JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1663–1669
© 2007 The Authors
Received May 2, 2007
Accepted August 20, 2007
DOI 10.1211/jpp.59.12.0008
ISSN 0022-3573

Coptidis Rhizoma attenuates repeated nicotine-induced behavioural sensitization in the rat

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Abstract

Repeated injections of nicotine can produce an increase in locomotor activity and the expression of immediate-early gene, c-*fos*, in the central dopaminergic areas. Many studies have shown that *Coptidis Rhizoma* (CR) and its main alkaloid compound, berberine (BER), have a suppressive effect on the central nervous system. We examined the influence of CR or BER on repeated nicotine-induced locomotor activity in rats and the change of c-Fos expression in the brain by using immunohistochemistry. Male Sprague–Dawley rats were given CR and BER before repeated injections of nicotine hydrochloride (0.4 mg kg^{-1}, s.c.) twice daily for 7 days. After 3 days withdrawal, rats received a challenge injection of nicotine. Pretreatment with CR (100 mg kg^{-1}, i.p.) and BER (100 mg kg^{-1}, i.p.) significantly inhibited the nicotine-induced locomotor activity and expression of c-Fos in the striatum and the nucleus accumbens. These results suggest that CR and BER may produce inhibitory effects of nicotine on behavioural sensitization by possibly reducing postsynaptic neuronal activation in the central dopaminergic systems.

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Funding: This study was supported by the Grant of the Korea Research Foundation (KRF-2006-311-H00001) and the SRC program of KOSEF (R11-2005-014), Republic of Korea.

Introduction

The dopaminergic mesolimbic system, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens, is critically involved in behavioural sensitization (DeBelleroche et al 1979; Clarke & Pert 1985; Marks et al 1992; McGehee & Role 1995; Brioni et al 1997), as shown by an enhanced locomotor response to a subsequent injection of an addictive drug, such as nicotine (Clarke & Pert 1985; Robinson & Becker 1986; Kalivas & Stewart 1991). Behavioural sensitization has been implicated in the development of drug addiction (Robinson & Becker 1986; Robinson & Berridge 1993) and drug-induced psychosis (Segal et al 1981; Post & Contel 1983; Robinson & Becker 1986). Many studies have focused on the role of the mesolimbic dopamine (DA) system, which is known to play an important role in the reinforcing effects of nicotine (Corrigall et al 1992), and it has been demonstrated that the development of psychostimulant-induced behavioural sensitization might be associated with alterations of neurotransmission in the mesolimbic dopamine pathways (Fitzgerald & Nestler 1995). Nicotine activates dopamine release in the nucleus accumbens and striatum (Imperato et al 1986; Pontieri et al 1996). Dopamine release in nicotine-sensitized rats is causally related to the locomotor effects of the drug (Corrigall & Coen 1991), which are blocked by dopaminergic antagonists and reduced by lesion of dopamine neurons in the nucleus accumbens (Kelly et al 1975; Clarke et al 1988).

Several studies have demonstrated that repeated nicotine exposure causes changes in gene expression within brain reward systems and elevates expression of the immediate-early gene, c-*fos*, in dopaminergic target areas, such as the nucleus accumbens, striatum and prefrontal cortex (Kiba & Jayaraman 1994; Panagis et al 1996; Mathieu-Kia et al 1998). Since many studies suggest that alterations in the dopamine terminal areas may be critical in the long-term effects of nicotine, it is likely that the behavioural sensitization to nicotine may reflect alterations in extracellular dopamine release or postsynaptic gene expression (Panagis et al 1996; Shim et al 2001).

Coptidis Rhizoma (*Coptis chinensis* Franch., CR), a Korean traditional herbal medicine, has been widely used for hundreds of years. CR consists of the major active alkaloid compound berberine (BER; 4~7%) and also small amounts of columbamine, coptisine,

groenlandicine, berberastine and thalifendine. BER ($C_{20}H_{18}CINO_4$) is an isoquinoline alkaloid; its salts are typically yellow and are widely distributed in many species of the Berberidaceae, Fumariaceae, Papaveraceae and other plant families (Li et al 2005; Lee et al 2007). It is known that CR and BER have a suppressive effect on the central nervous system (Yamahara 1976a; Hwang et al 2002; Wang et al 2005). It is reported that protoberberine alkaloids, such as BER, palmatine and coptisine, exhibit a wide variety of pharmacological and biological actions and in particular inhibit dopamine biosynthesis (Clement-Cormier et al 1979; Harsing et al 1988; Xu & Malave 2001). It is also known that BER reduces dopamine content in PC12 cells and exhibits depressant effects such as sedation (Hsu & Kin 1962; Sethi 1983; Tang & Eisenbrand 1992; Shin et al 2000). However, the effects of CR or BER on repeated nicotine-induced neurochemical and behavioural alterations have not been investigated. Therefore, in this study, we aimed to examine whether CR or BER could attenuate repeated nicotine-induced locomotor activity. The expression of c-Fos in the nucleus accumbens and striatum was also examined by using immunohistochemical methods to determine a possible mechanism underlying the suppressive effects of CR or BER on the repeated nicotine-induced behavioural sensitization in rats.

Materials and Methods

Animals

Male Sprague–Dawley rats, 260~270 g each, were obtained from Samtaco Animal Corp. (Seoul, Korea). The experiment began at least 7 days after their arrival. Rats had free access to food and water and they were maintained on a 12-h light–dark cycle (lights on at 0700 h) at an ambient temperature of 22–24°C with a controlled relative humidity of 55%. All the 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). A voucher specimen (No. KH-CR01) was 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 alkaloid compound, BER (4~7%), and also small amounts of columbamine, coptisine, groenlandicine, berberastine and thalifendine. Nicotine hydrochloride and BER (Sigma, St Louis, MO) were obtained from standard commercial suppliers. Nicotine hydrochloride was dissolved in 0.9% NaCl and the other drugs were dissolved in distilled water. The same samples, CR and BER, were used in this study as reported previously (Lee et al 2007).

Experimental design

The experiment consisted of three phases: a 7-day developmental phase, a 3-day withdrawal phase and a one-day challenging phase. Rats were divided into five groups. Two groups were pretreated with saline (0.9% NaCl, i.p., SAL group; n = 5) or nicotine (0.4 mg kg^{-1}, s.c., NIC group; n = 6) twice daily for seven consecutive days, after which time rats were challenged with the same dose of saline or nicotine, 72 h after the last treatment, respectively. The acute nicotine-treated group (0.4 mg kg^{-1}, s.c., CON group; n = 6) received saline for 7 days, after which time the rats were challenged with nicotine, 72 h after the last treatment. The other experimental groups were pretreated with CR (100 mg kg^{-1}, i.p., CR + NIC group; n = 6) and BER (100 mg kg^{-1}, i.p., BER + NIC group; n = 6), respectively, 30 min before the injections of nicotine during a 7-day development phase. The locomotor activity was measured for 1 h after every injection of nicotine or saline.

Measurement of locomotor activity

Rats were individually housed before behavioural testing. The locomotor activity was measured in a rectangular container (40 × 40 × 45 cm) that was equipped with a video camera above the centre of the floor as described previously (Chae et al 2004). The walls and floor were made of clear plastic and they were painted black. The locomotor activity was monitored by a video-tracking system using the S-MART program (PanLab, Barcelona, Spain). The rats were allowed to adapt themselves for 1 h in the container and the distance they travelled was recorded every 10 min throughout a 1-h baseline and for 1 h after treatment at 1000 h. Locomotor activity was measured in cm.

Immunohistochemistry for c-Fos

One hour after the last behavioural testing, the rats were deeply anaesthetized with sodium pentobarbital (80 mg kg^{-1}, i.p.) and then they were 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 and cryoprotected in 20% sucrose. The brains were cut by a cryostat as 30-μm coronal sections. The sections were obtained according to the rat atlas of Paxinos & Watson (1986) and they were stored in PBS for immunocytochemical processing. The sections were immunostained for Fos protein by the avidin–biotin–peroxidase method. The sections were rinsed three times for 5 min each in PBS and were then incubated for 72 h at 4°C with a primary polyclonal antiserum (rabbit anti-c-*fos*; Chemicon, Temecula, CA) at a titre 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-rabbit IgG antibody at a 1:200 dilution (Vector Laboratories, Burlingame, CA). Following a 90-min incubation in the Elite standard vecta stain avidin–biotin complex (ABC) reagent (Vector Laboratories, Burlingame, CA), the sections were again washed three times for 5 min each time in PBS, then they were incubated in a

medium containing 0.05% 3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO) 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 were individually mounted onto slides. After allowing the slides to air dry, they were cover-slipped. The sections were viewed at 100× magnification and the number of Fos-like immunoreactive cells was quantified in the shell and core of the nucleus accumbens and the striatum. Counts of Fos-labelled cells were made by an observer blind to the treatment within square grids of defined size (100×100 microns) that were placed over each area. Fos-labelled cells were counted only if they reached a defined darkness above the background. Counts from the striatum and nucleus accumbens were obtained according to the stereotaxic atlas (Paxinos & Watson 1986). The cells within the striatal and accumbal areas were counted on each of 3 sections for each rat.

Statistical analysis

The experimental results are expressed as means ± s.e. The behavioural data were analysed by analysis of variance with repeated measures using the SPSS program (Version 8.0). Statistical differences among groups were further analysed using Tukey's post-hoc test. The immunohistological data were calculated and analysed by one-way analysis of variance followed by the Tukey's post-hoc technique. $P<0.05$ was considered to be significant.

Results

Effect of CR and BER on the repeated nicotine-induced locomotor activity

When the rats that were given repeated nicotine treatments were then challenged with systemic administration of nicotine, their behavioural responses were significantly increased compared with those of the saline-treated rats (SAL group) or acute nicotine-induced rats (CON group). The locomotor activity across time for 1 h after the saline and nicotine challenges is shown in Figure 1. The behavioural response to nicotine challenge in the repeated nicotine-treated group was significantly higher than that in the saline-treated group ($P<0.001$) and acute nicotine-treated group ($P<0.001$). Analyses of variance (5×8, treatment×time) performed on the activity scores following the drug injections indicated a significant effect of drug injections ($F(4,24)=175.605$, $P<0.001$). Tukey's post-hoc comparisons indicated that the behavioural response to nicotine challenge in the repeated nicotine-treated group was significantly higher than that in the acute nicotine-treated group ($P<0.001$). The CR (100 mg kg^{-1}) and BER (100 mg kg^{-1})-treated groups did not show any change in locomotor activity when compared with the saline-treated group ($P=0.924$, $P=0.902$, respectively). Administration of CR and BER before the nicotine injection significantly blocked the effects of nicotine on the locomotor activity during the 60-min testing period compared with the NIC group (Figure 1). CR and BER administered 30 min before the nicotine injection decreased nicotine-induced locomotor activity to 4818.7 ± 238.8 cm ($P<0.001$) and 4749.6 ± 241.4 cm ($P<0.001$), respectively, when compared with the NIC group's locomotor activity of 7335.5 ± 482.0 cm ($F(4.24)=24.083$, $P<0.001$). CR and BER administered 30 min before the nicotine injection decreased nicotine-induced locomotor activity during the development phase, as seen in Figure 2. These behavioural effects were statistically significant after the fifth injection (Figures 1 and 2).

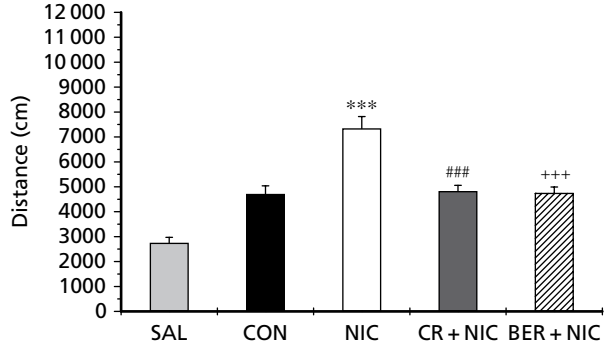

Figure 1 Effect of CR and BER on the locomotor activity in nicotine- or saline-pretreated rats during challenge phase. Rats were pretreated with saline (SAL) or nicotine (0.4 mg kg^{-1}, s.c., NIC) twice daily for seven consecutive days, after which time the rats were challenged with the same dose of saline or nicotine, 72 h after the last treatment. The acute nicotine-treated group (CON) received saline for 7 days, after which time the rats were challenged with nicotine, 72 h after the last treatment. The other experimental groups were pretreated with CR + NIC (100 mg kg^{-1}, i.p.) and BER + NIC (100 mg kg^{-1}, i.p.), 30 min before the injections of nicotine during development phase. ***$P<0.001$ vs SAL group; ###$P<0.001$; +++$P<0.001$ vs NIC group (Tukey's test following a repeated analysis of variance). The vertical lines indicate s.e.

Effect of CR and BER on the repeated nicotine-induced Fos-like immunoreactivity

The SAL group received saline instead of a test substance in this study because previous studies revealed that Fos-like immunoreactivity (FLI) was increased, due to the handling and injection stress, in a variety of brain areas. Following the systemic injections of nicotine, a massive amount of FLI was present in the striatum and the nucleus accumbens (Figure 3, 4).

In the lateral striatum, post-hoc comparisons revealed that the NIC group showed a marked increase in the FLI compared with the SAL group ($P<0.001$) (Figure 3). CR and BER administration 30 min before the nicotine injection decreased the numbers of Fos-like immunoreactive cells to 8.50 ± 0.79 ($P=0.248$) and 6.50 ± 0.61 ($P<0.01$), respectively, when compared with the NIC group's Fos-like immunoreactive cells of 11.11 ± 1.00 ($F(4, 86)=7.793$, $P<0.001$).

In the medial striatum, post-hoc comparisons revealed that the NIC group showed a marked increase in the FLI compared with the SAL group ($P<0.001$) (Figure 3). CR and BER administration 30 min before the nicotine injection

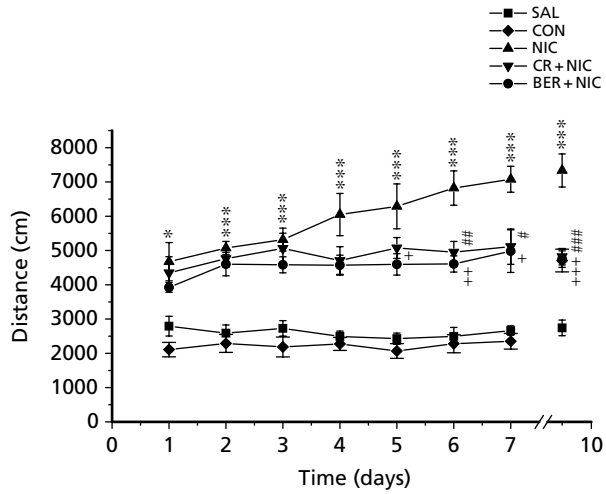

Figure 2 Effect of CR and BER on the locomotor activity in nicotine- or saline-pretreated rats during development phase. Rats were pretreated with saline (SAL) or nicotine (0.4 mg kg^{-1}, s.c., NIC) twice daily for seven consecutive days, after which time the rats were challenged with the same dose of saline or nicotine, 72 h after the last treatment. The acute nicotine-treated group (CON) received saline for 7 days, after which time the rats were challenged with nicotine, 72 h after the last treatment. The other experimental groups were pretreated with CR + NIC (100 mg kg^{-1}, i.p.) and BER + NIC (100 mg kg^{-1}, i.p.), 30 min before the injections of nicotine during development phase. *$P < 0.05$, ***$P < 0.001$ vs SAL group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ and +$P < 0.05$, ++$P < 0.001$, +++$P < 0.001$ vs NIC group (Tukey's test following a repeated analysis of variance). The vertical lines indicate s.e.

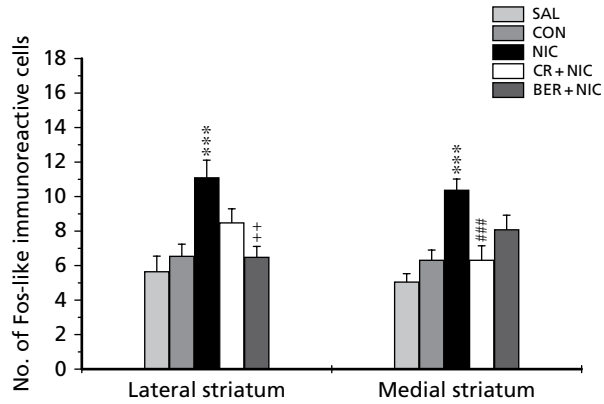

Figure 3 Expression of c-Fos in the striatum of rat brain after systemic injections of saline or nicotine with CR and BER. The results are presented as the mean ± s.e. total number of Fos-like immunoreactive neurons within a 100 × 100 μ grid over the areas at 100× magnification. ***$P < 0.001$ vs SAL group; ###$P < 0.001$, ++$P < 0.01$ vs NIC group (Tukey's test following a one-way analysis of variance). The cells within the striatal cell area were counted on each of 3 sections per rat.

decreased the numbers of Fos-like immunoreactive cells to 6.33 ± 0.82 ($P < 0.001$) and 8.00 ± 0.87 ($P = 0.094$), respectively, when compared with the NIC group's Fos-like immunoreactive cells of 10.39 ± 0.63 (F(4, 86) = 9.661, $P < 0.001$)

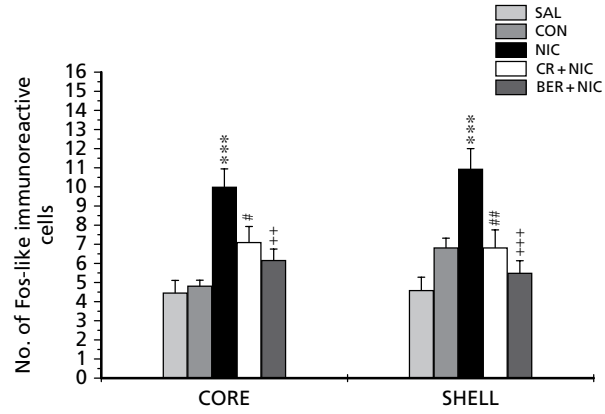

Figure 4 Expression of c-Fos in the nucleus accumbens of rat brains after systemic injections of saline or nicotine with CR and BER. The results are presented as mean ± s.e. total number of Fos-like immunoreactive neurons within a 100 × 100 μm grid over the areas at 100× magnification. ***$P < 0.001$ vs SAL group; #$P < 0.05$, ##$P < 0.01$, ++$P < 0.01$, +++$P < 0.001$ vs NIC group (Tukey's test following a one-way analysis of variance). The cells within the accumbal cell area were counted on each of 3 sections per rat.

In the core of nucleus accumbens, post-hoc comparisons revealed that the NIC group showed a marked increase in the FLI compared with the SAL group ($P < 0.001$) (Figure 4). CR and BER administration 30 min before the nicotine injection decreased the numbers of Fos-like immunoreactive cells to 7.11 ± 0.82 ($P < 0.05$) and 6.17 ± 0.58 ($P < 0.01$), respectively, when compared with the NIC group's Fos-like immunoreactive cell numbers of 10.00 ± 0.94 (F(4, 86) = 9.592, $P < 0.001$).

In the shell of nucleus accumbens, post-hoc comparisons revealed that the NIC group showed a marked increase in the FLI compared with the SAL group ($P < 0.001$) (Figure 4). CR and BER administration 30 min before the nicotine injection decreased the numbers of Fos-like immunoreactive cells to 6.83 ± 0.92 ($P < 0.01$) and 5.50 ± 0.64 ($P < 0.001$), respectively, when compared with the NIC group's Fos-like immunoreactive cells of 10.94 ± 1.06 (F(4, 86) = 9.260, $P < 0.001$).

Discussion

The results of this study demonstrated that systemic challenge with nicotine successfully produced a large increase in locomotor activity. It also produced FLI in the striatum and nucleus accumbens, which are the major projection areas of the central dopaminergic system. These results are in good agreement with other previous evidence (Panagis et al 1996; Shim et al 2001; Chae et al 2004). Our results also clearly showed that pretreatment with CR and BER significantly suppressed repeated nicotine-induced locomotor activity and increases in c-Fos expression in the striatum and nucleus accumbens in the central dopamine system. Therefore, our results suggest that pretreatment with CR and BER attenuated the development of locomotor activity in response

to nicotine by modulating the activation of postsynaptic dopamine receptors in the striatum and nucleus accumbens.

The nicotine-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 BER only-treated groups. The CR or BER only injections did not produce any significant increase in locomotor activity, indicating that CR and BER by themselves did not affect any significant behavioural changes. Meanwhile, CR or BER administered 30 min before the nicotine injection inhibited repeated nicotine-induced behavioural sensitization when compared with the repeated nicotine-treated group. These results suggest that CR and BER inhibit repeated nicotine-induced psychological dependence, as determined by the behavioural sensitization. However, more direct measures, such as nicotine self-administration or nicotine-induced conditioned place preference, should be tested in the future, to determine the inhibitory effects of CR and BER on nicotine-dependent behavioural paradigm.

We did a pilot dose–response experiment with small number of rats using CR and BER 50, 100 and 200 mg kg^{-1} and we found that CR and BER 100 mg kg^{-1} produced maximal effect. In addition, the dosage (100 mg kg^{-1}) selected in this study is a relatively standard one that previous workers have reported (Xu & Malave 2001; Lee et al 2007). A dosage of 100 mg kg^{-1} of BER is actually 6 times higher than that contained in a dosage of 100 mg kg^{-1} of CR and it is known that CR contains palmatine and coptisine as well as BER. Previous reports have shown that palmatine and coptisine reduced behavioural activity and dopamine biosynthesis (Xu et al 1989; Hsieh et al 1993; Shin et al 2000). Therefore, it may be possible that other alkaloids can affect nicotine-induced neuronal and behavioural alterations. Future study is needed to investigate the effects of these components on nicotine-induced neurochemical and behavioural sensitization.

Since many studies have shown that the behavioural and reinforcing effects of nicotine are mediated by the central dopamine systems (Imperato et al 1986; Marshall et al 1997; Lecca et al 2000), we compared the activity of Fos protein, encoded by *c-fos* proto-oncogene, with nicotine in the main dopamine terminal regions, the striatum and the nucleus accumbens. Pretreatment with CR and BER significantly inhibited accumbal and striatal FLI as well as the development of the nicotine-induced behavioural sensitization to subsequent nicotine challenge. In particular, BER reduced expression of FLI in the shell and lateral striatum much more than the core and medial striatum. These results suggest that the effect of BER on nicotine may be mediated through neuronal cells within the limbic structures, which are known to be involved in rewarding properties of drug abuse, rather than the nigrostriatal system, which is known to be associated with the control of motor function.

Our results also suggest that the inhibitory effect of CR and BER on behavioural activity is closely related to blockade of dopaminergic biosynthesis or transmission, shown by reduced post-synaptic neuronal activation in the dopaminergic terminals, the nucleus accumbens and striatum. This suggestion is very much strengthened by previous studies showing that BER suppressed dopamine biosynthesis in the brain (Vizi et al 1986; Lee & Kim 1996; Shin et al 2000).

BER can penetrate through the blood–brain barrier to reach the striatum, cortex and hippocampus, and it has been demonstrated that BER might directly act on brain nuclei to produce a neuroprotective effect (Clement-Cormier et al 1979; Wang et al 2005). Several studies have also shown that protoberberines have pharmacological effects on the central dopamine system (Lee & Kim 1996; Shin et al 2000). Other kinds of protoberberines, tetrahydroprotoberberines (THPBs), were previously shown to display the highest affinity to the D1- and D2-like receptors (Xu et al 1989; Jin & Sun 1995; Wu & Jin 1996, 1997) and THPBs showed a strong antagonistic action at D2 receptors (Marcenac et al 1986; Xu et al 1989; Wang et al 1997). THPBs are also known to have a variety of pharmacological effects on the central nervous system, such as sedation, hypnosis and analgesia (Hsu & Kin 1962; Xuan et al 1992; Yamahara 1976b). Consistent with the above studies, our results suggest that inhibitory effect of CR and BER on the repeated nicotine-induced hyperactivity may be closely related to the inhibition of the activated dopaminergic system produced by nicotine. The pharmacological actions of CR or BER on the central dopaminergic system should be investigated in the future, in addition to the clinical study of CR or BER on nicotine addiction.

In summary, the results of our study suggest that the inhibitory effects of CR and BER on the repeated nicotine-induced locomotor activity were closely associated with the reduction of dopamine biosynthesis and postsynaptic neuronal activity.

Conclusions

Coptidis Rhizoma and berberine could decrease nicotine-induced behavioural activity and they may modulate the nicotine-induced dopamine transmission at post-synaptic levels. These results suggest that CR and BER may be effective for inhibiting the behavioural effect of nicotine by possibly modulating the central dopaminergic system. Our data suggest that CR and BER may possibly be a useful therapeutic agent for nicotine addiction.

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