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Effects of *Coptis japonica* on Morphine-Induced Conditioned Place Preference in Mice

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Morphine, an analgesic with significant abuse potential, is considered addictive because of drug craving and psychological dependence. It is reported that repeated treatment of morphine can produce conditioned place preference (CPP) showing a reinforcing effect in mice. CPP is a useful method for the screening of morphine-induced psychological dependence. In the present study, we investigated the effect of the methanolic extract of Coptis japonica (MCJ) on morphine-induced CPP in mice. Furthermore, we examined *c*-fos expression in the parietal cortex, piriform cortex, striatum, nucleus accumbens, and hippocampus of the morphine-induced CPP mouse brain. Treatment of MCJ 100 mg/kg inhibited morphine-induced CPP. Expression of *c*-fos was increased in the cortex, striatum, nucleus accumbens, and hippocampus of the morphine-induced CPP mouse brain. These increases of expression were inhibited by treatment with MCJ 100 mg/kg, compared to the morphine control group. Taken together, these results suggest that MCJ inhibits morphine-induced CPP through the regulation of *c*-fos expression in the mouse brain.

Key words: Conditioned place preference, Coptis japonica, *c*-Fos, Immunocytochemistry

INTRODUCTION

Morphine is considered an addictive drug because drug-craving and psychological dependence are well-known features associated with its abuse. A single treatment with morphine in animals produces hyperactivity and stereotyped behaviors (Shuster *et al.*, 1963). Chronic treatment with morphine leads to the development of conditioned place preference (CPP) (Mucha *et al.*, 1982; Bardo et al 1984). The CPP paradigm has been used as a model for studying the reinforcing effect of drugs with dependence liability (van der Kooy, 1987). It has been suggested that the development of CPP induced by morphine is mainly associated with the enhanced dopaminergic transmission in dopaminergic synaptic terminals (van der Kooy, 1987; Koob, 1992) and the increased sensitivity of dopamine receptors (Kim *et al.*, 1996). Recently, suppression of *c*-fos induction in the nucleus accumbens prevented acquisition but not expression of morphine-induced CPP (Tolliver *et al.*, 2000). This result suggests that immediate early genes such as *c*-fos may also play an important role in the development of morphine-induced CPP.

Coptis japonica is a well-known, traditional oriental medicine. It has a wide range of pharmacological and biological activities, including anti-inflammatory (Ivanovska and Philipov, 1996) and antimicrobial (Schmeller *et al.*, 1997) effects. Hsieh *et al.* (2000) have reported that Coptis Chinese has an ameliorating effect on scopolamine-induced amnesia in rats. It is reported that protoberberine alkaloids from the roots of Coptis japonica inhibit the catecholamine biosynthesis in PC12 cells (Lee and Kim, 1996). Recently, it is reported that coptisine, a major component of Coptis japonica, inhibits MAO-A activity in the mouse whole brain (Ro *et al.*, 2001). We, therefore, presumed that inhibitors of catecholamine biosynthesis or MAO-A inhibitors could be a candidate for the treatment of drugs abuse. This assumption led us to study the effects of methanolic extract of *Coptis japonica* (MCJ) on morphine-induced CPP.

The present experiments were primarily undertaken to determine the effects of MCJ on the CPP induced by

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morphine. Also, *c*-fos immunoreactivity was measured in the morphine-induced CPP mouse brain to examine the neurochemical mechanisms underlying morphine-induced CPP.

MATERIALS AND METHODS

Animals and drugs

Male ICR mice (MJ Ltd., Seoul, Korea) weighing 18-24 g at the beginning of the experiment were used. They were housed 10 mice to a cage with water and food available ad libitum under an artificial 12 h light/dark cycle (light at 7:00 a.m.) and constant temperature (22±2°C).

The drug used was morphine hydrochloride (Je-il Pharm. Co., Seoul, Korea). MCJ was obtained from the Institute of Natural Medicine, Hallym University (Chuncheon, Korea). All drugs were dissolved in physiological saline just prior to the experiment.

Measurement of morphine-induced CPP

Apparatus

The CPP apparatus, made according to our previously reported methods (Kim *et al.* 1996), consisted of two square-based Plexiglas compartments (15×15×15 cm), one with white walls and the other with black walls joined by a gray tunnel (3×3×7.5 cm) which could be closed by guillotine doors. To provide a tactile difference between the compartments floors, the white compartment had a metal grid floor and the black compartment had a wire mesh floor. Removal of the guillotine doors during the pre-testing and the final testing phases allowed animals free access to both compartments, and the time spent by the mice in each of the two compartments was recorded for 15 min using a video camera. The time spent by the mice in the tunnel was ignored, since it comprised less than 5 % of the total time measured. All conditioning or test sessions were conducted under ambient light (20-30 Lux).

Procedures for place conditioning

Preliminary data from our laboratory indicated that native mice spent more time in the black compartment than in the white compartment when given free access to the entire apparatus for 15 min. Thus, to establish conditioning, we paired the morphine-administered mice with the initially non-preferred white compartment. The control mice received a subcutaneous injection of saline immediately before exposure to the black compartment. Morphine (5 mg/kg, s.c.) was given just before the mice were placed in the white compartment. To test the effect of MCJ (100 mg/kg, p.o.) alone or in combination with morphine, MCJ was administered 1 h prior to saline or morphine injections, respectively.

Pre-testing phase: On day 1, the mice were pre-exposed to the test apparatus for 5 min. The guillotine doors were raised and each animal was allowed to move freely between the two compartments. On day 2, baseline preference was determined for the non-preferred side vs. the preferred side for 15 min.

Conditioning phase: On days 3, 5, 7 and 9, the mice were injected with drug before confinement in the white compartment, non-preferred side, for 40 min. On days 4, 6, 8 and 10, the mice were injected with saline before confinement in the black compartment, preferred side, for 40 min.

Testing phase: On day 11, the guillotine doors were raised, the mice were placed in the tunnel in the central part of the apparatus, and the time spent by the mice in the two compartments was recorded for 15 min.

Place preference data were expressed as the difference between times spent in the testing and pre-testing phases in the white compartment. We also measured the number of crossings between white and black compartments in the testing phase.

Measurement of Immunocytochemistry

Brain section

Animals were anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), 24 h after the morphine-induced CPP paradigm. Mouse brains were removed and brain samples were sectioned coronally (45 μm) on a freezing microtome at -20°C.

Immunocytochemistry for *c*-fos expression

Floating sections of brains were processed as described previously by Baker & Farbman (1993). Briefly, the immunocytochemical procedure started with rinsing twice in 0.1 M PBS, followed by 2 h incubation to suppress non-specific absorption in the preincubation solution (0.1 M PBS containing 0.2% Triton X-100, 1% bovine serum albumin). To demonstrate *c*-fos immunoreactivity, we used the primary antiserum: rabbit anti-c-fos (1:1000, Santa Cruz Biotechnology, Inc) in a solution of 0.5% bovine albumin and preservative sodium azide in 0.1 M PBS. The sections were incubated in primary antiserum for 16 h at room temperature. On the following day, sections were incubated for 1 h in biotinylated rabbit secondary antibody obtained from Vector laboratories. After a short rinse with PBS, they were reacted by using the avidin-biotin peroxidase complex (ABC) method (Vector), and washed twice in 0.1 M PBS. The antigens were visualized by the solution containing 0.02% 3,3-diaminobenzidine tetrahydrochloride (DAB) and 0.0045% H_2O_2 at room temperature.

Statistics

The data were expressed as mean±S.E.M. Statistical analysis was carried out by one-way analysis of variance

(ANOVA). In the case of significant variation, the individual values were compared by the Student Newman-Keuls test. The criterion for significance was $p<0.05$ in all statistical analyses.

RESULTS

Effects of MCJ on morphine-induced CPP

Only the group treated with 100 mg/kg of MCJ did not show any CPP compared with the saline control group (data not shown). The morphine-treated group showed a significant psychological dependence producing CPP effect ($p<0.01$, Fig. 1). The group pretreated with 100 mg/kg of MCJ showed a significant inhibition of 5 mg/kg of morphine-induced CPP yielding a time difference between that spent in the testing and pre-testing phases in the white compartment of -13 sec, which was 129 sec less than the 116 sec of the morphine control group ($p<0.01$).

Effects of MCJ on crossing numbers in morphine-induced CPP mice

We measured the crossing numbers between white and black compartments in the testing phase. However, there was no significant difference in crossing numbers between the morphine treatment and saline groups (Table I). Also, there was no significant change in crossing numbers in the MCJ pretreatment groups.

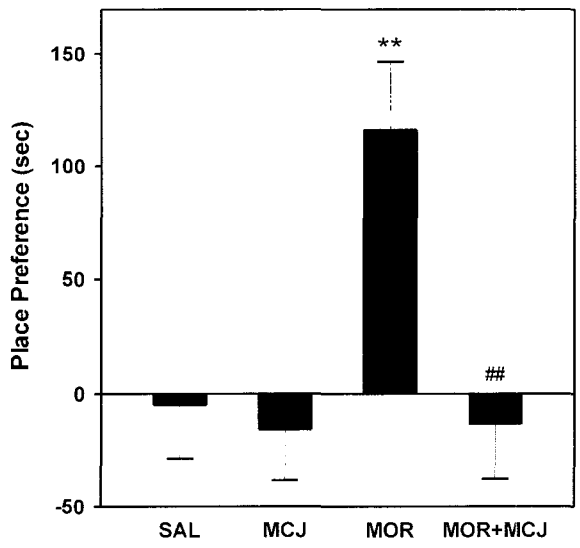

Fig. 1. Inhibitory effect of MCJ on morphine-induced CPP in mice. MCJ (100 mg/kg, p.o.) was administered 1 h prior to the injection of morphine (5 mg/kg). In the conditioning phase, the mice were injected with saline or morphine just before being confined in the black or white compartment for 40 min every day over 8 days. The scores were calculated from the differences between the testing and pre-testing phases (15 min) in the white compartment. **P<0.01, compared with that of the saline group. ##P<0.05, compared with that of the morphine group. Abbreviations: SAL, saline; MOR, morphine; MCJ, methanolic extract of *Coptis japonica*.

Table I. Effects of MCJ on numbers of crossing in morphine-induced CPP mice

Groups	Numbers of Crossing (Mean ± SE)	Numbers of Animals
Saline+Saline	52 ± 6.9	12
MCJ+Saline	44 ± 8.6	9
Saline+Morphine	52 ± 8.0	11
MCJ+Morphine	45 ± 9.3	9

MCJ (100 mg/kg, p.o.) was administered 1 h prior to the injection of morphine (5 mg/kg). In the conditioning phase, the mice were injected with saline or morphine just before being confined in the black or white compartment for 40 min every day over 8 days. The numbers of crossing between white and black compartments during the testing phase were counted (15 min).

Effects of MCJ on *c*-fos expression in morphine-induced CPP mice

The *c*-Fos expression was markedly increased in the parietal cortex, piriform cortex, striatum, nucleus accumbens, and hippocampus of mice with morphine-induced CPP (Fig. 2B, E, H, K, and N). However, pretreatment with 100 mg/kg of MCJ inhibited the *c*-fos expression of the morphine-induced CPP mouse brains which were measured in this study (Fig. 2C, F, I, L, and O).

DISCUSSION

Morphine indirectly stimulates the dopaminergic neurons by inhibiting GABAergic neurons (Johnson and North, 1992; Klitenick *et al.*, 1992). Morphine can activate mesolimbic DA release resulting in an activation of the mesolimbic DA pathway (Koob and Bloom, 1988; Wise and Rompre, 1989). The activation of the dopaminergic system appears to be involved in the rewarding and locomotor stimulant responses to morphine (Wise and Bozarth, 1987; Wise and Rompre, 1989). Accumulated evidence has suggested that the dopaminergic system plays a key role in the reinforcing effects of morphine (Bozarth, 1986). In support of this, dopamine receptor antagonists attenuated the reinforcing effects of morphine (Schwartz and Marchok, 1974; Shippenberg and Herz, 1987; 1988).

It has been reported that berberine and palmatine, protoberberine alkaloids from the roots of Coptis japonica, decrease dopamine content by reducing the tyrosine hydroxylase activity in PC12 cells (Shin *et al.*, 2000). It is also reported that berberine and palmatine also inhibit bovine adrenal tyrosine hydroxylase (Lee and Zhang, 1996; Lee *et al.*, 1996). Furthermore, coptisine, a major

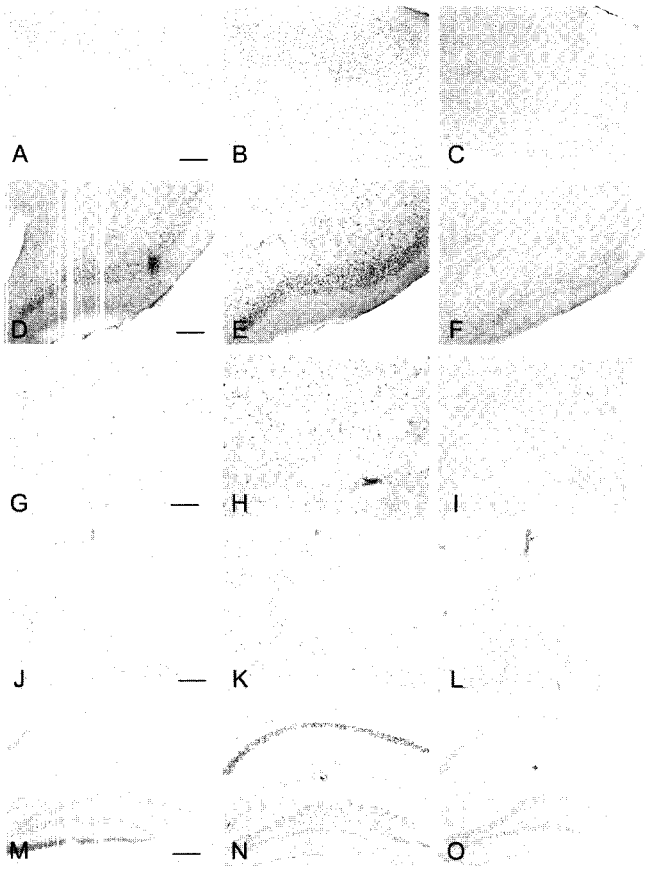

Fig. 2. Effects of MCJ on c-fos expression in morphine-induced CPP mice. Images showing the expression of c-fos immunoreactivity in the parietal cortex (A, B, C), piriform cortex (D, E, F), striatum (G, H, I), nucleus accumbens (J, K, L), and hippocampus (M, N, O) along the rostro-caudal axis. MCJ 100 mg/kg was administered to mice 1 h before morphine treatment (5 mg/kg), according to the CPP schedule. Mice were sacrificed at 24 h after the CPP schedule and brain samples were sectioned at a thickness of 45 μm. Abbreviations: SAL, saline; MOR, morphine; MCJ, methanolic extract of Coptis japonica. Scale bar = 200 μm.

component of Coptis japonica, inhibits MAO-A activity in the mouse whole brain (Ro *et al.*, 2001). In this study, pretreatment with MCJ inhibited morphine-induced CPP in mice. These results suggest that MCJ could inhibit morphine-induced CPP via modulation of the dopaminergic system. Therefore, further studies are needed to elucidate the involvement of dopaminergic systems in the inhibitory effect of MCJ on morphine-induced CPP.

Morphine and other addictive drugs induce the immediate-early gene *c*-fos in the nucleus accumbens and dorsal medial striatum (Graybiel *et al.*, 1990; Young *et al.*, 1991). It is proposed that *c*-fos expression in the nucleus accumbens is necessary for the acquisition, but not the expression, of morphine-induced CPP (Tolliver *et al.*, 2000). In this experiment, *c*-fos expression was increased in the parietal cortex, piriform cortex, striatum, nucleus accumbens, and hippocampus of the mouse brain, which produced morphine-induced CPP. These data suggest that the *c*-fos expression in the parietal cortex, piriform cortex, striatum, nucleus accumbens, and hippocampus plays a key role in producing morphine-induced CPP. We have noted that c-fos expression in the CA1 region of the hippocampus was more robust than that in the CA3 or dentate gyrus. These data are identical with the report of Frenois *et al.* (2002) that the CA1 region of the hippocampus is more responsible for the development of morphine addiction. Pretreatment with MCJ inhibited the expression of *c*-fos in these brain regions of morphine-induced CPP mice. This result suggests that MCJ inhibits morphine-induced CPP via blockade of the *c*-fos expression induced by morphine in these brain regions.

Taken together, it is concluded that MCJ, a methanolic extract of Coptis japonica, may be useful for the prevention and treatment of morphine addiction.

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