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 × 40 × 45 cm³) 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 µm 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% H₂O₂ for 1 min to reveal the immunoreactivity. Finally, the tissue was rinsed in PBS; this was followed by a brief rinse in dH₂O and the tissues mounted individually onto slides. After allowing the slides to air dry, they were cover-slipped. The tegmental cells were counted at ×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 ± 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 × 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 × 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 administrated 30 min before the cocaine injection decreased the cocaine-induced locomotor activity to
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 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, or \( ***P<0.001 \) versus SAL group and CON group, or \( ###P<0.001 \) versus COC group and \( +P<0.05, +++P<0.01 \) versus CR50 + COC group. The vertical lines indicate SE.

**CR and BER inhibited cocaine-induced TH-like immunoreactivity**

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 \( (P = 0.924, P = 0.902). \)

**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 \( ###P < 0.001 \) versus COC group and \( ++P < 0.05, +++P < 0.01 \) versus CR50 + COC group. The scale bar represents 25 μm.
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 modulat-
ing 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
protoberberines may have pharmacologic effects on
the central DA system (18,23,30,31), this is the first
demonstration of BER action on cocaine-induced beha-
vioral 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 prelimin-
ary 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 dopami-
nergic 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 trans-
mision 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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