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ORIGINAL INVESTIGATION

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Withdrawal from chronic morphine administration causes prolonged enhancement of immobility in rat forced swimming test

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Abstract *Rationale:* Opiate-dependent subjects experience severe depression as one of the subjective symptoms during withdrawal. No experimental work, however, has focused on the ability of opiate-withdrawal to produce depression-like behavior in dependent animal. *Objectives*. We therefore investigated whether withdrawal from chronic morphine treatment affects immobility in forced swimming test in rats. *Methods:* Morphine was administered in a dose escalation fashion using doses ranging from 20 to 140 mg/kg twice daily for 14 days, followed by 1–6 days of withdrawal, and their duration of immobility was assessed. *Results:* After the last morphine treatment, an increase in immobility occurred late on day 3 and persisted to at least day 6 of withdrawal without any change in ambulatory activity. *Conclusions:* The results suggest that the morphine withdrawal resulted in prolonged enhancement of depression-like behavior in drug-dependent laboratory animals.

Keywords Morphine · Opiate · Withdrawal · Depression · Forced swimming test

Introduction

In addition to neurological and vegetative signs, subjective symptoms, such as depression, anger and anxiety, is often associated with the abstinence in opiate-dependent patients (Haertzen and Hooks 1969; Haertzen et al. 1970; Kanof et al. 1992). This negative affective state, in particular depression, induced by opiate-withdrawal is a negative driving force in the production of drug craving and addiction (Childress et al. 1994).

An ability of opiates to induce motivational effects has been demonstrated by a large number of studies using operant and classical conditioning paradigms, e.g., intracranial electrical self-stimulation, drug self-administration, conditioned place or taste preference test. In these conditioning paradigms, opiates such as morphine and heroin exhibit rewarding properties (Deneau et al. 1969; Esposito and Kornetsky 1978; Phillips and Le Piane 1980; Mucha and Herz 1985). On the other hand, opiate withdrawal in dependent animal also elicits a robust aversive state (Pilcher and Stolerman 1976; Shaefer and Michael 1983, 1986; Mucha 1987). However it remains unclear whether such aversion induced by opiate withdrawal reflects a depressive state.

The forced swimming test in rats is a valid model to evaluate an antidepressant activity (Porsolt et al. 1979; Borsini and Meli 1988), in which a characteristic behavior, i.e., an immobile posture, is regarded as reflecting a state of lowered mood (Porsolt et al. 1978; Porsolt 1979). Studies have shown that endogenous opioid systems participate in the occurrence of behavioral immobility observed in the forced swimming test (Kastin et al. 1978; Amir 1982). Thus we reasoned that this model may also serve to assess the affective state of rats during withdrawal from chronic opiate treatment. We therefore investigated whether morphine-withdrawal in dependent rats induces enhanced immobility in the forced swimming test paradigm.

The schedule of morphine administration used in the present study has been reported to induce physical dependence, and severe and long-lasting suppression of mesolimbic dopaminergic neurotransmission, the neural substrate of reward, during drug withdrawal (Aquas and Di Chiara et al. 1992; Diana et al. 1995). Thus, this schedule is expected to produce an overt negative affective state.

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Materials and methods

Animals

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) weighing 260–310 g were used. The rats were housed in a temperature-controlled colony room on a 12-h light/dark cycle with lights

on at 7:00 a.m. All animals were housed in groups of four per cage, with food and water freely available.

Drugs and treatments

Rats were treated with morphine hydrochloride (Sankyo Pharmaceutical, Tokyo, Japan) dissolved in saline twice daily for 14 days according to the following schedule: the initial dose administered was 20 mg/kg and it was increased by 20 mg/kg every other day until day 14 of treatment. Doses of morphine up to 100 mg/kg were administered SC (0.2 ml/100 g), whereas higher doses were administered IP (1 ml/100 g). After the last morphine treatment (140 mg/kg, IP) on day 15, each rat was deprived of the drug for 1, 3 or 6 days. Our preliminary study confirmed that this schedule of morphine administration produces physical dependence by observing the signs of naloxone-precipitated withdrawal syndrome (e.g., wet-dog shakes and tooth chattering and jumping). Rats in control group were given saline instead of morphine for 15 days. The concomitant treatment with naloxone (20 mg/kg, IP, dissolved in saline) (Sigma, St Louis, Mo., USA) was done 15 min before the morphine administration. To exclude the possibility that the forced swimming-induced immobility is affected by the procedural stress due to repeated injection, we initially confirmed that the immobility in rats treated chronically with saline for 14 days was not different from that of non-injected rats.

Forced swimming test

A slight modification of the method described by Porsolt et al. (1978) was used. Rats were forced individually to swim in a Plexiglas cylinder (height: 40 cm; diameter: 15 cm) containing 17 cm of water maintained at 24±1°C. Total duration of immobility during a 10-min period was measured with a SCANET MV-10 apparatus (Toyo Sangyo, Toyama, Japan), which quantified struggling behavior by counting the interruptive numbers of infrared beams when the animal moved to escape from the water. To evaluate the enhancement of immobility time, the test was performed only once because animals rapidly become immobile during multiple trials (Porsolt et al. 1978).

Ambulatory activity test

Immediately before the forced swimming test, ambulation activity during a 10-min period was measured in an open field apparatus (length: 45 cm; side: 45 cm; height: 29 cm) with the SCANET MV-10 apparatus, which quantified spontaneous locomotion activity by counting the number of interruptions of the infrared beams.

Statistical analysis

Data from forced swimming and ambulatory activity test were analyzed by a two-way analysis of variance (ANOVA) or, when appropriate, a one-way ANOVA followed by multiple comparison test of Tukey.

Results

Immediately after the last morphine administration (day 0), a significant decrease in immobility was recorded ($P<0.01$ versus chronic vehicle), together with increasing ambulatory activity ($P<0.01$ versus chronic vehicle) (Fig. 1A, B). Typical stereotyped behaviors, e.g., gnawing and sniffing, were also observed on day 0. The decrease in immobility remained until the 1st day after withdrawal ($P<0.01$ versus chronic vehicle, Fig. 1A), although it was less intense than that of day 0.

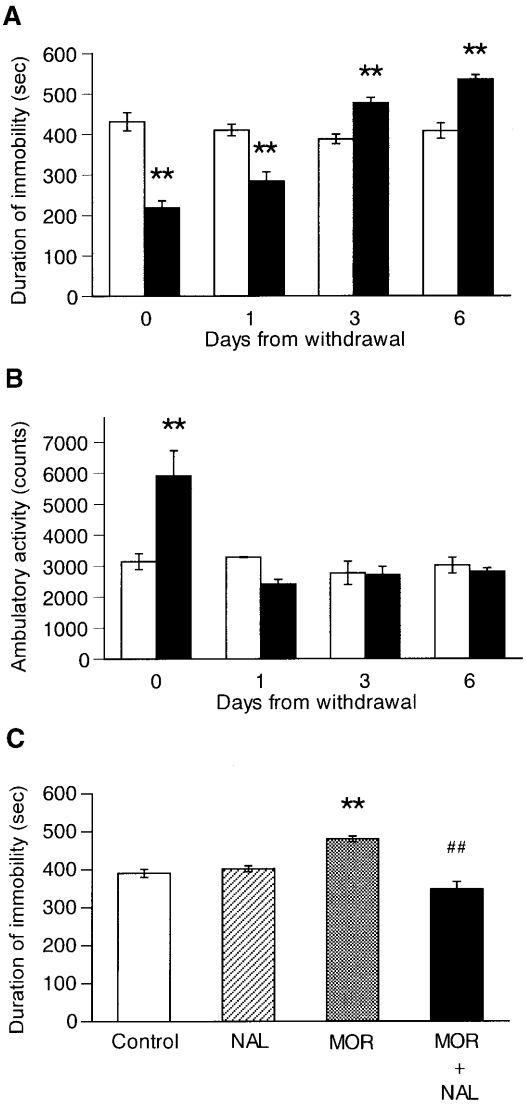

Fig. 1 Changes in immobile time in the forced swimming test (**A**) and ambulatory activity in the open field test (**B**) at different days after withdrawal from chronic vehicle (□) or chronic morphine (■) treatment. **C** Reversal by concomitant treatment with naloxone of the increase in immobility at 3 days of withdrawal. Each result represents the mean±SE (n=8). *Control* chronic vehicle, *NAL* chronic naloxone, *MOR* chronic morphine, *MOR+NAL* combination of chronic morphine and chronic naloxone. $^{**}P<0.01$ versus chronic vehicle. $^{\#\#}P<0.01$ versus chronic morphine

On day 3 following the cessation of morphine treatment, however, an opposite phenomenon, the enhancement of immobility without any influence on ambulatory activity, was observed ($P<0.01$ versus chronic vehicle, Fig. 1A, B). This phenomenon persisted for at least another 3 days ($P<0.01$ versus chronic vehicle, Fig. 1A). The difference of increased immobility between on days 3 and 6 was not statistically significant.

The increase in immobility at 3 days after morphine withdrawal was completely prevented by concomitant treatment with naloxone ($P<0.01$ versus morphine alone, Fig. 1C), without any influence on ambulatory activity (data not shown). Naloxone alone had no effect on the

immobility time (Fig. 1C) and the ambulatory activity (data not shown).

Discussion

In the present study, we demonstrated for the first time that withdrawal from chronic morphine administration increased the duration of immobility in the forced swimming test. Additionally, the immobility remained significantly elevated until at least day 6 of withdrawal. These findings suggest that morphine-withdrawal results in the prolonged enhancement of depression-like behavior in dependent rats.

Immediately after the last morphine administration (day 0), a marked decrease in immobility occurred. The schedule of morphine administration used in the present study causes behavioral sensitization as well as physical dependence (Aquas and Di Chiara 1992). Because the increased ambulatory activity and typical stereotyped behaviors, e.g., gnawing and sniffing, were observed after the last morphine challenge, such behavioral sensitization may have contributed to the decrease in immobility. Although there was no change in ambulatory activity, the decreased immobility was still significant on day 1 of withdrawal. The reason for this phenomenon is unclear, but activation of the endogenous opioid systems triggered by the forced swimming procedure may have decreased the immobility in sensitized rats. Indeed, forced swimming, as an uncontrollable stress, provokes an increase in the nociceptive threshold, which is mediated through endogenous opioid activation, known as stress-induced analgesia (Mogil et al. 1996; Suaudeau and Costentin 2000).

In the present study, the enhancement of immobility induced by morphine withdrawal was manifested in its delay and long-lasting nature. This profile appears to be consistent with the alteration of the mesolimbic dopaminergic system, which is a neural substrate of reward. Negative affective states due to opiate withdrawal has been linked to the suppression of dopaminergic neuronal activity in the mesolimbic area (Rossetti et al. 1992; Diana et al. 1995). Morphine withdrawal in dependent rats is known to cause a persistent and a progressive reduction of presynaptic dopamine release from striatal slices, while there is only transient sensitization of postsynaptic dopamine D_1 receptor-mediated adenylate cyclase activation (Tjon et al. 1994). Such temporal difference in adaptation (or recovery from adaptation) of pre- and postsynaptic dopaminergic neuron to chronic morphine may lead to a transient decrease, followed by a prolonged increase in immobility after the cessation of drug treatment.

Consistent with the previous reports on conditioning paradigms (Pilcher and Stolerman 1976; Shaefer and Michael 1983, 1986; Mucha 1987), the forced swimming test revealed that morphine withdrawal in dependent rats has the potential for inducing a negative affective state. In the clinical situation, the depression in opiate-dependent subjects is more severe than that in nondependent subjects (Maddux et al. 1987). Further, in human opioid-dependent subjects, precipitation by naloxone of an opioid withdrawal syndrome highly correlates with an increase in symptoms of dysphoria. Therefore, such negative affective state is considered as integral components of the opioid withdrawal syndrome (Kanof et al. 1992).

The duration of negative affective state induced by opioid withdrawal has not previously been investigated in detail. The duration of dysphoria that occurs during naloxone-precipitated withdrawal in human opioid dependent subjects has been reported to be brief (within 70 min after naloxone administration) (Kanof et al, 1992). However, the spontaneous (naturalistic) withdrawal syndrome, observed when opioid-dependent subjects stop their drug use, has a delayed onset and lasts for a long period as compared with naloxone-precipitated withdrawal syndrome (Blachly 1966; Kanof et al. 1992). Therefore, the possibility exists that under the spontaneous withdrawal condition, depressive (dysphoric) symptoms persist long after drug withdrawal as indicated by the present study. Among the many negative affective states associated with opioid withdrawal, depression can be a conditioned stimulus capable of eliciting drug craving (Childress et al. 1994). Here we demonstrate that morphine withdrawal induces depression-like behavior in rats in a manner similar to that in humans. Thus further analysis of depressive states induced by morphine withdrawal in dependent rats may provide a novel and suitable model to explore the mechanisms of drug craving in opiate addicts.

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