Formation of Morphine Tolerance in Offspring of Morphine-Tolerant Animals: Neurochemical and Neuroimmune Correlates

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We carried out a complex physiological, neurochemical, and neuroimmunologic study of the formation of tolerance to analgetic effect of morphine and analyzed enkephalinase A activity in different brain structures and serotonin antibodies in the serum. More early development of morphine tolerance and a sharp increase in serum antibody titer was found in the offspring of morphine-tolerant rats. This points to an imbalance in the neurotransmitter system and can serve as a diagnostic marker of pathology of the endogenous opioid system.

Key words: *morphine; tolerance; offspring; enkephalinase A; antiserotonin antibodies*

Pathology of neurobiological systems in the offspring of morphinized animals attracts now much attention. Our previous study [5] was devoted to the difficulties in obtaining offspring from morphine-tolerant or morphine-dependent animals. Morphine in high doses (25 mg/kg) administered throughout pregnancy cause 100% mortality of the offspring, while morphine treatment up to days 21 or 17 induces 65 and 31.8% mortality, respectively. Disturbances in parental behavior in 25-day-old rats and its normalization after naloxone treatment were described [12]. The authors conclude that the endogenous opioid system plays an important role in the regulation of parental behavior of young animals of both genders. There are data on increased antinociception in generation I male, but not female offspring of morphine-dependent animals, while in generation II animals this effect was present in both genders [11]. In this study only male rats were chronically morphinized with increasing morphine doses. However, there are no published data on the formation of morphine tolerance in the offspring of morphine-tolerant animals, which appears to be the most actual problem. Our previous study revealed pathology of the opioid system in the offspring of these animals [5]. On the other hand, an important role of antibodies to biologically active compounds, was recently established [2]. According to current concept antibodies can be considered as bioregulators with wide spectrum of physiological functions responsible for long-term correction of physiological and biochemical processes. Antibodies to neurotransmitters were found in various types of experimental and clinical neuropathology [1,3,4].

The aim of the present study was to investigate physiological, neurochemical, and neuroimmunologic peculiarities of the formation of tolerance to analgetic effect of morphine in the offspring of morphine-tolerant animals.

MATERIALS AND METHODS

The study was carried out on adult Wistar rats (5 males and 5 females) weighing 250-280 g and their 8-week-old offspring (n=16). Tolerance formation was described in detail [5]. On the next day after tolerance

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formation enkephalinase A activity in different brain regions of tolerant animals was assayed as described elsewhere [9]. Immediately after decapitation, blood was collected and the titer of antiserotonin (5-OT) antibody (maximum serum dilution yielding 1.5-fold difference from the control) were measured by enzyme-linked immunosorbent assay (ELISA) with 5-OT-BSA conjugate as the test antigen [3].

Two control groups were used: morphine-tolerant (n=10) and intact (n=8) offspring of the same age from normal parents not subjected to chronic morphinization. The sera from intact and morphine-resistant rats of the same age were used as the control sera.

The results were processed statistically using Student's t test.

RESULTS

In the offspring of morphine-resistant rats morphine tolerance developed more early than in controls: starting from the 2nd injection they needed higher doses of morphine in the nociceptive tail-flick test, since the latency of this response did not differ from the baseline. For instance, in only 7 animals the tolerance developed after increasing the dose of morphine by 0.5 mg/kg compared to the control (Fig. 1). However, these animals developed tolerance after 6 injections of 2.5 mg/kg, while the rats of control I and II groups developed tolerance after 14 and 9 injections of 2 mg/kg morphine, respectively (Fig. 2, *a*). In 12 rats (males and females) receiving 2 injections of 2.5 mg/kg morphine no analgesic effect was observed and these animals required higher doses (3 mg/kg: background — 11.3±0.2, after injection — 12.3±0.8; 4 mg/kg: background — 12±0, after injection — 20±0). In 4 animals, the tolerance appeared after 5 injections of 4 mg/kg morphine. In 6 animals (2 males and 4 females), the tolerance developed after 6 injections of 5 mg/kg morphine (Fig. 2, *b*), while other 5 animals (males and females) required 6 mg/kg. In these animals tolerance developed within 3 days (day 1: background — 11±0, after injection — 15.7±1.0; day 2: background — 10±0, after injection — 12±0; day 3: background — 10.5± 0.2, after injection — 10.5±1.3). It should be noted that, tolerance in females developed earlier than in males by 1-2 days, which agrees with the data on sex-related differences in the development of morphine tolerance in rats [7]. It was shown that males are more sensitive to antinociceptive effects produced by acute administration of 10-20 mg/kg morphine. Chronic morphine treatment disturbed estrous cycle in females. The increase in morphine dose probably results from pathology of the endogenous opioid system in the offspring of morphine-tolerant animals [5], which was confirmed by a sharp increase in enkephalinase A activity in structures of the brain antinociceptive system and elevated threshold of nociceptive response. High level of enkephalinase A activity resulting in the decreased endogenous opioid concentrations requires higher morphine doses for long-term manifestation of the analgetic effect. Enkephalinase A activity in various brain structures of experimental rats (tolerant offspring from tolerant animals) increased compared to control I (tolerant offspring from intact animals); however, these differences were insignificant (Table 1).

High enkephalinase A activity in structures of the antinociceptive system is a typical sign of morphine tolerance in both experimental and control animals. This confirms our previous data on activation of enkephalinase A in adult morphine-tolerant animals compared to morphine-sensitive animals receiving isotonic saline [6]. The content and incidence of anti-5-OT

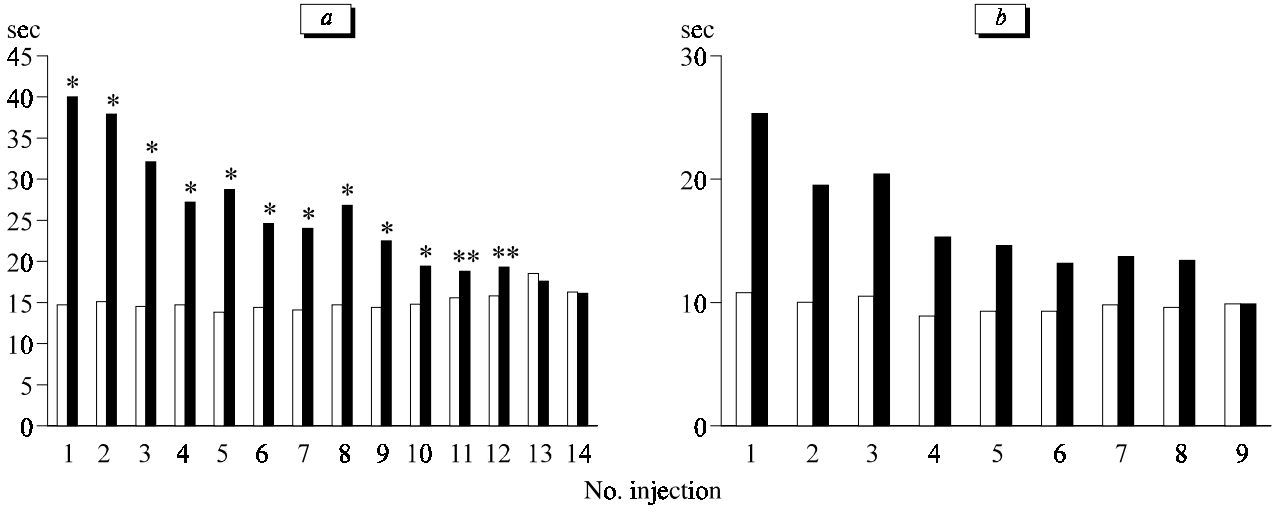

Fig. 1. Development of tolerance to analgesic effect of morphine in control II (*a*) and I (*b*). Here and in Fig. 2: ordinate: latency of tail flick response. Open bars: baseline, filled bars: morphine. *p<0.01, **p<0.05 compared to the baseline.

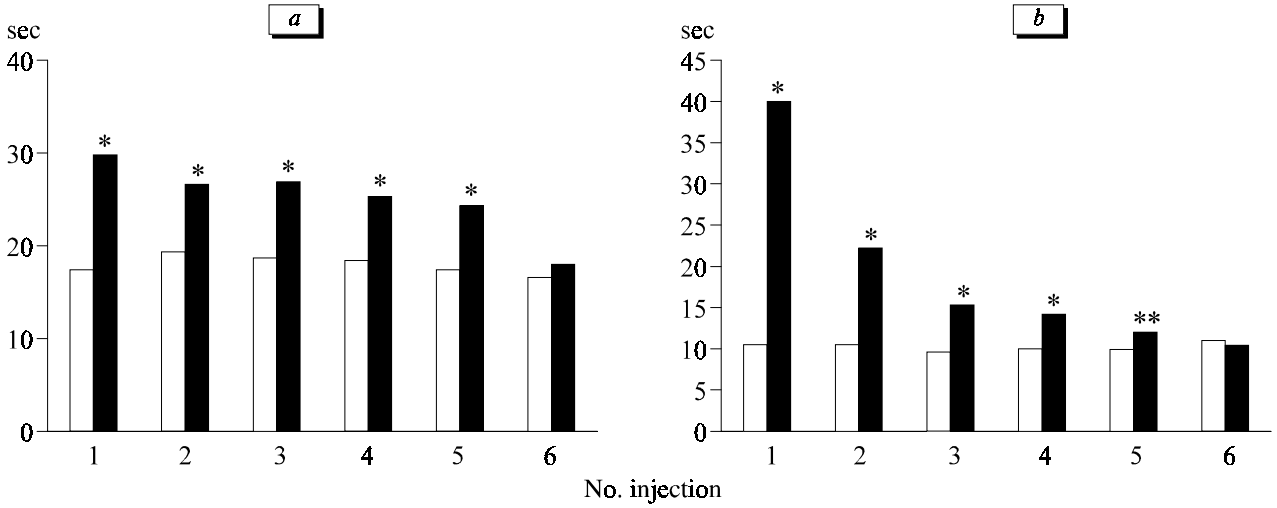

Fig. 2. Development of tolerance to analgesic effect of morphine in doses of 2.5 (*a*) and 5 mg/kg (*b*) in the offspring of morphine-tolerant animals.

antibodies increased in the offspring (males and females) of morphine-tolerant animals compared to the control. Thus, antibody titers in groups 1 and 2 offspring of morphine-tolerant animals were 1:64 and 1:64-1:128, respectively, whereas in control I and II the corresponding values were 1:16-1:32 or antibodies were not detected (in 20% animals of control II). These data confirm the involvement of antineurotransmitter antibodies in the neuroimmune regulation during pathology of the endogenous opioid system in offspring of morphine-tolerant animals.

Our findings agree with published data on the disturbances of estrous cycle in females [7] and reproductive function in males [10] treated with high morphine doses. Our experiments showed that even analgesic doses of morphine used for tolerance modeling cause pathology of the endogenous opioid system in the offspring, in particular, they promote the development of morphine tolerance. The presence of anti-5-OT antibodies in the serum indicates an imbalance in the neurotransmitter system and can be used as a marker of endogenous opioid system disturbances.

TABLE 1. Enkephalinase A Activity in Different Brain Structures (pmol/mg Protein per min, *M*±*m*)

Brain structure	Control	Experiment
Striatum	38.10±4.85	46.48±2.09
Hypothalamus	25.40±2.56	31.6±5.1
Midbrain	80.90±1.21	83.02±2.10
Hippocampus	44.45±4.26	46.10±4.87
Cortex	25.42±2.56	31.6±5.1

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