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Research report

Behavioral effects of fatty acid amide hydrolase inhibition on morphine withdrawal symptoms

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ABSTRACT

Chronic morphine exposure causes tolerance and dependence. The cessation of morphine consumption induces a withdrawal syndrome that may involve cannabinoids and is characterized by undesirable psychological and physical signs. The present study examined whether augmentation of the endocannabinoid system by inhibition of fatty acid amide hydrolase could suppress the morphine withdrawal syndrome in morphine-addicted rats.

Morphine dependency was induced by 7 consecutive days of morphine injection. The morphine-addicted rats received URB597 (1, 0.5, 0.3, 0.1, 0.03 mg/kg), a fatty acid amide hydrolase inhibitor, before the precipitation of morphine withdrawal syndromes by naloxone. Withdrawal symptoms including jumping, teeth chattering, paw tremor, wet dog shakes, face grooming, penis licking, standing, rearing, sniffing and percent of weight loss were recorded during 30 min after naloxone injection.

The results showed that the morphine withdrawal precipitated rats had significantly more withdrawal symptoms than naive control rats and the administration of URB597 (all doses except 0.03 mg/kg) reduced most of the morphine withdrawal symptoms. We conclude that the administration of URB597 modulated morphine withdrawal symptoms. This finding shows that endocannabinoids interact with the opioid system during the morphine withdrawal period and that potentiation of the endogenous cannabinoid system by URB597 may be a new target strategy for the management of morphine addiction.

1. Introduction

Previous studies have indicated that long-term repeated use of opiates, such as morphine, leads to dependence and tolerance both in humans and animals. Unexpected cessation of morphine or the administration of an opioid receptor antagonist precipitates withdrawal symptoms that include both somatic and affective components [19,20,32].

The mechanisms that subserve the opioid withdrawal syndrome remain incompletely understood. However, dopaminergic [4,8], serotonergic [18,22], noradrenergic [7,22,31], purinergic [2,27,46], glutamatergic [9,21,47] and cannabinoid [23,29,43,45] neurotransmitter systems have each been postulated to be involved.

The overlapping distribution of cannabinoid and opioid receptors in some neural structures supports the possibility of their interactions in physiological and pathological conditions, such as nociception and drug addiction [25,30,42]. Indeed, it has been reported that cannabinoid systems affect morphine withdrawal symptoms. For example, activation and blockage of cannabinoid receptor type 1 (CB1 receptors) decreased and increased withdrawal symptoms, respectively [24,29,45].

The endocannabinoid system consists of small molecules synthesized from arachidonic acid that contribute to many physiological processes such as nociception, cognition, learning and memory [36,44]. N-arachidonylethanolamide, also known as anandamide, is an endogenous cannabinoid that is released from neurons and acts on the CB1 receptor in the central nervous system. Anandamide is rapidly eliminated by the fatty acid amide hydrolase (FAAH) enzyme [3,13,14]. Administration of cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597), a selective FAAH inhibitor, increases endocannabinoid levels in the brain [6,16,34]. Interestingly, elevation of endocannabinoid levels by administration of URB597 reportedly causes few adverse effects, whereas treatment with exogenous cannabinoids induces effects

Abbreviations: FAAH, fatty acid amide hydrolase; URB597, cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester.

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such as dependence, hypothermia, catalepsy, hyperphasia and reward reinforcement [16,34,40,41]. Therefore, the present study examined the effects of a FAAH inhibitor, which lacks the adverse side effects of exogenous cannabinoids, on morphine withdrawal symptoms in the rat.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 150–200 g were obtained from the breeding colony at the Iran Pasteur Institute, Tehran. Four rats were housed per cage and were kept at a constant temperature of 20 ± 2 °C with a 12:12-h light/dark cycle (lights on at 7:00 A.M). Food and water were available ad libitum in the home cages. All procedures for the humane treatment of animals were approved by the research committee of the Hamadan University of Medical Sciences and were performed according to the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1985). All procedures and experiments were performed between 10:00 and 14:00 hours.

2.2. Induction of morphine dependence

The procedure to induce morphine dependence was as previously reported [5,35]. Briefly, the rats received one daily subcutaneous injection of morphine sulfate (Darou Pakhsh) at incrementally increasing doses (6, 16, 26, 36, 46, 56 and 66 mg/kg) for 7 consecutive days.

2.3. Measurement of withdrawal behaviors

Twenty-four hours after the last morphine injection, precipitated withdrawal symptoms were assessed following subcutaneous injection of the opioid antagonist, naloxone hydrochloride (Tolid Daru, 3 mg/kg, s.c.) on day 8.

Withdrawal signs were scored using scales as described before [30]. For behavioral assessment of opioid withdrawal, we studied the animals individually in a clear Plexiglas chamber (50 cm × 25 cm × 15 cm) that was placed in other dark chamber to avoid environmental perturbations. A digital camera connected to a recording computer was placed on the inner chamber to simultaneously show the rat behaviors. The reactions of each animal were evaluated by an observer who was not aware of the nature of the treatment received by that animal. The behaviors of all animals were evaluated by the same observer. Whenever necessary, the records were replayed for meticulous analysis. Ten distinct behaviors (scale behaviors: jumping, teeth chattering, paw tremor, wet dog shakes, face grooming, penis licking, standing, rearing and sniffing and percent of body weight loss) were scored during a 30-min period following the naloxone injection as behavioral signs of withdrawal. The number of bouts of jumping, teeth chattering, paw tremor, wet dog shakes, face grooming, penis licking, standing, rearing and sniffing was simply counted. Withdrawal syndrome induces diarrhea which causes weight loss. Body weight was measured before and 30 min after the administration of naloxone.

2.4. Experimental groups

Seventy-two animals were randomly divided into eight experimental groups ($n = 9$ per group), which included one non-dependent (control) and seven morphine-dependent groups. On the eighth day of the experiment, the control and one of the morphine-dependent groups received saline (saline group), but all other morphine-dependent groups received naloxone to precipitate morphine withdrawal symptoms. Naloxone-treated groups received intraperitoneal injections of saline (as control withdrawal symptoms) or URB597 (Sigma; 0.03, 0.1, 0.3, 0.5 or 1 mg/kg) 40 min before administration of naloxone to precipitate withdrawal.

2.5. Data analysis

Statistical comparisons among the experimental groups were made by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. All results are shown as the mean ± S.E.M, with statistical significance set at $p < 0.05$.

3. Results

Fig. 1 illustrates the effect of URB597, a FAAH inhibitor, on the number of jumps by morphine-dependent rats. As expected, administration of naloxone precipitated jumping in the morphine-dependent group (8.1 ± 1.54). Treatment with URB597 dose-dependently attenuated the number of naloxone-precipitated withdrawal jumps in morphine-dependent rats. The administration of URB597 (0.5 and 1 mg/kg) in the morphine-dependent group

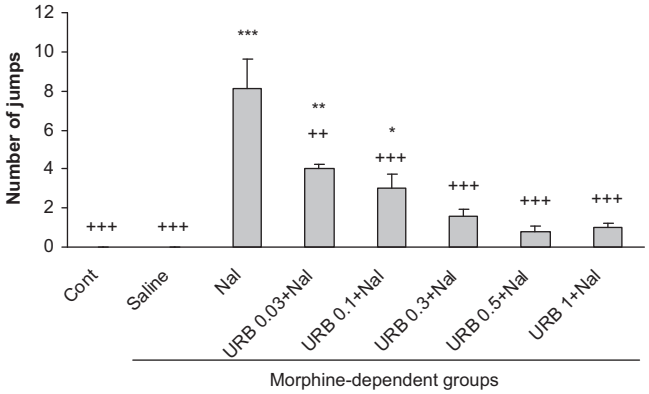

Fig. 1. The comparison of the number of jumps between the experimental groups. ***: $p < 0.001$; **: $p < 0.01$; and *: $p < 0.05$ compared to the control group. +++: $p < 0.001$ and ++: $p < 0.01$ compared to the morphine-dependent naloxone-treated group. Cont, control group; saline, morphine-dependent group that received saline; Nal, morphine-dependent naloxone-treated group; URB + Nal, morphine-dependent groups that received URB597 at doses of 0.03, 0.1, 0.5 and 1 mg/kg before naloxone injection; ordinate, mean + SEM.

significantly reduced the number of naloxone-precipitated withdrawal jumps (0.77 ± 0.27 and 1 ± 0.23, respectively) compared to that of the morphine-dependent group ($p < 0.001$ and $p < 0.001$, respectively). The doses did not differ in their ability to suppress withdrawal (Fig. 1).

Fig. 2 illustrates the ability of URB597 (0.03, 0.1, 0.3, 0.5 and 1 mg/kg) to suppress episodes of teeth chattering in the different animal groups. Low doses of URB597 (0.03, 0.1 and 0.3 mg/kg) attenuated teeth chattering in morphine-dependent rats (43.9 ± 7, 29.4 ± 6.9, 27.2 ± 6.4, respectively) compared to control morphine-dependent rats (49.6 ± 6.9), but the suppression did not achieve statistical significance ($p > 0.05$). However, higher doses of URB597 (0.5 and 1 mg/kg) significantly decreased teeth chattering in treated morphine-dependent rats compared to control ($p < 0.05$ and $p < 0.05$, respectively).

The effects of different doses of URB597 on the number of wet dog shakes in morphine-dependent rats are shown in Fig. 3. A low dose of URB597 (0.03 mg/kg) did not affect the number of wet dog shakes in morphine-dependent rats. However, larger doses (0.1, 0.3, 0.5 and 1 mg/kg) significantly inhibited the number of naloxone-precipitated withdrawal wet dog shakes in the morphine-dependent groups (all p-values < 0.001). The 1 mg/kg

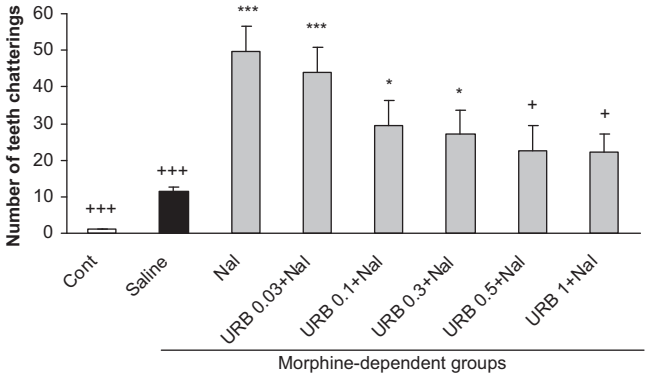

Fig. 2. The comparison of teeth chattering during a 30-min observation period between the experimental groups. ***: $p < 0.001$ and *: $p < 0.05$ compared to the control group. +++: $p < 0.001$ and +: $p < 0.05$ compared to the morphine-dependent naloxone-treated group. Cont, control group; saline, morphine-dependent group that received saline; Nal, morphine-dependent naloxone-treated group; URB + Nal, morphine-dependent groups that received URB597 at doses of 0.03, 0.1, 0.5 and 1 mg/kg before naloxone injection; ordinate, mean + SEM.