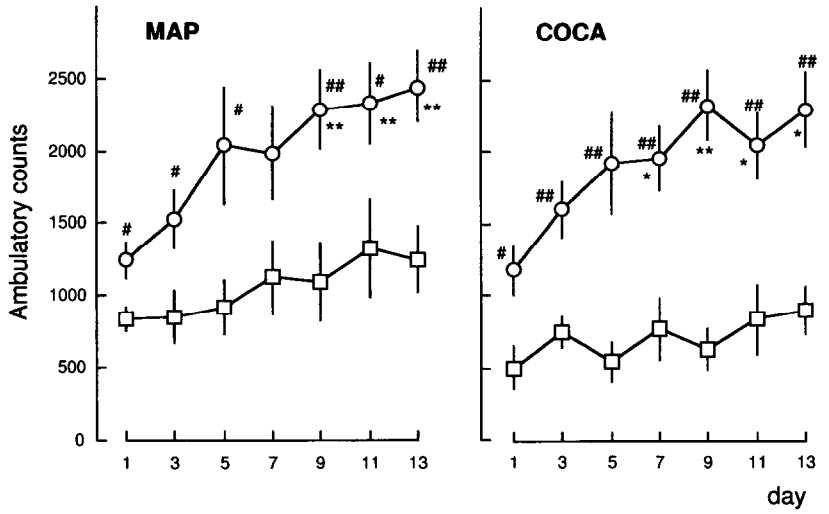

FIG. 2. Development of reverse tolerance to the ambulation enhancing effect of MA and cocaine. One mg/kg (□) or 2 mg/kg (○) of MA (left) or 10 mg/kg (□) or 20 mg/kg (○) of cocaine (right) was given IP, seven times at intervals 48 h apart. Each point is the mean ± SEM of the data obtained from six to eight mice. *$p < 0.05$, **$p < 0.01$, compared with the value on the first day (Dunnett's test). #$p < 0.05$, ##$p < 0.01$, compared with 1 mg/kg of MA or 10 mg/kg of cocaine group (Dunnett's test).

$F(6, 84) = 0.68$, $p = 0.67$. Similarly, for cocaine, ANOVA revealed an effect of group, $F(1, 84) = 115.38$, $p < 0.001$; an effect of time, $F(6, 84) = 2.90$, $p < 0.05$; and no group × time interaction, $F(6, 84) = 1.35$, $p = 0.24$. Meanwhile, the lower doses of MA and cocaine failed to induce reverse tolerance (Fig. 2). Thus, we employed the higher doses of MA + cocaine for examining the action of GE on stimulant-induced hyperactivity and sensitization.

As seen in Fig. 3, the reappearance of behavioral sensitization was observed when MA (2 mg/kg) or cocaine (20 mg/kg) were readministered, even after a 30-day discontinuation of drug injection. Repeated pretreatment with GE (200 mg/kg) 1 h before the injection of MA (2 mg/kg) or cocaine (20 mg/kg), at 48-h intervals for 13 days, significantly suppressed the development and expression of reverse tolerance to MA. In contrast, the lower dose of GE (100 mg/kg) had no effect on the development of reverse tolerance to MA and cocaine. Evaluation of the effects of GE upon the development of

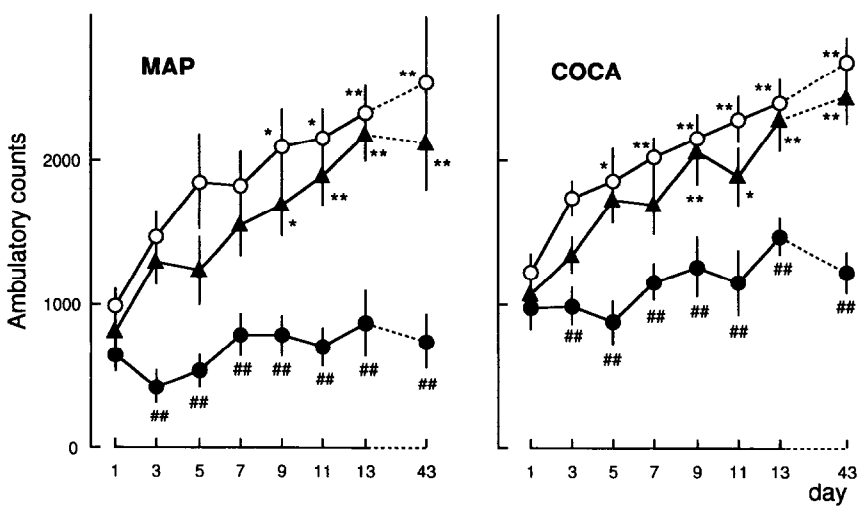

FIG. 3. Effect of GE on the development of reverse tolerance to the ambulation enhancing effect of MA and cocaine. Two mg/kg of MA (left) or 20 mg/kg of cocaine (right) with 100 mg/kg (▲) or 200 mg/kg of GE (●) was given IP, seven times at intervals 48 h apart. GE was given 1 h before MA or cocaine injection. The control group (○) received saline instead of GE. After 30 drug-free days, on the 43rd day, the same dose of MA or cocaine was readministrated. Each point is the mean ± SEM of the data obtained from 12–14 mice. *$p < 0.05$, **$p < 0.01$, compared with the value on the first day (Dunnett's test). ##$p < 0.01$, compared with MA or cocaine alone (Dunnett's test).

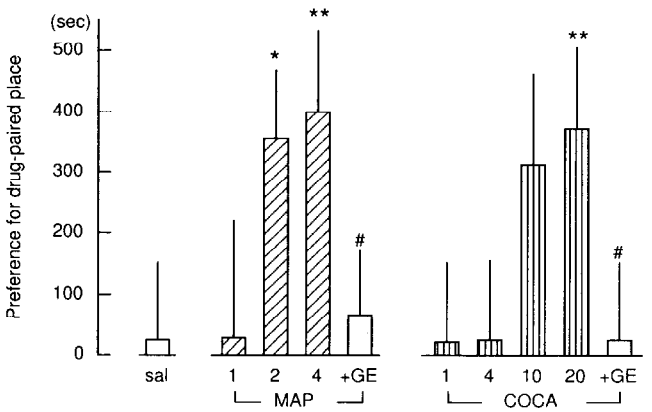

FIG. 4. Effect of GE on conditioned place preference induced by MA and cocaine. Conditioning was performed with 1, 2 and 4 mg/kg of IP MA (slanted rule column) or 1, 4, 10, and 20 mg/kg of IP cocaine (vertical rule column). GE 200 (dotted column) mg/kg IP was given 1 h before MA (4 mg/kg) or cocaine (20 mg/kg). The control group received saline (Sal., open column) instead of MA or cocaine. Conditioning scores represent the time spent in the drug-paired compartment minus the time spent in the saline-paired compartment. Each point is the mean ± SEM of the data obtained from 12–14 mice. $*p < 0.05$, $**p < 0.01$, compared with the value of the control group (Wilcoxon's test). $\#p < 0.05$, compared with respective MA or cocaine-treated groups (Wilcoxon's test).

reverse tolerance to MA and cocaine revealed significant group differences, $F(2, 280) = 75.31$, $p < 0.001$; $F(2, 280) = 63.75$, $p < 0.001$; effect of time, $F(7, 280) = 8.60$, $p < 0.001$; $F(7, 280) = 13.10$, $p < 0.001$, but no significant group × time interaction, $F(14, 280) = 1.42$, $p = 0.14$; $F(14, 280) = 1.51$, $p = 0.11$, respectively. In this case, neither a single injection nor chronic treatment (repeated injections at 48-h intervals for 13 days) with GE alone (200 mg/kg) influenced spontaneous motor activity of mice, $F(6, 28) = 1.25$, $p = 0.31$ (ambulatory count, mean ± SE: 75.2 ± 24.6 on the first day, 47.0 ± 12.3 on the 13th day).

In the experiment using the conditioned place preference paradigm, the saline-conditioned control mice exhibited no place preference, thus demonstrating that there were neither baseline biases for the black vs. white compartment, nor place conditioning effects of saline injection. Similarly, GE (200 mg/kg) alone induced neither significant place preference nor place aversion in mice (mean ± SE 26 ± 125 s, $n = 8$; NS, compared with saline control). In contrast, MA (1, 2, and 4 mg/kg) or cocaine (1, 4, 10, and 20 mg/kg) produced a place preference for the drug-conditioned compartment in a dose-dependent manner; that is, the time spent in the drug-paired compartment was increased and the time spent in the saline-paired place was decreased. The place preference induced by MA or cocaine was significantly reversed by pretreatment with GE, 200 mg/kg, 60 min prior to administration of those psychostimulants, $F(9, 114) = 1.99$, $p < 0.05$ (Fig. 4). Time spent in the middle compartment (neutral region) in the all experiments did not change significantly (data not shown).

DISCUSSION

The present studies have confirmed our previous results (30), suggesting that GE might be useful for the prevention of MA's psychotropic effects. Thus, the development of reverse tolerance to the ambulation-enhancing effect of MA was blocked by treatment with GE (200 mg/kg), while spontaneous motor activity was not affected by that dose of GE. We have further demonstrated that GE inhibits the development of reverse tolerance elicited by repeated cocaine injections administered at 48-h intervals, demonstrating that GE can block sensitizaton induced by psychostimulants with different neuropharmacologic profiles. Furthermore, GE suppresses the appearance of the altered activity levels observed at the time of the readministration of MA or cocaine after a 30-day discontinuation of drug administration. In addition, the development of tolerance to morphine-induced antinociception (12,13) and reverse tolerance to the ambulation-enhancing effects (12) of morphine have been reported to be blocked by GE. These latter findings, together with those of the present study, suggest that GE may be useful clinically for the prevention of adverse actions of several drugs with abuse liability.

Despite the clinical promise of GE, the mechanism underlying GE-induced inhibition of the development and expression of reverse tolerance to MA and cocaine remains unclear at the present time. It is widely accepted that the behavioral sensitization induced by repeated administration of amphetamines including MA (2,22) and cocaine (9,16,18) is attributable to dopaminergic hyperfunction in the central nervous system. Hence, it might be credible that the inhibitory effect of GE on the development of reverse tolerance to MA or cocaine is closely related to the recovery of dysfunction in the doperminergic system. In fact, Kim et al. (14) demonstrated that dopamine content is increased in some brain regions by ginseng saponin treatment, and Tsang et al. (31) have shown that the total ginsenoside fraction inhibits uptake of dopamine into rat brain synaptosomes. These findings have suggested that GE has the ability to modulate dopaminergic activity, although facilitation by ginseng saponin or ginsenoside on dopamine content is contrary to our explanation of the mechanism underlying GE-induced blockade of reverse tolerance to MA and cocaine. One possible resolution of this issue is that GE has a normalizing action on the abnormal state; that is, GE weakens the enhancement of dopaminergic activity induced by chronic administration of MA or cocaine, as the increase in dopamine content induced by ginseng treatment was observed in the normal state (14,31). Furthermore, it has been reported that daily injections of ginseng saponins in mice prevent the development of reverse tolerance to the locomotor-enhancing effect of morphine, and it was suggested that the suppressive effect of ginseng saponin on morphine-induced sensitization may be closely associated with GE's interaction with possible changes of presynaptic dopamine receptor function induced by the chronic morphine treatment (12).

In accordance with previous reports (8,13,17,18,26), MA and cocaine induced the preference for the drug injection-associated compartment, demonstrating that these psychostimulants possess a reinforcing effect. This effect of MA and cocaine was also inhibited by GE (200 mg/kg), which did not induce either a place preference or place aversion when administered alone. It is well known that the dopaminergic neurons of the ventral tegmental system are involved in reinforcement processes, suggesting that GE-induced blockade of MA and cocaine-induced place preference could be dependent on the modulatory action of GE on this neurochemical system (4,19). Because the reinforcing effect of psychostimulant drugs is believed to be at least one of the critical factors leading to abuse of and dependence on those drugs, GE might also be a useful pharmacotherapy for the prevention of MA and cocaine abuse and dependence.

The GE used in the present studies contains 18% ginseng

total saponins. However, it is unclear which active components of ginseng are responsible for the interactions between GE and psychostimulants in the present work. Ginsenosides have been reported to be the main active constituents of ginseng (36,37), and ginsenoside Rb1 suppresses aggression in mice (34,35). Recently, it has been demonstrated that the immune responses of mice injected with cocaine are depressed (33), and that cocaine modulates the immune system, especially interleukin-2 (IL-2) network (6). Furthermore, serum tumor necroses factor (TNF) levels have been reported to be decreased by cocaine administration, suggesting that macrophage activity may be suppressed by cocaine abuse (7). Indeed, the locomotor enhancing effect and the reinforcing effect of cocaine are blocked by lipopolysaccharide, which enhances endogenous poduction of TNF by activiation of macrophages (27). Interestingly, it has been demonstrated that in addition to ginsenosides, polysaccharides are found in ginseng root (36,37) and can markedly stimulate phagocytosis of the reticuloendothelial system and the production of antibodies (32). Furthermore, Qian et al. have reported that the numbers of plaque forming cells and specific rosette forming cells in tumor bearing mice are markedly increased after administration of ginseng polysaccharides (21). Taken together, GE-induced inhibition of the development of psychostimulant-induced sensitization and plaace preference may be partially due to the immune-activating effect of GE, which would counteracted the immune-suppressing effect of psychostimulants.

In conclusion, the present data show that the development of reverse tolerance and conditioned place preference to MA and cocaine, which are well known to be typical psychostimulant effects, are blocked by doses of GE that do not influence spontaneous motor activity or place preference. Abuse of MA or cocaine persists as a public health crisis that continues to cause significant problems. At the present time, there is no effective pharmacotherapy for these drug problems. It is well known that GE is a relatively nontoxic herbal drug and has a wide range of therapeutic actions. Accordingly, it may be beneficial to treat drug-dependent individuals with GE. Taken together, the results of this study indicate that GE may be useful for the prevention of MA and cocaine abuse and dependence. In addition, GE may possibly overcome intractable symptoms of other drugs with dependence liability.

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