Psychoimmunomodulatory Effect of Phenotropil in Animals with Immune Stress

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We studied the effect of phenotropil (25 mg/kg intraperitoneally, 5 days) on the immune and psychoemotional state of Wistar rats with LPS-induced immune stress. Hyperactivity of the immune system in animals after treatment with *Pseudomonas aeruginosa* LPS (100 μg/kg intraperitoneally, 3 days) manifested in a significant increase in the delayed-type hypersensitivity index, antibody titer in the reaction of passive hemagglutination, and phagocytic activity of peripheral blood neutrophils. Locomotor, orientation, and exploratory activities were reduced, while anxiety increased in animals with immune stress. Phenotropil exhibited the psychoimmunomodulatory effect under these conditions, which manifested in prevention of anxiety and fear response, increase in horizontal locomotion and exploratory behavior, and improvement of immunoreactivity.

Key Words: *phenotropil; lipopolysaccharide; immune stress; psychoimmunomodulation*

The search for new drugs modulating immunological reactivity of the body is an urgent problem of modern immunopharmacology. Much recent attention was paid to studying the role of neuroactive amino acids in immune processes [4,12]. Published data show that GABA and its pharmacological analogues affect various parameters of the immune system [2,5]. For example, administration of GABA contributes to an increase in the number of antibody-producing cells and serum agglutinin titer [1]. Our experiments on the model of immune deficiency showed that GABAergic agents (phenotropil, phenibut, baclofen, mephebut, *etc*.) exhibit immunomodulatory properties [6-9].

The necessity of immunocorrection with neurotransmitter derivatives is confirmed by the existence of cause-effect relationships between CNS dysfunction and immune imbalance. This state is often manifested in hyperactivity or hypoactivity of various components of immunogenesis [12].

Therefore, studying the immunotropic and psychotropic effects of phenotropil (one of the new medicines that belong to a pharmacological group of GABAergic drug) under conditions of LPS-induced immune stress is an urgent problem.

MATERIALS AND METHODS

Experiments were performed on male and female Wistar rats (n=90) weighing 250-300 g. The animals were maintained according to the rules of the International Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg, 1986). The rats were divided into the following three groups (n=10): control I, equivalent volume of physiological saline; control II, experimental immune stress; and treatment group, phenotropil in experimental immune stress. Phenotropil in a dose of 25 mg/kg was injected intraperitoneally for 5 days. Immune stress was induced by intraperitoneal injec-

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tions of *Pseudomonas aeruginosa* LPS in a dose of 100 μg/kg for 3 days.

The study was conducted in 3 series. Functional activity of the immune system and immunomodulatory effect of phenotropil were evaluated from the cellular delayed-type hypersensitivity reaction (DTHR, series I), humoral reaction of passive hemagglutination (RPHA, series II), and latex test for phagocytic activity of peripheral blood neutrophils (series III) [10]. Behavioral reactions of animals were studied in the open-field test and elevated plus-maze test [3].

The animals were immunized by a subcutaneous injection of sheep erythrocytes in a single dose of 2×10^8 cells (100 μl) to study DTHR. The challenge dose of sheep erythrocytes (10^8 cells in 20 μl) was administered subaponeurotically into the hindlimb ("treated" limb) on day 5 after sensitization. Physiological saline was injected into the contralateral limb ("control" limb). The local response was evaluated after 24 h. Index of DTHR was calculated as follows: $RI=(W_T-W_C)/W_C\times100\%$, where W_T is the weight of the "treated" limb and W_C is the weight of the "control" limb.

The animals were intraperitoneally immunized with sheep erythrocytes in a single dose of 10^8 cells (100 μl) to study RPHA. Antibody production was evaluated on day 7 after immunization. The titer of antibodies was expressed in geometrical mean values.

Phagocytic activity of peripheral blood neutrophils was studied with Melan-formaldehyde latex particles (1.5-2 μ, MinMedBioprom). Phagocytic activity of neutrophils was evaluated from the phagocytic index (PI, % of phagocytosis; number of latex-containing neutrophils per 100 cells) and phagocytic number (PN, number of latex particles per 100 cells).

The experiment was performed according to the international ethical and scientific standards for the planning and performance of animal studies. The results were analyzed by Student's *t* test (Statgraph).

RESULTS

The studied parameters of immunogenesis in animals with LPS-induced immune stress (control II) were higher than in saline-treated specimens (control I; Fig. 1).

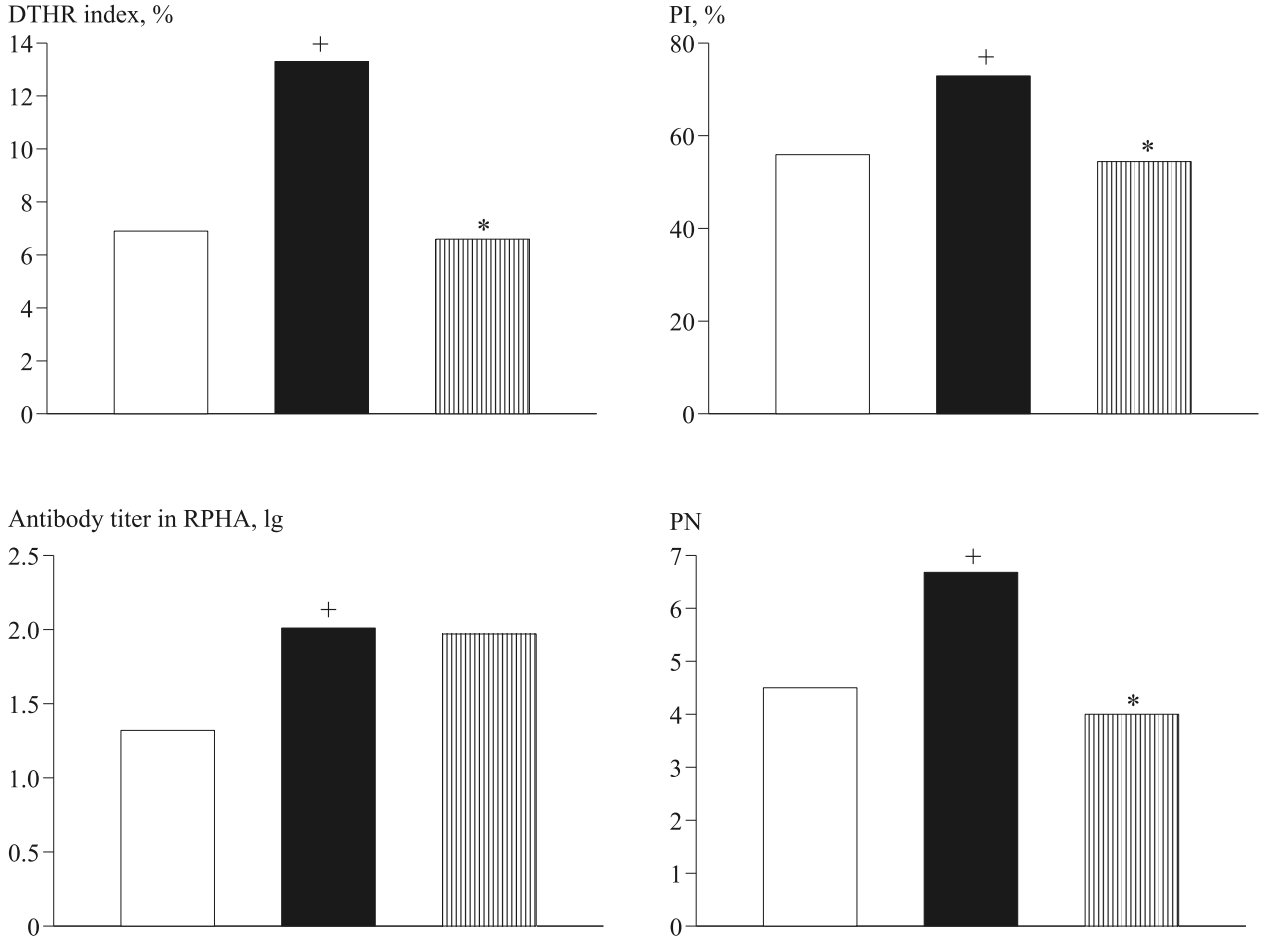

Fig 1. Effect of phenotropil on DTHR, RPHA, and phagocytic activity of peripheral blood neutrophils under conditions of LPS-induced immune stress. Here and in Fig. 2: light bars, control I (physiological saline); dark bars, control II (LPS, 100 μg/kg); shaded bars, phenotropil (25 mg/kg) and LPS (100 μg/kg). $p<0.05$: +compared to control I; *compared to control II.

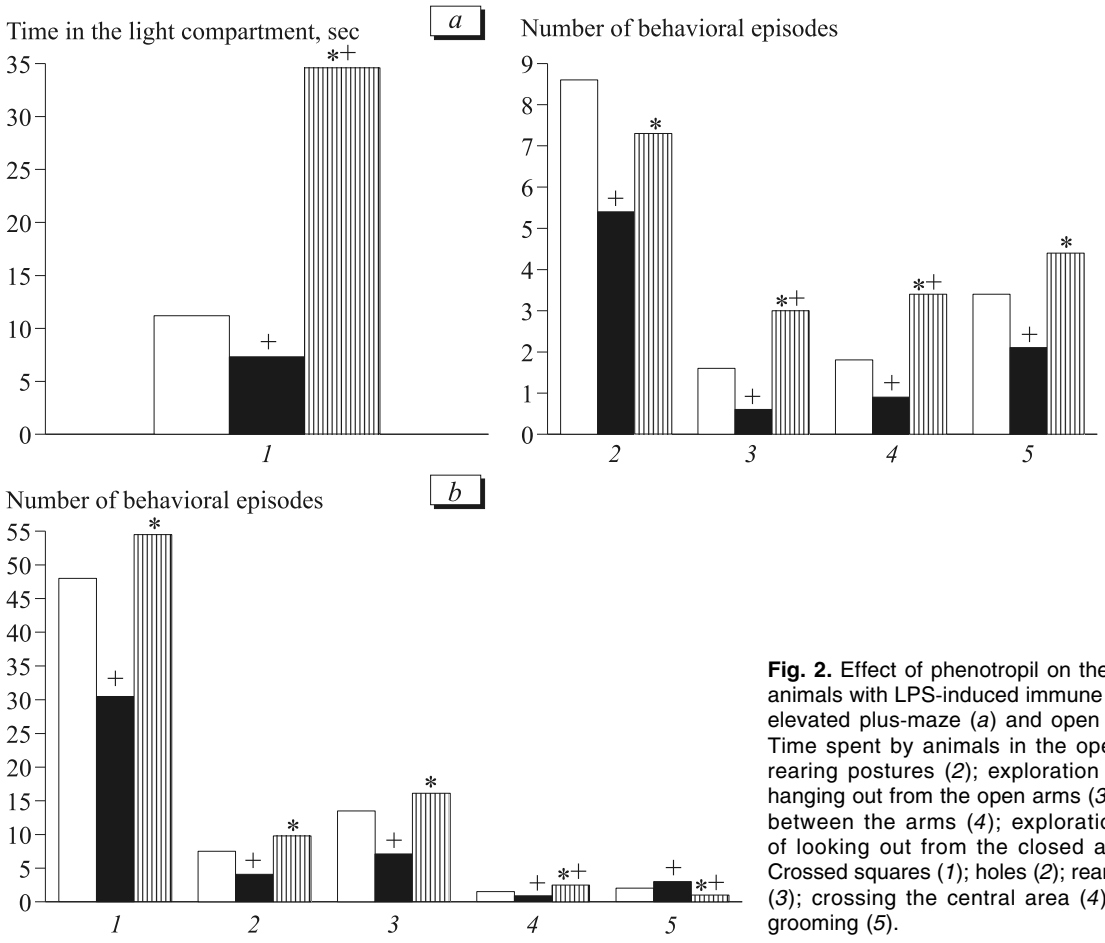

Fig. 2. Effect of phenotropil on the behavior of animals with LPS-induced immune stress in the elevated plus-maze (*a*) and open field (*b*). (*a*) Time spent by animals in the open arms (*1*); rearing postures (*2*); exploration episodes of hanging out from the open arms (*3*); transitions between the arms (*4*); exploration episodes of looking out from the closed arms (*5*). (*b*) Crossed squares (*1*); holes (*2*); rearing postures (*3*); crossing the central area (*4*); short-term grooming (*5*).

Administration of phenotropil to animals of the treatment group was followed by restoration of cellular immunity and nonspecific resistance. The index of DTHR and parameters of phagocytosis (PI and PN) were shown to decrease and did not differ from normal (control I, $p<0.05$). Anti-erythrocyte antibody titer (RPHA) in animals of the control group II was 1.5-fold higher than in rats of the control group I. This parameter remained high after treatment with phenotropil. Our results indicate that phenotropil has no effect on antibody production during immune stress (Fig. 1).

Analysis of the behavior of LPS-treated animals in the open-field test and elevated plus-maze test revealed reduced locomotor and exploratory activities and changes in ethological indexes for anxiety (Fig. 2). Phenotropil prevented the development of anxiety, fear, and sluggishness in animals with immune stress (control II). The slight psychostimulant effect of phenotropil was manifested in the increase of horizontal locomotion, vertical rearing postures, crossing the central area of the open field, and transition between the arms of the maze. Moreover, this drug increased the exploratory behavior of animals (hole exploration in the open filed, hanging out from the open arms, and exploration episodes of looking out from the closed arms in the maze).

We conclude that phenotropil produces a strong psychoimmunomodulatory effect in animals with immune stress after treatment with *Pseudomonas aeruginosa* LPS.

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