LEVAMIN AND CEREBROLYSIN AS IMMUNOSTIMULANTS

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In previous investigations [2-4] the writers showed that 9 of the 20 amino acids present in the composition of proteins (Asp, Asn, Glu, Cys, Thr, Trp, Ala, Val, and Ser) can induce Thy-1-antigen on bone marrow cells and can stimulate the thymus-dependent immune response in mice in the same way as the well-known thymus peptide – thymopeptin [2-4]. Individual immunoactive amino acids (Asp, Val) in the composition of thymopeptin also potentiated the immune response. However, the mixture of amino acids composing thymopeptin did not exhibit such activity [3]. The question arises whether only amino acids tested individually possess immunomodulating activity, or whether mixtures of individual amino acids also are active. To solve this problem, we tested the pharmacopeial preparations levamin and cerebrolysin, which are mixtures of individual immunoactive and inactive amino acids.

EXPERIMENTAL METHOD

Experiments were carried out on 270 male CBA mice weighing 14-16 g. Levamin 70 (from "Leiras," Finland) is a mixture of 12 amino acids (Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, His, Arg, Ala, Pro), whereas cerebrolysin (from "Ebave," Yugoslavia) is a brain tissue hydrolysate consisting of 18 amino acids. These substances were injected subcutaneously or given perorally by tube to the animals daily in different doses for 5 days, made up in pyrogen-free physiological saline (from "Polfa," Poland). To compare the immunomodulating action of levamin and cerebrolysin with the effect of the peptides, we tested thymopentin, a peptide from the thymus, synthesized by the method of classical synthesis in solution (Leningrad University). Thymopentin was administered by the scheme described below. Control animals received pyrogen-free physiological saline. The mice were then immunized intravenously with sheep's red blood cells (SRBC: $2 \cdot 10^6$ cells) or with Vi-antigen (0.001 μg per mouse). On the 4th day after immunization the number of IgM-antibody-forming cells (AFC) in the spleen of each mouse was determined by the method in [7]. To detect IgM-AFC to Vi-antigen, the latter in a concentration of 20 μg/ml was loaded on SRBC. To remove unbound Vi-antigen, the SRBC were washed no fewer than 8 times with physiological saline. The number of AFC was calculated per 10^6 splenic karyocytes.

Expression of Thy-1-antigen on bone marrow precursor T cells under the influence of the test preparations was assessed by a modified method in [5] after treatment of bone marrow cells with the preparations in vitro at 37°C for 1.5 h. The number of Thy-1^+ cells in the bone marrow cell population was determined by the complement-dependent cytoxicity test [1] using rabbit antiserum against cerebral cortical tissue of CBA mice, absorbed with mouse liver homogenate and mouse and sheep red blood cells [1]. After absorption from serum by reprecipitation with CO_2 the antigen—antibody complexes were removed. The antiserum was used in dilution of 1:50. In this concentration and in the presence of complement, in the form of fresh guinea pig serum (1:3) the antiserum caused death of 88.0 ± 1.3% of thymocytes and did not interact with CBA mouse bone marrow cells. No fewer than 200 cells, whose viability was tested with a 0.2% aqueous solution of trypan blue were counted. The experiment was repeated at least 2-3 times.

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TABLE 1. Effect of Levarnin, Cerebrolysin, and Thymopentin on Immune Response to SRBC (M ± m)

Preparation	Dose of preparation, μg/mouse/day					Administration of pyrogen-free physiological saline (control)
	1	$1 \cdot 10^{-2}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-8}$	
Levamin	24,5±3,5* (20)	21,2±3,4** (10)	22,6±1,2* (10)	25,1±1,7* (10)	12,1±1,4 (10)	11,0±1,6 (30)
Cerebrolysin	34,5±3,8* (10)	24,1±2,8* (10)	18,0±2,8** (10)	13,0±2,0 (10)	—	
Thymopentin	20,5±2,4* (11)	20,7±3,3* (8)	6,9±1,2 (12)	—	—	8,3±0,6 (20)

Legend. *$p < 0.01$, **$p < 0.05$: significant difference compared with corresponding parameter in control, —) no experiment carried out. Number of animals given in parentheses.

TABLE 2. Effect of Levamin, Cerebrolysin, and Thymopentin on Immune Response to Vi-Antigen (M ± m)

Preparation	Number of IgM-ACF per 10^6 splenic karyocytes
Levamin	8,9±0,7
Cerebrolysin	8,3±0,9
Thymopentin	9,1±0,8
Pyrogen-free physiological saline (control)	9,5±0,5

Legend. Preparations tested in concentration of $1 \cdot 10^{-2}$ μg/mouse/day. 10 animals studied in each group.

EXPERIMENTAL RESULTS

It follows from Table 1 that levamin, when administered to the animals within a dose range of $1\text{-}1 \cdot 10^{-6}$ μg/mouse, potentiated IgM-AFC production to SRBC by 2-2.2 times compared with the control, whereas cerebrolysin in the range $1\text{-}1 \cdot 10^{-4}$ μg/mouse increased their production by 1.6-3.1 times. Thymopentin exhibited activity only within the dose range of $1\text{-}1 \cdot 10^{-2}$ μg per animal, when it stimulated AFC production by 2.4-2.5 times. In a dose of $1 \cdot 10^{-4}$ μg thymopentin was inactive.

All the preparations tested had no effect on the level of the immune response to thymus-independent Vi-antigen (Table 2).

Administration of levamin or cerebrolysin ($1 \cdot 10^{-2}$ μg per animal) by tube for 5 days, just like injection of the preparation in the same dose, significantly ($p < 0.05$) stimulated the immune response to SRBC: the number of IgM-AFC was 13.3 ± 1.7 and 10.9 ± 1.4 respectively compared with 7.1 ± 1.2 in the control (20 mice were tested in each experimental group, 10 animals in the control group).

Treatment of bone marrow cells in vitro with levamin or cerebrolysin within the concentration range of $1\text{-}1 \cdot 10^{-6}$ μg/ml led to the appearance of Thy-1-antigen on their membranes. The increase in the number of Thy-1^+ cells varied from 16.6 ± 1.8 to 20.7 ± 2.8% compared with 0% in the control (cells in Hanks' solution).

The results show that not only separate amino acids [2-4], but also mixtures of them, like the thymus peptides, can induce Thy-1-antigen on precursor T-cells and can correspondingly potentiate the thymus-dependent immune response in the absence of any effect on the thymus-independent response. Potentiation by a mixture of amino acids (levamin and cerebrolysin), just as by thymopentin, of the thymus-dependent but not the thymus-independent immune response is evidence that their effect, like those of most thymus peptides, are connected with T-cell, but not B-cell function. The difference and the advantage of the amino-acid mixture, and in particular, the mixture of levamin and cerebrolysin which we tested, compared with thymus peptides is their ability, unlike the peptides in [6], to potentiate the immune response not

only to parenteral, but also to the peroral method of administration. This fact, and also the wider range of effective doses of levamin and cerebrolysin than of the thymus preparation thymopentin, makes their use for the correction of immunodeficiency states promising.

LITERATURE CITED

1. G. A. Belokrylov, Zh. Mikrobiol., No. 4, 23 (1976).
2. G. A. Belokrylov, Byull. Éksp. Biol. Med., No. 8, 213 (1986).
3. G. A. Belokrylov and I. V. Molchanova, Immunologiya, No. 3, 61 (1988).
4. G. A. Belokrylov, I. V. Molchanova, and E. I. Sorochinskaya, Dokl. Akad. Nauk SSSR, **286**, No. 2, 471 (1986).
5. R. S. Basch and G. Goldstein, Proc. Nat. Acad. Sci. USA, **71**, 1474 (1974).
6. P. Cazzola, P. Mazzanti, and N. M. Kouttab, Immunopharmacol. Immunotoxicol., **9**, 195 (1987).
7. N. K. Jerne and A. A. Nordin, Science, **140**, 405 (1963).

NEUROTRANSMITTER PROVISION FOR ORGANS OF THE IMMUNE SYSTEM DURING BENZPYRENE POISIONING

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The writers previously demonstrated the effect of benzpyrene, a toxic compound with low molecular weight, on immune homeostasis in the uterus–placenta–fetus system [1]. However, the fine mechanisms of the changes in the immune system during benzpyrene poisoning have not yet been explained. Since one important factor in the endogenous regulation of the immune function of the body is the monoamines of lymphoid tissue [3, 5, 6], it was decided to study the mediator background of the adrenergic nervous component of the immune organs in response to antenatal administration of benzpyrene.

EXPERIMENTAL METHOD

Female rats at the 10th-11th-12th day of pregnancy received an intraperitoneal injection of benzpyrene in a dose of 20 mg/kg body weight daily in a total dose of 60 mg/kg body weight. Another group of pregnant females, which received olive oil at the same times intraperitoneally, as the solvent of benzpyrene, served as the control. The mature offspring from the control and experimental (exposed to benzpyrene during pregnancy) females were divided into four groups. Groups 1 and 2 came from rats receiving olive oil during pregnancy, i.e., the offspring of the control group of females. Groups 3 and 4 came from rats receiving benzpyrene during pregnancy, i.e., the offspring of the experimental groups of females. Animals of groups 2 and 4 at the age of maturity received benzpyrene intraperitoneally in a dose of 30 mg/kg body weight for 2 days (i.e., they received a total dose of benzpyrene of 60 mg/kg body weight). Animals of groups 1 and 3 received olive oil as the control. The results of exposure to benzpyrene ex utero and in utero were read in the period of puberty, i.e., in the course

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