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Differential effects of acute morphine, and chronic morphine-withdrawal on obsessive–compulsive behavior: Inhibitory influence of CRF receptor antagonists on chronic morphine-withdrawal

S.N. Umathe [a,*], Y.R. Mundhada [a], P.S. Bhutada [b]

[a] Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Amravati Road, Nagpur 440033, Maharashtra, India
[b] Sinhgad College of Pharmacy, Department of Pharmacology, Vadgaon (Bk.), Pune 411041, Maharashtra, India

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ABSTRACT

Recent studies have provided convincing evidences for co-morbidity between opioid addiction and obsessive–compulsive disorder (OCD), and the involvement of the corticotrophin-releasing factor (CRF) in the effects of morphine-withdrawal. Some scanty evidences also point towards the role of CRF in OCD and related disorders. But, no evidence indicated the role of CRF in morphine withdrawal associated obsessive–compulsive behavior (OCB). Therefore, the present study investigated the role of CRF in morphine-withdrawal induced OCB in mice. Marble-burying behavior in mice was used to assess OCB as this model has good predictive and face validity. The results revealed that acute morphine dose dependently attenuated the marble burying behavior, whereas withdrawal of chronic morphine was associated with significant rise in marble burying behavior. This indicates the differential effect of acute morphine and chronic morphine-withdrawal on OCB. Further, acute treatment with CRF receptor antagonists like antalarmin (2 and 4 μg/mouse, i.c.v.) or astressin-2B (3 and 10 nmol/mouse, i.c.v.) dose dependently attenuated the peak morphine-withdrawal induced increase in marble burying behavior. Moreover, concomitant treatment with antalarmin (4 μg/mouse, i.c.v.) or astressin-2B (10 nmol/mouse, i.c.v.) along with morphine blocked the morphine-withdrawal associated exacerbation of OCB. These results indicate that OCB associated with morphine withdrawal state is partly mediated by the activation of central CRF receptors.

1. Introduction

Substantial lifetime co-morbidity between OCD and drug abuse has been reported (for review see, Gentil et al., 2009; Mancebo et al., 2009). OCD prevalence of 10–12% among psychoactive substance addicts have been reported (Friedman et al., 2000). It is suggested that the basic concepts underlying compulsive, impulsive and addictive behaviors overlap in their phenomenology, co-morbidity, family history and pathophysiology (for review see, Fontenelle et al., 2011). In addition, there is evidence that taking certain substances (e.g. cocaine, alcohol or methamphetamine) may exacerbate OCD symptoms, while others (e.g. opiates) may alleviate OCD symptoms (Koizumi, 1985; Satel and McDougle, 1991; Neziroglu et al., 1994; Nakazawa, 1999; Suzuki et al., 2002; Koran et al., 2005; Lima et al., 2005).

Evidence also suggests that the level of compulsivity and obsessionality in opioid dependence was higher and opioid addiction favors the higher incidence of OCD than the rate of OCD in the general population (Friedman et al., 2000). Moreover, OCD prevalence has been found to be four times higher than the rate in the general population (Friedman et al., 2000). OCD and long-term abstinent heroin addicts share a common impairment of working memory and/or attention involving or affecting the right prefrontal areas (Papageorgiou et al., 2003) and in OCD and opioid addiction cortico-limbic-basal-ganglia-thalamic circuits are involved (Mangold et al., 2000). This supports the hypothesis that the endogenous opioid system plays a role in the pathophysiology of both OCD and opioid addiction.

In addition, it is reported that oral morphine and tramadol caused transient relief of OCD symptoms in some patients (Shapira et al., 1997; Koran et al., 2005). Further, in animal studies, morphine and tramadol are also reported to reduce the obsessive–compulsive behavior (Nicolas et al., 2006; Rojas-Corrales et al., 2007). Conversely, de novo onset and worsening of OCD symptoms during methadone tapering have also been reported (Khazaal et al., 2006). Moreover, smaller doses of opioid antagonists showed improvement in OCD symptoms, whereas high-dose had no effect (Hamidi et al., 2007; Amiaz et al., 2008). These evidences indicate that no systematic study has been performed to indicate the exact

* Corresponding author. Tel.: +91 712 2500324; fax: +91 712 2500355.
E-mail address: umathesn@hotmail.com (S.N. Umathe).

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role of opioid in OCD. Therefore, present study was designed to investigate the effect of chronic morphine-withdrawal in animal model of OCD.

Convincing proof of evidences indicated that morphine-withdrawal is associated with various somatic and affective symptoms like anxiety, depression, cognitive dysfunction, hyperactivity, hyperalgesia, physical dependence, tolerance, dysphoria, seizures, jumping, tremors, sensitization, etc. and also the involvement of corticotrophin-releasing factor (CRF) type-1 and type-2 receptor in the modulation of multiple behavioral and neurobiological processes of morphine withdrawal (Hamlin et al., 2004; Lu et al., 2005; Stinus et al., 2005; Skelton et al., 2007; Lee et al., 2011; Navarro-Zaragoza et al., 2011; Garcia-Carmona et al., 2012). In addition, evidence suggests that CRF receptor (CRFR) signaling modulates obsessive–compulsive behavior. In male OCD patients, higher CRF concentrations were recorded in cerebrospinal fluid than men with panic disorder, generalized anxiety disorder and normal controls (Altemus et al., 1992; Fossey et al., 1996). Chappell et al. (1996) observed the higher levels of CRF in cerebrospinal fluid of Tourette's syndrome patients. Moreover, CRF-2 receptor antagonisms have been reported to exhibit anti-compulsive like effect in mice (Pelleymounter et al., 2002). However, the role of CRFR signaling in morphine withdrawal induced OCD is not reported. Therefore, present study determined whether CRF1 and 2 receptor antagonist influence morphine withdrawal induced obsessive–compulsive behavior in mice. Present study employed marble burying behavior of mice as animal model to screen obsessive–compulsive behavior as several previous studies employed this as an animal model to screen anti-OCD drugs, due to its high predictive validity (Joel, 2006).

2. Materials and methods

2.1. Animals

Adult male albino Swiss mice (24–30 g) were used in the present investigation. Mice were maintained at 23 ± 2 °C under 12:12 h light/dark cycle (light cycle: 08.00–20.00 h), with free access to rodent diet and tap water. Each experimental group comprised of six to eight mice. Behavioral studies were carried out between 09.00 and 14.00 h to minimize circadian influences (if any). All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.2. Drugs and solutions

Antalarmin, morphine and astressin-2B were purchased from Sigma–Aldrich, MO, USA. All the substances were dissolved in sterile saline except morphine. Morphine was dissolved in 2–3 drops of sodium hydroxide (3%) solution and diluted with saline as described previously (Kalange et al., 2007). The dose range was based on our observations and other reports (Pelleymounter et al., 2002; Nicolas et al., 2006; Umathe et al., 2008b; Bhutada et al., 2010).

2.3. Intracerebroventricular (i.c.v.) injection

The i.c.v. cannulation was carried out as described earlier (Umathe et al., 2011). Injections were made using a 5 μl Hamilton syringe (Hamilton, Nevada, USA) connected to an internal cannula (31 gauges) by polyethylene tubing with a volume of 1.0 μl was administered over a period of 60 s into the right lateral ventricle. The injection cannula was left in place for further 60 s before being slowly withdrawn to avoid back flow. Only data from animals showed uniform distribution of ink into lateral ventricles were used for statistical analysis. Less than 10% of the mice were eliminated from results because of inaccurate cannula placement or injection leakage.

2.4. Marble-burying behavior test

The marble-burying behavior test was carried out as described earlier (Umathe et al., 2008a, 2009, 2011). In brief, in plastic cages (40 × 28 × 14 cm) containing 5 cm thick saw dust bedding, 20 small glass marbles (~10 mm) were arranged evenly spaced in four rows of five. The cage was covered by a transparent plastic lead with line markings (3 × 2). Mice were individually placed in a marble-burying behavior apparatus with 20 glass marbles for 30 min. At the end, mice were removed, and unburied marbles were counted. A marble was considered 'buried' if more than two-thirds of its size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive–compulsive behavior. The total number of line crossings measured during 30 min was considered as locomotor counts for the animals.

2.5. Experimental groups and drug treatments

In acute morphine study, different groups of mice ($n = 6$) were injected subcutaneously with morphine (1.25, 2.5, 5 and 10 mg/kg) and 30 min after that subjected to marble burying behavior test. In a separate set, for the induction of morphine dependence, mice ($n = 6$) were injected twice daily (8.00 and 18.00 h) for 10 consecutive days with either saline or increasing doses of morphine specifically, on days 1 and 2 (5 mg/kg), on days 3 and 4 (7.5 mg/kg), on days 5 and 6 (10 mg/kg), on days 7 and 8 (15 mg/kg), on days 9 and 10 (20 mg/kg). This morphine dosage regimen was selected on the basis of our observation in present study and previous reports with slight modifications (Buckman et al., 2009), wherein demonstrated that such pattern induces significant tolerance, physical dependence, and an affective withdrawal signs. Marble-burying behavior was assessed at 2, 8, 24, 72 and 96 h time interval after morphine-withdrawal. The time interval at which mice exhibited maximum marble-burying behavior was recorded. To study the influence of CRF receptor antagonist on morphine dependent mice, separate set of mice ($n = 6$ or 7) were injected with CRF type-1 (antalarmin, 1, 2 and 4 μg/mouse, i.c.v.) and type-2 (astressin-2B, 1, 3 and 10 nmol/mouse, i.c.v.) receptor antagonist, 10 min prior to peak withdrawal hour and subjected to marble burying behavior test as described above. In a separate set of experiment ($n = 6$), to study the involvement of CRF, the CRF antagonist antalarmin (4 μg/mouse, i.c.v.) or astressin-2B (10 nmol/mouse, i.c.v.) were administered 5 min before each injection of morphine.

2.6. Statistical analysis

The data of morphine-withdrawal-induced changes in marble burying behavior and locomotor activity at various time intervals after chronic treatment with CRF antagonist were analyzed with two-way ANOVA followed by Bonferroni multiple comparison test. Data from acute CRF antagonist treated group were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test. The results are expressed as mean ± S.E.M. of 6–7 observations. $P < 0.05$ was considered statistically significant in all the cases.

3. Results

3.1. Effect of morphine on marble burying behavior in mice

One-way ANOVA revealed that acute administration of morphine significantly influenced the marble-burying behavior [$F(4,$