ACTION OF ε-AMINOCAPROIC ACID ON SOME SEROLOGICAL AND ENZYME REACTIONS

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The inhibitory activity of ε-aminocaproic acid (ε-AA) on the agglutination of bacterial vaccines and sheep's red cells and on the indirect hemagglutination reaction was investigated in vitro. In addition, the mechanism of the first phase of the immunological response was studied with the aid of luminescent antibodies. Inhibition of the immunological response did not occur in the systems described. ε-AA inhibits the precipitation reaction, the lytic activity of complement (in a dose of 12 mg/ml), and also the proteolytic activity of pepsin (3 mg/ml) and trypsin (25 mg/ml) relative to bovine serum albumin.

Substances not affecting the immunocompetent cells of the body are now used in addition to immunodepressants [5] for the treatment of a number of immunopathological states. One such substance is ε-aminocaproic acid (ε-AA), which prevents the development of anaphylactic reactions clinically and in experiments on animals and prolongs the survival of a heterologous skin graft [8, 10, 11], probably on account of its ability to inhibit the manifestation of some serological reactions [1, 4, 9, 12] and fibrinolysis [7].

The object of the present investigation was to study the effect of ε-AA on the result of interaction between antigen and antibody with the aid of models of serological reactions using soluble, corpuscular, and structural tissue antigens, and also lytic processes connected with the action of complement on sensitized cells and the reaction of proteolysis.

EXPERIMENTAL METHOD

Heat-killed vaccines of group C streptococci and Candida albicans, sheep's red cells, and horse serum albumin were used as the antigens. Sections of mouse kidney were cut on a cryostat at −20°C. Antibacterial, cytotoxic, and immune sera against horse protein were prepared in rabbits by the usual scheme [3]. Luminescent sera against guinea pig and rabbit globulin were obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

Agglutination of bacterial vaccines and sheep's red cells, the precipitation test in narrow tubes, and the hemolysis and indirect hemagglutination tests were carried out by Rezinkova's methods [6]. Staining of the sections with luminescent sera was carried out by the indirect method of Coons and Shephard [2]. Proteolysis of bovine sera albumin (30 mg/ml) was carried out with preparations pepsin (pH 5.0) and trypsin (pH 9.0) in a dose of 1 mg/ml. The intensity of proteolysis was determined from the change in the protein concentration in the solution after incubation for 30 min at 37°C (biuret reaction, measurements with FÉK-M1 photoelectric colorimeter). Preparations of ε-AA were added to the medium with which the antiserum or antigen was diluted. The presence of ε-AA in the medium was determined by the ninhydrin reaction on the SF-1 spectrophotometer, λ = 400 nm.

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TABLE 1. Effect of ε-AA on Serological and Enzyme Reactions

Reaction	Antigens and substrates used	Minimal dose of ε-AA inhibiting reaction (in mg/ml)
Agglutination	Vaccine of group C streptococci	–
	Vaccine of C. albicans	–
	Sheep's red cells	–
Indirect immunoluminescence	Vaccine of C. albicans	–
	Section of mouse kidney	–
Indirect hemagglutination	Horse serum albumin	–
Precipitation	The same	12.5
Hemolysis	Sheep's red cells	12.5
Proteolysis:		
by pepsin	Bovine albumin	3.0
by trypsin	The same	25.0

EXPERIMENTAL RESULTS

The action of ε-AA was investigated on the manifestation of a number of serological reactions (agglutination of bacterial vaccines and sheep's erythrocytes, indirect hemagglutination, precipitation, and hemolysis) and on the staining of tissue sections and films of bacterial cultures by luminescent sera by the indirect method of Coons and Shephard with the use of complement. The results showed that ε-AA, in a dose of 0.25-200 mg/ml, did not inhibit agglutination of vaccines of group C streptococci and Candida albicans, native sheep's red cells, or formalinized sheep's red cells loaded with horse albumin, by the corresponding immune sera. No difference was found in the character of luminescence of the basement membranes of the glomeruli in sections of the mouse kidney or cells of C. albicans vaccine in films when treated with homologous intermediate and labeled anti-rabbit sera containing ε-AA, and of control preparations treated with the corresponding immune sera without addition of ε-AA. Precipitation of horse albumin by the corresponding immune serum was inhibited on the addition of ε-AA to the medium, starting with a dose of 12.5 mg/ml (Table 1). These same concentrations of ε-AA blocked the lytic activity of complement when added to the immune complex (sheep's red cells plus hemolytic serum) in a solution of ε-AA. Complement fixation likewise was not found on immune complexes (sheep's red cells plus immune serum or C. albicans vaccine plus immune serum) in the presence of ε-AA when the preparations were tested by the immunoluminescence method using labeled serum against guinea-pig complement.

The addition of ε-AA (dose 100 mg/ml) to a mixture of sheep's red cells plus immune serum plus complement, previously incubated for 30 min at 4°C, did not inhibit the development of hemolysis. This showed that ε-AA is unable to inactivate complement when already adsorbed on the immune complex. Preliminary treatment of the sensitized red cells with ε-AA before contact with complement, followed by washing to remove all traces of the ε-AA, likewise did not inhibit the lytic activity of the complement, and complement fixation could have been detected by the immunoluminescence method. This last result indicated the possible absence of a strong bond between ε-AA and the immune antigen–antibody complex formed. The bond between ε-AA and complement also is labile: a mixture consisting of complement (1:2) and ε-AA (200 mg/ml), if first incubated for 30 min at 37°C, was easily separated on filtration through a column with Sephadex G-25 and washing with phosphate buffer (pH 7.2). Addition of ε-AA to the solution of bovine serum albumen inhibited the hydrolysis of protein by pepsin in concentrations of 3 mg/ml or higher in the medium, and blocked the activity of trypsin in a dose of 25-200 mg/ml (Table 1).

The results of these experiments show that visible manifestations of the agglutination of bacterial vaccines, of native sheep's red cells, and of sheep's red cells loaded with horse serum albumin by immune sera were not suppressed by ε-AA on its addition to the reacting mixture, by contrast with the action of ε-AA on the agglutination of human red cells and leukocytes [1, 4]. The observed inhibition of precipitation by ε-AA of horse serum albumin solutions by immune serum, in the absence of any effect on the agglutination of sheep's red cells loaded with the same antigen may perhaps be due, not to blocking of the formation of the antigen–antibody complex by the ε-AA, but to its action on the phase of

protein aggregation and on sedimentation of the precipitate. ε-AA inhibits proteolytic processes when added to the substrate of lytic activity of complement in immunological tests. Under these circumstances the ε-AA does not form a stable bond with complement, but prevents its sorption on the immune complex. These results suggest that ε-AA can be used to inhibit immunopathological processes due to the fixation of complement on an immune complex and to the action of proteases liberated by activation of lysosomes by an immune complex. The specific phase of combination of antigen with antibody likewise was not inhibited by ε-AA in systems consisting of C. albicans cells – antiserum and kidney sections – cytoxic serum on investigation by the luminescent antibody method.

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