ROLE OF PROTEOGLYCAN AGGREGATES IN CELL ADHESION

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Besides the protein-chondroitin-keratan sulfate (PCKS) fraction soluble at low ionic strength (nonreacting), various types of cartilage also contain large quantities of proteoglycan aggregates (PA). These PA are constructed from hyaluronic acid (HUA), to which macromolecules (60-100 or more) of aggregated PCKS, insoluble at low ionic strength, are attached along its length by the end of their protein cores (HUA-binding site) with the participation of binding protein, which stabilizes this compound [1, 7]. According to previous investigations, HUA and nonaggregating PCKS, besides many different and as yet incompletely studied biological functions, also behave in the body as factors reversibly restricting bell dispersion [3, 4].

In this connection it was considered necessary to study the ability of PA to undertake this function, which is a particularly interesting and important problem because the biological role of PA is insufficiently clear [1].

EXPERIMENTAL METHOD

PA and nonaggregating PCKS were isolated from the cartilaginous rings of the bovine trachea [5, 7], and HUA from human umbilical cords [2] in the form of potassium salts.

A suspension of rabbit erythrocytes in 0.1 M NaCl in the presence of phosphate buffer (1/15 M and pH 7.2) was used as model of a cell suspension. The method of quantitative determination of erythrocyte aggregation induced by proteoglycans was described previously [3, 4]. The determinations were made 4-8 times for each concentration of biopolymer tested.

EXPERIMENTAL RESULTS

Fractionation of a stable, evenly distributed suspension of erythrocytes in salt solution into phases of solution and accumulation of erythrocytes, i.e., their concentration in limited volume, arises in the presence of PA concentrations much lower than in the case of HUA and PCKS (Fig. 1). Aggregation of erythrocytes by 90% took place in the course of 30 min in the presence of concentrations of PA, HUA, and PCKS equal to 6.4, 40.0, and 128.0 nM respectively. In the control, i.e., in a suspension of erythrocytes in salt solution without any of these proteoglycans, no aggregation took place for 60 min or more. In their ability to displace erythrocytes from the spaces occupied by them in solution, i.e., to prevent their dispersion, PA are 6 and 20 times more active respectively than HUA and PCKS. The degree of erythrocyte aggregation was a linear function of logarithm of concentration for all three biopolymers studied (Fig. 1).

Erythrocyte aggregates induced by PA, HUA, and PCKS have different structures (Fig. 2). In the presence of PA, loose accumulations of erythrocytes are formed. Within these erythro-

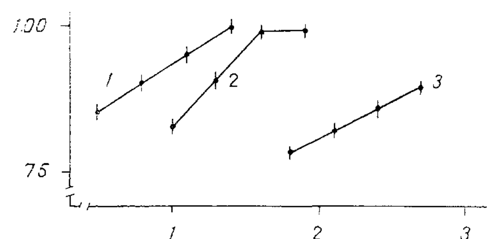

Fig. 1. Aggregation of erythrocytes as a function of log of concentration of PA (1), HUA (2), and PCKS (3). Abscissa, log of concentration (in nM); ordinate, % of erythrocytes aggregated in 30 min.

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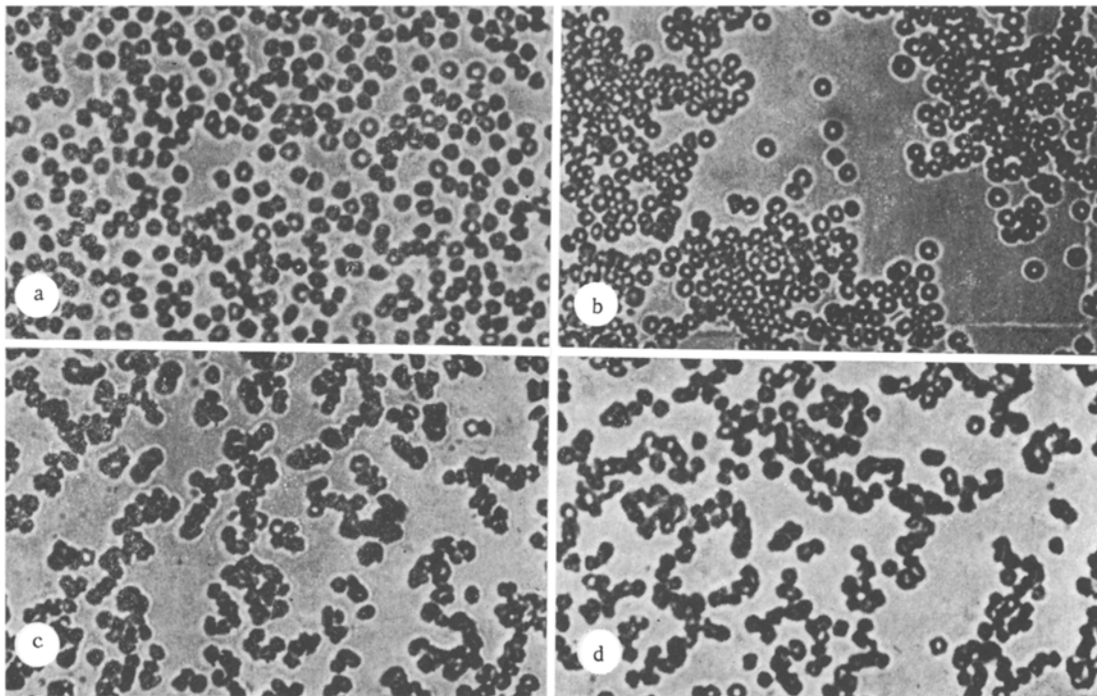

Fig. 2. Structure of erythrocyte aggregates: a) suspension of erythrocytes in 0.15 M NaCl (pH 7.2); b) loose aggregates of erythrocytes formed in the presence of salt solution by PA (6.4 nM); c) erythrocyte aggregates in the presence of HUA (40.0 nM) in salt solution; d) the same, in the presence of PCKS (128.0 nM), 200×.

cyte accumulations free spaces remain which persist even at high concentrations of PA (>25 nM), occurring beyond 100% aggregation of erythrocytes (Fig. 2b). A certain barrier hindering spontaneous contact of the erythrocyte membranes exists. The accumulations of erythrocytes resulting from the action of HUA and PCKS consist of dense aggregates within which free spaces between erythrocytes are not visible, nor the boundaries between them discernible (Fig. 2c, d).

The molecular weights of PA, HUA, and PCKS are 5.0×10^7 to 5.0×10^8, 8×10^6, and 1.0×10^6 to 4.0×10^6 daltons [6, 7]. PA also have an exceptionally large hydrodynamic volume [7]. Most probably the differences in the ability of PA, HUA, and PCKS to fractionate a uniformly distributed suspension of erythrocytes in salt solution into phases of solution and cells, discovered in these experiments, are due primarily to the values of their molecular weight, hydrodynamic volume, electric charge, and other physical properties of these proteoglycans. It may be that the giant size of the macromolecules and the well-marked hydrophilicity of this proteoglycan complex prevent the closer packing of the erythrocytes in their aggregates formed in the presence of PA.

It was shown previously that the degree of aggregation of erythrocytes is considerably greater in a mixture of HUA and PCKS (nonreacting fraction) than the combined values of aggregation of each of these proteoglycans acting separately in the same concentrations as in the mixture [4]. In the combined presence of HUA and PCKS in the solution more complex three-dimensional structures may arise than when only one of these proteoglycans is present in it, probably as a result of dipole orientation, for the protein component of PCKS contains arginine. In addition, since certain amounts of aggregated PCKS may be present in PCKS preparations, the possibility cannot be ruled out that unstable PA also may be formed in this case, through the absence of binding protein. Complex three-dimensional structures arising in solutions as a result of interaction in one way or another between HUA and PCKS have thus stronger ability to displace erythrocytes from the space they occupy. Macromolecules of PA, because of their exceptional physical parameters, can themselves form even more complex supramolecular three-dimensional systems in solutions, and this is expressed in their very high activity in this respect and the characteristic shapes of the erythrocyte aggregates.

Possibly the principal role of HUA, PCKS, and PA in cell adhesion is that these proteoglycans reversibly prevent dispersion of cells and bring their surfaces sufficiently close together to enable the specific factors of adhesion to operate.

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EFFECT OF IMIDAZOLE ON SOME METABOLIC PROCESSES IN WOUND TISSUES IN RATS

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The participation of cyclic nucleotides in cell proliferation and differentiation makes the study of their effect on repair processes and, in particular, during wound healing, particularly urgent. It was shown previously that administration of exogenous cAMP [2] and cGMP [3, 4] to animals after operations gives rise to a broad spectrum of changes in wound tissue metabolism, and thus evidently gives rise to an increase in the collagen content of the granulations. It has also been shown that a single injection of cGMP immediately after an operation in a near-physiological dose causes early activation of collagen biosynthesis in the granulations, skin, and muscles of wounds, activation of biosynthesis of various muscle proteins, and early mobilization of energy resources – glycogen metabolism and glycolysis [5]. The changes in metabolism thus observed contribute to the intensification of repair processes, as has been confirmed by morphological study and measurement of the area of wounds in control and experimental animals in the course of healing.

The facts so far obtained suggest that substances modulating the activity of the guanylate cyclase system may have a significant influence on metabolism and the regenerative capacity of injured tissues. One such substance is imidazole, an inactivator of 3',5'-AMP phosphodiesterase and an inhibitor of enzymic hydrolysis of 3',5'-GMP. Imidazole thereby alters the relative levels of cAMP and cGMP in the direction of an increase in concentration of the latter [7]. Imidazole also is interesting because it is a component of carnosine, which is known for its physiological activity [6] and its wound-healing action [9], and also of levamisole, which stimulates certain immune processes. The object of this investigation was to study the effect of imidazole administration on metabolic processes characterizing regeneration in wound tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male albino rats weighing 150-170 g with experimental wounds: a linear skin incision with a nichrome coil, inducing granulation tissue formation [4], implanted subcutaneously. The animals were divided into three groups with 10-12 rats in each group: 1) control animals (undergoing the operation), and 2 and 3) experimental animals. Imidazole was injected intraperitoneally into the experimental rats in 0.5 ml of 0.14 M NaCl solution 30 min after the operation in a dose of 5 mg (group 2) or 10 mg (group 3). The animals were investigated on the 5th day after the operation. The collagen content was measured [8] in the wound tissues (skin, muscles, and granulations). In addition, the content

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