# HLDF-6 Peptide Relieves Symptoms of Abstinence Syndrome during Experimental Opium Abuse

**S. V. Litvinova, A. L. Kalyuzhnyi, V. V. Aristova, V. V. Shul'govskii, I. A. Kostanyan*, N. N. Terebelina**, and L. F. Panchenko****

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Experiments were performed on rats with opium abuse induced by chronic administration of morphine in increasing doses. We studied the effect of HLDF-6 peptide on symptoms of naloxone abstinence. Repeated administration of HLDF-6 peptide in a dose of 0.2 mg/kg 24 and 0.5 h before naloxone relieved the major symptoms of the abstinence syndrome. A possible neurochemical mechanism underlying the effect of HLDF-6 peptide is inhibition of enkephalinase A in structures of the endogenous antinociceptive system.

**Key Words:** *morphine; abstinence syndrome; HLDF-6 peptide; enkephalinase A*

We studied the effect of HLDF-6 peptide on symptoms and severity of the abstinence syndrome (AS). The experimental model of opium abuse included the development of physical dependence on morphine in Wistar rats.

## MATERIALS AND METHODS

Experiments were performed on 32 male Wistar rats weighing 200-230 g and obtained from the nursery of Institute of Bioorganic Chemistry. The animals were kept under standard conditions (food and water *ad libitum*, temperature 18-24°C, humidity 30-70%, 12:12 h light/dark cycle; the litter was replaced twice a week). Experimental procedures were performed in accordance with the program of the Institute of Bioorganic Chemistry on humane attitude toward laboratory animals. Morphine in increasing doses of 5-60 mg/kg was administered twice a day at a 10-h interval for 7 days to produce physical dependence.

Department of Higher Nervous Activity, Biological Faculty, M. V. Lomonosov Moscow State University; *Laboratory for Proteins of Hormonal Regulation, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences; **Laboratory of Biochemistry, National Research Center for Narcology, Russian Ministry of Health, Moscow

Animal behavior was studied in several experimental situations. Before chronic morphinization each rat was tested in a round open field. We recorded horizontal and vertical activity, number of grooming reactions, count of entries and time spent in the central square (degree of anxiety), and other behavioral characteristics. Individual pain thresholds were estimated in the tail-flick test on an automatic analgesimeter (Columbus Instruments).

Pain thresholds were repeatedly measured after chronic morphinization. The animals were divided into 3 groups. Group 1 control rats ($n$=12) received 2 mg/kg naloxone 30 min after administration of 0.4 mg/kg 0.9% NaCl to provoke the withdrawal syndrome. Group 2 rats ($n$=10) received 2 mg/kg naloxone 30 min after administration of 0.4 mg/kg HLDF-6 peptide. Group 3 rats ($n$=10) received 2 mg/kg naloxone 30 min after the last treatment with 0.2 mg/kg HLDF-6 peptide (days 1 and 2). The open-field behavior of chronically morphinized animals was studied before naloxone administration.

Each rat was examined for AS symptoms over 15 min after naloxone administration. Then the animals were tested in the open field for 3 min. The severity of AS was estimated by the scale for abstinence symptoms [1]. We compared the mean total score of indexes for abstinence symptoms.

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After behavioral experiments the rats were decapitated. Enkephalinase A (EA) activity was measured in various brain structures [6].

The results were analyzed by Student's *t* test, Wilcoxon test, Mann—Whitney test, and Duncan test (ANOVA-2).

## RESULTS

HLDF-6 peptide had a potent effect on the major symptoms of AS. Group 1 rats were characterized by severe AS. The total score of physical dependence varied from 45 to 86 points (mean 59.2 points). In group 2 the total score of physical dependence was 12-24 points (mean 17 points). These data indicate that HLDF-6 peptide modulated the development of AS. Several major symptoms of AS were not observed in animals receiving HLDF-6 peptide. Diarrhea is one of the most important manifestations of AS. Only 2 rats of group 2 had moderate diarrhea. It should be emphasized that this symptom was present in all animals of group 1 (3-9 points). None of the rats attempted to escape the open field (jumping). Wet dog shakes were observed only in 2 animals. None of the rats exhibited forelimb shaking. The following symptoms of AS varied in degree (Table 1): dyspnea, ptosis (rare symptom), teeth-chattering (in some animals), and extension of hindlimbs (most rats). Locomotor activity in group 2 rats discontinued less than after 2 min. Aggressiveness markedly decreased in group 2 animals. The severity of this symptom was low in rats with naloxone-induced AS.

Pain thresholds sharply decreased in group 1 rats with AS. The animals were characterized by abstinent hyperalgesia (Fig. 1). Hyperalgesia was not revealed in group 2 rats. Pain thresholds in these animals were insignificantly lower than in the control (Fig. 1).

The severity of AS in group 3 rats was much lower than in group 2 animals. The total score of physical dependence varied from 8 to 14 points (mean 11 points). Severe manifestations of AS, including diarrhea (3-9 points), "wet dog shake", forelimb shaking, "corkscrew tail" posture, and ptosis, were absent in most animals. Some rats had only one of these symptoms (Table 1). One of the most severe symptoms of chronic morphinization and AS (diarrhea) was absent in group 3 rats receiving HLDF-6 peptide 1 day before the experiment. This disorder often causes death of animals at the stage of physical dependence before abstinence. Diarrhea was observed in 2 animals of group 3 receiving morphine in a dose of 50 mg/kg. The severity of diarrhea increased after treatment with 60 mg/kg morphine. It should be emphasized that morphine in this dose often causes death of animals. Administration of HLDF-6 peptide 1 day before the experiment prevented diarrhea. Moreover, diarrhea was not typical of rats with naloxone-induced AS. However, diarrhea was observed in all animals of group 1. Teeth-chattering was revealed in 5 rats of group 3.

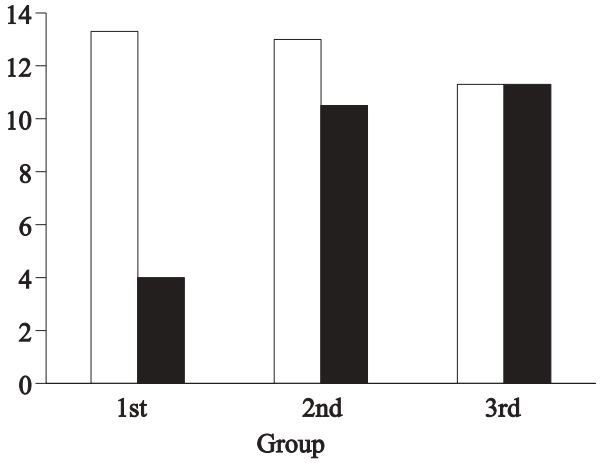


**Fig. 1.** Thermonociceptive thresholds in animals. Light bars: baseline level. Dark bars: thresholds in animals with naloxone-induced abstinence. Group 1, control: isotonic solution. Group 2: 0.4 mg/kg peptide 0.5 h before the experiment. Group 3: 0.2 mg/kg peptide 1 day and 0.5 h before the experiment.

Total locomotor activity of rats from different experimental groups was below normal (intact animals). These differences were most pronounced in group 1 rats, but less significant in group 2 animals. Activity of group 3 rats tended to normal. None of the animals of group 3 continued to move for more than 2 min. However, in non of the rats locomotor activity disappeared over the 1st minute (Table 1).

Group 3 rats did not exhibit aggressiveness typical of AS and observed in group 1 animals. These rats did

**TABLE 1.** Effect of HLDF-6 Peptide on Major Symptoms of AS (Percents are Shown in Brackets)

| Symptom of AS | Group | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Jumping | 6 (50) | 0 | 0 |
| Wet dog shake | 12 (100) | 2 (20) | 0 |
| Diarrhea | 12 (100) | 2 (20) | 0 |
| Gnash | 12 (100) | 6 (60) | 5 (50) |
| Ptosis | 8 (66.6) | 4 (40) | 1 (10) |
| Forelimb shaking | 12 (100) | 0 | 0 |
| Convulsions | 8 (66.6) | 0 | 0 |
| Time-to-termination of locomotor activity | | | |
| 40 sec-1 min | 9 (75) | 6 (60) | 0 |
| 1-2 min | 2 (16.6) | 4 (40) | 10 (100) |
| 2-2.5 min | 1 (8.3) | | |

**TABLE 2.** Effect of HLDF-6 Peptide on EA Activity in Brain Structures of Chronically Morphinized Rats (pmol/mg protein/min, $M \pm m$)

| Brain structure | Group | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Hippocampus | 55.3±6.7 | 46.1±3.8 | 56.6±8.1 |
| Hypothalamus | 65.7±4.8 | 70.8±4.8$^{+}$ | 51.6±5.8 |
| Midbrain | 82.5±3.9 | 62.9±6.9* | 57.1±5.2** |
| Striatum | 174.3±14.6 | 172.4±10.7$^{++}$ | 128.8±4.4* |
| Cortex | 57.7±7.1 | 68.8±6.3 | 73.2±3.4 |

**Note.** *$p<0.05$ and **$p<0.01$ compared to group 1; $^{+}p<0.05$ and $^{++}p<0.01$ compared to group 3.

not scuffle and were non-aggressive to the researcher during touching and measurement of pain thresholds.

Thermonociceptive thresholds in group 3 rats practically did not differ from the normal (Fig. 1).

In group 2 rats EA activity decreased in the midbrain, while in group 3 animals enzyme activity decreased in all structures of the endogenous antinociceptive system (Table 2). Our results confirm published data that HLDF-6 peptide acts as an enkephalinase inhibitor [3]. This is consistent with the hypothesis of S. V. Litvinova that EA plays a role in the development of morphine tolerance and dependence [2]. Inactivation of EA leads to an increase in enkephalin content, elevation of pain thresholds (suppression of abstinent hyperalgesia), and alleviation of AS. It should be emphasized that EA activity most significantly decreased in group 3 rats, which corresponded to pronounced alleviation of AS (compared to group 2 animals). These data are consistent with changes in endogenous opiate content and mRNA concentration in various brain structures during opium abstinence [7]. The content of pro-enkephalin mRNA in the caudal periaqueductal area increased during naloxone-induced and natural AS, but not after exposure to stress factors. These indexes returned to normal after disappearance of AS symptoms. Local administration of enkephalin analogue or peptidase inhibitor in this area alleviated symptoms of AS.

HLDF-6 peptide produces an analgesic effect and acts as an EA inhibitor in brain structures of the endogenous antinociceptive system [3]. This probably determines the ability of HLDF-6 peptide to relieve symptoms of AS in chronically morphinized animals. Our previous observations revealed increased total enkephalinase activity in blood plasma in patients with heroin abuse manifesting in a sharp decrease in leu-enkephalin half-life in the plasma. These changes serve as a motivational factor to continue narcotization [8]. Naloxone in low doses inhibits EA and decreases total enkephalinase activity [5], which has strong clinical effect in the abstinence and post-abstinence period [8]. Treatment with low doses of naloxone was most effective in the acute period of opiate withdrawal, which is consistent with manifestations of AS in experimental opium abuse. Our results indicate that HLDF-6 peptide is potent in modulating the system for synthesis and catabolism of endogenous opiates. An imbalance in these compounds contributes to intensification of catabolic transformations. Therefore, HLDF-6 peptide holds much promise to relieve symptoms of AS during opium abuse.

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