# Use of HLDF-6 Peptide for Correction of Disturbances in the Endogenous Opioid System in Offspring of Morphine-Tolerant Animals

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We studied the effect of bioactive peptide HLDF-6 on functional activity of the endogenous antinociceptive system in the offspring of morphine-tolerant animals. Disturbances in this system included changes in the thermonociceptive threshold and enkephalinase A activity in various brain structures. The peptide acted as a potent regulator of the homeostasis in systems responsible for the synthesis and catabolism of endogenous opioids. HLDF-6 effectively corrected disorders of the endogenous antinociceptive system.

**Key Words:** *nociception; morphine; tolerance; offspring; enkephalinase A; HLDF-6 peptide*

Growing incidence of drug addictions determines special importance of studies of functional disturbances in neurobiological systems in the offspring of morphine-tolerant animals. The search for new methods of their prevention and correction is an urgent problem [7,12,13]. Our previous studies demonstrated impairment of the reproductive function in adult morphine-treated animals [3], which is consistent with published data [5,7,9]. Pathological changes in the endogenous opioid system in the offspring of these animals manifest in the increase in nociceptive thresholds and enkephalinase A activity (EC 3.4.24.11) in brain structures of the endogenous antinociceptive system, when tolerance in parent animals was induced with analgesic doses of morphine [3]. Changes in nociceptive thresholds and activity of endopeptidases (*e.g.*, enkephalinase A) are the main criteria for functional activity of the endogenous opioid system. Dysfunction in this system required increased doses of morphine (5-6 mg/kg compared to 2 mg/kg) and accelerated the development of tolerance even at high concentrations of the preparation [4].

HLDF is a small glycolysated protein with a molecular weight of 8.2 kDa, which causes differentiation of parent H-60 cells into granulocytes [11]. HLDF-6, a fragment of HLDF consisting of 6 amino acids (TGENHR) initiates differentiation and blocks proliferation of parent cells similarly to the full-length protein [2]. An analogue of this peptide was synthesized at the M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry in 2000. The addition of HLDF-6 to the culture of aged HL-60 cells prevented their death. *In vitro* this peptide produced a positive effect on mouse embryos at the stage of division. The cells cultured in the presence of HLDF-6 are more resistant to exogenous factors. These data suggest that HLDF-6 acts as an adaptive factor. There are no specific receptors on the cell surface for this peptide. HLDF-6 affects physicochemical properties of the cell membrane and modulates binding of cytokines involved in proliferation [2]. Nonspecific interaction of HLDF-6 with lipids suggests that this peptide af-

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fects physicochemical properties of cell membranes, modulates physiological and adaptive processes at the cellular and organ levels, and changes the resistance of biological objects to factors causing a homeostatic imbalance.

Here we studied the effects of HLDF-6 on enkephalinase A activity in brain structures of the endogenous antinociceptive system to evaluate the neurochemical mechanism underlying its action on this system.

## MATERIALS AND METHODS

Experiments were performed on adult Wistar rats (5 males and 5 females, 200-220 g) and offspring of animals tolerant to the analgetic effect of morphine ($n$=18, 3-9 weeks' postnatal development) [3]. Experimental rat pups ($n$=10) received intraperitoneal injections of HLDF-6 in a daily dose of 0.2 mg/kg from the 4th to 9th week of postnatal development.

Control group 1 included F1 pups ($n$=10) of the same age. Eight parent Wistar rats (4 males and 4 females) received an equivalent volume of 0.9% NaCl instead of morphine. Control group 2 included F1 pups ($n$=8) from morphine-tolerant (MT) animals receiving daily injections of 0.9% NaCl instead of HLDF-6. Changes in nociceptive thresholds in the tail-flick test (NTTF) were studied on experimental and control rat pups aged 3-9 weeks.

The effects of HLDF-6 in a dose of 0.2 mg/kg on NTTF and enkephalinase A activity in various brain structures were studied on 10 adult morphine-sensitive (MS) Wistar male rats weighing 180-220 g. Control animals of the same age ($n$=10) received an equivalent volume of 0.9% NaCl instead of HLDF-6. The rats were decapitated after 14-day systemic treatment with test preparations. Enkephalinase A activity was measured in various brain structures [8].

The results were analyzed by Student's $t$ test, Wilcoxon test, and Mann—Whitney test.

## RESULTS

In rat pups of control group 1 aged 3-9 weeks NTTF little varied (insignificant within measurement error, Fig. 1). These findings are consistent with our previous results [3]. However, in the offspring of MT animals NTTF increased from the 6th to 9th weeks of postnatal ontogeny. NTTF slightly increased in experimental rat pups aged 6 weeks, but then progressively decreased staring from the 7th week and returned to baseline by the end of the 9th week. During this period NTTF in the offspring of MT rats not receiving the peptide surpassed that in 3-4-week-old pups (Fig. 1). Enkephalinase A activity increased in response to intensification of opioid synthesis in the endogenous antinociceptive system not related to the treatment with exogenous opioids. These changes reflect functional disturbances in the endogenous antinociceptive system in the offspring of MT animals [3]. The peptide corrected functions of the opioid system and normalized NTTF.

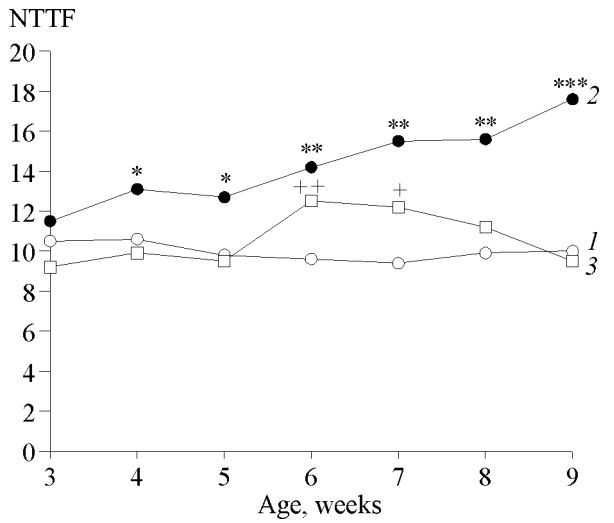


**Fig. 1.** Changes in thermonociceptive thresholds in the tail-flick test in offspring of morphine-sensitive and morphine-tolerant animals at various stages of postnatal development: control group 1 (*1*), control group 2 (*2*), and experimental group (*3*). *$p$<0.05, **$p$<0.01, and ***$p$<0.001 compared to *1*.

HLDF-6 acted as the enkephalinase inhibitor in structures of the endogenous antinociceptive system in adult MS animals (Table 1). Moreover, HLDF-6 produced the analgetic effect in the tail-flick test. These properties of the peptide probably contribute to its influence on NTTF in experimental rats. Under normal conditions the content of endogenous opioids is determined by the intensity of their biosynthesis and catabolism with peptide hydrolases (primarily enkephalinase) [1,6]. The increase in NTTF in the offspring of MT animals is followed by a sharp increase in enkephalinase A activity [3]. Abnormally increased release of endogenous opioids manifested in increased thresholds and induced catabolism by the negative feedback mechanism. Under these conditions the intensity of catabolic processes is above the normal.

**TABLE 1.** Effect of HLDF-6 on Enkephalinase A Activity in Various Brain Structures (pmol/mg protein/min, $M \pm m$)

| Brain structure | Control | Experiment |
|---|---|---|
| Hypothalamus | 93.7±2.2 | 42.4±3.2** |
| Midbrain | 87.2±3.5 | 82.6±3.9 |
| Striatum | 179.5±5.5 | 149.5±8.7* |
| Cortex | 73.7±4.1 | 72.5±5.1 |

**Note.** *$p$<0.05 and **$p$<0.001 compared to the control.

The content of opioids is low in adult tolerant animals [10], which is probably related to high activity of endopeptidases. As a result, catabolic processes prevail over the synthesis. Therefore, exogenous opioids should be used in high doses to produce the analgetic effect. Abnormalities in the synthesis and catabolism of opioids require treatment with higher doses of preparations and, therefore, accelerate the development of tolerance and dependence on these substances [4]. Enkephalinase inhibitor prevents disturbances in the interaction between systems synthesizing and catabolizing endogenous opioids in the offspring of MT animals. HLDF-6 maintains the homeostatic equilibrium impaired in the offspring of animals with chronic morphinization.

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