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INHIBITORY EFFECTS OF BERBERINE AGAINST MORPHINE-INDUCED LOCOMOTOR SENSITIZATION AND ANALGESIC TOLERANCE IN MICE

J.-H. YOO,[a1] E.-M. YANG,[a] J.-H. CHO,[a] J.-H. LEE,[b] S. M. JEONG,[b] S.-Y. NAH,[b] H.-C. KIM,[c] K.-W. KIM,[d] S.-H. KIM,[e] S.-Y. LEE[a] AND C.-G. JANG[a]*

[a]*Department of Pharmacology, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea*

[b]*Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea*

[c]*Neurotoxicology Program, College of Pharmacy, Korea Institute of Drug Abuse, Kangwon National University, Chunchon 200-701, Republic of Korea*

[d]*Department of Pharmacology, College of Medicine, Chonbuk National University, Chonju, Republic of Korea*

[e]*College of Physical Education, Kyunghee University, Youngin 449-701, Republic of Korea*

Abstract—We previously reported that a methanolic extract of *Coptis japonica*, which is a well-known traditional oriental medicine, inhibits morphine-induced conditioned place preference (CPP) in mice. Berberine is a major component of *Coptis japonica* extract, and it has been established that the adverse effects of morphine on the brain involve dopamine (DA) receptors. However, to our knowledge, no study has investigated the inhibitory effects of berberine on morphine-induced locomotor sensitization and analgesic tolerance in mice. Here, we investigated the effects of berberine on morphine-induced locomotor sensitization and on the development of analgesic tolerance. Furthermore, we examined the effects of berberine treatment on *N*-methyl-D-aspartate (NMDA) receptor channel activity expressed in *Xenopus laevis* oocytes. Berberine was found to completely block both morphine-induced locomotor sensitization and analgesic tolerance, and reduce D_1 and NMDA receptor bindings in the cortex. Moreover, berberine markedly inhibited NMDA current in *Xenopus laevis* oocytes expressing NMDA receptor subunits. Our results suggest that the inhibitory effects of berberine on morphine-induced locomotor sensitization and analgesic tolerance are closely related to the modulation of D1 and NMDA receptors, and that berberine should be viewed as a potential novel means of attenuating morphine-induced sensitization and analgesic tolerance.

Key words: morphine, analgesic tolerance, berberine, dopamine, *N*-methyl-D-aspartate, addiction.

Morphine is commonly used for pain relief, but the use of morphine as an analgesic is limited by its adverse effects, which include analgesic tolerance and addiction (Bozarth and Wise, 1984; Tzschentke, 1998; McNally, 1999; Trujillo, 2000). Morphine analgesic tolerance is probably not induced under controlled conditions, but for example, tolerance to morphine analgesia may develop when higher doses are administered to terminal cancer patients (Ueda et al., 2003). The majority of therapeutically useful opiates, including morphine, induce a euphoric effect that may support the self-administration of the drug both in human populations and in laboratory animals (Koob and Bloom, 1988). One of the behavioral effects of morphine in rodents is an altered locomotor activity. Locomotor sensitization is believed to be induced by the repeated use of drugs of abuse and to be produced by the incremental neuroadaptation of the neural system, rendering it increasingly, and perhaps permanently, hypersensitive to drugs (Robinson and Berridge, 1993).

At the cellular levels, there are two major systems involved in morphine addiction and analgesic tolerance. The first is the dopamine (DA) system. Considerable efforts have been directed toward characterizing the neuroadaptations that accompany reward and behavioral sensitization, and early interest was focused on members of the mesolimbic DA system (Pierce et al., 1995; Pierce and Kalivas, 1997). The activation of DA neurotransmission plays a crucial role in behavioral responses to drugs of abuse. In particular, increases in the extracellular levels of DA in the caudate putamen and nucleus accumbens (Di Chiara and Imperato, 1988; Spanagel et al., 1990) have been implicated in the reward and locomotor stimulatory properties derived from chronic morphine treatment. Moreover, previous studies suggest that DA receptor systems play an important role in the reward effect of morphine, because DA receptor antagonists inhibit morphine-induced conditioned place preference (CPP) and locomotor hyperactivity (Shippenberg and Herz, 1987, 1988; Shippenberg et al., 1993). Second, the *N*-methyl-D-aspartate (NMDA) receptor system may also be involved in morphine-induced neural and behavioral adaptations (Trujillo, 2000). A number of studies have suggested that NMDA receptor antagonists inhibit morphine-induced CPP, locomotor sensitization, and the development of analgesic tolerance (Trujillo and Akil, 1991, 1994), indicating that DA and NMDA receptors are involved in these morphine-induced adverse effects (Trujillo, 2000).

Coptis japonica is a well-known traditional oriental medicine and its roots are widely used as an anxiolytic in Asia, and are known to contain berberine (Fig. 1). Previous studies have shown that berberine has wide ranging pharmacological and biological activities, which include anti-

[1] Present address: Pharmacology Group, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.
*Corresponding author. Tel: +82-31-290-7780; fax: +82-31-292-8800.
E-mail address: jang@skku.ac.kr (C.-G. Jang).

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; DA, dopamine; DAT, dopamine transporter; DW, distilled water; CPP, conditioned place preference; IC_{50}, concentration of berberine required to inhibit control response to glutamate to 50% of maximum; NMDA, *N*-methyl-D-aspartate; RM, repeated measures; %MPE, percentages of maximum possible effects.

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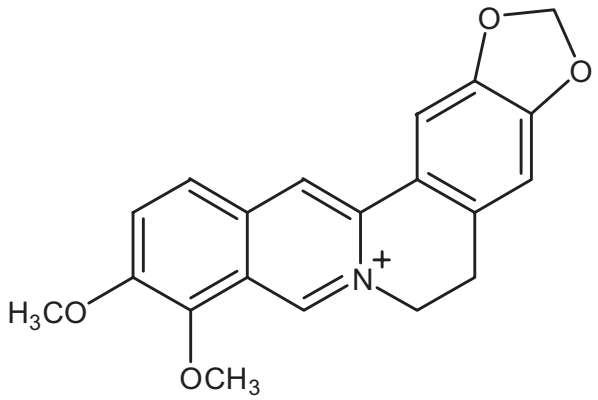

Fig. 1. The chemical structure of berberine.

inflammatory (Ivanovska and Philipov, 1996), anti-microbial (Schmeller et al., 1997), anti-amnesic (Peng et al., 1997), anti-malarial (Park et al., 2003), hypolipidermic (Kong et al., 2004), and anxiolytic-like effects (Peng et al., 1997). In addition, berberine is commercially used medically to treat diarrhea, and is considered to be an effective and non-noxious agent in this context (Rabbani et al., 1987). Moreover, previous evidence suggests that berberine alkaloids inhibit catecholamine biosynthesis by reducing tyrosine hydroxylase (TH) activity in PC 12 cells (Lee and Kim, 1996; Shin et al., 2000). Recently, we reported that a methanolic extract of *Coptis japonica* inhibits morphine-induced CPP in mice (Lee et al., 2003). However, the effects of berberine on morphine-induced locomotor sensitization or analgesic tolerance have not been investigated, which encouraged us to investigate the effects of berberine on morphine-related problems.

In the present study, we initially examined the effect of berberine on morphine-induced behavioral sensitization and analgesic tolerance in mice, and then we investigated whether berberine affects DA and NMDA receptor bindings, and NMDA receptor channel activity in *Xenopus laevis* oocytes expressing NMDA receptor subunits.

EXPERIMENTAL PROCEDURES

Animals

Male ICR mice (MJ Ltd. Co., Seoul, Korea) weighing 20–25 g were used in all experiments. All animals were acclimatized for 1 week prior to the experiments and were used only once. Mice were maintained in an animal room under a 12-h light/dark cycle at 22±2 °C. All animal care procedures were conducted in accordance with the U.S. National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Sungkyunkwan University. All efforts were made to minimize the number of animals used and their suffering. Animals were introduced to the test room 1 h before testing and all behavioral tests were performed between 9 a.m. and 5 p.m.

Drugs

Morphine hydrochloride (Macfarlan Smith Ltd., Edinburgh, UK) and berberine hemisulfate (Sigma Chemical Co., St Louis, MO, USA) were used in the present study. Morphine was dissolved in physiologic saline, and berberine was dissolved in distilled water (DW). NMDA receptor subunit (NR1-1a and NR2A) cDNAs were kindly provided by Professor S. Nakanishi (Kyoto University, Japan).

Locomotor activity

Based on the results of preliminary experiments, morphine-induced locomotor sensitization was measured for 1 h immediately after administering 10 mg/kg of morphine s.c. Locomotion was evaluated in transparent activity cages (opaque plastic, 30×30×30 cm), and video-tracking was conducted under dim illumination (25 lux), as described previously, with minor modifications (Yoo et al., 2003, 2004). Animals (n=9–11/group) were introduced to the test room 1 h before testing. In order to examine the effects of berberine on the development of morphine-induced sensitization, mice were pretreated with DW or berberine (1 or 2 mg/kg, p.o.), 1 h before morphine administration (10 mg/kg, s.c.) or saline once a day for five consecutive days in home cages. On day 6, 30 minutes after pretreatment with DW or berberine, each mouse was allowed to habituate in test cages for 30 min and then injected with saline or morphine (10 mg/kg, s.c.) in activity cages. In our preliminary study, this confinement during drug administration for five consecutive days better produced behavioral sensitization. Locomotor activity was then measured 60 min after saline or morphine administration. Data were analyzed using a computer-based video-tracking system (NeuroVision, Pusan National University), which monitors animal position four times per second. Activity cages and floor surfaces were thoroughly cleaned with 70% ethanol between tests.

Hot-plate test

Based on our preliminary experiments, morphine analgesia was measured 90 min after administering 5 mg/kg of morphine (s.c.). Initially we examined the effects of berberine administration on the antinociceptive effect of morphine. On the test day, basal nociceptive response was determined for each mouse using a hot plate apparatus in a plastic cylinder (height: 20 cm, diameter: 14 cm). Mice were placed individually on the hot plate (52 °C) and the time taken for a mouse to lick a hind paw or jump was recorded (latency). A cutoff time of 30s was set to prevent tissue damage, as described previously (Bohn et al., 1999). Twenty minutes after measuring baseline latency, mice were injected with either DW or berberine (1, 3, or 10 mg/kg, i.p.), and 30 min later were injected with saline or morphine (5 mg/kg). They were then retested after delays of 30, 60, or 90 min. The dosages and time schedules used were determined by preliminary our study.

The second part of this study involved an examination of the effects of repeated berberine pretreatment on analgesic tolerance to long-term morphine administration. Again preliminary experimentation showed that morphine analgesic tolerance develops better at 10 mg/kg than 5 mg/kg s.c. Thus, morphine (10 mg/kg, s.c.) or saline was administered to mice once a day for 6 days. Mice were pretreated with berberine (1, 3, or 10 mg/kg, i.p.) or DW 30 min before morphine injection on each occasion. On day 7, the effects of berberine on analgesic tolerance to morphine (5 mg/kg, s.c.) were evaluated using the hot-plate testing method described above.

Antinociceptive responses were calculated as percentages of the maximum possible effect (% M.P.E): % $M.P.E=[(T_t-T_0)/(T_c-T_0)]\times100$, where T_0 and T_t are hot-plate paw-licking or jumping latencies before and after morphine injection. The cutoff time (T_c) was set at 30 s. Effects were determined by calculating areas under the curves (AUC) obtained by plotting percentages of maximum possible effects (%MPE) as ordinate and time (min) as abscissa, and were expressed as percentages of the effects obtained in control animals treated only with morphine 5 mg/kg (Kaneto et al., 1982).

Receptor autoradiography

For ligand-binding studies ([^{3}H]SCH23390, [^{3}H]raclopride, [^{3}H]mazindol, and [^{3}H]MK-801), 1 day after locomotor activity