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Involvement of pCREB Expression in Inhibitory Effects of *Coptis japonica* on Morphine-induced Psychological Dependence

Seung-Hwan KWON, Ri-Ra HA, Seok-Yong LEE and Choon-Gon JANG*

Department of Pharmacology, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

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Abstract – Morphine is a potent analgesic with significant abuse potential, 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. Previously, we have reported the inhibitory effect of the methanolic extract of Coptis japonica (MCJ) on morphine-induced CPP in mice. The present study was employed whether p-CREB expression is involved in the inhibitory effect of MCJ on the morphine-induced CPP in the mouse hippocampus. Repeated administration of MCJ 100 mg/kg inhibited morphine-induced CPP. Expression of p-CREB was increased in the dentate gyrus of the hippocampus that had undergone morphine-induced CPP. This increase of expression was significantly inhibited by administration of 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 p-CREB expression in the mouse dentate gyrus of the hippocampus.

Keywords: Conditioned place preference; Coptis japonica; p-CREB; Immunohistochemistry

INTRODUCTION

Morphine is a useful analgesic, but considered an addictive drug because drug-craving and psychological dependence. 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 reported that drug addiction is strongly associated with learning and memory (Robbins and Everiff, 1999; Berke and Hyman, 2000). Learning and memory depend on activation of the transcription factor cAMP-response element binding protein (CREB). They are accompanied by alterations in neural plasticity at glutamate synapses. CREB, a transcription factor in particular has been implicated in opiates addiction (Nestler, 2001) and CPP (Gao *et al.,* 2003). This result suggests that CREB may play an important role in the development of morphine-induced CPP.

*Corresponding author
Tel: +82-31-290-7780, Fax: +82-31-292-8800
E-mail: jang@skku.ac.kr

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 psychological dependence or withdrawal syndrome, respectively. Previously we have reported that inhibitory effects of MCJ on the CPP induced by morphine. This inhibition was involved in the inhibition of increase in the c-fos expression of morphine CPP mouse brains. This result implicates that MCJ might regulate other gene expression, such as CREB which is implicated in opiates addiction (Nestler, 2001; Hyman, 1996). Therefore, the present experiments were undertaken to determine whether p-CREB expression is involved in the inhibitory effects of MCJ on the CPP induced by morphine to examine the neurochemical mechanisms.

MATERIALS AND METHODS

Animals and drugs

Male ICR mice (MJ Ltd., Seoul, Korea) weighing 18-24 g were used. They were housed 10 mice to a cage with water and food available ad libitum under an artificial 12 hr light/dark cycle (light at 7:00 a.m.) and constant temperature ($22 \pm 2^{o}C$).

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 ($15 \times 15 \times 15$ cm), one with white walls and the other with black walls 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 to freely 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. All conditioning or test sessions were conducted under ambient light (20-30 Lux).

Procedures for place conditioning

Preliminary data from our laboratory indicated that naive 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 hr 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 Immunohistochemistry

Brain section

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

Immunohistochemistry for p-CREB expression

Floating sections of brains were processed as described previously by Baker and Farbman (1993). Briefly, the immunocytochemical procedure started with rinsing twice in 0.1M PBS, followed by 2 h incubation to suppress non-specific absorption in the preincubation solution (0.1M PBS containing 0.2% Triton X-100, 1% bovine serum albumin). To demonstrate p-CREB immunoreactivity, we used the primary antiserum: rabbit anti-p-CREB (1:1000, Santa Cruz Biotechnology, Inc) in a solution of 0.5% bovine albumin and preservative sodium azide in 0.1M PBS. The sections were incubated in primary antiserum for 16h 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 0.1M 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

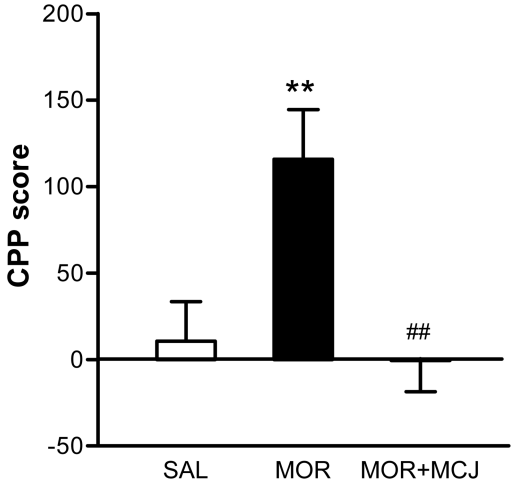

Fig. 1. Effect of MCJ on morphine-induced psychological dependence in mice. MCJ (100 mg/kg, p.o.) was administered 1 hr prior to the injection of morphine (5 mg/kg). In the conditioning phase, the mice were injected with saline or morphine just before being confined in the black or white compartment 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<0.01, compared with that of the morphine group. Abbreviations: SAL, saline; MOR, morphine; MCJ, methanolic extract of Coptis japonica.

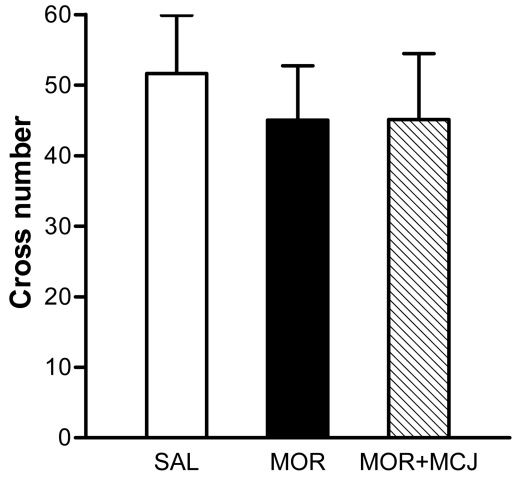

Fig. 2. Effects of MCJ on numbers of crossing in morphine-induced psychological dependence in mice.
MCJ (100 mg/kg, p.o.) was administered 1 hr prior to the injection of morphine (5 mg/kg). In the conditioning phase, the mice were injected with saline or morphine just before being confined in the black 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).

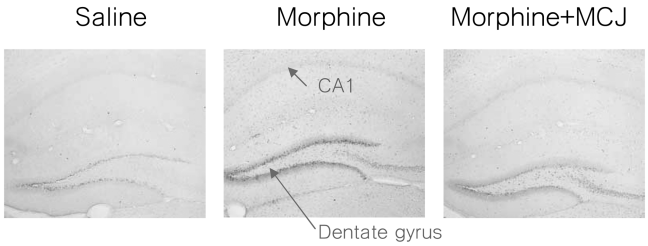

Fig. 3. Effects of MCJ on p-CREB expression in morphine-induced psychological dependence in mice. Images showing the expression of p-CREB immunoreactivity are in the dentate gyrus of the hippocampus along the rostro-caudal axis. MCJ 100 mg/kg was administered to mice 1 h before morphine treatment (5 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.

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

As reported previously, we confirmed that 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 -0.6 sec, which was 116.5 sec less than the 115.9 sec of the morphine control group ($p<0.01$).

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

As reported previously, we confirmed that there was no significant difference in crossing numbers between the morphine treatment and saline groups in the crossing numbers between white and black compartments in the testing phase (Fig. 2). Also, there was no significant change in crossing numbers in the MCJ pretreatment group.

Effects of MCJ on p-CREB expression in morphine-induced CPP mice

The p-CREB expression was markedly increased in the dentate gyrus of the hippocampus of mice with morphine-induced CPP (Fig. 3). However, pretreatment with 100 mg/kg of MCJ inhibited the increased p-CREB expression in the dentate gyrus of the hippocampus of

the morphine-induced CPP mouse which was measured in this study.

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 effects 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). However, the molecular mechanisms underlying morphine rewarding effects are not fully understood. Recently, several researchers have been focused on the gene expressions in specific brain regions of morphine dependent mice. It is reported that 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). Furthermore, we have previously reported that 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 (Lee *et al.,* 2003). A transcription factor, CREB, in particular has been implicated in opiates addiction (Nestler, 2001) and CPP (Gao *et al.,* 2003) because its activation was a consequence of upregulation of the cAMP pathway, one of the well established adaptations to drug abuse (Hyman, 1996).

In present experiment, p-CREB expression is increased in morphine-induced CPP mouse hippocampus. Treatment with MCJ inhibited the increase of p-CREB expression in morphine-induced CPP mouse hippocampus. CERB has been implicated in the formation of long-term memory and CREB-mediated transcription in the hippocampus is related to learning and memory (Deisseroth *et al.,* 1996). Also it is well known that hippocampus play a key role in tolerance, dependence, and withdrawal (Fan *et al.,* 1999; Lou *et al.,* 1999). The development of CPP is deeply related to a kind of the memory process combined with environmental cue and drug. Therefore, our result suggests that MCJ inhibits morphine-induced CPP via of the p-CREB expression induced by morphine in these brain regions. It has been reported that berberine, palmatine, and 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, berberine, a major component of Coptis japonica, inhibited morphine-induced CPP in mice (Yoo *et al.,* 2006). Therefore, further studies are needed to elucidate the molecular mechanisms and involvement of active components in the inhibitory effect of MCJ on morphine-induced CPP. Taken together, it is concluded that MCJ, a methanolic extract of Coptis japonica, may be useful for the prevention and treatment of morphine-induced psychological dependence.

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