Short communication

The Effect of Ionic Strength on the Age Dependent Stability of Rat Erythrocyte Membranes

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Many of the membrane functions are influenced by physical variables such as temperature, osmotic pressure, pH, ionic strength, etc. Certain electrolyte properties are better characterized by ionic strength than by ionic concentration because the former also allows for the interionic electrostatic forces. The principal processes in the living organism mostly require exactly defined physico-chemical conditions to operate. Ionic strength and pH of the medium were observed to affect the conformation changes in membrane proteins and formation of crystalline structures (Benga and Holmes 1984). These factors considerably influence that membrane fluidity which, in turn, affects many transport functions of the membrane (Coakley and Deeley 1980). Changes in rheological properties of erythrocytes determine its passage through narrow spaces in the microcirculation. Increased rigidity of the erythrocyte membrane is associated with reduced supply of oxygen and nutrients the tissues. In addition to physico-chemical factors, there are some other parameters that affect the elasticity of the erythrocyte membrane. All these effects are interrelated.

The aim of the present work was to obtained further information of the age dependent changes in erythrocyte membrane stability at various ionic strength of the medium.

Stability of the erythrocyte membranes was determined based on the hemolytic effect of brilliant cresyl blue (BCB) in isotonic medium; erythrocyte disintegration under these conditions depends on the elasticity of the erythrocyte membrane.

Blood samples were taken from 20 animals aged 90—105, 340—360, and 690—720 days each. Male rats of the Wistar strain were kept in standard conditions. The samples were taken by incision of the tail ends. Twenty five microliters of blood were aspirated into a heparinize capillary and added to 4975 microliters of the incubation medium of 7.4 pH and various ionic strength. The erythrocytes were incubated for 4 hours at 37 °C. One mmol BCB was then added to the solutions. The erythrocyte counts were determined by the classical

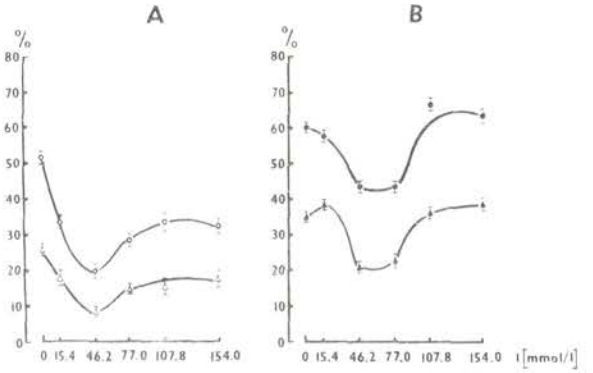

Fig. 1. The effect of ionic strength on the stability of erythrocyte membrane of 90—105 days old rats. *Ordinate*: Erythrocyte disintegration in %; *Abscissa*: Ionic strength of the incubation solution (mmol/l). *A*. Disintegration of erythrocytes in the absence of BCB, *B*. in the presence of BCB. Both after 2 hours of incubation (triangles) and after 4 hours (circles).

method before incubation (100 %) and after 2 and 4 hours of incubation.

Solutions of various ionic strength were prepared by mixing isotonic NaCl solution (154 mmol/l) with isotonic glucose solution (2775 mmol/l). BCB-induced disintegration of erythrocytes the 340—360 and 650—720 days old animals from studied in solutions with the following ionic strength: 0.0; 38.5; 77.0; 115.5; and 154.0 mmol/l. The ionic strengths user for the erythrocytes from 90—105 days old animals were as follows: 0.0; 15.4; 30.8; 40,2; 61.6; 77.0; 92.4; 107.8; 123.2; 138.0 and 154.0 mmol/l. Erythrocyte disintegration was measured both in the presence and absence of 1 mmol/l BCB. Mean values and their confidence intervals were calculated for each experimental run. The statistical significance of differences between the sets was tested by Student's *t*-test.

Fig. 1*A* and 1*B* show the results of the effect of ionic strength erythrocyte lysis in solutions with and without BCB obtained for the group of 90—105 days old animals. In the absence of BCB, erythrocyte disintegration depended on the ionic strength of the incubation medium. The disintegration effect was in pure isotonic glucose solution strongest with increasing ionic strength in decreased to reach a minimum at 46.2 mmol/l NaCl with a subsequent increase up 107.8 mmol/l NaCl. Further increasing ionic strength had no effect on erythrocyte disintegration.

In the presence of BCB, erythrocyte disintegration decreased sharply with increasing ionic strength up to 46.2 mmol/l NaCl. The minimum was obtained at 77.0 mmol/l NaCl; then, disintegration was sharply enhanced reaching a

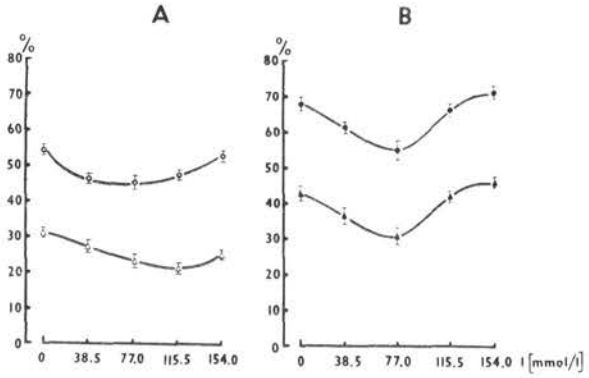

Fig. 2. The effect of ionic strength on the stability of erythrocyte membrane of 340—360 days old rats. For conditions and symbols see legent to Fig. 1.

maximum at 107.8 mmol/l NaCl. Again, further increasing ionic strength had no effect erythrocyte disintegration.

Fig. 2*A* and 2*B* show the effect of ionic strength of the incubation medium erythrocyte lysis for 340—360 days old rats. In the absence of BCB the erythrocyte disintegration decreased only moderately with the increasing ionic strength with a minimum at 77.0 mmol/l NaCl; subsequently, disintegration was moderate by enhanced reading a maximum at 154.0 mmol/l NaCl. The maxima after 2 and 4 hours of incubation are identical with that measured in solution containing isotonic glucose only. In the presence of BCB the decreased erythrocyte disintegration with increasing ionic strength was more remarkable; with a minimum at 77 mmol/l NaCl. Further increasing ionic strength resulted in an enhancement of the disintegration effect up to a maximum readed at 115.5 mmol/l NaCl; as comparised with the 90—105 days group however, this increase was less sharp. Raising the ionic strength to 154.0 mmol/l NaCl causes was associated with an only very slight enhancement of erythrocyte disintegration.

Fig. 3*A* and 3*B* show same plots for 690—720 days old rats. In the absence of BCB erythrocyte disintegration decreased only very moderately with increasing ionic strength, reading a minimum at 115.5 mmol/l NaCl. Raising ionic strength to 154.0 mmol/l NaCl slightly enhanced disintegration at both time intervals used. The values were similary to those obtained in pure glucose solutions; in the presence of BCB increasing ionic strength moderately decreased disintegration with a minimum at 115.5 mmol/l NaCl. Further increa-

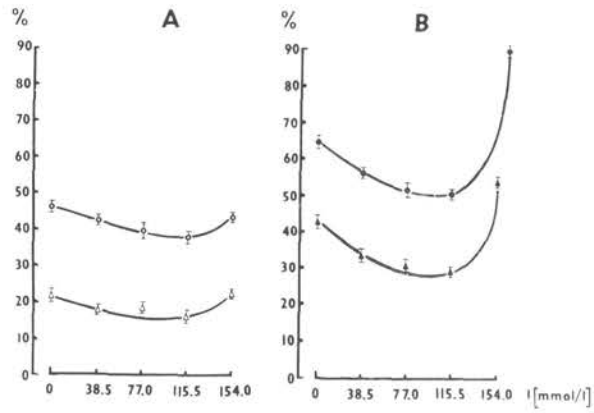

Fig. 3. The effect of ionic strength on the stability of erythrocyte membrane of 690—720 days old rats. For conditions and symbols see legend to Fig. 1.

sing ionic strength to 154.0 mmol/l NaCl however, strongly enhanced disintegration with the values exceeding these measured in pure glucose solution.

Erythrocyte membrane stability is determined by interaction between molecules of the individual membrane constituents i.e. phospholipids and proteins. Membrane fluidity reflects the relative freedom of lipid molecules to move in the membrane. An important factor of membrane fluidity is the vertical medium influences first of all the conformation of membrane proteins which, in turn significantly determines membrane fluidity (Benga and Holmes 1984). The ionic strength of blood is determined predominantly by sodium and chloride ions, and reads 160 mmol/l. The ionic strength of the intracellular medium is effectively controlled by the organism with minimum fluctuations occurring under normal conditions.

In our experiments the ionic strength of the incubation medium was varied between physiological values and zero. These variations have been expected to influence basically membrane fluidity and, consequently, also the lysis of erythrocytes in isotonic solutions of NaCl both without and with BCB; the presence of BCB enhanced the lysis. However minimal erythrocyte disintegration occurred at the same ionic strength values both in the presence and absence of BCB. The presence of BCB in the solution remarkably enhanced the influence of ionic strength on erythrocyte lysis. Our experiments have shown that optimum ionic strength, at which membrane was highest stability (and then erythrocyte disintegration is the lowest), changes with age gradually increasing from young to old rats: for 90—106 days old animals lowest disintegration was observed between

46.2—77.0 mmol/l NaCl while it was at 115.5 mmol/l for 690—720 days old rats. This could be explained by an optimum membrane fluidity reached at a certain ionic strength given by optimum hydration of the membrane components, in particular membrane proteins. Based on our results it may be assumed that the erythrocyte membrane undergoes qualitative changes during postnatal development. Similar conclusions could be drawn in our previous papers (Nicák 1986, 1987). Several method are presently being used for the determination of membrane fluidity. They allow to measure the fluidity of phospholipids in the outer membrane layer. The method used in this work, based on erythrocyte lysis by BCB, enables to measure fluidity of the membrane as a whole. At optimum membrane fluidity the membrane also has optimum stability as reflected by minimal erythrocyte lysis by BCB.

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