Changes in Thermonociceptive Thresholds and the Role of Enkephalinase A in Homeostasis in Morphine-Tolerant Rat Offspring

S. V. Litvinova, V. V. Aristova, V. V. Shulgovskii, N. N. Terebilina*, and L. F. Panchenko*

Translated from *Byulleten' Eksperimental'noi Biologii I Meditsiny*, Vol. 129, No. 5, pp. 560-562, May, 2000
Original article submitted December 3, 1999

The dynamics of thermonociceptive thresholds as a marker of the state of the endogenous opioid system was studied in the offspring of morphine-tolerant rats. Significant, age-dependent increase in thermonociceptive thresholds and higher levels of enkephalinase A in structures of the endogenous antinociceptive system were observed in the offspring compared with the control. These findings attest to disturbances of the opioid system in the progeny of morphine-tolerant rats and confirm the key role of enkephalinase A in the maintenance of homeostasis disturbed by chronic prenatal morphine treatment.

Key words: *enkephalinase A*; *nociception*; *morphine*; *tolerance*; *progeny*

The level of endogenous opioids depends on the intensity of opioid biosynthesis and the degree of their inactivation with peptidehydrolases [1,5]. Considering the tolerance to narcotic drug (to morphine, for example) characterised by specific neurochemical and immune alterations as the basic mechanism of the development of physiological dependency, we hypothesised that endopeptidases and, in particular, enkephalinase A (EA; EC 3.4.24.11) play a role in this process [3]. Regional and intracellular distribution of these enzymes in the brain coinciding with the localization of opiate receptors [11] and the ability of selective EA inhibitors to induce met-enkephalin accumulation in the striatum and modulate the pharmacological effects of enkephalin [8] confirm participation of EA in opioid catabolism. At the same time, increased thermonociceptive thresholds after target disruption of the EA gene [12] and antinociceptive and antidepressant effects of the EA antagonist BL-2401 [10] were observed. However, little is known about opioid-endopeptidase interactions in the offspring of morphine-tolerant animals. We assume that impairment of normal homeostatic interaction between these systems is a neurochemical mechanism responsible for the development of drug abuse. Altered dynamics of nociceptive thresholds and shifts in EA activity in different brain structures are specific signs of this process.

Our aim was to examine the dynamics of nociceptive thresholds (NT) as a marker of the state of the endogenous opioid system in the offspring of morphine-tolerant rats in postnatal ontogeny and to evaluate EA activity in various brain structures in these animals until postpubertal.

MATERIALS AND METHODS

Adult male and female Wistar rats weighing 250-300 g were tested for morphine sensitivity using the tail-flick test. NT were measured with an automatic Ugo Basile algesimeter. Morphine-sensitive animals were selected and the tolerance to the analgesic effect of morphine was induced according to a standard scheme [2-4]. Morphine hydrochloride (1%) was injected intra-

Department of Higher Nervous Activity, Biological Faculty, M. V. Lomonosov Moscow State University; *Laboratory of Neurochemistry, Institute of Narcology, Ministry of Health of the Russian Federation, Moscow. ***Address for correspondence:*** shulg@protein.bio.msu.ru. Shulgovskii V.

0007-4888/00/0005-474$25.00

muscularly in a dose of 2.5 mg/kg both to male and female animals. After reaching the tolerance, the rats were mated and their offspring formed the test group. The control group included the offspring of morphine-sensitive rats which were not morphinized (they were injected with the same volumes of 0.9% NaCl). Changes in NT were examined in both groups at the age of 3, 4, 5, 6, 7, 8, and 9 weeks. At the age of 8-9 weeks, the rats were decapitated, and EA activity was determined in various brain regions as described previously [7].

The data were statistically analyzed by Student's *t* test and by nonparametric Wilcoxon—Mann—Whitney test.

RESULTS

Chronic administration of morphine sharply reduced reproductive function: 4 couples had no progeny, other couples had 3 or 4 pups (compared with 6-11 in the control), 1 or 2 of them usually died. Our findings agree with other evidence on high mortality in the offspring of morphinized animals [6].

In 3-week-old rats of the test group, the mean NT to a thermal stimulus (n=14) was 12.3±0.7 sec. In 4-, 5-, 6-, 7-, 8-, and 9-week-old rats it was 13.1±0.4, 12.7±0.04, 13.4±0.3, 15.5±0.8, 15.6±0.6, and 17.1±0.5 sec, respectively. Thus, the thermonociceptive thresholds in the offspring of morphine-tolerant rats increased with age, while in controls (n=15) this parameter remained unchanged in the postnatal ontogeny (Fig. 1).

EA activity in the main structures of the endogenous antinociceptive system in the offspring in morphine-tolerant rats was significantly higher than in controls. Similar relationships were not observed in brain structures not related to the endogenous antinociceptive system (Fig. 2).

These results are consistent with previous data on long-lasting effects of prenatal morphinization on the opioid system in the offspring [6]. Enhanced EA activity in the morphine-tolerant offspring probably represent a homeostatic response of the neurochemical enzyme systems catabolyzing opioids to their increased levels, which was manifested in elevated thermonociceptive thresholds.

The age-related increase in thermonociceptive thresholds (not induced by exogenic opioids) is the marker of enhanced activity of the endogenous opioid system. EA activity increased in response to disturbances in this system. Our findings confirm the important role of EA in the induction of tolerance to the analgesic effect of morphine [3]. Low content of opioids in adult morphine-tolerant animals [9] together with high EA activity [4] are transformed in the offspring, probably, due to impaired regulation of the opioid system, since high nociceptive thresholds indicate an increase in the concentration of opioids in the antinociceptive structures in the offspring.

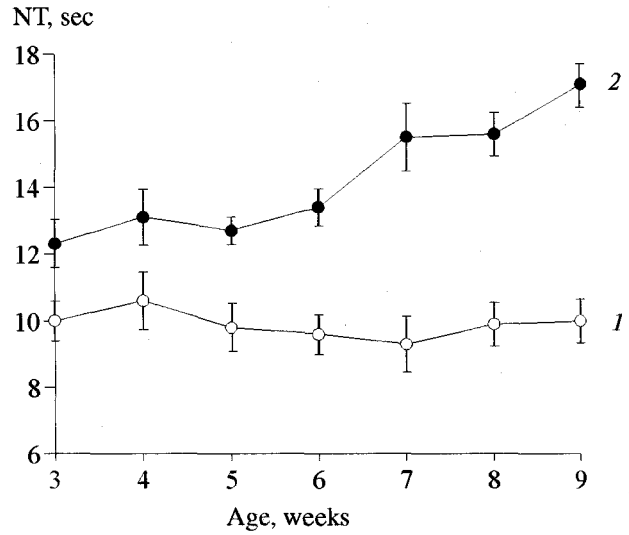

Fig. 1. Changes in thermal nociceptive thresholds (NT, tail-flick test) in offspring of morphine-sensitive (*1*) and morphine-tolerant (*2*) rats in postnatal ontogeny.

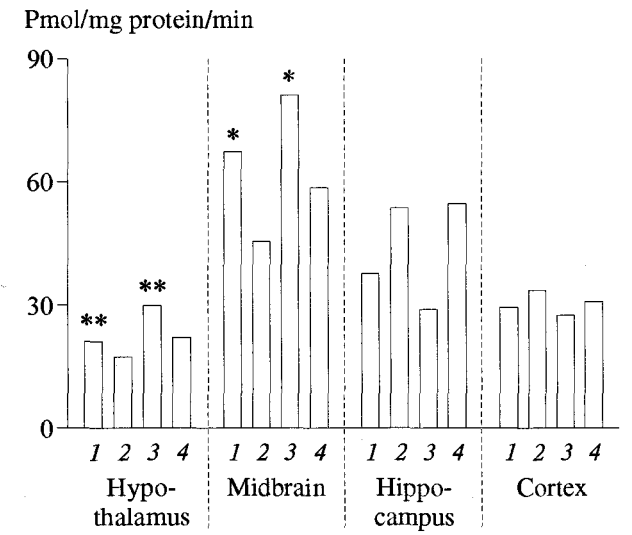

Fig. 2. Activity of enkephalinase A in various brain structures in morphine-tolerant (*1*, 3) and morphine-sensitive (*2*, *4*) rats. *1*, *2*: males; *3*, *4*: females; *p<0.01, **p<0.05 compared to morphine-sensitive rats.

The study was supported by "Universities of Russia – Fundamental Research" Program.

REFERENCES

1. A. V. Azaryan, *Peptide Hydrolases in Nervous System and Their Biochemical Functions* [in Russian], Erevan (1989), p. 207.
2. L. V. Kalyuzhnyii, M. A. Gruden', S. V. Litvinova, *et al.*, *Byull. Eksp. Biol. Med.*, **116**, No. 7, 6-9 (1993).
3. S. V. Litvinova, A. Yu. Kozlov, and L. V. Kalyuzhnyii, *Ibid.*, **116**, No. 7, 54-56 (1993).
4. S. V. Litvinova, V. V. Shulgovskii, L. F. Panchenko, *et al.*, *Vopr. Narkol.*, **4**, 49-53 (1997).

5. S. De la Baume, C. Gros, C. Yi, *et al.*, *Life Sci.*, **31**, No. 16-17, 1753-1756 (1982).
6. P. S. Eriksson and L. Ronnback, *Drug Alcohol Depend.*, **24**, 187-194 (1989).
7. D. Florentine, A. Sassi, and B. Roques, *Anal. Biochem.*, **553**, 284-290 (1984).
8. M. C. Fournie-Zaluski, *Neurochem. Int.*, **12**, No. 3, 375-383 (1985).
9. K. Gudehithlu, G. Tejwani, and H. Bhargava, *Brain Res.*, **553**, 284-290 (1991).
10. A. Kita, K. Imano, Y. Seto, *et al.*, *Jpn. J. Pharmacol.*, **75**, No. 4, 337-346 (1997).
11. B. Malfrey, J. Swerts, C. Liorens, *et al.*, *Neurosci. Lett.*, **11**, No. 3, 329-334 (1979).
12. A. Saria, K. Hauser, and H. Traurig, *Ibid.*, **31**, No. 1, 27-30 (1997).