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Spinal kappa-opioid system plays an important role in suppressing morphine withdrawal syndrome in the rat

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Abstract

To explore the possible involvement of spinal κ-opioid receptor in modulating morphine withdrawal syndrome, rats were made dependent on morphine by multiple injections of morphine HCl for 5 days. They were then given intrathecal administration (i.t.) of a κ-opioid receptor agonist *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide hydrochloride (U-50,488H, 2.5–10 μg) or its antagonist nor-binaltorphimine (nor-BNI, 1.25–5 μg), followed by intraperitoneal administration (i.p.) of naloxone (0.5 mg/kg), and the withdrawal syndrome was scored for 60 min. U-50,488H produced a dose-dependent suppression, whereas nor-BNI a dose-dependent potentiation in withdrawal syndrome. The latter result implies that an endogenous kappa receptor agonist, most probably dynorphin, exerts a tonic suppressive effect on morphine syndrome at spinal level.

Keywords: κ-Opioid receptor; Morphine withdrawal syndrome; *Trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide hydrochloride; Nor-binaltorphimine; Spinal cord; Naloxone

It has been reported that dynorphin attenuated morphine withdrawal symptoms in morphine-dependent animals [1,18] and heroin addicts [15,24], and the site of action has been located at spinal cord [5]. Auriacombe et al. [2] demonstrated that transcutaneous electrical stimulation with an intermittent high-frequency current effectively attenuated the abstinence syndrome of the rat after abrupt cessation of morphine administration. We have also shown that 100 Hz electroacupuncture (EA) stimulation was capable of suppressing morphine withdrawal syndrome in rats [10] and 100 Hz transcutaneous electrical nerve stimulation (TENS) was very effective in ameliorating morphine abstinence syndrome in heroin addicts [9]. One hundred Hz EA or TENS has been shown to accelerate the release of dynorphin from the spinal cord of the rats [8] and humans [7]. While dynorphin has been shown to be the endogenous ligand of the κ-opioid receptor by Chavkin et al. [3], no direct evidence is available to ascertain if κ-opioid receptor in spinal cord is indeed involved for the suppression of morphine withdrawal syndrome. If this hypothesis is true, then the morphine withdrawal syndrome should be dose-dependently suppressed by intrathecal administered κ-opioid receptor agonist and potentiated by its antagonist. This was tested in the present study. The pharmacological tools used were *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide hydrochloride (U-50,488H) and nor-binaltorphimine (nor-BNI) that have been recognized as the classical κ-opioid receptor agonist [21] and antagonist [17], respectively. In this study intrathecal injection was used as route of administration according to the work of Green and Lee [5].

Adult male Wistar rats weighting 200–250 g were obtained from the Experimental Animal Center, Peking University, and caged individually, with food and water available ad libitum in the home cages. The rats were injected subcutaneously with morphine hydrochloride three times a day (08:00, 13:00 and 18:00 h) at the following doses per injection: 5 mg/kg on the first day, followed by 10, 20, 40 and 50 mg/kg from day 2–5. On day 6, an injection of 50 mg/kg was given at 08:00 h and the naloxone precipitated withdrawal was tested 6 h later.

On day 5, rats were anesthetized with chloral hydrate (300 mg/kg intraperitoneal (i.p.)) and were implanted with PE-10 tubing by a procedure modified from Yaksh and Rudy [25]. A 12.5 cm length PE-10 tubing that had a knot 7.5 cm from the tip was inserted into the spinal subarachnoid space through an incision made on the atlanto-occipital membrane. The tip of the tubing lay in the region of the lumbar enlargement. The rats were allowed to recover for 24 h, and were injected via the PE-10 tubing on day 6. U-50,488H or nor-binaltorphimine (nor-BNI) was dissolved in sterile 0.9% NaCl (normal

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saline, NS) and injected in 10 μl volume, the tubing was filled with NS after drug injection. The animals in control group were i.t. administered with 10 μl NS. The i.t. injection was finished within 20 s.

The morphine-dependent rats with i.t. catheters were randomly divided into three groups, injected i.t. with U-50,488H, nor-BNI or NS, respectively. Fifteen min after the i.t. injection, the rats in each group was administered with naloxone (NX, 0.5 mg/kg, i.p.). Each rat was then individually placed in a plastic cage, and the presence of withdrawal signs was evaluated at 15 min intervals for 1 h. In this study we scored two counted signs (wet shakes, escape attempts) and one checked sign (teeth chattering), and the percent of body weight loss (weight loss/initial body weight × 100%) was measured before and 60 min after the NX injection.

Morphine HCl is a product of Qinghai Drug House (PR China). U-50,488H and naloxone HCl were obtained from Sigma Inc, nor-BNI was a generous gift from Dr P.S. Portoghese, University of Minnesota, MN.

The effect of i.t. U-50,488H on naloxone-precipitated morphine withdrawal signs: 26 morphine-dependent rats were randomly divided into four groups (n = 6-7). Three groups were given i.t. injection of U-50,488H (2.5, 5 and 10 μg) and one group with NS. After 15 min the rats were given naloxone (0.5 mg/kg, intraperitoneal (i.p.)), and the four withdrawal signs were scored every 15 min for 1 h. U-50,488H attenuated three out of four signs (Fig. 1A–C) during the 4 × 15 min period. The difference was most prominent in the second observation period, i.e. 15–30 min after naloxone treatment. A dose-dependent relation-

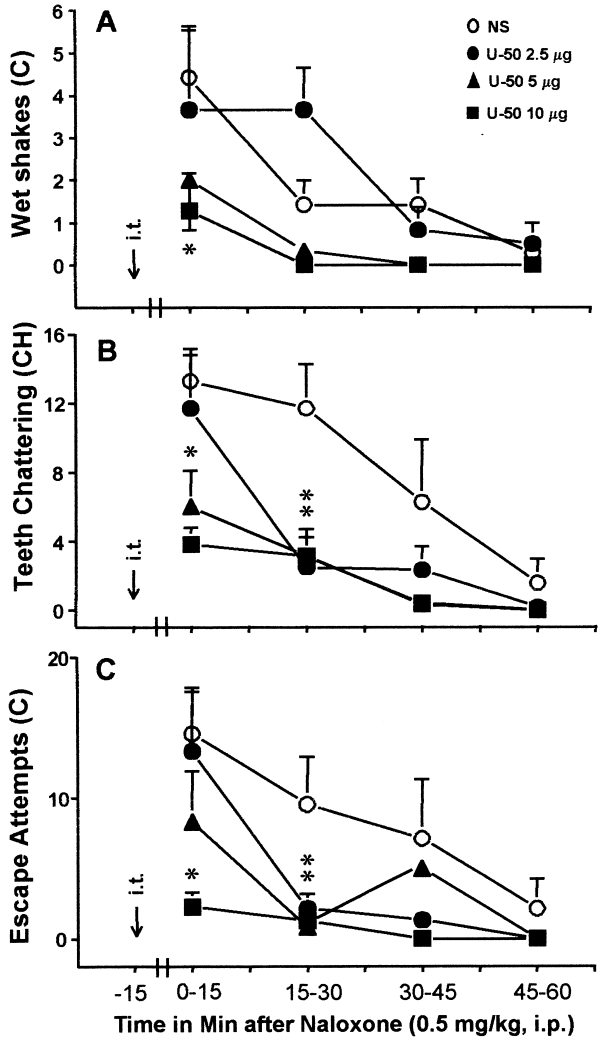

Fig. 1. Effect of i.t. U-50,488H on naloxone-precipitated withdrawal signs in rats injected with morphine for 5 days. NS (n = 6), U-50,488H 2.5 μg (n = 7), 5 μg (n = 6) and 10 μg (n = 7) were given 15 min prior to naloxone (0.5 mg/kg, i.p.) injection. The withdrawal signs are expressed as scores, either counted (C) or checked (CH), over four time periods of 15 min each. The values (vertical lines represent SE of the mean) of wet shakes (C), teeth chattering (CH) and escape attempts (C) were analyzed via two-way analysis of variance (ANOVA). The results are shown in A–C. *$P < 0.05$; **$P < 0.01$, compared with NS control group by Duncan test.

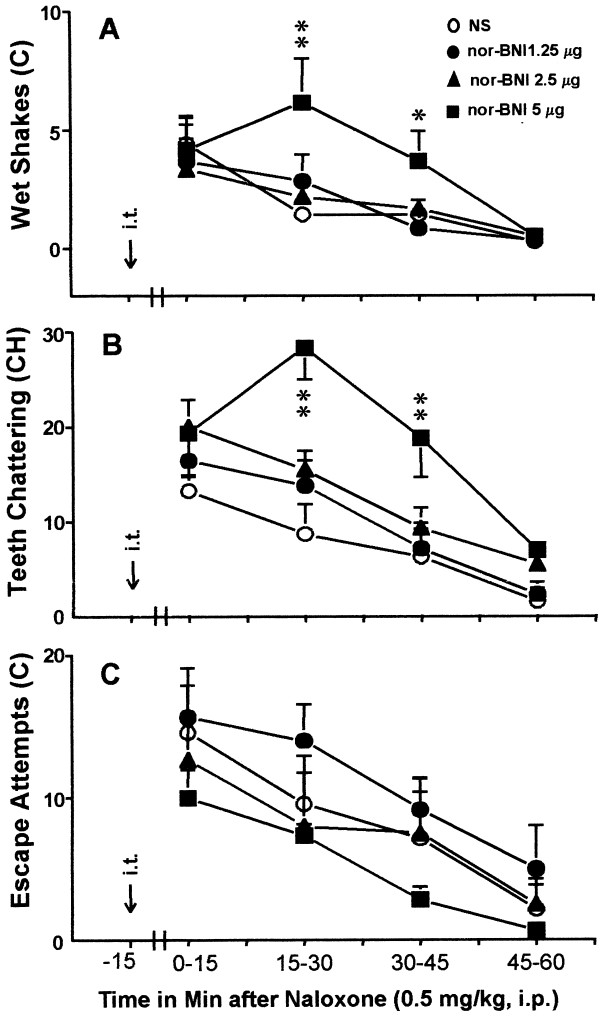

Fig. 2. Effect of i.t. nor-BNI on naloxone-precipitated withdrawal signs in rats injected with morphine for 5 days. NS (n = 6), nor-BNI 1.25 μg (n = 7), 2.5 μg (n = 6) and 5 μg (n = 7) were given 15 min prior to naloxone (0.5 mg/kg, i.p.) injection. Other annotations are same as in Fig. 1.

ship can be more clearly shown when the scorings were calculated on 60 min basis (Fig. 3A–C). The weight loss of morphine withdrawal rats was slightly attenuated by U-50,488H at 10 μg dose, but the difference did not reach statistically significant level (Fig. 3D).

Twenty-six morphine-dependent rats were randomly divided into four groups ($n = 6$-7) and i.t. administered with nor-BNI (at 1.25, 2.5 and 5 μg doses) or NS, respectively. Naloxone (0.5 mg/kg, i.p.) precipitated withdrawal signs were scored for a period of 60 min as were stated above. At the dose of 5 μg/kg nor-BNI increased the number of wet shakes (Fig. 2A, $P < 0.01$), and teeth chattering (Fig. 2B, $P < 0.01$). The effect was more prominent in the second and third observation periods (15–45 min after naloxone treatment). A dose-dependent relationship can be more clearly shown in Fig. 3B,D) when the total scores in 60 min were taken into account. The escape attempts were not markedly affected by nor-BNI compared with the control group (Figs. 2C and 3C). The weight loss of morphine withdrawal rats was significantly aggravated by nor-BNI at 2.5 and 5 μg doses (Fig. 3D).

The naloxone-precipitated morphine withdrawal syndrome in rats includes a battery of signs, such as vocalization, irritability, wet shakes, teeth chattering, diarrhea, ptosis, nosebleed, escape attempts (or jumping), salivation, weight loss, penile licking (or self-stimulation), etc. In the present study we took four of them into account, i.e. wet shakes, teeth chattering, escape attempts, and weight loss, that are easy to characterize and have been used by most research groups [4,22,23]. It has been repeatedly demonstrated that intrathecal injection of U-50,488H [12,13] or nor-BNI [14,20] per se at the doses used in the present study does not affect motor functions in the non-morphine dependent rats.

Results of the present study demonstrated that three out of four withdrawal signs were dose-dependently suppressed by the κ-opioid receptor agonist U-50,488H. Failure of the U-50,488H to attenuate body weight loss during morphine withdrawal might be accounted for by the fact that U-50,488H has a marked diuretic effect [11] that may cause weight loss. In other words, if it were not the diuretic effect which cause reduction of body weight, the weight loss induced by morphine withdrawal would have been almost abolished. In addition, it is interesting to note that while μ-opioid receptors have been made tolerant to morphine in the present paradigm, κ-opioid receptors remain sensitive to κ agonist. In fact, Gulati and Bhargava [6] even found an up-regulation of spinal κ-opioid receptors in morphine tolerant or dependent mice.

The purpose of using nor-BNI in the present study was to assess whether endogenously released κ-opioid receptor agonist is involved in self-limitation of opiate withdrawal syndrome. Results shown in Figs. 2 and 3 clearly indicate that nor-BNI exacerbates three out of four withdrawal signs, suggesting that endogenous κ-opioid receptor agonist, most probably dynorphin, does play a role in ameliorating morphine withdrawal syndrome. We have no rational explanation why nor-BNI was not able to potentiate escape attempts. The possibility that nor-BNI by itself may interfere with motor functions has been ruled out [14,20], at least in morphine-naive rats.

Recently Tao et al. [19] reported that coadministration of

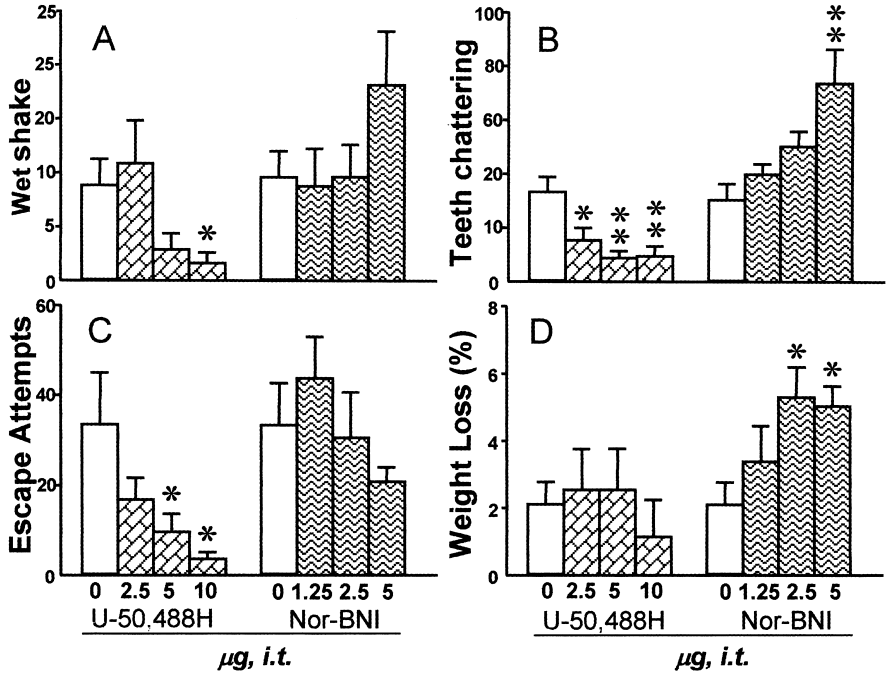

Fig. 3. Effect of U-50,488H or nor-BNI on the four withdrawal signs during the whole 60-min observation period. The four withdrawal signs over the time course of the data collection (vertical lines represent SE of the mean) were assessed by one-way ANOVA. The results are shown in (A–D). *$P < 0.05$; **$P < 0.01$, compared with NS control group by Duncan test.

8 mg/kg U-50,488H (i.p.) with morphine twice daily for 6 days attenuated naloxone-precipitated morphine withdrawal syndrome, suggesting that systemic administration of U-50,488H may also be effective in suppressing morphine abstinence syndrome. In addition, Suzuki et al. [16] demonstrated that pretreatment with nor-BNI (5 mg/kg, subcutaneous (s.c.) on the 1st and 4th days) in the mice during chronic morphine treatment (5 days regime) significantly aggravated the naloxone-induced body weight loss, but failed to affect the incidence of other withdrawal signs. These results, in general, seem to support the point that activation of κ-opioid receptors suppresses morphine withdrawal. However, since both studies used systemic (i.p. or s.c.) route of administration and single dosage of U-50,488H or nor-BNI, that makes the profile difficult to analyze. The results shown in the present study using i.t. route of administration and relatively wide dose rage of the κ-opoid receptor agonist and its antagonist provide site-specific, dose-related and time-related information on the modulatory effect of spinal κ-opioid receptors on the development of morphine abstinence syndrome.

Since the present study clearly demonstrates that activation of κ-opioid receptors in spinal cord suppresses morphine withdrawal syndrome, and blockade of spinal κ-opioid receptors aggravates the syndrome, it would naturally lead to the consideration of the use of physical means that can accelerate the release of endogenous κ-opioid agonist dynorphin in spinal cord for the treatment of morphine withdrawal syndrome. High frequency (100 Hz) electroacupuncture and TENS have been shown to increase the dynorphin release in spinal cord in rats [8] and humans [7], that, indeed, are the currently available measures to suppress opiate withdrawal in rats [10] and humans [9], respectively.

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