This fact suggests that pulmonary receptors do not play an essential role in the stimulating action of naloxone and TRH on breathing.

The results of this investigation do not contradict the limited information present in the literature on the stimulating effect of opioid antagonists on respiration during hypoxia. Disturbances of respiration arising during the development of acute hypoxia due to blood loss are evidently due to a definite extent to the action of endogenous opioid peptides, whose inhibitory effect on respiratory center neurons is abolished by naloxone and TRH. This hypothesis does not rule out other possible mechanisms of the stimulating action of opioid antagonists on respiration in actute hypoxia.

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TWO FUNCTIONS OF PROTEOGLYCANS IN ERYTHROCYTE AGGREGATION AND ADHESION

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KEY WORDS: proteoglycans; aggregation; adhesion; erythrocytes

The writers' previous investigations showed that normal potassium salts of hyaluronic acid (HUA), of soluble protein-chondroitin-keratan sulfate (PCKS), and proteoglycan aggregates (PA) of cartilage, which are highly important components of different kinds of cells and the matrix of connective tissue, besides their many other funtions, also play the role of factors preventing dispersion of cells and other tissue elements, displacing them from the space occupied by proteoglycans, and concentrating them in a limited volume. Thus these biopolymers promote aggregation and subsequent and nonspecific adhesion of cells and also the formation of certain extracellular tissue structures [5-8]. Investigations of the aggregating action of proteoglycans on cells of the retina and other tissues, by a number of workers, have completely confirmed the above theory [6]. It has, moreover, been suggested that the ability of proteoglycans to displace cells nonspecifically into the smallest possible space is of essential important for morphogenesis also [12]. Howsever, the nonspecific and reversible stereochemical action of HUA, PCKS, and PA on cell aggregation does not rule out

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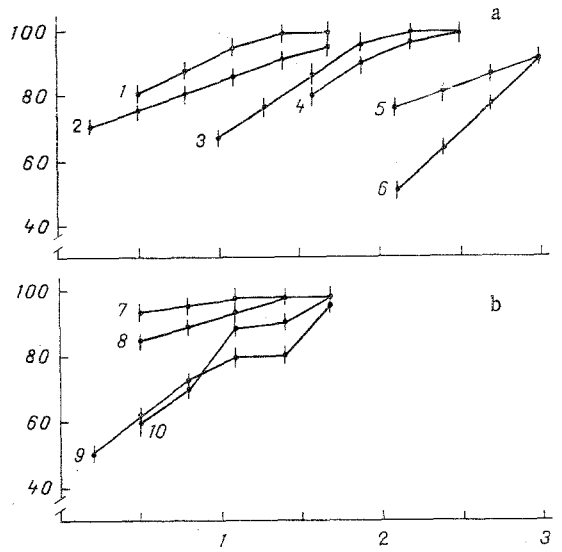

Fig. 1. Dependence of degree of aggregation (in percent of total number of erythrocytes in 30 min) of NE and FE on logarithm of concentration of PA, HUA, and PCKS. Abscissa, log of concentration (in nM); ordinate, degree of aggregation (in percent). 1) NE + PA; 2) FE + PA; 3) NE + HUA; 4) FE + HUA; 5) NE + PCKS; 6) FE + PCKS; 7) NE + PA + 20 nM HUA: 8) NE + 60 nM PCKS; 9) NE + $(Ca^{++})_n$-PA; 10) NE + $(Mg^{++})_n$-PA.

the possibility that definite chemical structures (domains), specifically bound to some degree or other with HUA, PCKS, and PA macromolecules, are present on the outer surface of cell membranes and also in tissue formations.

The aim of the present investigation was to demonstrate the presence of such domains, to define their role in cell aggregations together with the nonspecific action of proteoglycans as factors limiting dispersion of cells by their own displacing action, using suspensions of erythrocytes in salt solution as the experimental model. The advantage of such a model is that it makes it unnecessary to use proteolytic enzymes, which can destroy structures necessary for interaction with HUA, PCKS, and PA, on the surface of the cell membranes, as is the case when cells are isolated from tissues and tissue cultures.

EXPERIMENTAL METHOD

Methods of obtaining normal potassium salts of HUA, PCKS, and PA, and of normal Ca- and Mg-salts of PA, and of quantitative determination of erythrocyte aggregation were described previously [6-8]. To demonstrate the presence of chemical groups binding with proteoglycans on the surface of the rabbit's erythrocytes, the cells were suspended in 0.15M NaCl (pH 7.2, phosphate buffer) and were treated with 3.0% formaldehyde solution [10] for 18 h at 37°C with constant stirring. The excess of formaldehyde was removed from the mixture by washing the erythrocytes with the above-mentioned salt solution. The suspension of formalinized erythrocytes (FE; 10%) in buffered salt solution (pH 7.2) was kept at 4°C. Commercial formalin was treated with the HO^--form of an anionic exchange resin (Amerlite IRA-400, from Serva, West Germany) to remove formic acid from it, as was shown by development of a neutral reaction of the solution.

EXPERIMENTAL RESULTS

Formaldehyde-treated erythrocytes, suspended in 0.15 M NaCl (pH 7.2, phosphate buffer), preserved their ability to undergo aggregation in the presence of PA, HUA, and PCKS, but they were aggregated much less strongly than intact erythrocytes under the same conditions and with the same concentration of proteoglycans. It was shown that PA do not induce complete aggregation of formalinized erythrocytes in the presence of concentrations of the given polymer at which normal erythrocytes (NE) are completely aggregated. In the presence of HUA complete aggregation of FE took place in higher concentrations of HUA than similar aggregation of NE. PCKS caused complete aggregation of both kinds of erythrocytes in the same concentration, but in lower concentrations of this proteoglycan weakening of aggregation of FE was much more clearly defined (Fig. 1).

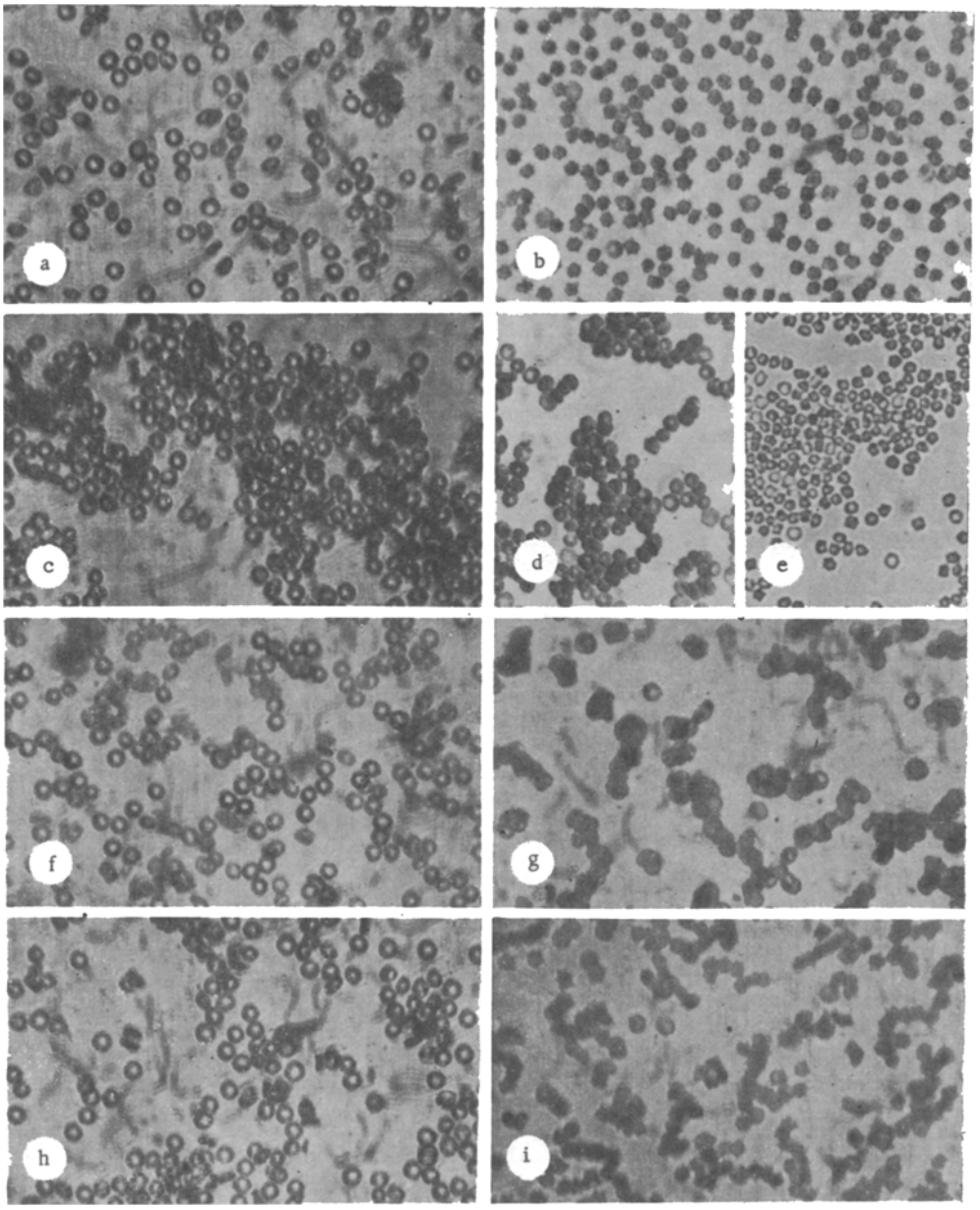

Fig. 2. Structure of aggregates of NE and FE. a) Suspension of FE in 0.15 M NaCl (pH 7.2), b) suspension of NE in 0.15 M NaCl (pH 7.2), c) aggregates of FE in salt solution containing 25 nM PA, d) aggregates of NE under the same conditions, e) the same, in the presence of 1.6 nM PA, f) aggregates of FE in salt solution containing 160 nM HUA, g) aggregates of NE under the same conditions, h) aggregates of FE in salt solution containing 500 nM PCKS, i) aggregates of NE under the same conditions, magnification 200.

If PA and HUA were both present in the suspension of NE in salt solution (pH 7.2) potentiation of erythrocyte aggregation was observed, up to 100% in PA in a concentration of 30 mM and HUA in a concentration of 20 nM, but this did not happen in the same concentrations of PA and HUA but acting separately. Potentiation of aggregation of NE also was observed in a mixture of PA with PCKS, but the effect of PCKS was quantitatively weaker than that of HUA, in agreement with the weaker aggregating action of PCKS itself.

Dependence of aggregation of intact erythrocytes on the concentration of normal Ca- and Mg- salts of PA is more complex than in the case of the potassium salt of PA. During aggregation of erythrocytes induced by normal salts of PA of these bivalent cations, aggregation rose steeply at higher concentrations of these salts, and this was followed by total aggregation (Fig. 1). Although the molecular weights of these salts of PA were not determined, it

can be stated that Ca^{++} and Mg^{++}, combining with PA polyanions, create giant complexes, in a semidispersed state in solution, and this evidently is reflected in the shape of the curve of dependence of degree of erythrocyte aggregation on concentration of these polymers.

Differences are observed in macrostructure of NE and FE aggregates arising in the presence of PA, HUA, and PCKS (Fig. 2). In salt solution FE (control) appear a little swollen. If PA is present in such a solution, these erythrocytes form aggregates in which the separate cells are much less densely packed together than in aggregates of intact erythrocytes in the same concentration of PA, in which these cells form denser accumulations, but with clearly distinguishable separate erythrocytes and boundaries between them. The macrostructure of NE aggregates in PA in a concentration of 1.6 nM, i.e., many times less than in the experiment with FE mentioned above, is similar to that of the aggregates in this experiment. HUA and PCKS also form loosely packed aggregates from FE, in concentrations of these proteoglycans in which (or even much lower) concentrations NE are bound into dense accumulations with indistinguishable separate cells.

Since treatment of erythrocytes with formaldehyde reduces their ability to be aggregated in salt solution containing PA, HUA, and PCKS, which follows from the macrostructure of aggregates of these erythrocytes, it can be concluded that formaldehyde, reacting primarily with the amino groups of the protein components of the erythrocyte membranes [9], modified the arrangement of the domain binding with these proteoglycans on the surface. The possibility cannot be ruled out that for every proteoglycan studied there exist domains, which are to a certain degree particular, but which probably overlap one another. Summation of the values of erythrocyte aggregation in salt solution containing AP together with HUA and AP together with PCKS can be explained by additional binding of HUA and PCKS with free domains. Concentrations of HUA and PCKS added in these experiments are too low and by themselves insufficient for their action of displacing erythrocytes from the liquid phase, especially in the presence of high concentrations of PA which are exceptionally active in this respect. Nevertheless, it is hardly possible to completely rule out any effect of added HUA and PCKS on this supplanting effect. Addition of small quantities of these proteoglycans to salt solutions containing PA can induce the formation of 3-dimensional complex structures with the ability to exert particularly powerful displacing action, and also the space filled with them relative to the cells, in the solution. Erythrocytes perhaps do not have particular domains which bind relatively specifically with PA, for PA macromolecules are constructed from hyaluronate, along which (with the participation of binding protein) PCKS are attached (insoluble fraction) [5]. For that reason PA may probably make contact with erythrocytes through domains specific for PCKS, since HUA, as a component of PA, is blocked by the last proteoglycan, in agreement with the very small increase in aggregation of intact erythrocytes induced by PA by the addition of PCKS to the medium.

Weakening of erythrocyte aggregation induced by HUA and PCKS by means of heparin, discovered previously [1, 6], is perhaps the result of blocking by heparin of domains with HUA and PCKS, for these domains may be overlapping.

The results suggest that the mechanism of aggregation of erythrocytes, and also of various other cells, by PA, HUA, and PCKS, may include interaction of these proteoglycans with definite chemical groups on the outer surfaces of the membranes, besides their principal action, i.e., displacement of the cells from the space in the medium occupied by proteoglycans and their concentration within a certain minimal volume. The problem of the quantitative ratio between the contributions of these two factors to aggregation is a matter for special investigation. Nevertheless it can be postulated that aggregation of FE, when domains binding proteoglycans are modified, takes place entirely on account of displacement of these erythrocytes from the space in solutions occupied by PA, HUA, and PCKS. This is in good agreement with the fact that PA, as the biocomplex with the highest molecular weight, is also the most active as regards aggregation of NE and FE.

It follows from this dependence of aggregation of intact erythrocytes on valency of the cations of normal salts of PA that the aggregating power of PA and also, evidently, of other simpler proteoglycans, is determined by conformational factors in the macromolecules of these biopolymers, for the conformation of salts of PA and other proteoglycans depends on valency of the cation [3, 4]. The question accordingly arises of the role of Ca^{++} in aggregation and adhesion as the direct binding component, in the form of what are called calcium bridges. This cation is perhaps essential for the formation of salts of proteoglycans.

Biological systems always contain several different proteoglycans [5, 12]. Probably, therefore, during aggregation and adhesion of cells the action of one of them, bound relatively specifically with the outer surface of the cell membrane, is reinforced by the nonspecific stereochemical (displacing) effect of other proteoglycans contained in the given system, as revealed by model systems and tissue cultures [2, 11]. The interactions noted above may be of essential importance in processes of morphogenesis, when sharp changes take place in the absolute content of the proteoglycans and also in the relations between the quantities of these biopolymers and glycoproteins [1, 5, 11, 12].

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CHANGES IN PASSIVE ELECTRICAL PROPERTIES OF ERYTHROCYTES DURING HEMOPERFUSION

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612.111.014.423

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The widespread use of hemoperfusion in clinical practice has necessitated the development of rapid methods of monitoring the state of the blood cells actually during hemoperfusion changes take place in the chemical composition and physical characteristics of the plasma membranes of the blood cells [1, 3]. One such method that may be suggested is to measure the passive electrical properties of blood cells and blood plasma, which depend on the functional state of the cells and tissues and may be drastically changed during the development of various pathological states (aging, malignant transformation, low temperature injury, and so on) [7-9].

The aim of this investigation was to study the electrical characteristics of erythrocytes (total impedance, capacitance and conductance of the membrane, conductance of the intra- and extracellular medium) under hemoperfusion conditions on intact rabbits *in vivo* and on erythrocytes from normal human blood donors *in vitro*.

EXPERIMENTAL METHOD

Hemoperfusion was performed on intact rabbits with an anteriovenous circuit, using columns with a capacity of 50 cm^3, packed with synthetic SKN-2K activated charcoal (Medical

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