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Short communication

Involvement of corticotropin-releasing factor receptor subtype 1 in morphine withdrawal regulation of the brain noradrenergic system

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Abstract

Effects of pretreatment with the selective corticotropin-releasing factor (CRF) subtype 1 (CRF_1) receptor antagonist, 2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethyl-amino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine (CRA1000) on the behavioral and biochemical changes after naloxone-precipitated morphine withdrawal were examined in ICR mice. Mice were chronically treated with morphine (8–45 mg/kg) for 5 days. Naloxone (3 mg/kg, s.c.) precipitated jumping, diarrhea, and body weight loss in morphine-dependent mice. In addition, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and noradrenaline turnover (MHPG/noradrenaline) levels in the cerebral cortex were increased following naloxone challenge in morphine-dependent mice. However, 5-hydroxytriptamine turnover did not alter the increase following naloxone challenge in morphine-dependent mice. Pretreatment with CRA1000 (20 mg/kg, i.p.) attenuated the incidence of withdrawal signs and naloxone-precipitated increases in noradrenaline turnover. These results suggest that the activation of CRF_1 receptor may play an important role in the elevation of noradrenaline transmission, but not in 5-hydroxytriptamine transmission, in the cerebral cortex, which projects from the locus coeruleus during morphine withdrawal.

Keywords: Corticotropin-releasing factor (CRF); Dependence; Morphine; Noradrenaline

1. Introduction

Corticotropin-releasing factor (CRF) receptors are members of the G protein-coupled receptor superfamily. Recent studies have identified two CRF receptor subtypes, designated CRF subtype 1 (CRF_1) receptor and CRF subtype 2 (CRF_2) receptor, both of which are positively coupled to the cyclic AMP second messenger system (Chen et al., 1993, 2000; Lovenberg et al., 1995). Many lines of evidence indicate that the central CRF system is involved in expression of morphine withdrawal signs. Thus, a peptide CRF receptor antagonist alpha-helical CRF_{9-41} and nonpeptide selective CRF_1 receptor antagonist CP-154,526 attenuated several behavioral signs of naloxone-precipitated morphine withdrawal, including jumping, body weight loss, and naloxone-induced place aversion in morphine-dependent animals (Heinrichs et al., 1995; Iredale et al., 2000; Lu et al., 2000). CRF receptor is reported to contribute to the somatic, anxiogenic, and aversive symptoms of withdrawal from opioids (Heinrichs et al., 1995; Iredale et al., 2000; Lu et al., 2000).

The central noradrenergic system has been hypothesized to play an important role in the expression of morphine withdrawal signs. In biochemical investigations, the levels of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), the major metabolite of noradrenaline in the cerebral cortex, which projects from the locus coeruleus, increased in the subsequent naloxone challenge in morphine-dependent animals (Crawley et al., 1979; Funada et al., 1993). However, the involvement of CRF_1 receptor in the interaction between morphine withdrawal and the central noradrenergic system has not been similarly documented.

Recently, Okuyama et al. (1999) identified the novel nonpeptide CRF_1 receptor antagonist, 2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine

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(CRA1000). In the present study, the effects of pretreatment with CRF_1 receptor antagonist CRA1000 on naloxone-precipitated withdrawal signs and on the naloxone-induced elevation of noradrenaline turnover were evaluated in morphine-dependent mice.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g) were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The mice were maintained on a 12 h light/dark (lights on 8 AM to 8 PM), and laboratory mouse chow and water were provided ad libitum. All procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Effect of CRA1000 on naloxone-precipitated morphine withdrawal signs

Morphine was injected s.c. daily at 9 AM and 7 PM. According to the schedule described by Funada et al. (1993) using mice, the morphine dose was increased progressively from 8 to 45 mg/kg over a period of 5 days. The doses of morphine (mg/kg) injected at 9 AM and 7 PM were as follows: 1st day (8, 15), 2nd day (20, 25), 3rd day (30, 35), 4th day (40, 45), and 5th day (45 at 9 AM only). Withdrawal signs were precipitated by injecting naloxone (3 mg/kg, s.c., in a volume of 10 ml/kg) 2 h after final morphine administration that has been shown to produce severe withdrawal (Funada et al., 1993). Selective CRF_1 receptor antagonist CRA1000 (20 mg/kg, i.p.) or vehicle (8% dimethylsulfoxide and saline) was given 5 min prior to the s.c. injection of naloxone or saline to morphine-dependent mice. Dose of CRA1000 was selected based on existing literature (Okuyama et al., 1999) and the anti-withdrawal effects of the CRF_1 receptor antagonist CP-154,526 that was observed in previous reports (Iredale et al., 2000; Lu et al., 2000). After the naloxone challenge, mice were immediately placed in a Plexiglas cylinder (18-cm diameter × 31-cm height). Behavioral changes were recorded using a digital video camera (DCR-PC100, Sony) for 15 min. Body weight loss was measured 5 min before, and at 15, 30, 45, and 60 min after naloxone challenge.

2.3. Neurochemical analysis

Using high performance liquid chromatography (HPLC) with electrochemical detection (ECD), the concentration of noradrenaline, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 5-hydroxytriptamine, and 5-hydroxyindoleacetic acid (5-HIAA) were determined as in the previous paper (Funada et al., 1993). CRA1000 (20 mg/kg, i.p.) or vehicle (8% dimethylsulfoxide and saline) was given 5 min prior to the s.c. injection of naloxone (3 mg/kg) or saline to morphine-dependent mice. The mice were sacrificed 60 min after s.c. injection of naloxone. The brain was quickly removed, and the cerebral cortex was dissected out onto an ice-cold glass plate, as described previously (Funada et al., 1993). The tissues were frozen to −80 °C and stored until analysis. The frozen sample was homogenized in 500 ml of 0.2 M perchloric acid containing 100 mM EDTA (2Na) and 100 ng isoproterenol (as an internal standard). The homogenates were centrifuged at 120,000 × *g* for 20 min at 4 °C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. Solution samples (20 μl) were analyzed by the HPLC and ECD systems, respectively. The HPLC system consisted of a delivery system (EP-300, Eicom, Kyoto, Japan), an analytical column (SC-5ODS, Eicom, Kyoto, Japan), and a guard column. The electrochemical detection system (EC-300, Eicom, Kyoto, Japan) employed a graphite electrode, and was used at a voltage setting of +0.75 V, and Ag/AgCl was used as a reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer, pH 3.9, containing 15% methanol, sodium 1-octanesulfonate, and ethylenediaminetetraacetic acid disodium salt (EDTA-2Na). The flow rate was set to 0.23 ml/min with a column temperature of 25 °C (ATC-300, Kyoto, Japan).

2.4. Drugs

Morphine H_2SO_4 (Takede, Tokyo, Japan) and naloxone hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) were purchased commercially, and were dissolved in saline. CRA1000 was synthesized in the Laboratories of Taisho Pharmaceutical, (Saitama, Japan). CRA1000 was dissolved in 8% dimethylsulfoxide and saline. These drugs were injected in a volume of 10 ml/kg. Noradrenaline, MHPG, 5-hydroxytriptamine and 5-HIAA (Sigma-Aldrich) were dissolved in 0.02 N acetic acid for HPLC.

2.5. Statistical analysis

2.5.1. Behavioral data

The number of jumps was analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Dunnett's test for comparison between experimental and control groups. For the data of the changes in body weight loss, two-way ANOVA with time as the repeated measures was used to compare the drug-treated and control groups. Post hoc comparisons using the Dunnett's test were used to determine differences between the groups at each of the 15-min time points before and after the naloxone challenge. The incidence of naloxone-precipitated diarrhea,

ptosis, or wet-dog shakes was statistically evaluated using Fisher's probability test.

2.5.2. Biochemical data

The noradrenaline turnover was determined as the noradrenaline ratio, which was calculated as: noradrenaline ratio = MHPG/noradrenaline. The 5-hydroxytriptamine turnover was determined as the 5-hydroxytriptamine ratio, which was calculated as: 5-hydroxytriptamine ratio = 5-HIAA/5-hydroxytriptamine. Data were analyzed using one-way ANOVA followed by post-hoc Dunnett's test for comparison between experimental and control groups.

3. Results

3.1. Effect of CRA1000 on naloxone-precipitated somatic signs of morphine withdrawal

As shown in Fig. 1A, naloxone precipitated jumping behavior in the vehicle-pretreated morphine-dependent mice. Similarly, vehicle-pretreated morphine-dependent mice showed marked body weight loss after naloxone challenge (Fig. 1B). Vehicle-pretreated morphine-dependent mice showed naloxone-induced diarrhea (incidence, 10/10), ptosis (incidence, 10/10) and wet-dog shakes (incidence, 10/10). These behavioral events in vehicle-pretreated morphine-dependent mice did not significantly differ from that in vehicle-untreated mice (jumping, 134.2 ± 13.8 counts/15 min; body weight loss at 60 min after naloxone injection, −4.8 ± 0.55%; diarrhea, 10/10; ptosis, 10/10; wet-dog shakes, 10/10). Pretreatment with CRA1000 (20 mg/kg) significantly suppressed the naloxone-precipitated jumping and body weight loss (Fig. 1). Two-way repeated measures ANOVA indicated significant effects of CRA1000 ($F(1, 19) = 16.5$, $P = 0.007$), time ($F(3, 57) = 47.6$, $P < 0.0001$) and interaction ($F(3, 57) = 15.0$, $P < 0.0001$) in Fig. 1B. In CRA1000-pretreated morphine-dependent mice, incidence of naloxone-induced diarrhea was significantly reduced (incidence, 1/11). On the other hand, vehicle-pretreated morphine-dependent mice also showed naloxone-induced ptosis (incidence, 10/10) and wet-dog shakes (incidence, 10/10). Pretreatment with CRA1000 (20 mg/kg) did not alter the expression of these behavioral events (ptosis, 10/11; wet-dog shakes, 11/11).

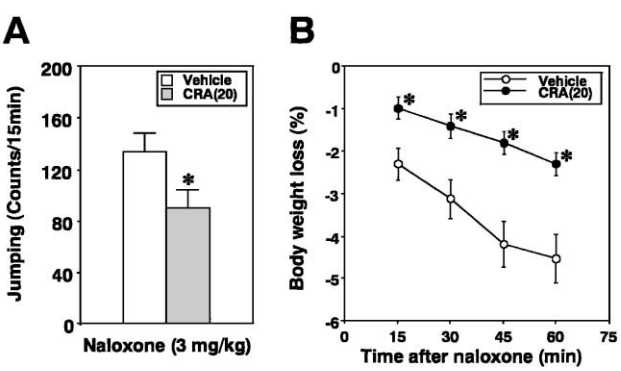

Fig. 1. Effect of pretreatment with CRA1000 on the incidence of naloxone-precipitated withdrawal signs in morphine-dependent mice. Pretreatment with CRA1000 (CRA, 20 mg/kg, i.p.) or vehicle was carried out 5 min before naloxone (3 mg/kg, s.c.) injection. (A) The withdrawal symptom of jumping was observed to last for 15 min. Each column represents the mean ± S.E.M. of 10–11 animals. (B) Effect of CRA1000 on the time course changes in naloxone-precipitated body weight loss in morphine-dependent mice. Body weight in CRA1000-treated mice did not significantly differ from that in vehicle-treated mice (CRA1000-treated group = 30.6 ± 0.53 g, vehicle-treated group = 31.0 ± 0.59 g) at 5 min before naloxone injection. Each point represents the mean ± S.E.M. of 10–11 animals. * $P < 0.05$ vs. vehicle group.

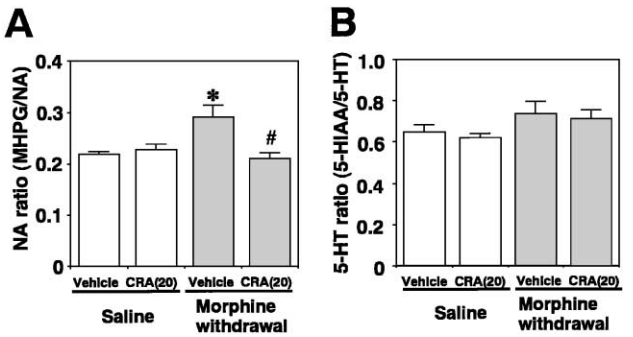

Fig. 2. (A) Effect of pretreatment with CRA1000 on noradrenaline turnover during naloxone-precipitated morphine withdrawal in mouse cerebral cortex. (B) Effect of pretreatment with CRA1000 on 5-hydroxytriptamine turnover during naloxone-precipitated morphine withdrawal in mouse cerebral cortex. Pretreatment with CRA1000 (CRA, 20 mg/kg, i.p.) or vehicle was carried out 5 min before naloxone (3 mg/kg, s.c.) treatment. Mice were sacrificed 60 min after naloxone treatment. Noradrenaline (NA), 5-hydroxytriptamine (5-HT), and NA-and 5-HT-related substances, respectively, were measured using the HPLC–ECD system. Each column represents the mean ± S.E.M. of five to six animals. Open column represents naloxone-induced neurochemical changes in the chronic saline-treated group. Closed column represents naloxone-induced neurochemical changes in the chronic morphine-treated group. * $P < 0.05$ vs. vehicle-treated saline group. # $P < 0.05$ vs. vehicle-treated morphine dependent group.

3.2. Effect of CRA1000 on naloxone-induced noradrenaline turnover elevation in the cerebral cortex

As shown in Fig. 2A, the s.c. administration of naloxone (3 mg/kg) significantly elevated the cortical noradrenaline ratio in morphine-dependent mice ($F(2,13) = 7.12$, $P = 0.008$). Pretreatment with CRA1000 (20 mg/kg) significantly suppressed the elevation of naloxone-induced noradrenaline turnover in the cerebral cortex. On the other hand, the administration of naloxone did not alter the levels of cortical 5-hydroxytriptamine ratio in vehicle- or CRA1000-pretreated morphine-dependent mice (Fig. 2B, $F(2,13) = 0.81$, $P = 0.46$). Administration of CRA1000 (20 mg/kg) did not alter the cortical noradrenaline and 5-hydroxytriptamine ratios in saline-treated mice.

4. Discussion

The present study demonstrated that the naloxone-precipitated somatic signs of morphine withdrawal were attenuated by pretreatment with the selective CRF_1 receptor antagonist CRA1000. The anti-withdrawal pattern of CRA1000 is similar to that observed in previous reports using the CRF_1 receptor antagonist CP-154,526 (Iredale et al., 2000; Lu et al., 2000). Microinjection of the quaternary opioid antagonist methylnaloxonium into several brain regions has shown that the most sensitive site for the expression of jumping as a symptom of morphine withdrawal is the locus coeruleus (Maldonado et al., 1992). Further, the central administration of methylnaloxonium did not induce withdrawal diarrhea in morphine-dependent animals (Maldonado et al., 1992). It has been assumed that the diarrhea, which results from morphine withdrawal, is caused by an increase in gastrointestinal motility (Brown et al., 1988). In consideration of the findings in these reports, our results suggest that blockades of central and peripheral CRF_1 receptor may be involved in the suppressive effects of CRA1000 on morphine withdrawal signs.

The present findings demonstrated that the administration of naloxone-produced marked morphine withdrawal signs in morphine-dependent mice, and significantly elevated the noradrenaline ratio in the cortex, indicating that morphine withdrawal can enhance noradrenaline neurotransmission in the cortex, which projects from the locus coeruleus. These results are consistent with those previous reports on morphine-dependent animals (Funada et al., 1993). On the other hand, findings in the present study indicated that administration of naloxone did not alter the cortical 5-hydroxytriptamine ratio in morphine-dependent mice. These results indicate that cortical noradrenaline transmission, but not 5-hydroxytriptamine transmission, may be activated by naloxone-precipitated morphine withdrawal.

The central noradrenergic system has been hypothesized to be involved in the expression of morphine withdrawal signs. For example, the firing rate of locus coeruleus neurons increases during morphine withdrawal precipitated by an opioid antagonist (Rasmussen et al., 1990). Moreover, a naloxone-induced increase in noradrenaline turnover has been shown in cerebral cortex innervated by the locus coeruleus in morphine-dependent animals (Crawley et al., 1979; Funada et al., 1993). Milanes et al. (1998) showed that hyperactivations of central CRF and noradrenaline systems may be involved in morphine withdrawal. A previous study has indicated that the application of CRF into the locus coeruleus stimulates noradrenaline release in the prefrontal cortex (Smagin et al., 1995). In addition, it has been shown that i.c.v. administration of endogenous opioid peptide β-endorphin increases the expression of c-*fos* and of CRF mRNA (Wang et al., 1996). Recently, the neuroanatomical distribution of CRF_1 receptor in mouse brain was characterized in detail using anti-CRF_1 receptor antiserum (Chen et al., 2000). In the locus coeruleus, very dense distribution of neurons expressing CRF_1 receptor was shown. The findings in these reports taken together with the present results support the possibility that the activation of CRF_1 receptor may be involved in the expression of morphine withdrawal signs.

In summary, the present findings indicate that pretreatment with the CRF_1 receptor antagonist CRA1000 attenuated the incidence of withdrawal signs and naloxone-precipitated increases in cortical noradrenaline turnover. These results suggest that activation of the central CRF system, especially activation of CRF_1 receptor, may be involved in the expression of morphine withdrawal signs. The CRF receptor antagonists, particularly the CRF_1 receptor antagonist, might be of some value in the treatment of morphine withdrawal.

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