# Methylnaloxone Suppresses the Development of Withdrawal Syndrome in Morphine-Dependent Rats

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We studied the effect of peripheral opioid receptor antagonist methylnaloxone on the development of withdrawal syndrome in morphine-dependent rats. Intraperitoneal injections of methylnaloxone iodide in a daily dose of 2 mg/kg over 3 days after morphine withdrawal reduced the severity of withdrawal symptoms. The mean total score of withdrawal syndrome in treated rats (3.20±0.13) was 2-fold lower compared to the control, mainly due to less pronounced wet dog shake behavior, limb and head shakes, dyspnea, ptosis, and teeth chattering. Methylnaloxone iodide in the specified dose had no effect on such symptoms of withdrawal syndrome as diarrhea and writhing. Our results indicate that modulation of the peripheral opioid system can reduce the severity of opioid withdrawal syndrome. Methylnaloxone-induced variations in the function of peripheral opioid receptors are probably accompanied by changes in the central nervous system, which prevents the development of withdrawal syndrome.

**Key Words:** *methylnaloxone; physical dependence; morphine withdrawal syndrome; peripheral opioid receptors*

Chronic opioid consumption is followed by the appearance of several phenomena. The major phenomena are tolerance and physical dependence, which are associated with drug-induced rearrangement of intraneuronal processes [5]. These changes are accompanied by a decrease in the effect of opioids. Drug withdrawal results in the development of withdrawal syndrome. The higher is the degree of physical dependence, the greater is the severity of withdrawal syndrome. Neurochemical processes in brain neurons accompanyng various stages of chronic opioid consumption (from variations in the function of opioid receptors [6] to changes in gene expression [3]) are studied in detail.

It is undoubtedly that long-term opioid consumption produces strong effect on peripheral organs and systems. The most significant changes occur in μ-opioid receptors that are present in practically all peripheral tissues. Peripheral interactions are less studied than central processes despite great contribution of the peripheral opioid system into the development of physical dependence and withdrawal syndrome. Probably, the influence on the peripheral compartment of the endogenous opioid system is always accompanied by changes in the function of the central compartment that plays a role in the development of dependence.

Methylnaloxone, a derivative of opioid receptor antagonist naloxone, cannot cross the blood-brain barrier due to high polarization and low lipophilicity. As distinct from naloxone, methylnaloxone does not cause "precipitation" of withdrawal syndrome in morphine-dependent subjects [7]. Methylnaloxone in high doses antagonizes the analgesic and depressive effects of opioids [4].

Here we studied the effect of peripheral treatment with methylnaloxone on withdrawal syndrome in morphine-dependent rats.

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## MATERIALS AND METHODS

Experiments were performed on 80 male Wistar rats weighing 180-200 g. The rats were kept in cages (8-10 specimens per cage) under 12:12-h light/dark regimen and had free access to standard mixed food and water.

The animals received morphine hydrochloride in increasing doses of 10-60 mg/kg for 14 days. Morphine hydrochloride was given 2 times a day with 12-h intervals. Forty control rats ($n$=40) received an equivalent volume of NaCl instead of morphine (2 times a day).

The animals were divided into 8 groups (10 rats per group): 4 control groups receiving no morphine and 4 treatment groups comprising morphine-dependent rats. Twenty-four hours after the last injection of morphine or isotonic NaCl, morphine-untreated and morphine-dependent animals received 3 intraperitoneal injections of isotonic NaCl (1 ml/kg, control) or methylnaloxone iodide (0.5, 2.0, and 10.0 mg/kg) at 24 h intervals. One hour after the last injection, specific withdrawal symptoms in each rat (shakes, dyspnea, ptosis, writhing, teeth chattering, piloerection, rhinorrhea, posture change, and diarrhea) were evaluated in an automatic open-field device for 3 min.

The differences were significant at $p$<0.05 (unpaired Student's $t$ test).

## RESULTS

The signs for morphine withdrawal syndrome of different severity were revealed in morphine-dependent animals of the control group (administration of isotonic NaCl 24 h after the last injection of morphine). The mean total score of general behavioral characteristics and specific reactions in control animals was 6.17±0.41. The mean total score in rats receiving isotonic NaCl instead of morphine (1.54±0.12) was determined by such nonspecific signs as shaking of limbs and head and, more rarely, ptosis and teeth chattering. Methylnaloxone iodide had no effect on the total score in control rats not receiving morphine (Fig. 1). The signs of withdrawal syndrome in morphine-dependent animals injected with methylnaloxone in a dose of 2 mg/kg were less pronounced than in rats receiving isotonic NaCl instead of methylnaloxone. The mean total score in treated rats was 2-fold lower than in the control (3.20±0.13, $p$<0.05, Fig. 1).

The decrease in the mean total score was mainly associated with modification of wet dog shake behavior, shaking of limbs and head, dyspnea, ptosis, and teeth chattering. Methylnaloxone iodide in the specified dose had no effect on such components of withdrawal syndrome as diarrhea and writhing (Fig. 2). This substance increased vertical locomotor activity (number of rearing postures), which was reduced in morphine-dependent animals. Methylnaloxone in other doses had little effect on the test parameters (Fig. 1).

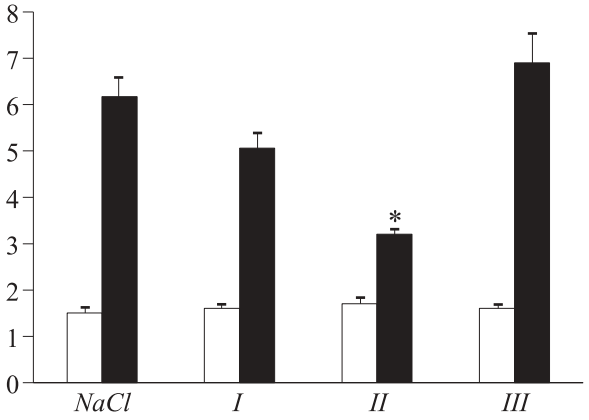


**Fig. 1.** Total score of withdrawal syndrome in control (light bars) and morphine-dependent animals (dark bars) after administration of methylnaloxone iodide in doses of 0.5 (*I*), 2.0 (*II*), and 10.0 mg/kg (*III*). *$p$<0.05 compared to rats receiving NaCl.

Administration of naloxone in a wide dose range sharply increased withdrawal syndrome in mor-

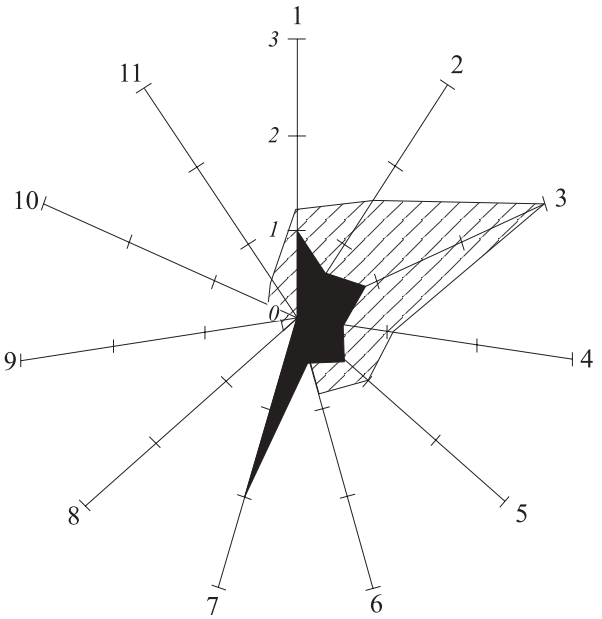


**Fig. 2.** Structure of withdrawal syndrome in morphine-dependent rats under control conditions (shaded area) and after administration of methylnaloxone iodide in a dose of 2 mg/kg (dark area). Symptoms of withdrawal syndrome: wet dog shakes (*1*), shaking of limbs (*2*), shaking of head (*3*), dyspnea (*4*), ptosis (*5*), teeth chattering (*6*), diarrhea (*7*), piloerection (*8*), posture disorders (*9*), writhing (*10*), and rhinorrhea (*11*). Axes *1*, *2*, and *3*: mean frequency of symptoms in the group.

phine-dependent subjects (precipitation phenomenon), which mainly resulted from its influence on central opioid receptors [1,2]. We found that administration of peripheral opioid receptor antagonist decreases the severity of withdrawal syndrome. These results indicate that the severity of opioid withdrawal syndrome can be modulated by affecting the peripheral opioid system. Methylnaloxone-induced variations in the function of peripheral opioid receptors are probably accompanied by changes in the central nervous system, which prevents the development of withdrawal syndrome. Methylnaloxone does not prevent the development of peripheral withdrawal signs (*e.g.*, diarrhea). It can be hypothesized that increasing the dose of methylnaloxone to 10 mg/kg can leads to its partial penetration through the blood-brain barrier, which can determine its ability to provoke withdrawal syndrome.

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