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EFFECT OF THYMALIN ON THE CYCLIC NUCLEOTIDE SYSTEM IN THE MOUSE SPLEEN

I. S. Gavrilenko, V. Kh. Khavinson, V. G. Morozov, and Yu. Yu. Ivnitskii

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The ability of humoral factors of the thymus to activate the immunoreactive systems of the body has been demonstrated recently [1, 4, 7, 8]. However, insufficient attention has been paid to the study of the biochemical basis of the mechanism of the biological activity of these substances. Parameters reflecting the state of the cyclic nucleotide system in the tissues following administration of thymus preparations *in vivo* are very interesting in this respect. Considering the universality of functions of the cyclic nucleotides as intracellular mediators, it is natural to expect that definite changes in their concentration will be found under these circumstances.

The object of this investigation was to study the time course of changes in components of the cyclic nucleotide system in the mouse spleen under the influence of the thymus preparation thymalin [5].

EXPERIMENTAL METHOD

Noninbred albino mice weighing 18-20 g were used. Animals of the experimental group were given thymalin in physiological saline by intraperitoneal injection in a dose of 50 μg/g body weight. Choice of this dose was based on the results of experiments in which its administration led to a marked rise of antibody titers in mice [1]. Animals of the control group received physiological saline. Intact animals constituted a separate group.

There were two series of experiments. In series I there were 115 mice, divided into the three groups mentioned above. The animals were decapitated 1, 3, and 10 days after injection of thymalin. The spleen was quickly removed and weighed and the concentrations of cyclic AMP (cAMP) and GMP (cGMP) in it was determined by means of a cyclic AMP assay kit and cyclic GMP RIA kit (Amersham, England) respectively. The radioactivity of the samples was determined in a Mark II liquid scintillation counter (Nuclear Chicago, USA).

The experiments of series II were carried out on 99 mice, killed 1, 3, and 10 days after injection of thymalin. Activity of adenylate cyclase (AC, [6]) and cyclic AMP phosphodiesterase (PDE, [2]) was determined in the splenic tissue. The results were subjected to statistical analysis by Student's and Wilcoxon–Mann–Whitney tests.

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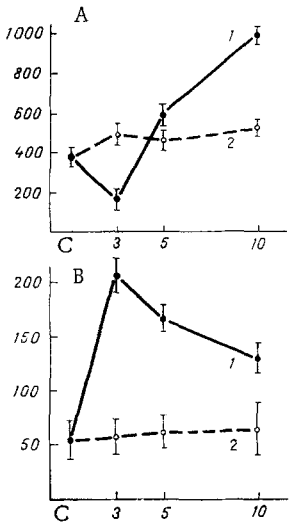

Fig. 1

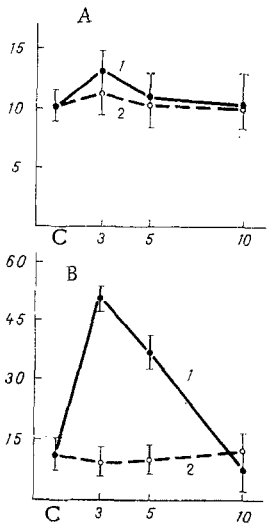

Fig. 2

Fig. 1. Time course of changes in cAMP (A) and cGMP (B) concentrations in mouse spleen after injection of thymalin. Abscissa, time (in days) after injection; **ordinate, concentration** (in pmoles/g tissue). 1) Mice receiving thymalin; 2) changes in control tests. C) Control (intact mice).

Fig. 2. Time course of changes in AC (A) and PDE (B) activity in mouse spleen after injection of thymalin. Ordinate, AC activity (in pmoles cAMP/mg protein/min) and PDE activity (in nmoles cAMP/mg protein/min). Remainder of **legend** as to Fig. 1.

EXPERIMENTAL RESULTS

After injection of thymalin marked changes in the levels of cyclic nucleotides and AC and PDE activity were found in the splenic tissue of the mice (Figs. 1 and 2).

The cAMP concentration in the mouse spleen 3 days after injection of thymalin was significantly lower ($P < 0.05$) than in intact animals (150 ± 29 compared with 391 ± 28 pmoles/g tissue). Later, under the influence of thymalin there was a significant ($P < 0.05$) rise in the cAMP level to 605 ± 27 and 986 ± 30 pmoles/g on the 5th and 10th days, respectively. In the control group a tendency was noted for the cAMP level to rise ($P > 0.5$) at these times of the investigation. The time course of the cGMP level in the splenic tissue of mice of the experimental group was opposite in character to that of cAMP. The greatest increase in the cGMP concentration was observed 3 days after injection of thymalin (210 ± 17 compared with 61 ± 18 pmoles/g in the control, $P < 0.01$). Later the cGMP level fell, but remained higher than in the intact animals (167 ± 13 and 136 ± 16 pmoles/g on the 5th and 10th days of the experiment, respectively). Values for the cGMP concentration in the spleen of animals of the control group (61 ± 18, 65 ± 17, and 65 ± 20 pmoles/g on the 3rd, 5th, and 10th days of the experiment, respectively) differed significantly from those in intact animals (55 ± 16 pmoles/g).

Determination of activity of the enzymes for cAMP synthesis and hydrolysis revealed a definite time course for PDE but no significant changes in AC activity at the same times. PDE activity rose to the highest level on the 3rd day (50.7 ± 3.8 compared with 8.3 ± 2.2 **nmoles/mg protein/min** in the control, $P < 0.01$). Later PDE activity fell, and on the 10th day it was 6.5 ± 1.9 compared with 11.5 ± 2.8 nmoles/mg protein/min in the control. PDE activity in the intact animals was 11.1 ± 2.5 nmoles/mg protein/min.

Changes in the components of the cyclic nucleotide system in the mouse spleen under the influence of thymalin were thus biphasic in character. In the early stages (3 days) after injection thymalin lowered the cAMP level and raised the cGMP level, which is characteristic of the T_2 lymphocyte subpopulation. In the late stages (10 days after injection) the cAMP concentration in the spleen was considerably higher, possibly connected with the action of the thymus factor on less mature T_1 lymphocytes, which participate directly in the realization of the immune response [3]. Elevation of the cAMP level promotes maturation of the cells and enhances their functional activity. Correspondingly, the changes observed under the influence of thymalin in the cAMP and cGMP concentrations may depend on the degree of differentiation

and the functional state of the cell population studied. The dynamics of the cyclic nucleotides revealed by these experiments evidently is caused by changes in PDE activity (an early rise and late fall), and this may indicate the possible point of application of the biological activity of thymus factor.

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EXPERIMENTAL STUDY OF THE EFFECT OF PERORAL ADMINISTRATION OF NITROSODIMETHYLAMINE ON FUNCTION AND ENZYMIC ORGANIZATION OF PULMONARY ALVEOLAR MACROPHAGES

R. V. Merkur'eva, N. N. Litvinov, B. V. Aulika, L. Kh. Mukhambetova, L. I. Bushinskaya, S. I. Dolinskaya, E. R. Yakimova, I. N. Konstantinova, and A. V. Votyakov

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The pulmonary alveolar macrophages, as a component of the mononuclear phagocytic system, determines to some degree the resistance of the organism to the unfavorable action of external environmental factors. The important role of the functional state of the alveolar macrophages in the defensive reactions of the body against inhaled chemical pollutants (nitric oxide, hydrogen sulfide, ozone, sulfur dioxide, carbon tetrachloride, etc.) has been demonstrated previously [1, 3, 4]. Meanwhile the possible role of alveolar macrophages in the development of defensive and compensatory mechanisms against the peroral uptake of pollutants, including chemical carcinogens belonging to the nitroso compounds, which are widespread in the environment [5, 6], has not yet been settled. Previous investigations showed that nitrosodimethylamine (NDMA), acting systemically, injures the membranes of the vital intracellular organelles of the liver [8].

It was accordingly decided to undertake a comparative cytological and biochemical study of the function and enzymic organization of alveolar macrophages in the early stages of development of the biological action of NDMA.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats (of which 12 were controls) 12, 24, and 48 h after intragastric administration of NDMA (30 mg/kg body weight). A combination of cytological and biochemical methods of investigation, described previously [3, 7], was used. Together with analysis of the cell composition and counting the total number of macro-

Laboratory of Biochemistry, A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow.

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