Variation in Antiinfectious Nonspecific Resistance of the Organism Caused by Cholinergic Stimulation

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Cholinergic stimulation (acetylcholine, aceclidine, armin) is found to improve antiinfectious nonspecific resistance in mice. The effect depends on the dose of cholinomimetic (armin), is maximal during the first 18 hours of the infectious process, and is determined by serum antibacterial activity, lysozyme activity, and the function of neutrophils and natural killers.

Key Words: *cholinergic stimulation; antiinfectious resistance; nonspecific factors of the organism's resistance; natural cytotoxicity*

There is an urgent need to study the interrelationships between the neuroendocrine and immune systems [10,11,13]. Nevertheless, despite growing interest in the effect of cholinergic stimulation on immunocompetent cells and immune reactions [2,4, 13,14], the changes in antiinfectious nonspecific resistance for activation of the parasympathetic nervous system are still poorly understood. This problem is not only of theoretical, but also of practical importance, since apart from aiding our understanding of the role of the cholinergic system in the regulation of immune homeostasis, it opens up the possibility of using cholinomimetic preparations as immunostimulators. The aim of the present study was to determine the effect of cholinergic stimulation on changes in the antiinfectious nonspecific resistance of the organism in different periods of an experimental infectious process.

MATERIALS AND METHODS

The experiments were performed on mice weighing 18-22 g. The preparations used for activation of the cholinergic system were: armin, an irreversible inhibitor of cholinesterase, in doses of 0.05-1.0 LD_{50}, acetylcholine (20 mg/kg), and the M-cholinomimetic aceclidine (1 mg/kg). The preparations were injected subcutaneously. The antiinfectious nonspecific resistance of the organism was determined one day after cholinergic stimulation according to mortality after 18 and 36 hours of experimental peritonitis induced in the mice by intraperitoneal injection of *E. coli* in a dose of 2×10^9 microbial bodies. Nonspecific factors of the organism's resistance (serum antibacterial activity - SAA, serum content of lysozyme, and phagocyte-metabolic activity of neutrophils by

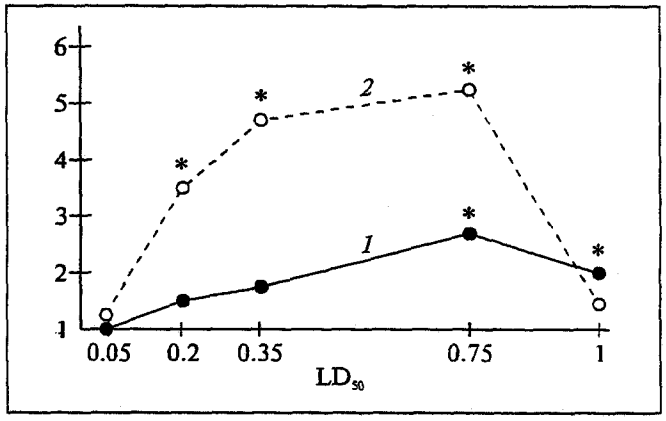

Fig. 1. Decrease in mortality from experimental infection in mice as a function of armin dose. Ordinate: ratio of the decrease in deaths in comparison with the control. Effect up to 36 (*1*) and 18 hours (*2*). Here and in Figs. 2 and 3: *$p<0.05$ in comparison with the control.

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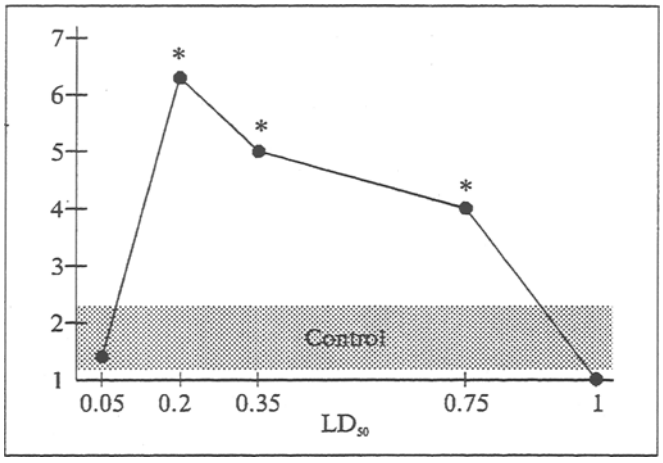

Fig. 2. Ratio of mortality from experimental infection in mice between 18 and 36 h to that before 18 h as a function of armin dose. Ordinate: ratio of the increase in mortality. *C*: control.

the test with nitroblue tetrazolium) were assessed one day after armin injection by routine methods [5]. Activity of natural killers (natural cytotoxicity) was determined spectrophotometrically 24 hours after injection of armin (1.0 LD_{50}) and acetylcholine (20 mg/kg) as described previously [2]. The data were processed statistically using the Student *t* test.

RESULTS

Cholinergic stimulation was found to reduce mortality from experimental infection in mice in a dose-dependent manner within a concentration range of armin from 0.05 to 0.75 LD_{50} (Table 1). This dependence manifested itself most strongly when deaths during the first 18 hours were recorded.

When the dose of armin was increased to 1.0 LD_{50}, the decrease in mortality became less expressed, but still exceeded the control level (Fig. 1.). It should be noted that practically no lethal outcomes from the experimental infection were observed after 36 hours. Our attention was drawn to an interesting phenomenon: when the preparation was injected in doses of 0.2-0.75 LD_{50}, deaths from infection increased between 18 and 36 hours against the background of an overall increase in viability.

The dependence of this effect on the armin dose is demonstrated in Fig. 2. When we used acetylcholine as a cholinomimetic agent, we found that mortality in the experimental and control groups was 66.6±6.9 and 38.5±7.8% ($p<0.01$), respectively, after 18 hours and 91.5±4.0% (control) and 74.5±7.0% (experiment) ($p<0.05$) after 36 hours. It is seen that cholinergic stimulation most markedly reduced deaths from infection between 18 and 36 hours. The same pattern was observed for aceclidine: mortality after 18 hours was 66.7±11.1 and 12.5± ±7.5%,and after 36 hours 95.0±5.1 and 37.4±15.6% in the control and experimental groups, respectively.

When we studied the effect of cholinergic stimulation of nonspecific factors of the organism's resistance, it was found that SAA, the serum lysozyme content and the phagocyte-metabolic activity of neutrophils rose as the dose of armin increased from 0.05 to 0.75 LD_{50} (Fig. 3) and dropped at 1.0 LD_{50}. The serum lysozyme activity, though substantially reduced, exceeded the control level. Comparison of the nature of the studied relationships presented in Figs. 1, 2, and 3 revealed a similar dependence in changes in mortality and factors of nonspecific resistance under conditions of cholinergic stimulation. In the presence of acetylcholine (20 mg/kg) and armin (1.0 LD_{50}) natural cytotoxicity accounted for 38±3 (n=10) and 14±2% (n=9), respectively, *vs.* 29±3% in the control (n=10, $p<0.05$). The marked drop in deaths from experimental infection, especially during the first 18 hours, is evidently due to the activation of SAA, serum lysozyme activity, phagocyte-metabolic activity of neutrophils, and function of natural killer lymphocytes.

It may be assumed that armin increased SAA, lysozyme, and phagocyte-metabolic activity of neutrophils through activation of M-cholinoreactive structures [3]. The direct effect of cholinotropic drugs on M-cholinoceptors of neutrophils is also possible [6], which results in the release of anti-

TABLE 1. Effect of Armin on Mortality from Experimental Infection (*E. coli*) in Mice after 18 and 36 h

Dose, LD_{50}	Mortality, %			
	18 h		36 h	
	control	experiment	control	experiment
0.03	70.4±9.0 (27)	66.7±9.8 (24)	77.8±8.1 (27)	83.3±7.8 (24)
0.20	19.4±6.6 (36)	5.5±3.8* (36)	47.2±8.3 (36)	33.3±7.9 (36)
0.35	35.0±7.8 (37)	5.0±3.4* (40)	54.0±8.2 (37)	25.0±6.8* (40)
0.75	43.5±12.6 (23)	8.8±4.9* (34)	91.3±5.9 (23)	32.3±8.0* (34)
1.00	36.0±9.8 (25)	28.6±8.7 (28)	56.0±10.1 (25)	28.6±8.7* (28)

Note. Figures in parentheses indicate the number of animals; *$p<0.05$ in comparison with the control

bacterial enzymes from cells and enhanced phagocytosis due to an increased intracellular content of cGMP [12]. The same mechanism underlies the cholinergic stimulation-induced (acetylcholine) increase in the activity of natural killers [8,15], which are known to be one of the main factors of antiinfectious nonspecific resistance. The decrease in the organism's nonspecific resistance with an increase of the dose of armin to 1.0 LD_{50} is related to inactivation of esterases of neutrophils [9] and lymphocytes [7]. However, the boosting effect of acetylcholine on the nonspecific resistance surpasses the suppressive effect mediated through inhibition of blood cell esterases.

The improvement of antiinfectious nonspecific resistance produced by cholinergic stimulation, maximally expressed during the first 18 hours, suggests the possibility of using cholinomimetics for emergency activation of antibacterial resistance in various infectious diseases.

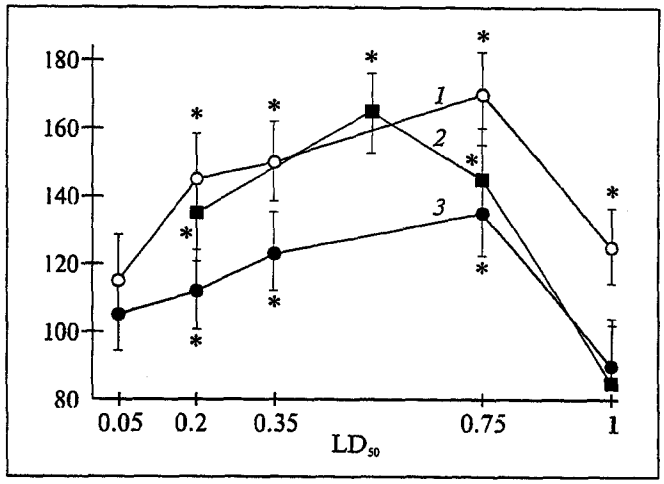

Fig. 3. Change in nonspecific factors of the organism's resistance as a function of armin dose. Ordinate: activity, % of control (100%). *1*) serum lysozyme content; *2*) phagocyte–metabolic activity of neutrophils; *3*) SAA. Each series comprised 15–20 animals.

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