

Original Article

Inhibitory Effects of *Coptidis rhizoma* and Berberine on Cocaine-induced Sensitization

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Substantial evidence suggests that the behavioral and reinforcing effects of cocaine can be mediated by the central dopaminergic systems. Repeated injections of cocaine produce an increase in locomotor activity and the expression of tyrosine hydroxylase (TH) in the main dopaminergic areas. Protoberberine alkaloids affect neuronal functions. *Coptidis rhizoma* (CR) and its main compound, berberine (BER) reduced the dopamine content in the central nervous system. In order to investigate the effects of CR or BER on the repeated cocaine-induced neuronal and behavioral alterations, we examined the influence of CR or BER on the repeated cocaine-induced locomotor activity and the expression of TH in the brain by using immunohistochemistry. Male SD rats were given repeated injections of saline or cocaine hydrochloride (15 mg/kg, i.p. for 10 consecutive days) followed by one challenge injection on the 4th day after the last daily injection. Cocaine challenge (15 mg/kg, i.p) produced a larger increase in locomotor activity and expression of TH in the central dopaminergic areas. Pretreatment with CR (50, 100, 200 and 400 mg/kg, p.o.) and BER (200 mg/kg, p.o.) 30 min before the daily injections of cocaine significantly inhibited the cocaine-induced locomotor activity as well as TH expression in the central dopaminergic areas. Our data demonstrate that the inhibitory effects of CR and BER on the repeated cocaine-induced locomotor activity were closely associated with the reduction of dopamine biosynthesis and post-synaptic neuronal activity. These results suggest that CR and BER may be effective for inhibiting the behavioral effects of cocaine by possibly modulating the central dopaminergic system.

Keywords: cocaine–berberine–*Coptidis rhizome*–locomotor activity–tyrosine hydroxylase–ventral tegmental area.

Introduction

Repeated exposure to psychostimulants induces sensitization to their behavioral stimulant effects. Behavioral

sensitization is the enhanced motor-stimulant response that occurs with repeated exposure to such psychostimulants as cocaine or amphetamine (1,2).

Cocaine is a potent and widely abused psychostimulant that exerts behavioral and neuropharmacologic effects; these effects may be mediated by the central dopaminergic systems. Especially, the mesolimbic system from the ventral

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tegmental area (VTA) to the nucleus accumbens (NAc), one of the main central dopaminergic systems, has been implicated in the processes of drug addiction and this includes behavioral sensitization (3,4). For example, many studies reveal that the reinforcing effects of cocaine are related to the blockade of the dopamine (DA) reuptake systems (5) and the consequent increases in binding of DA to its post-synaptic receptors (6). The inhibition of DA reuptake has been demonstrated in several brain nuclei such as the NAc, the striatum, the VTA and the medial prefrontal cortex (3,7–9). DA receptor antagonists also block the cocaine-induced increases of locomotor activity and stereotypy, the decrease of food intake (10) and they block the cocaine-induced reinstatement of drug seeking behavior (11,12). In addition, several studies have shown that repeated exposure to cocaine produced the expression of tyrosine hydroxylase (TH) enzyme activity for DA biosynthesis in mesolimbic DA pathways (13,14).

Coptidis rhizoma (*Coptis chinensis* FRANCH, CR) has been widely used as a Korean traditional medicine for hundreds of years. CR exerts therapeutic effects on disorders such as anxiety, depression and substance abuse in human and animal studies (15,16) and CR and its main compound, berberine (BER) produced a variety of biological effects on the central nervous system (17–20). In particular, protoberberine alkaloids such as BER, palmatine and coptisine inhibited DA biosynthesis (21,22). BER reduces the dopamine content in PC12 cells (18,23).

However, the effects of CR or BER on cocaine-induced neurochemical and behavioral alterations have not been investigated. Therefore, in the present study, we determined to examine whether CR or BER could affect the repeated cocaine-induced locomotor activity. The expressions of TH in the VTA were also examined by using immunohistochemical methods in order to determine a possible mechanism underlining the suppressive effects of CR and BER on cocaine-induced behavioral sensitization in rats.

Methods

Subject

Male Sprague–Dawley rats weighing 260–270 g each were obtained from Samtaco Animal Corp. (Seoul, Korea). The experiment began at least 7 days after their arrival. Rats were maintained on *ad libitum* food and water and they were maintained on a 12 h light-dark cycle (lights on at 7:00) at an ambient temperature of 22–24°C with a controlled relative humidity of 55%. All experiments were approved by

the Kyung Hee University Institutional Animal Care and Use Committee.

Preparation of the Drugs and the Methanol Extracts of *Coptidis Rhizoma*

CR was purchased from an oriental drug store (Jungdo Inc. Seoul, Korea). The voucher specimens (no. KH-CR01) are deposited at the herbarium located in the College of Oriental Medicine, Kyung Hee University. CR (100 g) was cut into small pieces and extracted three times in a reflux condenser for 24 h each time using 85% methanol. The solutions were combined, filtered through Whatman no. 1 filter paper and concentrated using a rotary vacuum evaporator, this was followed by lyophilization. The yield was 12.8% (w/w). CR consists of the major active component, berberine (4–7%) and also of small amount of columbamine, coptisine, groenlandicine, berberastine and thalifendine. Cocaine hydrochloride (Macharlan, Smiss Limit, UK) and berberine (Sigma, St Louis MO, USA) were obtained from the standard commercial suppliers. Cocaine hydrochloride was dissolved in 0.9% saline and CR and berberine were dissolved in distilled water.

Experimental Design

The experiment consisted of three phases: a 10 day developmental phase, a 3 day withdrawal phase and a 1 day challenging phase. The experiment was designed to investigate the effect of CR and BER on the behavioral sensitization produced by repeated injection of cocaine. The rats were divided into eight groups. The two groups were pre-treated with saline [0.9% NaCl, i.p. saline-treated rats (SAL) group; $n=4$] or cocaine [15 mg/kg, i.p. cocaine-induced rats (COC) group; $n=7$] one daily for 10 consecutive days, after which time the rats were challenged with the same dose of saline or cocaine, 72 h after the last treatment, respectively. The acute cocaine treated group (15 mg/kg, i.p. CON group; $n=5$) received saline for 10 days, after which time the rats were challenged with cocaine, 72 h after the last treatment. The other experimental groups were pre-treated with CR 50 mg/kg (CR50 + COC group, p.o. $n=5$), CR 100 mg/kg (CR100 + COC group, p.o. $n=4$), CR 200 mg/kg (CR200 + COC group, p.o. $n=8$), CR 400 mg/kg (CR400 + COC group, p.o. $n=5$) and berberine 200 mg/kg (BER200 + COC group, p.o. $n=5$), respectively, 30 min before the injections of cocaine during a 10 day development phase. We did a pilot dose–response experiment with using BER 50, 100 and 200 mg/kg and we found that BER 200 mg/kg produced a maximal effect. Also, the dosage (200 mg/kg) chosen in

this study is a relatively standard one that other workers have reported on in a previous animal study (24). Their locomotor activity was measured for 1 h after every injection of cocaine or saline.

Measurement of Locomotor Activity

The rats were individually housed prior to behavioral testing. Locomotor activity was measured in a rectangular container ($40 \times 40 \times 45 \text{ cm}^3$) that was equipped with a video camera above the center of the floor as described previously (25). The walls and floor were made of clear plastic and they were painted black. Locomotor activity was monitored by a videotracking system using the S-MART program (PanLab, Barcelona, Spain). Rats were allowed to adapt themselves for 1 h in the container and the distance they traveled was recorded every 10 min during a 1 h baseline and during a 1 h after treatment. The measure of locomotor activity was indicated by cm.

Immunohistochemistry for Tyrosine Hydroxylase

One hour after the last behavioral testing, rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.) then perfused through the ascending aorta with normal saline (0.9%) and this was followed by 800 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were removed, post-fixed overnight, cryoprotected in 20% sucrose, and cut by a cryostat as $30 \mu\text{m}$ coronal sections. The sections were obtained according to the rat atlas of Paxinos and Watson (26) and stored in PBS solution for immunocytochemical processing. Sections were immunostained for TH protein by the avidin-biotin-peroxidase method. Sections were rinsed three times for 5 min each in PBS, then incubated for 72 h at 4°C with a primary polyclonal antiserum (sheep anti-TH; Chemicon, Temecula, CA, USA) at a titer of 1: 2000 in PBS containing 0.3% Triton-X100 (PBST). The sections were washed for 5 min in PBST and then incubated for 120 min in PBST containing biotinylated goat anti-sheep IgG antibody at a 1: 200 dilution (Vector Laboratories, Burlingame, CA, USA). Following a 90 min incubation in the Elite standard vecta stain avidin-biotin complex (ABC) re-agent (Vector Laboratories, Burlingame, CA, USA), the sections were again washed three times for 5 min each time in PBS, then incubated in a medium containing 0.05% 3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO, USA) with 0.01% H_2O_2 for 1 min to reveal the immunoreactivity. Finally, the tissue was rinsed in PBS; this was followed by a brief rinse in dH_2O and the tissues mounted individually onto slides. After allowing the slides to air dry, they were cover-slipped. The

tegmental cells were counted at $\times 400$ magnifications using a microscope rectangle grid that measured 50×50 microns, according to the rat atlas (26). The cells within the tegmental areas were counted on each of three sections per animal.

Statistical Analysis

Experimental results were expressed as means \pm SE. The behavioral data were analyzed by one-way ANOVA using the SPSS program (Version 8.0). Statistical differences among groups were further analyzed using Tukey's *post hoc* test. The immunohistologic data were calculated and analyzed by one-way ANOVA followed by the Tukey's *post hoc* technique. *P* values < 0.05 were considered to be significant.

Results

Cocaine-induced Locomotor Activity Higher in the Chronic Group

When the rats that were given repeated cocaine treatments were then challenged with systemic administration of cocaine, their behavioral responses were significantly increased compared with those of the saline-induced rats (SAL group) or acute cocaine-induced rats (CON group). The locomotor activity across time for 1 h after saline and cocaine challenges is shown in Fig. 1. The behavioral response to cocaine challenge in the repeated COC group was significantly higher than that in the saline-treated group ($P < 0.001$) and acute cocaine-treated group ($P < 0.001$). ANOVAs (8×11 , treatment \times time) performed on the activity scores following the drug injections indicated a significant effect of a group difference [$F(7, 35) = 79.506$, $P < 0.001$], effect of day [$F(10, 350) = 1.602$, $P < 0.05$], but not group \times day interaction [$F(70, 350) = 0.913$, $P = 0.672$]. Tukey's *post hoc* comparisons indicated that the behavioral response to cocaine challenge in the repeated cocaine-treated group was significantly higher than that in the acute cocaine treated group ($P < 0.001$).

CR and BER Significantly Blocked Chronic Cocaine-induced Locomotor Activity

Administration of CR or BER before the cocaine injection significantly blocked the effects of cocaine on locomotor activity during the 60 min testing period compared with the COC group, as is seen in Fig. 1. CR50, CR100, CR200, CR400 mg/kg and BER 200 mg/kg administered 30 min before the cocaine injection decreased the cocaine-induced locomotor activity to

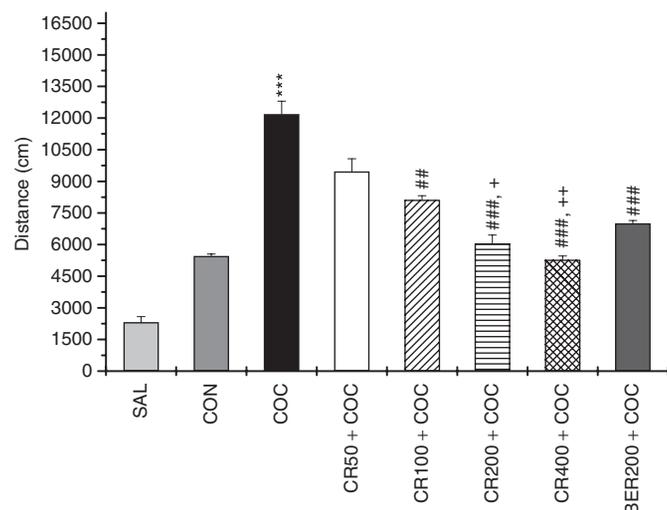


Figure 1. Effect of CR and BER on the locomotor activity in cocaine- or saline-pre-treated rats. Rats were pre-treated with saline (SAL group) or cocaine (15 mg/kg, i.p. COC group) one daily for 10 consecutive days, after which time the rats were challenged with the same dose of saline or cocaine, 72 h after the last treatment. The acute cocaine treated group (15 mg/kg, i.p. CON group) received saline for 10 days, after which time the rats were challenged with cocaine, 72 h after the last treatment. The other experimental groups were pre-treated with CR 50 mg/kg (CR50+COC group), CR 100 mg/kg (CR100+COC group), CR 200 mg/kg (CR200+COC group), CR 400 mg/kg (CR400+COC group) and berberine 200 mg/kg (BER200+COC group), 30 min before the injections of cocaine during a 10 days development phase. Significance with Tukey's test following a repeated ANOVA is indicated as *** $P < 0.001$ versus SAL group and CON group, or ## $P < 0.01$, ### $P < 0.001$ versus COC group and + $P < 0.05$, ++ $P < 0.01$ versus CR50+COC group. The vertical lines indicate SE.

9463.6 \pm 606.7 ($P = 0.154$), 8115.8 \pm 201.4 ($P < 0.05$) 6052.1 \pm 399.2 ($P < 0.001$), 5273.8 \pm 186.6 ($P < 0.001$) and 7000.3 \pm 142.9 ($P < 0.001$), respectively, when compared with the COC group's locomotor activity numbers of 12183 \pm 618.1 [$F(7,35) = 15.404$, $P < 0.001$]. In a pilot study it was shown that the only CR (100 mg/kg) or BER (100 mg/kg)-treated groups did not show any change in locomotor activity when compared with the saline-treated group ($P = 0.924$, $P = 0.902$).

CR and BER inhibited cocaine-induced TH-like immunoreactivity

The SAL group received saline instead of a test substance here because the previous studies revealed that TH-like immunoreactivity was increased due to the handling and injection stress in a variety of brain areas. Following the systemic injections of cocaine, a massive amount of TH was present in the ventral tegmental area (Figs 2 and 3).

Measures of one-way ANOVAs on the numbers of TH-like immunoreactive cells revealed a significant difference among the groups [$F(7,141) = 32.930$, $P < 0.001$]. *Post hoc* comparisons revealed the COC

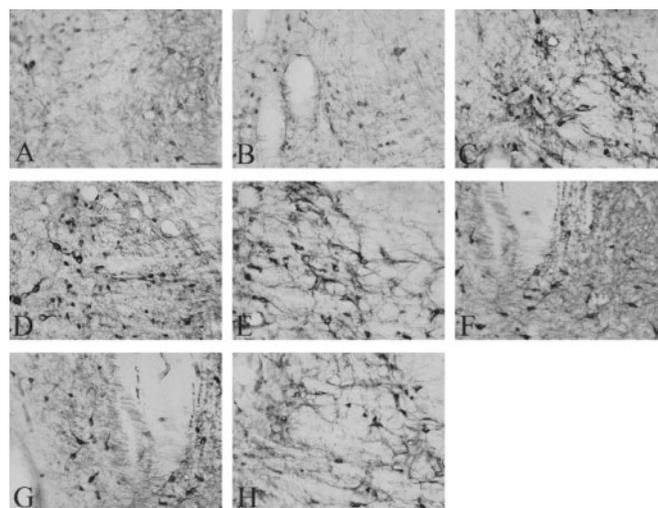


Figure 2. Representative photographs showing the TH expression in the ventral tegmental area of SAL (A), CON (B), COC (C), CR50+COC (D), CR100+COC (E), CR200+COC (F), CR400+COC (G) and BER200+COC (H) groups. The scale bar represents 25 μ m.

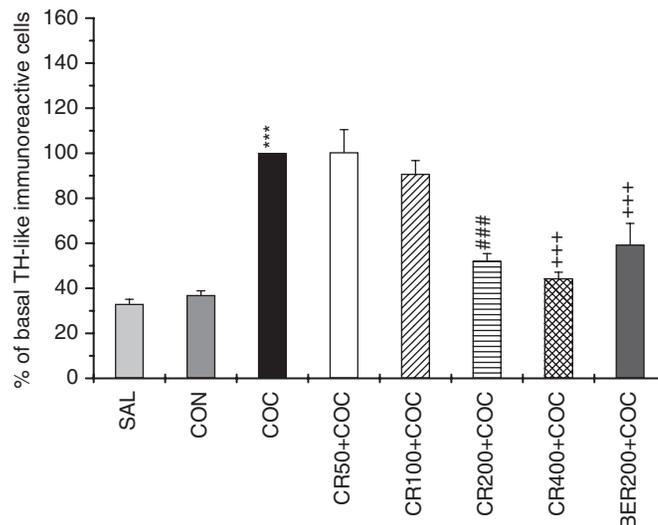


Figure 3. Expression of TH in the ventral tegmental area after systemic injections of saline or cocaine with *Coptidis rhizoma* and Berberine. The results are presented as the mean \pm SE. The total number of TH-like immunoreactive neurons within a (50 \times 50 μ m) grid over the areas at $\times 400$ magnification. Significance with Tukey's test following a one-way ANOVA is indicated as *** $P < 0.001$ versus SAL group and CON group, or ### $P < 0.001$ and +++ $P < 0.001$ versus COC group. The cells within the tegmental cell area were counted on each of three sections per animal.

group showed more increase in the TH expression than that of the SAL group ($P < 0.001$) and CON group ($P < 0.001$) (Fig. 2). CR50, CR100, CR200, CR400 mg/kg and BER 200 mg/kg administration 30 min before the cocaine injection decreased the numbers of TH-like immunoreactive cells to 100.39 \pm 10.11 ($P = 1.000$), 90.83 \pm 5.90 ($P = 0.897$), 52.07 \pm 3.32 ($P < 0.001$), 44.39 \pm 2.77 ($P < 0.001$) and 59.41 \pm 9.39 ($P < 0.001$),

respectively, when compared with the COC group's TH-like immunoreactive cells of 100 ± 0 .

Discussion

Systemic challenge with cocaine successfully produced a significant increase in locomotor activity and TH immunoreactivity in the ventral tegmental area, which is the major projection area of the central dopamine system. The results of present study demonstrated that repeated daily injections of cocaine produced a higher increase in locomotor activity to a subsequent systemic challenge with cocaine. Current results also clearly showed that a pre-treatment with CR and BER significantly suppressed the chronic cocaine-induced sensitization of locomotor activity and reduced the chronic cocaine-induced increases of TH expression in the VTA in the central DA pathways. These findings suggest that a pre-treatment with CR and BER reduces the development of locomotor activity in response to cocaine by modulating dopamine synthesis in the VTA. It is well known that the mesolimbic system from the VTA to the NAc mediates the behavioral and reinforcing effects of cocaine (27). In the present study, repeated injections of cocaine produced increases in locomotor activity and increases in the TH-like immunoreactivity in the central DA systems. These results agree with the other previous evidence (12,28).

The cocaine-treated group showed a significant increase in locomotor activity, when compared with the saline-treated group. However, there was no significant difference in locomotor activity between the CR and berberine only-treated groups. The CR or BER injections did not produce any significant changes in locomotor activity, indicating that CR or BER did not produce any significant increase in behavioral activity. Meanwhile, the CR and BER administered 30 min prior to the cocaine injection inhibited chronic cocaine-induced sensitization of locomotor activity when compared with the chronic cocaine-treated group. These results suggest that CR and BER inhibit the chronic cocaine-induced psychologic dependence, as determined by the behavioral sensitization paradigm.

Current results demonstrated that pre-treatment of CR 200 mg/kg and CR 400 mg/kg produced a significantly higher inhibitory effect on activity than BER 200 mg/kg. Since it is known that CR contains plamatine and coptisine as well as BER and previous reports have shown that palamatine and cotisine reduced behavioral activity and DA biosynthesis (18,29,30), perhaps other alkaloids may affect cocaine-induced neuronal and behavioral alterations. Future analyses are needed to investigate neurochemical and behavioral effects of these components on cocaine-induced sensitization.

We found that a pre-treatment of CR and BER significantly inhibited development of the cocaine-induced behavioral sensitization and tegmental TH to subsequent cocaine challenge. The present results also suggest that pre-treatment of CR and BER inhibition of the repeated cocaine-induced hyperactivity is closely related with the inhibition of the activated dopaminergic systems that is produced by cocaine. Therefore, the inhibitory effect of CR and BER on behavioral activity reflects the blockade of dopaminergic biosynthesis or transmission. This suggestion is strongly strengthened by previous studies showing that treatment of BER suppressed DA biosynthesis in the brain (18,31,32).

Even though several studies have indicated that protoberberins may have pharmacologic effects on the central DA system (18,23,30,31), this is the first demonstration of BER action on cocaine-induced behavioral and neurochemical activity. Little is currently known about the action of BER on the central DA system at cellular or molecular levels. Other kinds of protoberberines, the tetrahydroprotoberberines (THPBs), were previously shown to display the highest affinity for the D1- and D2-like receptors (30,33–36). Our preliminary data suggest that BER may bind to DA-receptor sites (unpublished observations). In addition to the clinical study of CR or BER on cocaine addiction, further pharmacologic actions of BER on the central dopaminergic systems should be investigated in the future.

Taken together, CR has been empirically prescribed as a traditional medicine for the treatment of substance abuse, but its major therapeutic actions have not been well known. However, our results demonstrated that CR and BER have central effects and thus contribute to the restoration of a normal biochemical balance in the dopaminergic system. Therefore, our results might provide a mechanistic rationale for herbal treatments including CR for treatment of drug abuse.

In summary, the present results demonstrated that CR and BER inhibit chronic cocaine-induced sensitization and they may modulate the cocaine-induced DA transmission at both the pre- and post-synaptic levels. These results suggest that CR and BER may be effective for inhibiting the behavioral effects of cocaine by modulating the central dopaminergic system.

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