ROLE OF LIPID PEROXIDATION IN COMPLEMENT-DEPENDENT HEMOLYSIS

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The molecular mechanisms of complement-induced damage to the membranes of target cells have not been finally elucidated. In the final stages of activation of complement, its last five serum components are combined into a macromolecular complex C5b-9, which is incorporated into the membrane of the damaged cell and forms a transmembrane channel [2, 7]. Further damage to the membrane may be caused by colloid osmotic shock [6], by conformational changes in the membrane lipids [3], and by activation of phospholipases [9]. At the same time, we know that many hemolytic agents exert their action indirectly through activation of lipid peroxidation (LPO) in erythrocyte membranes [8].

In this investigation the role of LPO in complement-dependent lysis of erythrocytes was studied.

EXPERIMENTAL METHOD

Sheep's erythrocytes were washed and suspended in Hanks' solution in a concentration of $2 \cdot 10^4$ cells/μl. The erythrocytes were sensitized with rabbit blood serum in a final titer of 3:1. Guinea pig serum in a final dilution of 1:320 was used as complement. Hemolysis was carried out with constant mixing of the erythrocyte suspension at 37°C for 1 h. The suspension without complement served as the control. Samples from the experimental and control suspensions were taken before and during hemolysis. The LPO level was estimated from concentrations of malonic dialdehyde (MDA) and Schiff bases. The MDA concentration was determined in suspension medium after sedimentation of unlysed erythrocytes at 4000 rpm for 5 min by a fluorescence test with 2-thiobarbituric acid [10]. The concentration of Schiff bases was determined after extraction of lipids with a mixture of chloroform and methanol (2:1) by measuring natural fluorescence (excitation 360 nm, emission 440 nm) [4] on a Hitachi MPF-4 (Japan) spectrofluorometer. The hemoglobin concentration in the suspension medium was measured spectrophotometrically. The experiment was repeated 8 times.

EXPERIMENTAL RESULTS

On the addition of complement, leading to lysis of the sensitized erhthrycytes, a significant increase in the concentration of MDA and Schiff bases took place. Comparison of the kinetic curves of MDA formation and of hemoglobin outflow during complement-dependent hemolysis shows that the increase in MDA concentration preceded hemolysis. We know that LPO products, by increasing permeability and reducing the mechanical stability of the membrane, can cause hemolysis of erythrocytes [8]. This suggests that when hemolysis is initiated by complement, this happens through activation of LPO of the erythrocyte membranes. The maximal MDA concentration in fact coincides in time with the maximal rate of outflow of hemoglobin, and arrest of hemolysis coincided with the minimum of the MDA concentration (Figs. 1 and 2).* The decrease in MDA concentration was perhaps due to the reaction of the dialdehyde with amino groups of proteins and phosphatidylethanolamine, with the formation of Schiff bases [4, 5]. This is confirmed by the continuous increase in concentration of the latter during complement-dependent hemolysis (Fig. 2). A second increase in LPO activity did not lead to lysis of erythrocytes. At this time membranes of cells already hemolyzed were probably undergoing oxidation. The phenomenon of autooxidation of cell membranes, destroyed through the action of various factors, has been described in the literature [1].

*Figures 1 and 2 were omitted from the Russian original – Publisher.

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The kinetics of the change in MDA concentration thus reflects superposition of three processes: activation of LPO of the erythrocyte membranes under the influence of complement, spontaneous oxidation of erythrocyte ghosts, and the reaction of MDA with amino groups of proteins and phospholipids.

These results are evidence that LPO of erythrocyte membranes participates in the mechanism of complement-dependent hemolysis.

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GLUCOCORTICOIDS IN INTRACELLULAR CATABOLISM OF MYOSIN AND ACTIN

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Glucocorticoids are known to increase the proteolytic activity of skeletal muscles [8] and to stimulate, in the first place, degradation of myofibrillary proteins [10, 12]. The atrophy developing under these circumstances, as well as the increase in alkaline proteinase activity in them, depends on the type of skeletal muscle [1, 11]. Considering that nonlysosomal proteinases also participate in the intracellular catabolism of muscle proteins [3], and also that the number of lysosomes in skeletal muscles is very small and that inhibition of lysosomal proteinases does not inhibit myosin degredation [7], it can be concluded that there are good grounds for the opinions of those workers who consider that the initial step in muscle protein degredation does not involve the participation of lysosomal proteinases [7]. It has been shown, for instance, that thiol proteinases, which are activated at neutral pH values by Ca^{++} ions, degrade troponin I and T [7], whereas serine proteinases, at the same pH values, destroy both structural and regulatory proteins of the contractile system of muscles [9, 13]. This suggests that glucocorticoids, by increasing proteolytic activity of alkaline proteinases in the muscle cell, may initiate degradation of myofibrillary proteins.

The aim of this investigation was to study connections between proteolytic activity of alkaline proteinases and the intensity of their synthesis, and also the degradation of actin and myosin in muscle cells when the blood glucocorticoid level is raised.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 16-17 weeks, kept under the conditions described previously [11]. Alkaline poteinases were isolated from m. gastroc-

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