0.29 mg/dl in the control (n=5, p<0.01). It is known that synthesis of the acute phase proteins in the liver is induced by the products of activated phagocytes [9].

These results suggest that the previously described NK-induced changes in the blood system are associated not only with the stress-realizing systems but also with activation of phagocytizing cells.

REFERENCES

1. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, *Stress and Blood System* [in Russian], Moscow (1983).
2. E. V. Gubler and A. A. Genkin, *Application of Nonparametric Statistical Criteria in Biomedical Investigations* [in Russian], Leningrad (1973).
3. I. I. Dolgushin, A. V. Zurochka, and L. Ya. Ebert, *Byull. Eksp. Biol. Med.*, **106**, No. 9, 330-331 (1988).
4. A. V. Zurochka, *Immunobiological Properties of Neutrophil Secretory Products (Neutrophilokines)*, DM Thesis [in Russian], Chelyabinsk (1991).
5. O. L. Kolesnikov, I. I. Dolgushin, I. A. Volchegorskii, and A. V. Zurochka, *Eksp. Onkol.*, No. 5, 36-38 (1993).
6. O. L. Kolesnikov, I. A. Volchegorskii, V. E. Tseilikman, *et al.*, *Byull. Eksp. Biol. Med.*, **117**, No. 3, 257-258 (1994).
7. A. N. Mayanskii and D. N. Mayanskii, *Neutrophil and Macrophage* [in Russian], Novosibirsk (1983).
8. F. R. Ballkwill, M. S. Naylor, and S. Malik, *Eur. J. Cancer.*, **26**, No. 5, 641-644 (1990).
9. H. Baumann and J. Gauldie, *Immunol. Today*, **15**, No. 2, 74-80 (1994).
10. M. A. Jagels and T. E. Hugli, *J. Immunol.*, **148**, No. 4, 1119-1129 (1992).
11. A. Marusic, I. Djikic, and M. Marusic, *Agents Actions*, **31**, No. 3-4, 280-284 (1990).

Immunotherapy of Experimental Drug Addiction with Antibodies to Serotonin and Dopamine

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Systemic administration of antiserotonin and antidopamine antibodies to chronically morphinized animals reduces the major manifestations of the withdrawal syndrome induced by naloxone injection or discontinuation of morphine.

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

Immunization of chronically morphinized animals with protein conjugates of serotonin (5-HT) and dopamine (DA) suppresses to different extents the manifestations of the withdrawal syndrome [7]. Experimental data suggest that systemic administration of antibodies to the above-mentioned neurotransmitters is a physiologically adequate therapy for drug addiction. The detection of autoantibodies to neurotransmitters in drug addicts [1] and in chronically morphinized animals [2] supports this suggestion. In addition, it was demonstrated that antiserotonin antibodies have a pronounced protective effect in alcoholism, a pathology similar to drug addiction [6].

Our objective was to explore the possibility of suppressing the major manifestations of withdrawal syndrome in chronically morphinized animals by systemic administration of antibodies to 5-HT and DA.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice weighing 20 g. Two models of withdrawal syndrome were used. In the first series, 40 mice were chronically morphinized by injecting rising doses of morphine (from 20 to 80 mg/kg body weight, subcuta-

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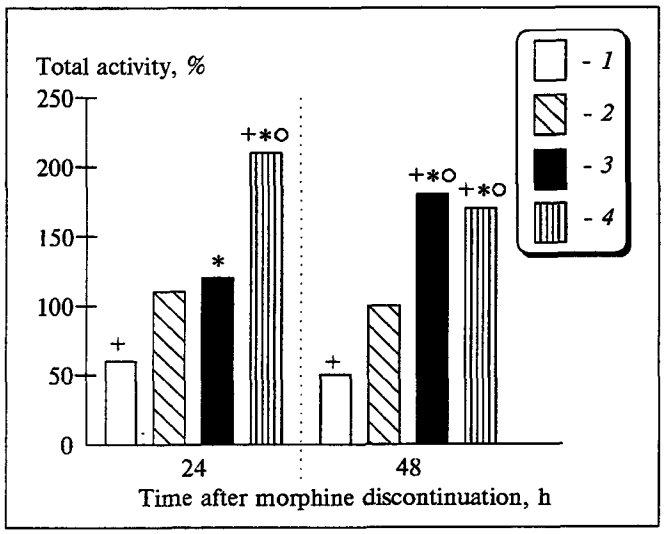

Fig. 1. Effect of antibodies to neurotransmitters on the behavioral reactions of chronically morphinized mice during the withdrawal syndrome. Motor activity prior to morphine discontinuation is assumed as 100%. $p<0.05$: +compared with the parameters prior to morphine discontinuation, *compared with group 1, °compared with group 2. Here and in Fig. 2: *1*) administration of normal saline (morphinized control); 2) administration of nonimmune rabbit γ-globulin; 3) antibodies to 5-HT; *4*) antibodies to DA.

neously) and forced watering with 0.1% morphine in sucrose solution, after which the animals had free choice between sucrose solution with and without morphine [7,8]. Daily dose of morphine (mg/kg) was calculated from the amount of sucrose solution drunk by the mice. Change in the analgetic effect of the test-dose of morphine (5 mg/kg intraperitoneally) in comparison with intact controls served as the primary parameter of drug dependence. The analgetic effect of morphine (EM) was calculated from the following formula: $EM=[LP_2-LP_1)/LP_1]\times 100\%$, where LP_1 is the latency period of licking the hind leg during nociceptive thermal stimulation in the hot-plate test (55°C) without the test-dose of morphine and LP_2 is the same parameter 30 min after administration of the test-dose.

In the second series of experiments, 40 mice were morphinized during a 16-day period by injecting morphine twice daily at a 12-h interval subcutaneously in doses increasing from 10 to 60 mg/kg.

Antibodies to DA and 5-HT were obtained by rabbit immunization with the corresponding conjugates prepared as described elsewhere [2]. The antibody titer determined in immunoenzyme assay varied from 1:2000-1:4000. The γ-globulin fractions from sera of immunized and intact rabbits were isolated by ammonium sulfate precipitation, dialyzed, lyophilized, and stored at 4°C.

The behavior reactions were studied in the open field test with the determination of the total activity index for 3 min [3]. In both series, mice were divided into two control and experimental groups. Experimental animals (groups 3 and 4) were injected antibodies to 5-HT and DA, respectively (50 mg protein/kg body weight in 0.2 ml normal saline, intraperitoneally). Group 2 mice were injected the same dose of nonimmune rabbit γ-globulin, and group 1 mice received 0.2 ml normal saline.

In the first series, mice were immunized two times at an 8-day interval two weeks after the end of morphinization (free choice between morphine and sucrose solution). The withdrawal syndrome was modeled by discontinuation of morphine for 48 h on day 6 after the second immunization.

In the second series, mice were given one injection of the antibodies. The withdrawal syndrome was modeled by intraperitoneal injection of 20 mg/kg naloxone 24 h after immunization. The preparation was synthesized at the A. N. Nesmeyanov Institute of Element-Organic Compounds, Russian Academy of Sciences. Before and immediately after the administration of naloxone, the open field test was performed, and the pain sensitivity was evaluated in the hot plate test in a Ugo Basile apparatus.

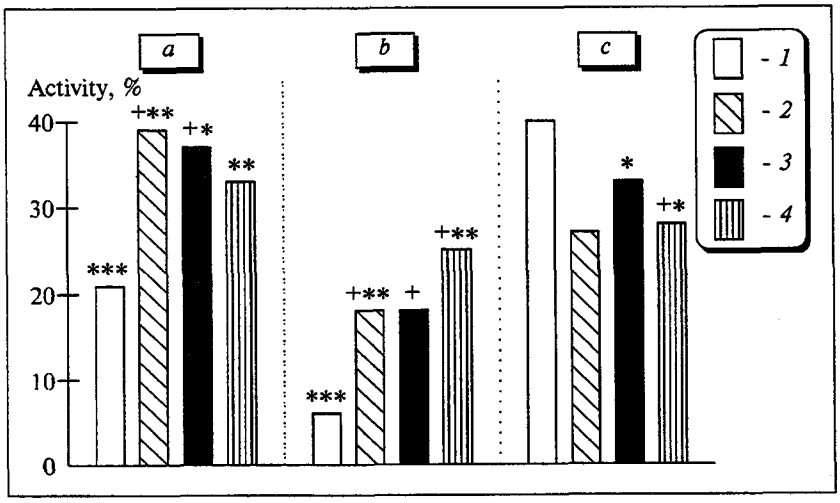

Fig. 2. Effect of antibodies to 5-HT and DA (passive immunization) on the behavioral reactions of mice (open field test) under conditions of the naloxone-induced withdrawal syndrome. Motor activity prior to naloxone administration is assumed as 100%. *a*) horizontal activity; *b*) vertical activity: *c*) exploring activity. +$p<0.05$ compared with group 1; *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the initial activity.

The results were analyzed using the Student's and Fischer's tests.

RESULTS

In the first series, chronic administration of morphine to mice induced the development of drug dependence. The mean daily consumption of morphine increased from 13.7±1.0 to 26.4±2.6 mg/kg. All the mice developed tolerance to morphine: the analgetic effect of morphine decreased 3.5-fold in comparison with the baseline value (from 104.3±5.9 to 29.5± 12.0%, $p<0.001$).

Twenty-four and 48 h after discontinuation of morphine, the open field motor activity of untreated mice dropped dramatically (Fig. 1), which is a typical manifestation of the withdrawal syndrome in mice [11]. Antidopamine antibodies elicited an appreciable protective effect manifesting itself as an increase in the motor activity 24 and 48 h after morphine discontinuation. Antiserotonin antibodies also markedly increased the motor activity, the effect being more pronounced 48 h after the drug discontinuation.

In control mice (groups 1 and 2), the pain sensitivity increased 24 and 48 h after morphine discontinuation, and the latent period of the response to thermal stimulation decreased 2-fold, indicating the development of the withdrawal hyperalgesia [4]. The pain sensitivity did not increase in mice treated with the antibodies. The antibodies produced a pronounced protective effect, manifesting itself as the lowering of withdrawal hyperalgesia particularly 48 h after morphine discontinuation.

In the second series, chronic morphinization also led to the development of drug dependence, as evidenced by a decrease in the analgetic effect of the test-dose of morphine from 133.1±7.0 to -6.8±5.8% ($p<0.001$). Blockade of opiate receptors by naloxone induced pronounced symptoms of the withdrawal syndrome: substantial decrease in vertical and horizontal motor activities, lowering of exploring activity (Fig. 2), and emergence of some specific manifestations such as shaking, grooming, tremor of head and body, and ptosis (Fig. 3).

A single intraperitoneal administration of antibodies to 5-HT markedly increased horizontal and vertical motor activities compared with the control and decreased the number and frequency of specific manifestations of withdrawal syndrome, particularly of shakings, which are known to be associated with the 5-HT mechanisms [4]. Antibodies to DA stimulated horizontal and vertical motor activities, inhibited (to a smaller extent compared with antibodies to 5-HT) the number and frequency of shakings and

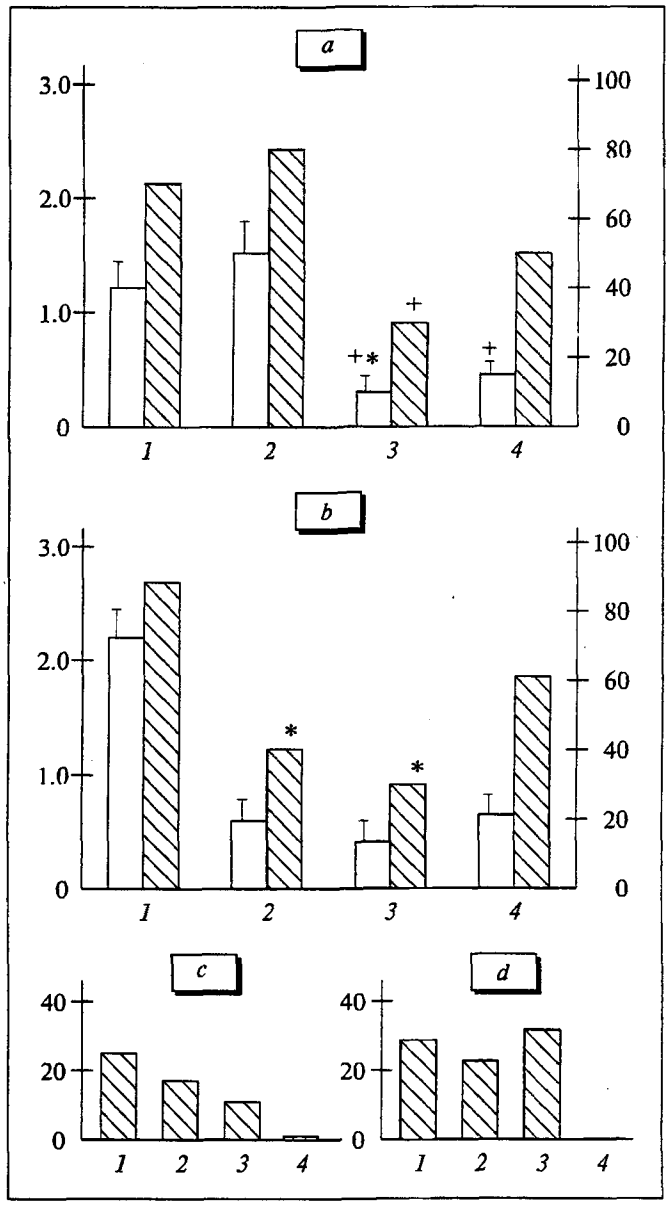

Fig. 3. Effect of passive immunization of mice with antibodies to 5-HT and DA on specific manifestations of the naloxone-induced withdrawal syndrome. *a*) shaking; *b*) grooming; *c*) tremor of the head and body; *d*) ptosis. Ordinates (*a*, *b*): left, parameter (absolute units); right, frequency (%). White bars: parameter; shaded bars: frequency. Ordinates (*c*, *d*): frequency (%); *1*) control (normal saline); *2*) nonimmune rabbit γ-globulin; *3*) antibodies to 5-HT; *4*) antibodies to DA. $p<0.05$: *compared with the control; +compared with γ-globulin.

grooming, and prevented the development of tremor and ptosis (Fig. 3).

After administration of naloxone, in control animals (group 1) the latency period increased significantly by 47.4±0.6% ($p<0.01$), indicating an increase in the pain sensitivity. Antibodies to 5-HT elicited a protective effect, reducing the withdrawal hyperalgesia in the hot plate test: the latency decreased by 33.8±2.5% ($p<0.05$) compared with the control. Antibodies to DA had no effect on the

naloxone-induced withdrawal syndrome: hyperalgesia was preserved in all mice.

The "natural" withdrawal syndrome was abolished by antiserotonin and antidopamine antibodies (Fig. 1), while the naloxone-induced syndrome was partially suppressed by nonimmune rabbit γ-globulin (Figs. 2 and 3). However, the specific manifestations of the syndrome (shaking, tremor, and ptosis) were abolished only by antibodies to the neurotransmitters.

It should be emphasized that the morphine withdrawal syndrome is a combination of various symptoms (hyperalgesia, aggressiveness, tremor, shakings, etc.) associated with various neuroregulatory processes in the CNS [4]. Other neurotransmitters may be involved in this syndrome together with the major catecholaminergic mechanisms [1]. For example, it has been shown that precursors of 5-HT and DA potentiate, while inhibitors of neurotransmitter synthesis and the correspondent receptors blockers attenuate, the major manifestations of the morphine withdrawal syndrome [4]. This accounts for the unilateral effect of antibodies to 5-HT and DA. Presumably, antibodies to the neurotransmitters affect the functional state of the corresponding receptors, which was confirmed experimentally [10]. In addition, by changing the concentrations of neurotransmitters in the circulation these antibodies may modify the functional state of the immune system cells that produce lymphokines, which are known to act as modulators of the CNS. For example, the cytokines α-interferon and interleukin alleviate the morphine withdrawal syndrome [11]. Meanwhile, these results and the evidence that antibodies to neurotransmitters decrease the tolerance to morphine [5] indicate that antibodies to 5-HT normalize nociceptive receptors both during the development of morphine dependence and the withdrawal hyperalgesia, elevating the pain sensitivity threshold in both cases.

Thus, our findings show a possibility of reducing and preventing morphine dependence and major manifestations of the withdrawal syndrome by administration of antibodies to 5-HT and DA, the latter being less effective.

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REFERENCES

1. I. P. Anokhina, N. B. Gamaleya, T. P. Nebarokova, *Byull. Eksp. Biol Med.*, **104**, No. 10, 460-461 (1987).
2. L. A. Basharova, V. A. Evseev, L. A. Vetrile, *et al.*, *Ibid.*, **115**, No. 5, 469-471 (1993).
3. S. Yu. Buslovich, A. I. Kotelenets, and O. M. Fridlyand, *Zh. Vyssh. Nervn. Deyat.*, No. 1, 168-171 (1981).
4. A. V. Val'dman, E. A. Babayan, and E. E. Zvartau, *Psychopharmacological, Medical, and Legal Aspects of Drug Addiction* [in Russian], Moscow (1988).
5. V. A. Evseev, L. A. Basharova, L. A. Vetrile, *et al.*, *Vopr. Narkol.*, No. 1, 33-39 (1995).
6. V. A. Evseev, T. V. Davydova, V. G. Fomina, *et al.*, *Byull. Eksp. Biol. Med.*, **116**, No. 12, 582-584 (1993).
7. V. A. Evseev, N. A. Trekova, L. A. Basharova, *et al.*, *Ibid.*, **119**, No. 5, 526-529 (1995).
8. S. E. Metkalova, *Effect of Immunization with Protein-Morphine Conjugate on Morphine Dependence. Abstr. PhD Thesis* [in Russian], Moscow (1983).
9. N. A. Patkina and E. E. Zvartau, *Farmakol. Toksikol.*, No. 1, 19-23 (1980).
10. N. Bobkova, L. Plakhinas, L. Basharova, *et al.*, in: *Studies in Neuroscience. Signal Molecules and Behavior*, Manchester (1991), pp. 205-212.
11. Dafny N., Wagel V., and D. Droth, *Life Sci.*, **3**, 1721-1726 (1985).