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EFFECT OF LOW CONCENTRATIONS OF CHLORPROMAZINE ON HUMAN ERYTHROCYTE DISTRIBUTION BY SPHERICAL INDEX AND ON TIME PERIOD OF HYPOTONIC HEMOLYSIS

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The possible consequences of chlorpromazine (CP) effect at low concentrations after its incorporation into erythrocyte membranes were analysed. Erythrocyte distribution by spherical index and the rate of hemolysis in hypotonic medium were studied in 0.125 mM and 0.25 mM CP. The investigation showed the non-uniform response of erythrocytes from different donors to CP introduced into suspension. Samples were divided into two groups according to their reaction to CP. In the first group of donors the CP introduction invoke full or partial shift of the distribution curves towards larger values of spherical index. In the second group CP introduction resulted in the rise of cell density in low spherical index interval. Thus the interaction of amphiphile substances with a membrane bilayer can evoke controversial effects. One of the possible effects of CP is intercalation of alkyl regions into the membrane bilayer. This can lead to an increase of membrane surface. On the other hand amphiphile incorporation into lipid bilayer can lead to the formation of inverted micelles. In this case the membrane surface diminishes comparing to the native one at the expense of transition of a part of membrane material to the described phase. Thus both the phenomena of stretching and constriction of membrane surface are equally probable in the studied CP concentration interval and depend on the population state of a donor.

Key words: erythrocytes, spherical index, chlorpromazine.

Amphiphilic substances under low concentrations are known to protect erythrocytes against hypotonic, hypertonic or mechanically induced lysis [1]. Presence of both stomacytogenic and echinocytogenic amphiphiles under high concentration results in erythrocyte spherification and lysis. Mechanism of antihemolytic effect of amphiphiles has not been completely elucidated yet. One considers that during building of amphiphilic substance into a lipid bilayer the membrane surface area augments. Therefore cell gains higher volume prior to lysis [1,2]. The other hypothesis considers an increased potassium leakage from cells during incorporation of amphiphilic molecules into membranes to be a possible protective mechanism [3].

Recently [4] we have proposed the method for determination of erythrocyte distribution density by spherical index, i.e. by the characteristics, uniformly associated to an erythrocyte surface-volume ratio. Spherical index is determined as the ratio of erythrocyte and sphere volumes with equal surface area (V_{sph}/V_{er}). For spherocyte this parameter makes 1, but for normocytes (biconcave disks) it gains the values, higher than 1. According to our data [4] an average value of spherical index for erythrocytes of healthy adult donors makes 1.48. The method proposed enables to determine not only an average value but the whole spectrum of this parameter in erythrocyte population.

We have selected a minimum CP concentration at hematocrit of ≈30% when erythrocyte shape did not visibly change, and this made 0.125 mM, or $2.3 \cdot 10^7$ CP molecules per one erythrocyte [5]. This value is close to that, calculated by Seeman [6] for different lipid-soluble and amphiphilic solutions under 50% protection against hypotonic hemolysis (maximum protection against hemolysis was observed under twice higher concentration [1]). Under 0.125 mM CP concentration the shape of majority of erythrocytes does not practically change, but single erythrocytes with one smoothened cavity (initial stage of transfer towards stomatocyte shape), as well as single stomatocytes-I are observed. With augmentation of CP concentration up to 0.25 mM the amount of stomatocytes-I increases and the stomatocytes-II appear [5].

Research was aimed to study the possible consequences of incorporation of chlorpromazine at low concentrations into erythrocyte membranes. Erythrocyte distribution on spherical index and rate of hemolysis in hypotonic medium were studied under 0.125 mM and 0.25 mM CP concentrations.

MATERIALS AND METHODS

The study was performed in donor blood erythrocytes, preserved with "Glygitsir" solution. Physiological solution and hypotonic solution of non-penetrative substance were prepared with sodium chloride. Chlorpromazine {(2-Chloro-10 [3-dimethylaminopropyl] phenothiazine) hydrohloride} (Sigma) was added to red blood cell suspension (30% hematocrit) at final concentrations 0.125 or 0.25 mM. The stock solution of 10 mM CP in physiological solution was used for dilutions.

Rate of hemolysis was measured by the small-angle light scattering method. At the initial moment, 3 μl of erythrocyte suspension were added to 3 ml of hypotonic aqueous solution of the non-penetrating substance (NaCl) in the chamber of the device for measuring of the intensity of the scattered light. The time dependence of the light scattered by the cell suspension was registered at 9° towards the reference beam (k = 1000 nm).

Density of erythrocyte distribution by spherical index was determined using the method, described elsewhere [4]. It is based on the physical and mathematical model for the membrane transport occurring in cells placed into the hypotonic solution of non-penetrating substance and uses the small-angle light scattering method for obtaining experimental curves of osmotic fragility.

RESULTS AND DISCUSSION

The investigation showed the non-uniform reaction of erythrocytes from different donors to CP introduction into suspension. Densities of erythrocyte distribution by spherical index for different donors are presented in Fig. 1 and 2. Fig. 1 represents the distribution by spherical index in the erythrocyte samples, there CP introduction invoke full or partial shift of the curves towards the larger values of spherical index. Centre of the distribution on Fig. 1a shifts towards a higher spherical index at 0.125 mM CP. With augmentation of CP concentration up to 0.25 mM the curve shift to the left, but the cell density in the area of low values of spherical index remained below the control population density.

Additional peak in distribution (erythrocyte fraction with low spherical index) on Fig. 1b shifts towards higher spherical indices both at 0.125 mM and 0.25 mM CP. The shift augments with rise in CP concentration. The distribution densities in Fig.1c and 1d have no additional distribution maxima in the low spherical index interval.

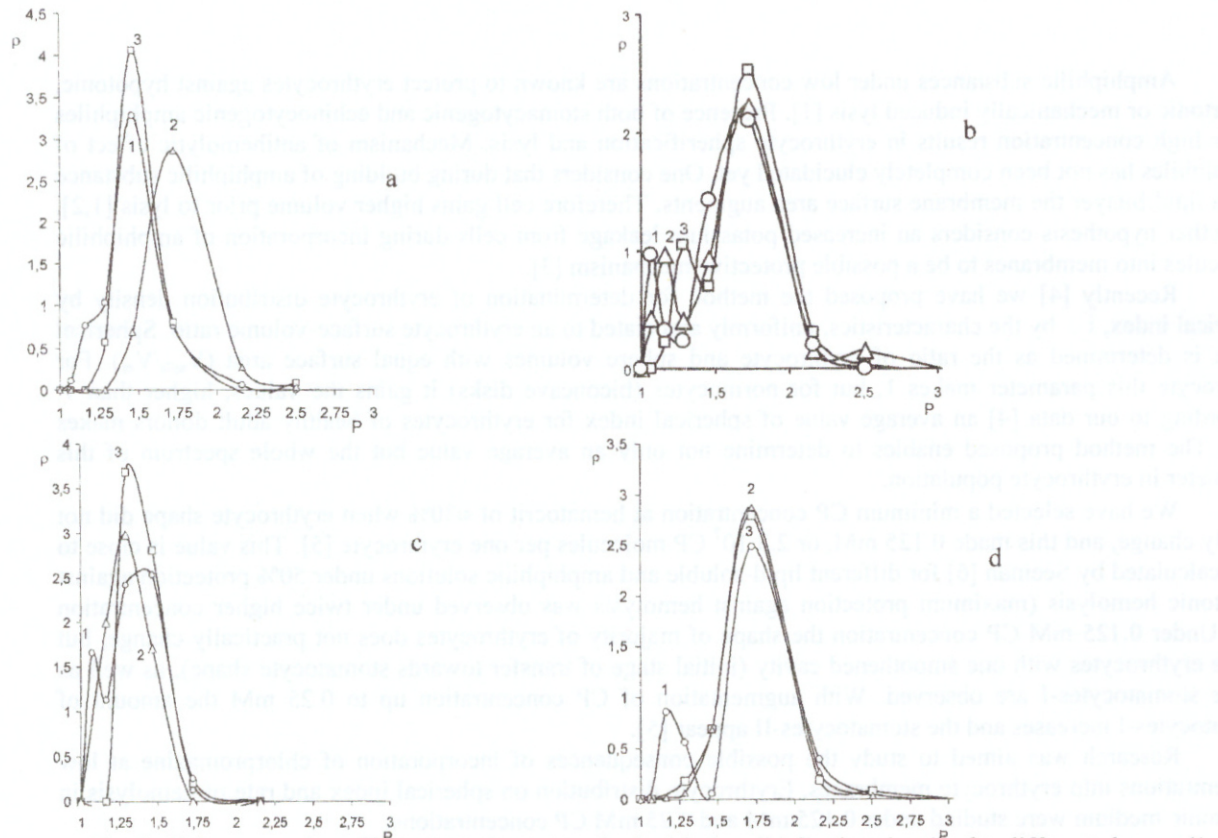

Fig. 1. Chlorpromasine effect on erythrocyte spherical index distribution density for different donors (1 – initial distribution; 2 – after addition of 0.125 mM CP; 3 – after addition of 0,25 mM CP): group A (with increased spherical index after CP addition).

Fig. 2 shows the distributions for erythrocyte population groups, where CP introduction usually resulted in a rise of cell density in the low spherical index interval. Distributions on Fig. 2a and 2b present the diminishing of cell density in the low spherical index interval after introducing of 0.125 mM CP. Vice versa the increasing of CP concentration up to 0.25 mM resulted in an augmentation of that parameter, manifested in appearing of additional cell fraction with low spherical index. In population shown on Fig. 2c, adding both 0.125 and 0.25 mM CP resulted in appearance of cell fraction with low spherical index rising in number with increase of CP concentration. Fig. 2d presents the population with a full shift of cell distribution towards the low spherical index interval when using both CP concentrations.

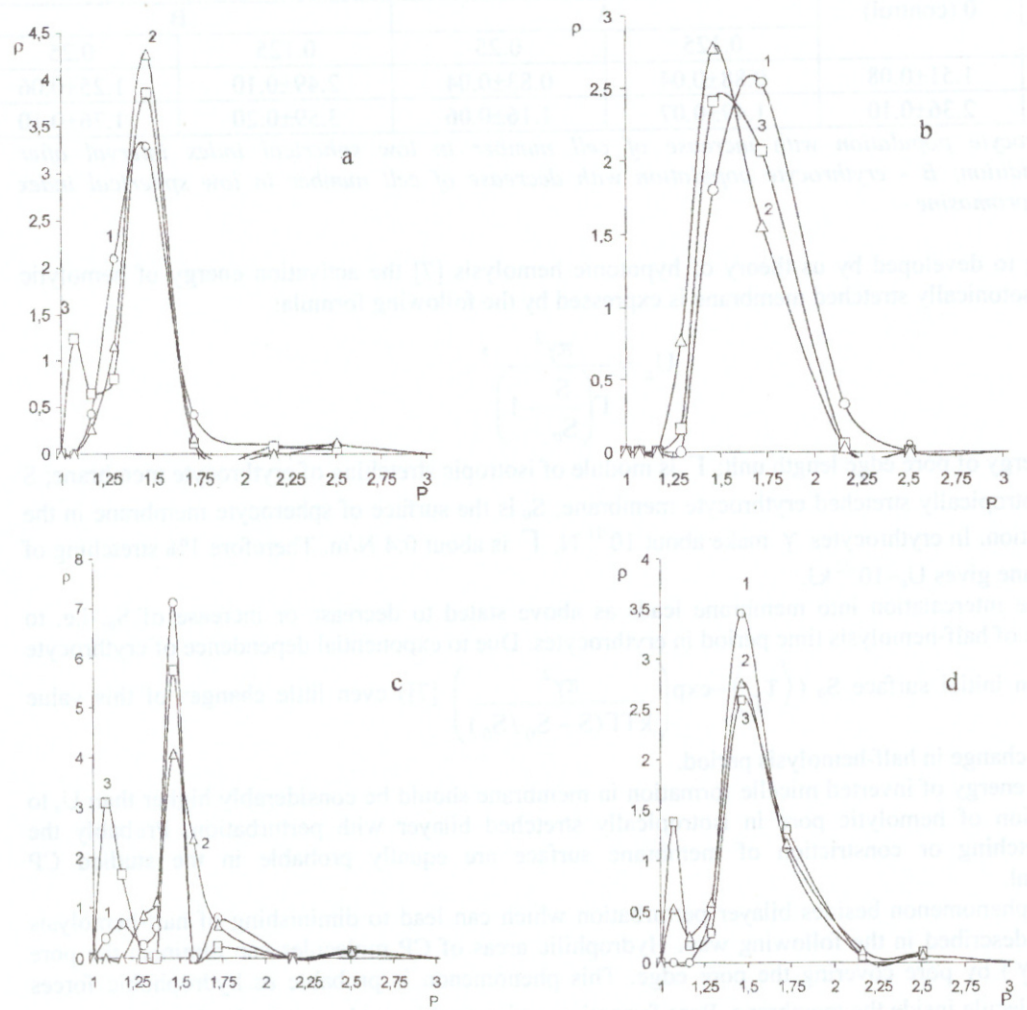

Fig. 2. Chlorpromasine effect on erythrocyte spherical index distribution density for different donors (1 – initial distribution; 2 – after addition of 0.125 mM CP; 3 – after addition of 0,25 mM CP): group B (with decreased spherical index after CP addition).

Investigation of a period of erythrocyte hemolysis in hypotonic solutions of sodium chloride did not reveal a clear result. When analysing the experimental data the erythrocyte populations were divided into two groups: those where CP addition led to increase of cell density in low spherical index interval (group A) and those with the inverse effect (group B) (Table). It is worth to note that if adding 0.125 mM CP the half-hemolysis period in group A diminished and in group B increased, than adding 0.25 mM CP the period decreased in both groups, but in group B it was less considerable.

Thus the interaction of amphiphile substances with bilayer membrane can evoke controversial effects. On the one hand intercalation of alkyl regions into bilayer can lead to increase of membrane surface. On another hand amphiphile incorporation into lipid bilayer can lead to formation of inverted micelles (H_{II}-phase), composed of lipid molecules and membrane intercalated amphiphile molecules, and located between two monolayers of bilayer membrane [1]. Such a micelle formation eliminates apparently a misbalance between monolayers expanding due to amphiphile intercalation. In this case the membrane surface can diminish

comparing to the native one because of transition of a part of membrane material to the described phase. In the first of stated cases the critical volume, when hypotonic hemolysis starts, increases, in the second case – it decreases.

Table. Period of erythrocyte half-hemolysis ($\tau_{1/2}$, sec) in hypotonic solutions of sodium chloride

NaCl concentration	Chlorpromasine concentration, mmol				
	0 (control)	A		B	
		0.125	0.25	0.125	0.25
0.3%	1.51±0.08	0.88±0.04	0.83±0.04	2.49±0.10	1.25±0.06
0.4%	2.36±0.10	1.49±0.07	1.16±0.06	3.59±0.20	1.76±0.10

Note: A – erythrocyte population with increase of cell number in low spherical index interval after chlorpromasine addition; B - erythrocyte population with decrease of cell number in low spherical index interval after chlorpromasine

According to developed by us theory of hypotonic hemolysis [7] the activation energy of hemolytic pore formation in isotonically stretched membrane is expressed by the following formula:

$$U_a = \frac{\pi\gamma^2}{\Gamma\left(\frac{S}{S_0} - 1\right)},$$

where γ is free energy of pore edge length unit; Γ is module of isotropic stretching of erythrocyte membrane; S is the surface of isotropically stretched erythrocyte membrane; S_0 is the surface of spherocyte membrane in the absence of deformation. In erythrocytes γ make about 10^{-11} N, Γ is about 0.4 N/m. Therefore 1% stretching of erythrocyte membrane gives $U_a \sim 10^{-22}$ kJ.

Amphiphile intercalation into membrane leads as above stated to decrease or increase of S_0, i.e. to decrease or increase of half-hemolysis time period in erythrocytes. Due to exponential dependence of erythrocyte hemolysis period on initial surface S_0 ($\langle \tau_p \rangle \sim \exp\left(\frac{\pi\gamma^2}{kT\Gamma(S - S_0/S_0)}\right)$ [7]) even little changes of this value result in substantial change in half-hemolysis period.

Activation energy of inverted micelle formation in membrane should be considerably higher than U_a to achieve the formation of hemolytic pore in isotropically stretched bilayer with perturbation. Probably the phenomena of stretching or constriction of membrane surface are equally probable in the studied CP concentration interval.

One more phenomenon besides bilayer perturbation which can lead to diminishing of half-hemolysis time period can be described in the following way. Hydrophilic areas of CP molecules can diminish the pore edge free energy (γ) by pore covering the pore edge. This phenomenon is probable as hydrophobic forces endeavor the CP molecule inside the membrane. Pore formation owing to this can be energetically advantageous as it diminishes the energy of CP molecule hydrophilic parts pushing out of membrane. The non-intercalated into membrane amphiphile part of the molecule covers the membrane surface and increases the module of its isotropic stretching (Γ) proportionally to the width of CP layer adsorbed on membrane.

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