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EFFECT OF DIFFERENT PROTEINS ON PROTEOGLYCAN INDUCED ERYTHROCYTE AGGREGATION

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The ability of proteoglycans to exclude cells, cellular organelles, and compartments, and also other structural elements of tissues from the space occupied by them, to concentrate all this in a limited volume, and to prevent their dispersion has been studied in considerable detail in model experiments and on tissue cultures [3-7, 10-13]. However, in none of these investigations has the effect of the protein components of the medium on steric exclusion and aggregation of cells induced by proteoglycans been studied. The aim of the present investigation was to study effects of protein components of the blood plasma on steric exclusion and aggregation of erythrocytes in their suspension in salt solution, used as a model of isolated cells, realized by hyaluronic acid (HUA) and a natural protein — chondroitin — keratan sulfate (PCKS) complex. The need for such research is dictated by the fact that proteoglycans act in the tissues as steric exclusion factors always in the presence of various proteins.

EXPERIMENTAL METHOD

An equilibrium suspension containing 30% of rabbit erythrocytes by volume was made up from erythrocytes washed free from blood plasma with salt solution (0.15 M NaCl, pH 7.2, phosphate buffer) in the same solution. In experiments with whole blood, stabilized by citrate, the volume of the erythrocytes also was made up to 30%. Quantitative determination of erythrocyte aggregation was carried out by measuring the rate of separation of the suspension and blood into phases of solution (or plasma) and blood cells at 20°C [3]. The initial rate of this separation was determined as the tangent of the angle of slope of the curve to the abscissa. Highly purified and high-polymer preparations of HUA and PCKS were obtained in the form of the normal potassium salts, by methods suggested by ourselves [1, 2]. A total fraction of albumins, ($\alpha + \beta$)-globulins, and γ-globulin were isolated from rabbit serum (the same serum as that from which the erythrocytes

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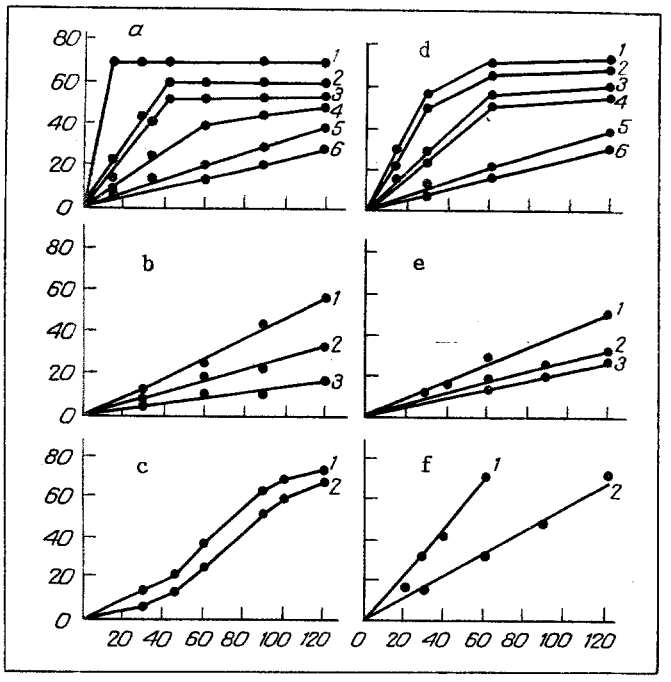

Fig. 1. Effect of various proteins on rate of fractionation of erythrocyte suspension in salt solution into phases of solution and cells, induced by HUA and PCKS. Abscissa, time (in min); ordinate, relative volume (in % of layer of solution above aggregated erythrocytes). Abbreviations in caption: suspension — erythrocyte suspension in salt solution; alb. — albumins, IR) initial rate of separation of suspension into phases of solution and cells (in % of relative volume of solution above erythrocytes, in $mm^3 \cdot min^{-1}$) a: 1) Suspension + HUA (2.0 $mg \cdot ml^{-1}$), IR 4.3; 2) blood + HUA (2.0 $mg \cdot ml^{-1}$), IR 1.3; 3) suspension + HUA (1.0 $mg \cdot ml^{-1}$), IR 1.9; 4) blood + HUA (1.0 $mg \cdot ml^{-1}$), IR 0.5; 5) suspension + HUA (0.5 $mg \cdot ml^{-1}$), IR 0.3; 6) blood + HUA (0.5 $mg \cdot ml^{-1}$), IR 0.2; b: concentration of HUA in all experiments in series b and c 1.0 $mg \cdot ml^{-1}$. 1) Suspension + HUA + alb. (4.0 $mg \cdot ml^{-1}$. 1), IR 0.3; 2) the same + HUA + γ globulin (1.0 $mg \cdot ml^{-1}$), IR 0.7; 3) the same + HUA + (α + β)-globulins (1.0 $mg \cdot ml^{-1}$), IR 0.1; c: 1) suspension + HUA (α + β)-globulins (1.0 $mg \cdot ml^{-1}$) + alb. (4.0 $mg \cdot ml^{-1}$), IR 1.6; d: 1) blood + PCKS (7.0. $mg \cdot ml^{-1}$), IR 1.7; 2) suspension + PCKS (7.0 $mg \cdot ml^{-1}$), IR 1.5; 3) blood + PCKS (3.5 $mg \cdot ml^{-1}$) IR 1.0; 4) suspension + PCKS (3.5 $mg \cdot ml^{-1}$), IR 0.8; 5) blood + PCKS (1.6 $mg \cdot ml^{-1}$), IR 0.3; 6) suspension + PCKS (1.6 $mg \cdot ml^{-1}$), IR 0.2; e: concentration of PCKS in all experiments of series e and f is 7.0 $mg \cdot ml^{-1}$. 1) Suspension + PCKS + alb. (4.0 $mg \cdot ml^{-1}$), IR 0.5, 2) the same + PCKS + γ-globulin (1.0 $mg \cdot ml^{-1}$), IR 0.3; 3) the same + PCKS + (α + β)-globulins (1.0 $mg \cdot ml^{-1}$), IR 0.3; f: 1) suspension + PCKS + (α + β)-globulin (1.0 $mg \cdot ml^{-1}$) + alb. (4.0 $mg \cdot ml^{-1}$), IR 1.0; 2) the same + PCKS + γ-globulin (1.0 $mg \cdot ml^{-1}$) + alb. (4.0 $mg \cdot ml^{-1}$), IR 0.5.

were obtained) by fractionation with salts, variation of pH, and treatment with ethanol at a low temperature (—5 to —6°C), followed by further purification and determination of homogeneity by moving boundary electrophoresis [8, 9].

EXPERIMENTAL RESULTS

The control experiments showed that albumins, (α + β)-globulins, and γ-globulin, in the concentrations tested, and in the absence of HUA and of PCKS, did not bring about fractionation of the erythrocyte suspension in salt solution into phases of solution and cells at any time during the experiment. HUA in the same concentration and in the same relative volume of erythrocyte fraction in salt solution and of whole blood had a much more effective action in the suspension than in whole blood as regards separation into phases of liquid and cells, as was shown by the higher initial velocity of this fractionation in the erythrocyte suspension than in blood (Fig. 1a). This occurred with all HUA concentrations studied. The presence of blood serum albumins in the erythrocyte suspension containing 1.0 mg · ml^{-1} of HUA sharply reduced the rate of separation of that suspension into solution and erythrocytes. An even more marked decrease in rate of this separation was produced by (α + β)-globulins and γ-globulin, the former being more active than the latter (Fig. 1b). The presence both of albumins and of (α + β)-globulins initially slowed, then sharply accelerated separation of the suspension into solution and erythrocytes, until the largest possible volume of these cells taken in salt solution was reached. Similar fractionation of the suspension also took place in the combined presence of albumins and γ-globulin in the salt solution, the difference being that the curve reflecting the time course of fractionation lay a little below that in the first case, but ultimately maximal fractionation was achieved within the same time (Fig. 1c). The presence both of albumins and of the above-mentioned globulins in the erythrocyte suspension led to mutual neutralization of the inhibitory effect on the action of HUA exerted by these proteins when acting independently.

Separation by PCKS of whole blood and a suspension in salt solution into phases of liquid and erythrocytes did not differ significantly in the initial rate of separation. Within the limits of the PCKS concentrations studied, this separation took place rather faster in whole blood than in erythrocyte suspension in salt solution (Fig. 1d). Albumins, and also (α + β)-globulins and γ-globulin delayed fractionation in suspension into solution and erythrocytes, globulins having a significantly stronger action than albumins, but no difference was observed in this action between the types of globulins mentioned above (Fig. 1e). If both albumins and (α + β)-globulins, or albumins and γ-globulins were present together in the solution, the rate of separation into phases increased, and the increase was significantly greater in the case of the first combination of protein fractions (Fig. 1f).

Definite quantitative differences were observed as regards the effect of the proteins tested on the action of HUA and PCKS as steric exclusion factors. The action of HUA was manifested by a much greater degree in the erythrocyte suspension in salt solution than in whole blood, whereas the same action of PCKS in whole blood was slightly greater than its action in the suspension of erythrocytes in salt solution. Activity of HUA was inhibited to a greater degree by (α + β)-globulins than by γ-globulin, but in the case of PCKS both these fractions equally depressed the activity of this proteoglycan. The time course of separation of the erythrocyte suspension into phases during the action of HUA in the presence of a combination of albumins and globulins followed an S-shaped curve, whereas in the case of PCKS under the same conditions, dependence on time was linear.

Differences observed between the effects of the various proteins on steric exclusion of erythrocytes, realized by HUA and PCKS, are probably determined by the much higher electrical charge on the PCKS macromolecule than on the HUA macromolecule. The molecular mechanism of the effect of albumins, (α + β)-globulins, and γ-globulin, which themselves do not displace erythrocytes from salt solution, on this action of HUA and PCKS is very complex, and its elucidation requires special investigations. Changes in the rate of separation of the erythrocyte suspension into phases in the presence of albumins, (α + β)-globulins, and γ-globulin is perhaps connected with the influence of these proteins on the size and shape of aggregates formed through the action of HUA and PCKS [5]. The possibility cannot be ruled out that abolition of the inhibitory effect on the action of HUA and PCKS when the test proteins are present together can also be explained by a change in the size and shape of the erythrocyte aggregates. The fact that the effect of protein fractions on steric exclusion of erythrocytes from the liquid phase under the influence of HUA and PCKS is itself of essential interest. Data obtained by the use of an erythrocyte suspension in salt solution as a model of isolated cells are fully confirmed by tissue culture experiments [12, 13]. The regulating effect of proteins on the action of HUA and PCKS as factors concentrating the cells in a definite volume plays a role also in periods of growth of the organism, morphogenesis, tissue regeneration, and other similar processes.

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LUMINOL- AND LUCIGENIN-DEPENDENT CHEMILUMINESCENCE DURING AUTOOXIDATION OF 6-HYDROXYDOPAMINE

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Endogenous 6-hydroxydopamine (6-OHDA), a minor product of secondary metabolism of dopamine, is one of the most powerful neurotoxins, for it can disturb the structural and functional integrity of the neuronal membranes of the brain [5, 6]. The neurotoxicity of 6-OHDA is linked primarily with its ability to accumulate in large amounts in the central and peripheral catecholaminergic neurons, damaging them and causing the development of "parkinsonism" in experimental animals [3, 4]. It has been demonstrated on model systems and systems in vitro that one of the most likely mechanisms for the realization of the pathological action of 6-OHDA is its ability to undergo autooxidation, with the formation of O_2^- and H_2O_2, which are powerful activators of free-radical oxidation of membrane lipids and proteins. Indeed, the fact that 6-OHDA can activate lipid peroxidation (LPO) of brain membranes was demonstrated recently [7, 10]. However, stimulation of LPO of neuronal membranes by autooxidation of 6-OHDA has a number of special features: first, the stimulating effect depends ultimately on the 6-OHDA concentration; second, superoxide dismutase (SOD) which, as we know, usually inhibits LPO in biomembranes, potentiates 6-OHDA-induced LPO of brain membranes [7]. In this connection, in order to study the mechanism of initiation of LPO under the influence of 6-OHDA, the features of its autooxidation were studied by the luminol- and lucigenin-dependent chemiluminescence (ChL) method.

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