Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 1, pp. 23-27, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00224-X

Blockade by Ginseng Total Saponin of the Development of Cocaine Induced Reverse Tolerance and Dopamine Receptor Supersensitivity in Mice

HACK-SEANG KIM,*[1] JIN-GOO KANG,* YEON-HEE SEONG,†
KI-YEUL NAM‡ AND KI-WAN OH*

*Department of Pharmacology, College of Pharmacy, †Department of Veterinary Pharmacology, College of Agriculture, Chungbuk National University, Cheongju 360-763, Korea
‡Korea Tobacco and Ginseng Research Institute, Taejeon 320-345, Korea

Received 11 January 1994

KIM, H.-S., J.-G. KANG, Y.-H. SEONG, K.-Y. NAM AND K.-W. OH. *Blockade by ginseng total saponin of the development of cocaine induced reverse tolerance and dopamine receptor supersensitivity in mice.* PHARMACOL BIOCHEM BEHAV **50**(1) 23-27, 1995. – Daily repeated administration of cocaine (15 mg/kg, over a 7-day period) developed reverse tolerance to the ambulation-accelerating effect of cocaine. Intraperitoneal administration of ginseng total saponin (GTS, 100 and 200 mg/kg of body weight) prior to and during chronic administration of cocaine inhibited the development of reverse tolerance. Dopamine receptor supersensitivity was also developed in reverse tolerant mice that had received the same cocaine. The development of dopamine receptor supersensitivity was evidenced by the enhanced hypothermic response to apomorphine (1 mg/kg) and the enhanced ambulatory activity of apomorphine (4 mg/kg). GTS also prevented the development of dopamine receptor supersensitivity induced by the chronic administration of cocaine. These results provide that GTS may be useful for the prevention and therapy of the adverse action of cocaine. It is concluded that the development of reverse tolerance to the ambulation-accelerating effect of cocaine may be associated with the enhanced dopamine receptor sensitivity because both phenomena were blocked by GTS.

Reverse tolerance Cocaine Ginseng total saponin (GTS) Dopamine receptor supersensitivity

GINSENG is well known as a herbal medicine and has been used in therapy for thousands of years. Recently, its chemical and pharmacological studies have been reported by many investigators of many countries. Many reports made it evident that ginseng has various effects on the nervous system. Ginseng not only has stimulative and inhibitive effects on the central nervous system (21,25,26,28), but also regulates the effects of sedatives, hypnotics, and convulsants (20). It also acts as a modulator of neurotransmission in the brain (12,33).

Dopaminergic and noradrenergic neurons in the central nervous system play important roles in the behavioral effects of drugs. Cocaine acts as a stimulant of the central nervous system through an inhibition of uptake of dopamine and norepinephrine (1,16,18). Tatum and Seevers (29) first reported an enhancement of the motor-accelerating effect of cocaine after repeated administration in dogs. This phenomenon is referred to as sensitization or reverse tolerance. It was also reported that rats sensitized to cocaine showed an enhanced response to apomorphine, a direct dopamine receptor agonist, suggesting the development of dopamine receptor supersensitivity (5,17,27). It has been demonstrated that the behavioral sensitization after repeated administration of cocaine is attributable to the dopaminergic hyperfunction in the central nervous system (10,22,24,30).

On the other hand, there are reports of blockade by ginseng extract on the development of reverse tolerance to the dependence-liable drugs. Kim et al. (11) have reported that G115 [trademark for the standardized ginseng extract containing

[1] To whom requests for reprints should be addressed.

4% ginsenoside (Pharmaton Ltd., Lugano-Bioggio/Switzerland)] inhibits the development of reverse tolerance to the locomotor-accelerating activity of morphine and the development of dopamine receptor supersensitivity to morphine. Tokuyama et al. (31) have shown that standardized ginseng extract prevents the development of reverse tolerance to the ambulation-accelerating effect of methamphetamine.

For these reason, the present experiments were undertaken to determine the effects of GTS, as an active component fraction of ginseng extract, on the development of reverse tolerance to the ambulation-accelerating effect of cocaine in mice. Furthermore, the effects of GTS on the dopamine receptor supersensitivity induced by cocaine were also examined.

METHOD

Animals and Drugs

ICR male mice weighing 20–30 g in a group of 10–20, were used in all experiments. They were housed in the acrylic fiber cage in a controlled room (22 ± 2°C), and were freely given the solid diet and tap water.

The drugs used were cocaine hydrochloride (Han-Saem Pharm. Co., Korea), apomorphine (Sigma, St. Louis, MO), and GTS [saponins mixture containing at least 10 glycosides known as ginsenosides from Panax Ginseng, extracted and purified by Namba et al.'s Method (19), and supplied by Korea Ginseng and Tobacco Research Institute]. Except for apomorphine, the drugs were dissolved in physiological saline. Apomorphine was dissolved in saline containing 0.1% ascorbic acid, just prior to the experiment. Cocaine was administered to mice subcutaneously (SC). GTS and apomorphine were administered to mice intraperitoneally (IP).

Measurement of the Development of Reverse Tolerance to the Ambulation-Accelerating Effect

Because the development of reverse tolerance is variable, dependent upon such factors as drug dose, route of administration, and duration of treatment, for each end point investigated parameters were selected empirically to produce a significant degree of reverse tolerance within a period of 7 days or less. For example, the rate of development of reverse tolerance is generally dose dependent; therefore, the daily drug doses were designed to produce a significant change in end point within the limited duration of the study. Furthermore, in the mouse, reverse tolerance to cocaine develops more expeditiously to SC than IP administration; hence, the former route of administration was used to procedure reverse tolerance in these experiments.

The ambulatory activity of mice was measured by the tilting-type ambulometer (AMB-10, O'Hara & Co., Japan). Each mouse was placed in the activity cages of 20 cm in diameter, 18 cm in height, and drug administration was carried out after an adaptation period of 10 min. Daily ambulatory activity was measured for 1 h after cocaine administration. Cocaine 15 mg/kg were administered to mice once a day for 7 days. The development of reverse tolerance was evidenced by measuring the enhanced ambulation-accelerating activity with the repeated administration of cocaine.

The consistent and reliable inhibitory effect of GTS was observed dose dependently in mice pretreated with 1 h prior to cocaine administration when the combined effect of cocaine and GTS was investigated at various time intervals. Therefore, mice were pretreated with GTS 100 or 200 mg/kg 1 h prior to cocaine injection.

Measurement of the Development of Dopamine Receptor Supersensitivity

The development of dopamine receptor supersensitivity was determined by measuring the enhanced hypothermic response to and enhanced ambulatory activity of apomorphine (a dopaminergic receptor agonist) in mice treated with cocaine.

Additional groups of mice that had received the same chronic cocaine and GTS as in the measurement of the development of reverse tolerance in the previous test were used to determine the effects of these treatments on the hypothermic response to apomorphine. The hypothermic response to an intraperitoneal injection of apomorphine 1 mg/kg (23), a dosage enough to produce hypothermia, was determined 24 h after the final injection of cocaine. Body temperature was determined by using a rectal probe (inserted 2.5 cm into the rectum) and a telethermometer. The measurements were made just prior to and 30 min after apomorphine injection. Data are expressed as the difference between the measurements before and after the injection of apomorphine.

Other additional groups of mice that had received the same chronic cocaine and GTS in the previous test were used to determine the effects of these treatments on the apomorphine induced ambulation-accelerating activity. The ambulation-accelerating activity of apomorphine was measured 24 h after the final injection of cocaine. Mice were first allowed to preambulate for 10 min followed by a 20-min test period. After an interval of 30 min, mice were given apomorphine 4 mg/kg, a dosage that produced a significant increase of ambulatory activity (4). Just after the administration, the 10-min preambulation period and the 20-min test period were repeated. Data are expressed as the difference between the test activity counts before and after the injection of apomorphine.

Statistics

The data were expressed as mean ± SE. The significant differences were first analyzed by variance (ANOVA). In the case of significant variation, the individual values were compared by Student's *t*-test.

RESULTS

Inhibitory Effects of GTS on the Development of Cocaine-Induced Reverse Tolerance to the Ambulation-Accelerating Effect

Figure 1 showed the mean overall ambulatory activity after repeated administration of cocaine (15 mg/kg). The ambulation-accelerating activities of cocaine were enhanced by repeated treatment; that is, reverse tolerance was developed to the ambulation-accelerating effects of cocaine. Single or chronic treatment with GTS or saline alone did not influence the spontaneous motor activity of mice.

Cocaine-induced ambulation-accelerating activity was markedly enhanced by repeated administration of the drug showing 2,300 counts on day 6, compared with the count of 1,250 on day 1. GTS, given 1 h before cocaine injection, dose dependently suppressed the development of reverse tolerance to cocaine. In mice pretreated with GTS 200 mg/kg, the activity count on day 6 was around 1,100, about 1,200 less than that of only cocaine pretreated group (Fig. 1).

These results suggest that GTS inhibits the development of reverse tolerance to the ambulation-accelerating effects of cocaine.

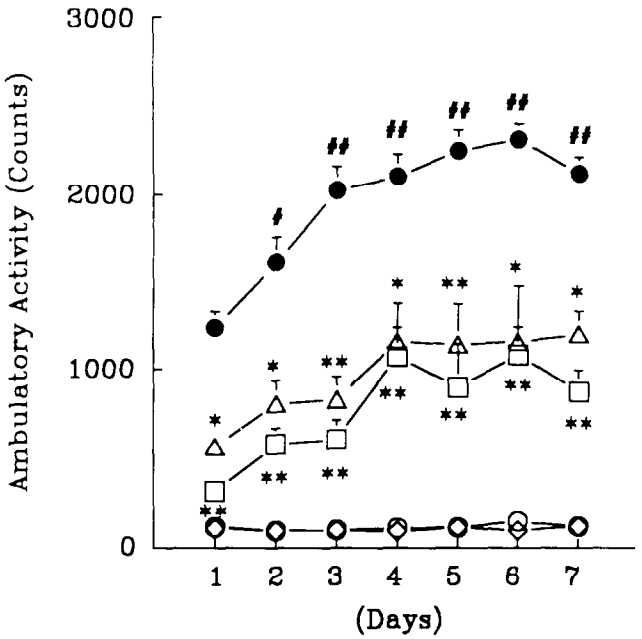

FIG. 1. Effect of GTS on the development of reverse tolerance to the ambulation-accelerating effect of cocaine. Cocaine 15 mg/kg (SC) was administered to mice once a day for 7 days. GTS 100 or 200 mg/kg (IP) was administered to mice 1 h prior to the injection of cocaine. The ambulatory activity was measured for 1 h after the cocaine administration. #$p < 0.05$, ##$p < 0.01$, compared with the count on day 1; *$p < 0.05$, **$p < 0.01$, compared with that of the cocaine group. ○—Saline; ●—Cocaine 15 mg/kg; ◇—GTS 200 mg/kg; □—Cocaine 15 mg/kg + GTS 200 mg/kg; △—Cocaine 15 mg/kg + GTS 100 mg/kg.

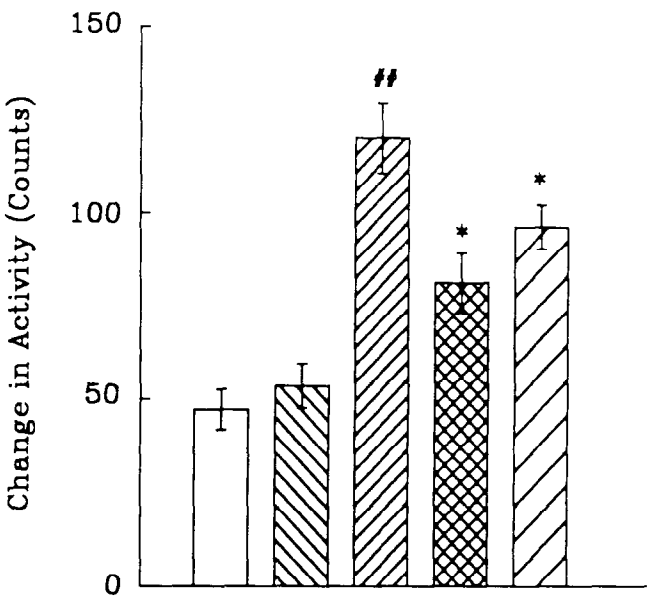

FIG. 2. Effect of GTS on the enhanced ambulatory activity of apomorphine in mice treated with cocaine. The ambulatory activity of apomorphine 4 mg/kg (IP) was determined 24 h after the final injection of cocaine. ##$p < 0.01$, compared with that of saline group; *$p < 0.05$, compared with that of cocaine group. Open column (1st col): Saline; right-to-left slanted line column (2nd col): GTS 200 mg/kg; left-to-right slanted line column (3rd col): cocaine 15 mg/kg; cross-hatched column (4th col): Cocaine 15 mg/kg + GTS 200 mg/kg; wide slanted rule column (last col): Cocaine 15 mg/kg + GTS 100 mg/kg.

Inhibitory Effects of GTS on the Development of Dopamine Receptor Supersensitivity

The hypothermic response to apomorphine were enhanced in mice treated with cocaine, compared with the saline treatment group. Cocaine-treated mice that had received chronic injection of GTS 100 or 200 mg/kg 1 h prior to the injection of cocaine did not exhibit an enhanced hypothermic response to apomorphine (Table 1). Single or chronic treatment with GTS or saline alone did not influence the hypothermic response to apomorphine.

Apomorphine, at a dose of 4 mg/kg, produced a significant increase of ambulatory activity in chronic cocaine-treated mice, compared with the increase observed in saline-treated mice. The enhanced ambulatory activity of apomorphine in

TABLE 1

EFFECT OF GTS ON THE ENHANCED HYPOTHERMIC RESPONSE TO APOMORPHINE IN MICE TREATED WITH COCAINE

Group	Body Temperature, (°C)		Temperature Change (°C)
	At 0 min	At 30 min	
Saline	38.10 ± 0.22	37.06 ± 0.24	−1.04 ± 0.19
GTS	38.23 ± 0.22	37.28 ± 0.17	−0.95 ± 0.14
Saline + Cocaine	38.25 ± 0.17	36.33 ± 0.20	−1.92 ± 0.18*
GTS 100 + Cocaine	38.24 ± 0.10	36.99 ± 0.17	−1.25 ± 0.17†
GTS 200 + Cocaine	38.17 ± 0.11	37.01 ± 0.21	−1.16 ± 0.15†

The hypothermic response to apomorphine 1 mg/kg IP was determined 24 h after the final injection of cocaine. Rectal temperature was measured before and 30 min after the apomorphine injection. Changes were expressed as the differences of temperature between before and after the injection.

*$p < 0.05$, compared with that of saline group.

†$p < 0.05$ compared with that of cocaine group.

chronic cocaine-treated mice was inhibited by GTS 100 mg/kg or 200 mg/kg pretreatment 1 h prior to the injection of cocaine (Fig. 2).

These results show another evidence that chronic administration of cocaine develops dopamine receptor supersensitivity and GTS blocks the development of dopamine receptor supersensitivity.

DISCUSSION

It has been reported that the standardized ginseng extract G115 inhibited the development of reverse tolerance to ambulatory accelerating effects of morphine and methamphetamine, and independently of these, the development of dopamine receptor supersensitivity induced by morphine (11,31). However, in the present experiment, GTS separated from ginseng extract also inhibited the development of both phenomena induced by cocaine. So, it is presumed that GTS is an active compound fraction of ginseng extract on the inhibition of the development of reverse tolerance to the ambulation accelerating effect of cocaine and the dopamine receptor supersensitivity induced by cocaine. The present experiments show that cocaine increases the ambulatory activity of mice, and the effects are progressively enhanced by repeated administration of cocaine, indicating the development of reverse tolerance. This result is identical with those reported previously (5,9,14,24,27). The phenomenon of reverse tolerance is a model for studying the psychotoxicity of dependence-liable drugs (2). The inhibitory effect of GTS on the phenomenon in mice is useful for the therapy of the adverse actions of cocaine.

Cocaine inhibits the reuptake of catecholamines at presynaptic terminals. Chronic exposure to cocaine produces a functional depletion of dopamine synthesis (13,18,32). In association with these facts, it has been presumed that the behavioral sensitization produced by chronic administration of cocaine is accompanied by the change of dopaminergic neuronal activity. In support of this, it has been demonstrated that such sensitization is blocked by neuroleptics (3,6,7).

Then, the enhanced responses to apomorphine, a direct-acting dopamine receptor agonist, should be enhanced because the postsynaptic dopamine receptor supersensitivity develops after the repeated administration of cocaine (5,17,27). In the present experiments, the enhancement of the hypothermic response to and ambulatory activity of apomorphine was also confirmed in groups treated with cocaine, compared with that of control group, suggesting the development of dopamine receptor supersensitivity. GTS inhibited the development of reverse tolerance to ambulation-accelerating effect of cocaine. And GTS also inhibited the development of dopamine receptor supersensitivity by the chronic treatment of cocaine. In support of this, Kim et al. (11) demonstrated that standardized ginseng extract inhibited the development of reverse tolerance to the locomotor accelerating activity of morphine and the development of morphine-induced dopamine receptor supersensitivity, and suggested that the inhibitory effect of ginseng extract on this action may be associated with the interruption of chronic morphine action at the presynaptic dopamine receptors. Tokuyama et al. (31) demonstrated that standardized ginseng extract suppressed the development of reverse tolerance to the ambulation-accelerating effect of methamphetamine, and presumed that the inhibitory effect of ginseng extract on methamphetamine-induced reverse tolerance was related to the recovery of dysfunction in the dopaminergic system.

However, the possible mechanisms underlying the inhibition by GTS of the development of reverse tolerance and dopamine receptor supersensitivity to cocaine remains unclear. Kim et al. (12) and Tsang et al. (33) have shown that dopamine content is increased by ginseng saponin treatment, and the ginseng saponin inhibits the uptake of dopamine into rat brain synaptosomes, suggesting that GTS has the ability to modulate the dopaminergic activity preferentially. It may be, therefore, plausible that the inhibitory effects of GTS on the development of reverse tolerance and dopamine receptor supersensitivity to cocaine may be related to the recovery of the dysfunction at the presynaptic dopamine receptors because the cocaine action on dopamine receptors is indirect as Gawin (6), Giannini (7), and Moore (18) suggested.

Moreover, the high doses of 4 mg/kg and 1 mg/kg of apomorphine acting as a postsynaptic dopamine agonist were used in the tests of the enhanced ambulatory activity and the enhanced hypothermic response to apomorphine, respectively. In addition, Henry and White (8), and White et al. (15) have demonstrated a postsynaptic increase in D_1 dopamine receptor sensitivity following the chronic administration of cocaine. Therefore, it should not be excluded that the inhibitory effects of GTS on the development of reverse tolerance and dopamine receptor supersensitivity to cocaine could be simultaneously having a postsynaptic modulatory effect via direct and indirect effects.

The present results have demonstrated that GTS may be useful for prevention and therapy of the adverse action of cocaine. Repeated administration of cocaine developed reverse tolerance to the ambulation-accelerating effect of cocaine as well as dopamine receptor supersensitivity. Administration of GTS inhibited the development of reverse tolerance to the ambulation-accelerating effect of cocaine and the supersensitivity of dopamine receptors. So it is tempting to speculate that the development of reverse tolerance to the ambulation-accelerating effect of cocaine may be closely associated with the development of dopamine receptor supersensitivity because both phenomena were blocked by GTS.

ACKNOWLEDGEMENT

This study was supported by Fund from Korea Tobacco and Ginseng Research Institute (1993).

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