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Ambiguity of the Effect of High and Low Doses of Amino-Acid Preparations on the Immune Response and Phagocytosis in Mice

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It is shown that the effect of amino-acid preparations (levamine-70-70, cerebrolysin, and aviamine) is dose-dependent. Thus, levamine-70 and cerebrolysin at 65 mg/kg do not affect the immune response but stimulate phagocytosis. Aviamine at 65 mg/kg inhibits the immune response but stimulates phagocytosis and in a dose of 6.5×10^{-2} mg/kg boosts both processes.

Key Words: *levamine-70; cerebrolysin; aviamine; immune response; phagocytosis*

The amino-acid preparations levamine-70 and cerebrolysin stimulate the thymus-dependent immune response when administered subcutaneously in mice in a range of low doses (6.5×10^{-2}-6.5×10^{-8} mg/kg) during 5 days [1]. The effect of these preparations on phagocytosis, a nonspecific index of resistance, has not been studied.

Under clinical conditions levamine-70 and cerebrolysin are usually used in significantly higher doses, 0.65 mg/kg and 12.5 mg/kg, respectively.

The aim of the present investigation was a comparative assessment of the effects of high and low doses of amino-acid preparations used in medical (levamine-70 and cerebrolysin) and veterinary (aviamine) practice on the thymus-dependent response and phagocytic activity of neutrophils (PAN) in mice.

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MATERIALS AND METHODS

Experiments were carried out on 285 male CBA mice weighing 14-16 g. Levamine-70 (Leiras, Finland), a mixture of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine, arginine, alanine, proline, and glycine; cerebrolysin (Ebeve, Austria), a hydrolysate of brain tissue containing 18 amino acids, and aviamine (Drug Plant, St. Petersburg), a hydrolysate of hen blood casein containing 18 amino acids, were used.

Cerebrolysin and aviamine were dosed by the protein content, which constituted 1 g/ml and 0.032 g/ml, respectively, whereas levamine-70 was dosed by the total content of amino acids (0.053 g /ml) and converted to kg of mass.

The preparations dissolved in apyrogenic saline (Polfa, Poland) were injected s.c. in a range of doses 65.0-6.5×10-[10] mg/kg during 5 days. Then some of the treated mice were immunized with

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TABLE 1. Effect of Amino-Acid Preparations on the Immune Response ($M \pm m$)

Preparation	Control	Number of IgM-APC per 10^6 karyocytes of mouse spleen after application of preparations in doses, mg/kg/day					
		65.0	6.5×10^{-2}	6.5×10^{-4}	6.5×10^{-6}	6.5×10^{-8}	6.5×10^{-10}
Levamine-70	11.0±1.6 (20)	8.4±2.2 (8)	24.5±3.5* (20)	21.2±3.4** (10)	22.6±1.2* (10)	25.1±1.7* (10)	12.0±1.4 (10)
Cerebrolysin	11.0±1.6 (20)	18.4±6.1 (8)	34.5±3.8* (10)	24.1±2.8* (10)	18.0±2.8* (10)	13.0±2.0 (10)	–
Aviamine	11.1±1.0 (8)	6.5±1.5** (8)	19.0±2.3** (8)	35.9±8.7** (8)	14.6±2.2 (8)	11.4±3.2 (8)	12.5±2.6 (8)

Note. Here and in Table 2 asterisks mean a reliable difference as compared to the control (the administration of apyrogenic physiological saline): *$p<0.01$; **$p<0.05$. The number of animals is in parentheses; a dash means the test was not performed.

sheep erythrocytes (2×10^6 cells once intravenously), and on the 4th day after immunization the number of IgM-antibody-producing cells (APC) was determined in the spleen of each mouse according to a method described elsewhere [5].

The number of APC was converted to 10^6 spleen karyocytes.

For PAN assessment exudates were obtained from mice treated with the preparations 2.5 h after i.p. administration of 10% sterile peptone solution. The final concentration of cell exudate was 12.5 mln/ml and consisted 98% of neutrophils. A 24-h culture of *St. aureus* with a final concentration of 250 mln/ml served as a test microbe. The indexes determined were as follows: the phagocytic index, evaluated as the percentage of phagocytizing neutrophils, and the phagocytic number, estimated as the arithmetic mean of microbial cells in one leukocyte [2]. A minimum of 900-1000 neutrophils were counted.

RESULTS

Levamine-70 boosts the production of APC as compared with the control in the range of doses 6.5×10^{-2}-6.5×10^{-8} mg/kg (Table 1). A tenfold increase of the levamine-70 dose (to 0.65 mg/kg) results in an increase of the number of APC (18.4±2.7 versus 11±1.6 in the control, $p<0.01$, $n=12$), which is reliably ($p<0.05$) lower than for levamine-70 injection at 6.5×10^{-8} mg/kg. At 65 mg/kg the preparation is inactive. Cerebrolysin is active in a lesser range of doses: 6.5×10^{-2}-6.5×10^{-6} mg/kg. When the dose is increased to 65 mg/kg, the preparation loses the ability to stimulate the immune response. Aviamine boosts APC production only in the dose range of 6.5×10^{-2}-6.5×10^{-4} mg/kg, whereas in a dose of 65 mg/kg it significantly ($p<0.05$) depresses the production of APC.

The effect of the amino-acid preparations levamine-70, cerebrolysin, and aviamine on PAN differs from their influence on the immune response. Thus, the administration of levamine-70 and cerebrolysin in a dose of 6.5×10^{-2} mg/kg markedly boosts the immune response while not affecting the phagocytosis of staphylococcus by neutrophils. However, a pronounced stimulation of the phagocytic process is observed at a 1000-fold increase of the dose, to 65 mg/kg, at which levamine-70 and cerebrolysin do not affect the immune response. Aviamine is active at 65 mg/kg and 6.5×10^{-2} mg/kg as a stimulator of phagocytosis, i.e., unlike levamine-70 and cerebrolysin, aviamine boosts PAN in a low dose (6.5×10^{-2} mg/kg) (Table 2).

Under the influence of the test doses of levamine-70 and aviamine the phagocytic number does not vary and ranges from 2±0.07 to 2.3±0.03 vs. 2.2±0.07 in the control. For a high dose of cerebrolysin (65 mg/kg) the phagocytic number rises from 2.2±0.07 in the control to 2.8±0.12 ($p<0.01$).

The data presented show that, depending on the dose, the amino-acid preparations variously affect the immune response and phagocytic process. Levamine-70 tested in mice in a dose of 0.65 mg/kg, which has a trophic effect in clinical practice, boosts the immune response reliably less than in a significantly lower dose (6.5×10^{-8} mg/kg). A hundredfold dose of levamine-70 (65 mg/kg) loses the ability to stimu-

TABLE 2. Effect of Amino-Acid Preparations on PAN ($M \pm m$)

Preparation	Control	Phagocytic index, % after application of preparations in doses, mg/kg/day			
		65.0	6.5×10^{-2}	6.5×10^{-4}	6.5×10^{-6}
Levamine-70	17.5±0.7 (6)	24.0±0.4* (5)	18.2±3.4 (6)	19.6±0.8 (6)	20.6±1.7 (5)
Cerebrolysin	17.5±0.7 (6)	25.0±0.6* (6)	18.0±1.5 (5)	20.3±1.4 (5)	20.2±1.9 (6)
Aviamine	17.5±0.7 (6)	24.6±0.4* (5)	27.9±0.9* (5)	14.8±1.0 (4)	18.3±0.2 (5)

late the immune response but acquires a phagocytosis-stimulating property which is not exhibited by low doses (6.5×10^{-2}-6.5×10^{-4} mg/kg). A similar characteristic is inherent in cerebrolysin as well. Aviamine tested in a dose of 65 mg/kg (used in poultry raising and producing a trophic effect) [3] inhibits the immune response but, like levamine-70 and cerebrolysin, boosts PAN. A decrease of the dose to 6.5×10^{-4} mg/kg results simultaneously in a loss of the phagocytosis-stimulatory effect and a boost of the immune-stimulatory action.

Thus, the abolition of the immune-stimulatory property for the use of a high (trophic) dose attests to a reverse dependence of the immune-stimulatory and trophic functions. The effect of high doses of preparations on phagocytes, unlike their influence on lymphocytes, probably stems from phylogenetically determined trophic properties of neutrophils.

The differences in the action of various doses of amino-acid preparations as well as in the effects of certain amino acids and peptides [4] on the immune response and phagocytosis attest to the relative autonomy of the specific and of nonspecific components of defense.

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Immunological Responses during the Organism's Adaptation to a Dosed Thermal Factor

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Overheating of mice over the course of 10 and 20 days suppresses the proliferative activity of splenic cells in response to stimulation with phytohemagglutinin, concanavalin A, lipopolysaccharide, pokeweed mitogen, and alloantigens. The number of antibody-producing cells in the spleen drops on day 5 of overheating and is still low on days 10-20. Forty days after the start of overheating the functional activity of lymphocytes is restored. Overheating of animals does not change the colony-forming activity of hemopoietic stem cells.

Key Words: *overheating; lymphocyte; immunity; adaptation; immune system*

A high temperature of the external environment is an unfavorable physical factor frequently acting on the human organism under natural conditions and in a specific industrial setting. It has been shown that exposure of the organism to exogenous hyperthermia leads to disturbances in the state of different organs and systems [2,4]. This provides an incentive to study the functional systems of the organism during hyperthermia and adaptation to heat and to seek ways of raising the organism's resistance under given conditions. Published data on the functions of the immune system during adaptation of the organism to thermal factors are limited in many respects.

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