Immunization with Neurotransmitters (Serotonin, Dopamine, Noradrenalin) Conjugated with Protein Suppresses the Manifestations of Morphine Withdrawal Syndrome

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A protective effect of immunization with neurotransmitters (serotonin, dopamine, noradrenalin) conjugates with protein during reproduction of the withdrawal syndrome was demonstrated in two different models of opium narcomania. In the first experiment rats were immunized before morphinization, and in the second morphine dependence was induced in mice before immunization. Similar effects were observed in immunization of rats and mice: antibodies to serotonin caused the greatest suppression of symptoms of the withdrawal syndrome.

Key Words: *neurotransmitters; immunization; antibodies; withdrawal syndrome*

Dysfunction of the catecholaminergic and serotoninergic neurotransmitter systems of the brain is known to play an important role in the pathogenesis of narcomania and alcoholism [1]. Previously we showed that both types of neuropathology are associated with increased production of autoantibodies to neurotransmitters (NT), which is observed both experimentally and clinically in patients with alcoholism [7] and narcomania [3]. In addition, antibodies to serotonin and the catecholamines dopamine and noradrenalin have been found capable of inhibiting the alcohol withdrawal syndrome (WS) and at the same time of modulating the analgetic action of morphine and the development of tolerance to it [4]. A study of the possible neuromodulating action of antibodies to NT on the manifestation of morphine WS was a logical continuation of this research.

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MATERIALS AND METHODS

Seventy male Wistar rats weighing 200 to 230 g and 45 male C57Bl/6 mice weighing 18 to 20 g were used in the experiments. Two series of experiments were carried out. In the first series the animals were preimmunized with NT conjugated with protein (bovine serum albumin, BSA), after which they were chronically exposed to morphine. In the second series animals with full-blown narcotic dependence were immunized.

Conjugates for immunization were synthesized using modifications of methods described elsewhere [15,16]. The animals were immunized with ascending doses of the antigens as described previously [2]. Antibodies to NT in the blood sera were titrated by solid-phase enzyme immunoassay on polystyrene plates sensitized with the respective test antigens [3].

In the first series of experiments narcotic dependence was simulated after active immunization of rats with NT-BSA conjugates: morphine hydro-

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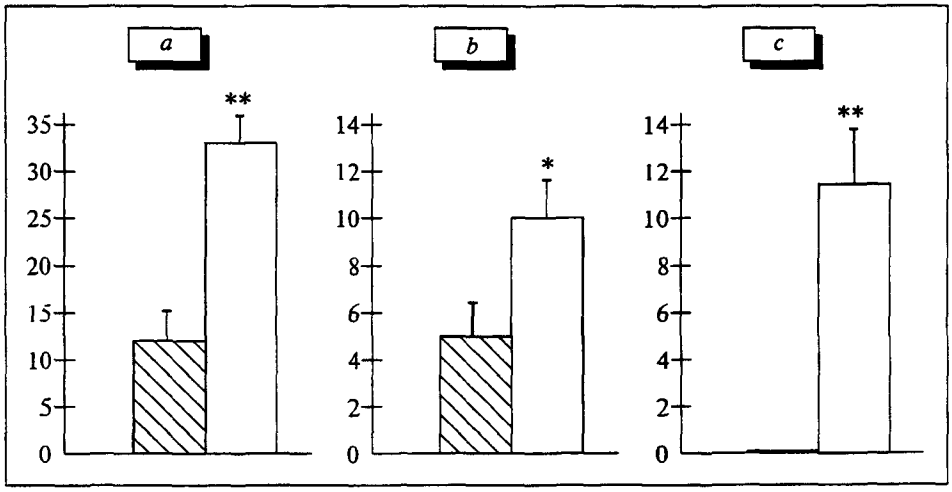

Fig. 1. Behavior of nonimmunized rats in the open field before and after naloxone injection in experimental opium narcomania. Ordinate: absolute values of behavioral parameters during 4-min testing: *a*) number of crossed squares; *b*) number of stand-stills; *c*) number of jumps. Here and in Fig. 2, black bars: before naloxone, white bars: after naloxone administration. One asterisk shows $p<0.05$, two asterisks $p<0.001$.

chloride was intraperitoneally injected twice a day at 12-h intervals in doses increasing every 3 days, from 10 to 50 mg/kg b.w., for 14 days.

In the second series of experiments opium narcomania was simulated in mice as described previously [9]. Morphine hydrochloride was injected subcutaneously twice a day at 12-h intervals, in a dose increasing every 3 days, from 20 to 80 mg/kg b.w. The animals were then fed a 0.1% morphine solution in 10% sucrose for 10 days, after which they were given the choice for 21 days between 0.05% morphine solution in 5% sucrose and a 5% sucrose solution. The time course of daily consumption of liquids was recorded and the mean daily consumption of morphine was calculated in mg/kg b.w.

WS was reproduced in rats by intraperitoneal injection of naloxone in a dose of 1 mg/kg, and in mice by withdrawal of morphine for 48 h. WS was assessed using a complex of behavioral parameters in the open field test. This complex included both specific manifestations of WS occurring in animals (jumps, chattering and grinding of the teeth, and wet-dog syndrome) and nonspecific behavioral disorders such as increased horizontal and vertical activities, a greater number of peeping through holes, etc. The relationship between antibodies to NT and behavioral reactions of animals was assessed by the total index of activity [8], which included the following components of behavior during the open-field test: number of square crossings, peeping through holes, and grooming acts. Animals were subjected to the open-field test before and after WS reproduction. Results were statistically processed using Student's and Fisher's tests and Spearman's ranked correlation coefficient.

RESULTS

Naloxone blocking of opiate receptors in nonimmunized rats with opium narcomania led to the manifestation of WS symptoms, which was expressed by a reliable increase of horizontal and vertical motor activities and the appearance of jumps and grinding of the teeth (Fig. 1). Increased production of antibodies to NT led to an appreciable reduction of WS manifestations in immunized groups, which was expressed as a reduced number of jumps and of motor and exploratory activities. The most pronounced reduction of the total index of activity (Fig. 2) and of the total number of jumps was observed in rats immunized

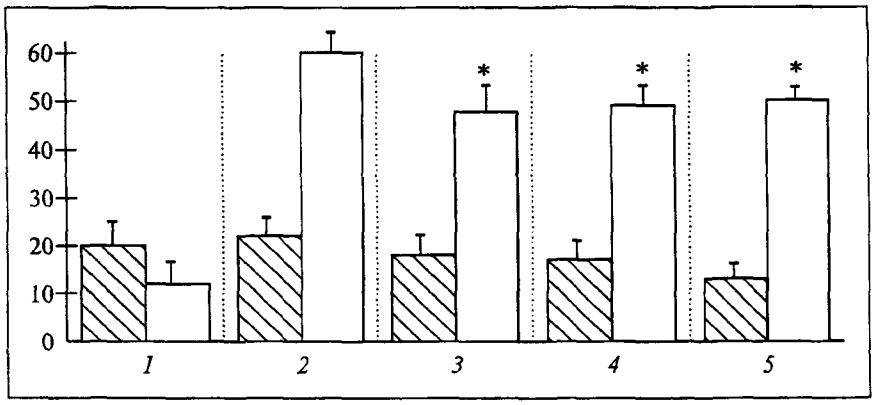

Fig. 2. Effects of antibodies to neurotransmitters on the manifestation of WS in rats with experimental opium narcomania. Ordinate: total index of activity (absolute values). *1*) control; *2*) morphine-treated control; morphine-treated animals with antibodies to serotonin (*3*), dopamine (*4*), and noradrenalin (*5*). An asterisk shows $p<0.001$ *vs.* group *2*.

with serotonin-BSA conjugate. Statistical analysis demonstrated a negative correlation between the level of antiserotonin antibodies and the total index of activity (r=-0.71, p<0.01).

These results were confirmed in the second series of experiments, when the effects of immunization with NT-BSA conjugates on an already developed morphine dependence were studied in mice. Changes in two parameters were followed up: the time course of morphine consumption under conditions of free choice of a liquid and the expression of WS caused by withdrawal of the narcotic without naloxone administration. A group of 15 intact mice not subjected to forced narcotization served as controls.

Chronic morphinization of animals led to the development of narcotic dependence, which was assessed by the daily consumption of morphine under conditions of free choice of liquid. Mice consumed morphine in a dose of 29±2.6 mg/kg b.w., on average, this constituting 19.4±1.8% of the total volume of liquid they drank. In control non-narcotized animals the mean daily consumption of the narcotic was 10.9±1.1 mg/kg or 6.6±0.3% of the total amount of liquid they drank (p<0.001). Intensified production of antibodies to dopamine and noradrenalin led to a reliable reduction of morphine production: by 49 and 40%, respectively (p<0.01) in comparison with the level of narcotic consumption before immunization, the amount of liquid drunk daily remaining unchanged. Immunization of mice with serotonin-BSA conjugate did not appreciably alter the level of morphine consumption.

Withdrawal of the narcotic in the group of nonimmunized animals (morphine-treated control) led to the manifestation of WS symptoms. This was expressed in an appreciable increase of motor and exploratory activity; the total index of activity increased by 73% in comparison with the previous testing (p<0.001). Moreover, the "Straub-tail" symptom characteristic of WS was observed in 50% of animals. Immunization of animals with NT-BSA resulted in a reliable depression of WS manifestations, a 72% reduction (p<0.01) of the total index of activity in the group of animals with high titers of antibodies to serotonin being the most noticeable sign, and the protective effect of antibodies to catecholamines being less marked (Fig. 3). The "Straub-tail" symptom was not observed in any of the immunized animals.

Hence, the results indicate the possibility of suppressing the principal manifestations of WS with antibodies to NT. Such a suppression was observed both under conditions of preliminary

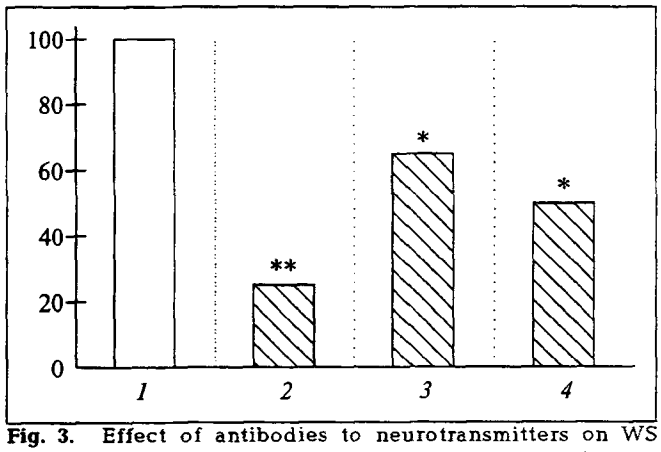

Fig. 3. Effect of antibodies to neurotransmitters on WS manifestation in mice. Ordinate: difference between absolute values of total index of activity during withdrawal and before morphine withdrawal in % (the value in the group of morphine–treated control taken as 100%). *1*) morphine–treated control; morphine–treated animals with antibodies to serotonin (*2*), dopamine (*3*), and noradrenalin (*4*). One asterisk shows p<0.05, two asterisks p<0.01 *vs.* group *1*.

(before narcotization) immunization of animals with NT-BSA conjugates and after immunization following the development of morphine dependence. This agrees with our previous findings on the protective action of antibodies to NT in a similar neuropathological entity, experimental alcoholism [6]. The greatest neuroimmunomodulating effect is associated with the production of antibodies to serotonin. Antibodies to catecholamines exert a less marked effect on the main manifestations of WS, but in contrast to antiserotonin antibodies they cause a reliable reduction of consumption of the narcotic under conditions of free choice.

Dafny *et al.* [12-14] note the ambiguous effects of immunomodulators on the development of morphine tolerance and the intensity of WS manifestation. Evidently, this has to do with the large variety of direct and mediated effects of immunomodulators such as anti-NT antibodies. On the one hand, they can modify the sensitivity of receptors to NT directly or indirectly as a result of NT binding in the circulation [10], a fact which confirms our previous reports about changed functional activity of the D_1/D_2 brain receptors in animals immunized with dopamine-BSA conjugates [11]. On the other hand, antibodies to NT may change the function of immune cells and provoke the corresponding immunocyte populations into producing a number of factors (interleukins, interferon, etc.) which can modulate the functions of the central nervous system [13].

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