Effect of Lysophosphatidylcholine on Salt Permeability through the Erythrocyte Membrane under Haemolytic Conditions

S. Eskelinen

Department of Physiology, University of Oulu, SF-90220 Oulu, Finland

Abstract. Human erythrocytes were incubated in haemolytic salt or sucrose media and the amount of potassium and haemoglobin released were monitored. In hypotonic NaCl and KCl solutions potassium release and haemolysis increased with time showing that the cell membrane had been injured and became permeable to intra- and extracellular cations which, due to intracellular haemoglobin, causes water influx and continuous haemolysis. Both potassium release and haemolysis remained, however, at their 2-minute level in the presence of LPC. Thus, LPC could reseal the membrane and prevent continuous salt fluxes. It protected erythroctes from hypotonic haemolysis and the protection was more efficient in NaCl than in sucrose media. This suggests that the increase in the critical volume of erythrocytes caused by LPC occurs both in electrolyte and sucrose media, and the additional protection observed in electrolyte media is due to the resealing of the injured cell membrane by LPC. The repairing mechanism was mediated via the membrane lipids or integral proteins, since the time-course of haemolysis of erythrocytes swollen in NaCl media at the spectrin-denaturing temperature of 49.5 °C was similar to that at room temperature with and without LPC. LPC did not protect erythrocytes from colloid osmotic haemolysis caused by ammonia influx in an isotonic NH_4Cl medium, but protected the cells from colloid osmotic haemolysis caused by sodium influx through nystatin-channels in NaCl media without any area or volume increase. Hence, LPC could not prevent ammonia influx through the lipid bilayer, but suppressed sodium influx through nystatin-channels presumably via LPC interference with cholesterol.

Key words: Cytoskeleton — Lipid bilayer — Membrane injury — Membrane recovery — Osmotic swelling

Introduction

Many detergents and drugs prevent hypotonic haemolysis of erythrocytes. The expansion of the cell membrane and, thus, an increase of the critical cell volume has been suggested as an explanation (Seeman et al. 1969; Seeman 1972). Before lysis, the swollen cells remain spherical from 1.5 s to several minutes, the period depending on the osmotic pressure of the medium (Jay and Rowlands 1975; Jay 1978; Bowdler and Chan 1969; Chan et al. 1975). During this period the cells leak potassium ions (Jay and Rowlands 1975; Jay 1978) and, in NaCl media, also sodium ions enter the cells (Chan et al. 1975). The ability of detergents to prevent haemolysis by repairing the membrane injury during this leaking period has not been investigated, even though it has been shown that, e.g. lysophosphatidylcholine (LPC) enters the inner leaflet of the cell membrane during haemolysis (Lange et al. 1982) and many detergents can reseal the erythrocyte ghosts (Johnson 1982). In the present work the ability of LPC to repair the erythrocyte membrane during the transient leaking period of swollen cells in hypotonic electrolyte and sucrose media or during the continuous influx of ions or molecules during colloid osmotic lysis of human erythrocytes has been investigated.

Materials and Methods

Materials: Fresh human blood with a citrate-phosphate-dextrose solution as an anticoagulant was supplied by the Finnish Red Cross Blood Transfusion Service, Oulu, and the experiments were started within two hours of donation. Before the experiments, the erythrocytes were washed once with an isotonic NaCl medium, the buffy coat was removed and the cells were resuspended in the plasma to obtain a haematocrit of 50 %.

The incubation media were phosphate-buffered NaCl or KCl (various concentrations of NaCl or KCl, 5.8 mmol·l^{-1} phosphate, pH 7.4), phosphate-buffered NaCl-sucrose (various concentrations of NaCl, 27 mmol·l^{-1} sucrose, 5.8 mmol·l^{-1} phosphate, pH 7.4), Tris-HCl-buffered sucrose (various concentrations of sucrose, 15 mmol·l^{-1} Tris(hydroxymethyl)-aminomethan, pH 7.4) solutions or an isotonic ammonium chloride solution (150 mmol·l^{-1} NH_4Cl, pH 7.4 with NaOH), the osmolalities of which were checked with a Knauer's freezing point osmometer. Lysophosphatidylcholine (Sigma Chemical Co., USA) was dissolved in absolute ethanol to a concentration of 5 mmol·l^{-1} (2.5 mg/ml) and nystatin (Sigma Chemical Co.) in methanol to a concentration of 5 mg/ml, and both were diluted with the incubation media to obtain the concentrations desired.

Haemolysis experiments: Washed erythrocytes resuspended in the plasma were diluted 1:1 with an isotonic NaCl solution and preincubated for 30 minutes at room temperature for the experiments at room temperature, and at 44—45 °C for the experiments at elevated temperatures. The experiments for rapid haemolysis at room temperature were performed by pipetting 20 µl of the preincubation mixture into 5 ml of isotonic or hypotonic medium. After incubation for a given time period (1 to 60 minutes) haemolysis was stopped by adding 5 ml of a corresponding hypertonic medium, which rendered the medium isotonic. The unhaemolyzed cells were immediately removed by centrifugation at

2000 × *g* for 10 minutes (Parpart et al. 1947; Eskelinen and Bernhardt 1984; Eskelinen and Saukko 1984).

For the experiments at elevated temperatures, the incubation media were preincubated at 44—45 °C in test tubes, with the sample temperature being monitored using a thermistor. The tube and its thermistor was immersed in another water bath set at 77—78 °C, and 20 µl of the preincubation mixture was pipetted into the medium in the test tube once the target temperature (spectrin denaturing temperature 49.5 °C, Brandts et al. 1977) had been reached. The tube was then removed from the hot water bath and rapidly cooled to room temperature in a third water bath (Coakley et al. 1983). Incubation was contifued at room temperature and stopped after various time periods by adding the hypertonic media. The unhaemolyzed cells were centrifuged out as above. Osmotic swelling of erythrocytes occurs within 1—2 seconds (Jay 1978; Eskelinen and Coakley 1986) and thus the cells had swollen at the desired temperature before cooling; the subsequent incubation, however, was always carried out at room temperature. Because of rapid swelling the cells did not fragment, as normally happens to discocytes exposed to the temperature of 49.5 °C (Eskelinen et al. 1985).

In the experiments with LPC the hypotonic incubation media contained 4 µmol·l^{-1} LPC, a concentration giving maximum protection against hypotonic haemolysis (Eskelinen and Saukko 1984). Otherwise, the experiments were carried out as above.

In the experiments with nystatin the erythrocytes were pretreated with nystatin before the swelling experiments to open the cation channels of the membrane (Cass and Dalmark 1973; Freedman and Hoffmann 1979). An aliquot of 8 ml of washed blood was diluted with 100 ml of an isotonic NaCl-sucrose medium (138.6 mmol·l^{-1} NaCl, 27 mmol·l^{-1} sucrose, 5.8 mmol·l^{-1} phosphate, pH 7.4) containing 50 µg/ml nystatin, and incubated for 30 minutes at + 4 °C in the dark. The cells were washed once with the same medium and resuspended in the same isotonic NaCl-sucrose solution containing 50 µg/ml nystatin to obtain a haematocrit of approx. 50 %. This suspension was stored at + 4 °C and used on the same day. The preincubation mixture for the experiments with nystatin-treated cells consisted of an isotonic NaCl-sucrose solution with 50 µg/ml nystatin diluted 1:1 with the nystatin-treated cell suspension. The haemolysis experiments were carried out as above, except that all the incubation media contained 50 µg/ml nystatin.

The haemoglobin content was determined from the absorbance of the supernatants obtained in the haemolysis experiments at 540 nm (Parpart et al. 1947), and the potassium content by atomic absorption spectrophotomery. The absorbance for the potassium measurements in the NaCl and sucrose media were corrected in order to be mutually comparable by reference to KCl standards produced in isotonic NaCl and sucrose solutions (Eskelinen and Saukko 1984).

Scanning electron microscopy: For scanning electron microscopy, an aliquot of 20 µl of the preincubation mixture was pipetted onto a piece of Nuclepore filter floating in a test tube in 5 ml of the medium to be investigated. The same volume (5 ml) of 2 % glutaraldehyde (GA) prepared with phosphate-buffered water (pH 7.4), containing the same concentrations of LPC or nystatin as did the incubation medium, was then added slowly to the cell suspension drop by drop to prevent stomatocytosis of the swollen cells induced by GA (Eskelinen and Saukko 1983). The method developed by Wollweber and his co-workers (1981) for postfixation was used to prevent cell shrinkage during critical point drying. The cells attached to the Nuclepore filter and fixed with GA for 2 hours were postfixed in 0.5 % OsO_4 solution for 45 minutes, in 1 % tannic acid solution for 1 hour and in 0.5 % uranyl acetate solution in 10 % ethanol for 1 hour. After postfixation the cells on the filter were dehydrated with an ascending ethanol series, critical point dried with CO_2 and coated with a gold-palladium mixture by sputtering. They were examined in a JEOL JSM-35 scanning electron microscope (SEM) operated at 15 kV.

The dimensions of the swollen cells with or without LPC treatment were determined from calibrated SEM micrographs photographed at a magnification of × 4000 (Eskelinen and Saukko 1983).

Results

Haemolysis in hypotonic media

Figs. 1*a* and 2*a* confirm the observations by Bowdler and Chan (1969) and Chan et al. (1975) of a continuous increase in potassium release and haemolysis as a function of time in hypotonic NaCl media, and their constancy in sucrose media. The time needed for potassium release or haemolysis to increase in the NaCl media were considerably longer than the 1—2 seconds needed to reach an osmotic equilibrium across the cell membrane after a change in the ambient osmotic pressure (Jay 1978). Hence, the continued increase in haemolysis cannot be due to the primary water influx, and there must be a cell population which becomes injured during sphering to permit potassium efflux and sodium influx. With the cell membrane permeable to extracellular cations intracellular haemoglobin molecules induce in NaCl media an excess water influx and haemolysis. Sodium influx in conjuction with osmotic swelling has been confirmed experimentally by Chan et al. (1975). The behaviour of erythrocytes in KCl media was similar to that in NaCl media (Fig. 3*a*); consequently the membrane becomes permeable not only to sodium, but also to other small extracellular ions as a result of osmotic swelling (Bowdler and Chan 1969).

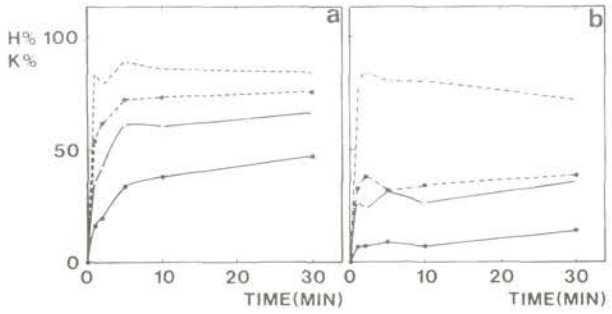

Fig. 1. Degree of haemolysis (H %) and potassium release (K %) calculated as percentages of haemolysis and potassium release in water as a function of time at room temperature (*a*) in 65 mmol·l^{-1} NaCl solution (H %, ●—●; K %, ○—○) or 61 mmol·l^{-1} NaCl solution (H %, ●—●; K %, ○- -○) without LPC, and (*b*) in 65 mmol·l^{-1} NaCl solution (H %, ●—●; K %, ○—○) or 56 mmol·l^{-1} NaCl solution (H %, ●-.-●; K %, ○-.-○) with 4 μmol·l^{-1} LPC.

The potassium release or haemolysis in the presence of 4 μmol·l^{-1} LPC did not continue to increase as a function of time as clearly as it was the case without LPC in electrolyte media (Figs. 1 and 3). Hence, LPC may be assumed to have a resealing effect on the membrane and to close the membrane to electrolyte fluxes which induce water influx and maintain continuous haemolysis. This is confirmed

by the greater differences between haemolysis observed in NaCl than in sucrose media with and without LPC, especially after 30 minutes incubation (Figs. 1—2, Table 1). The solutions compared in Table 1 (65 mmol·l^{-1} NaCl vs. 88 mmol·l^{-1} sucrose, and 56 mmol·l^{-1} NaCl vs. 82 mmol·l^{-1} sucrose) were chosen so that the degree of haemolysis after 30 minutes incubation in these solutions without LPC will be at the same level (47 % in 65 mmol·l^{-1} NaCl solution, 84 % in 56 mmol·l^{-1} NaCl solution, 51 % in 88 mmol·l^{-1} NaCl sucrose and 77 % in 82 mmol·l^{-1} sucrose solution, respectively). Hence, the haemolytic conditions were comparable regardless of the different osmolalities of the NaCl and sucrose solutions used. LPC also prevented continuous haemolysis in KCl media (Fig. 3*b*) showing that the ability of LPC to reseal the membrane injury was not specific to sodium.

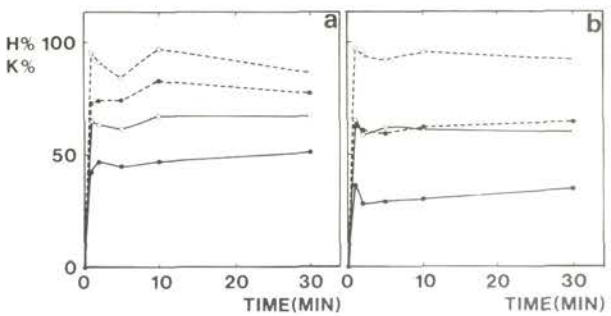

Fig. 2. Degree of haemolysis and potassium release as a function of time at room temperature (*a*) in 88 mmol·l^{-1} sucrose solution (H %, ●—●; K %, ○—○) or 82 mmol·l^{-1} sucrose solution (H %, ●- -●, K %, ○- -○) without LPC, and (*b*) in 88 mmol·l^{-1} sucrose solution (H %, ●—●; K %, ○—○) or 82 mmol·l^{-1} sucrose solution (H %, ●- -●; K %, ○- -○) with 4 μmol·l^{-1} LPC.

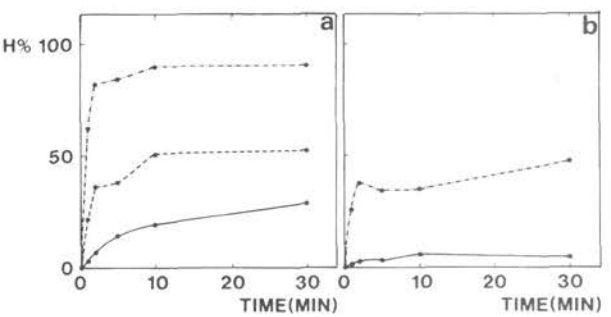

Fig. 3. Degree of haemolysis calculated as a percentage of haemolysis in water as a function of time at room temperature (*a*) in 69 mmol·l^{-1} KCl solution (●—●), in 65 mmol·l^{-1} KCl solution (●- -●) or 56 mmol·l^{-1} KCl solution (●-.-●) without LPC, and (*b*) in 69 mmol·l^{-1} KCl solution (●—●) or in 56 mmol·l^{-1} KCl solution (●-.-●) with 4 μmol·l^{-1} LPC.

Table 1. Differences in the degree of haemolysis of erythrocytes (H%) in the absence or presence of 4 μmol·l^{-1} LPC after 2 or 30 minutes' incubation in hypotonic NaCl or sucrose media.

Medium	$^{H\%}$no LPC$^{-H\%}$ LPC *after 2 minutes'* incubation	$^{H\%}$no LPC$^{-H\%}$ LPC *after 30 minutes'* incubation
65 mmol·l^{-1} NaCl	13.0	33.4
56 mmol·l^{-1} NaCl	37.5	44.3
88 mmol·l^{-1} sucrose	18.7	17.2
82 mmol·l^{-1} sucrose	13.8	12.2

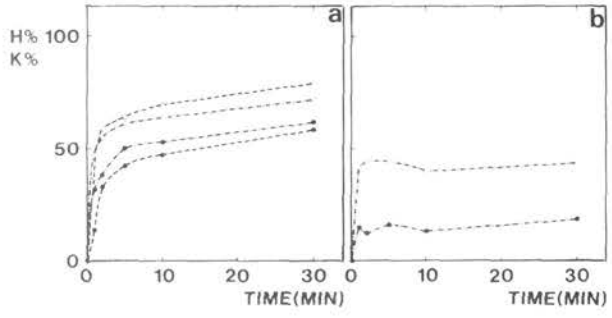

Fig. 4. Degree of haemolysis and potassium release as a function of time at 49—50 °C (*a*) in 61 mmol·l^{-1} NaCl solution (H %, ●- -●; K %; ○- -○) or 56 mmol·l^{-1} NaCl solution (H %, ●-.-●; K %, ○-.-○) without LPC, and (*b*) in 56 mmol·l^{-1} NaCl solution with 4 μmol·l^{-1} LPC (H %, ●-.-●; K %, ○-.-○).

The site of LPC action in the membrane was investigated in experiments on erythrocytes with spectrin denatured by heating to 49—50 °C during swelling (Brandts et al. 1977). Since, after swelling at 49—50 °C, haemolysis in NaCl media was time-dependent, the membrane had been rendered irreversibly permeable to salts (Fig. 4*a*). LPC prevented continuous haemolysis as at room temperature (Fig. 4*b*) suggesting that the influence of LPC was mediated via the lipid bilayer and integral proteins and was independent of the state of the cytoskeleton.

Colloid osmotic haemolysis

Preincubation of erythrocytes in an isotonic NaCl-sucrose medium with nystatin for 30 minutes at +4 °C opened the cation channels, since the degree of haemolysis was lower in the media with 27 mmol·l^{-1} sucrose as compared with NaCl media (Fig. 5). The fact that haemolysis increased with time suggests a continuous influx of sodium and water (Fig. 6).

LPC protected the erythrocytes from haemolysis in all the isotonic and hypotonic NaCl media used and over all the given time periods (Figs. 5 and 6). This prevention of haemolysis by LPC must have been due to a reduced salt permeability of the membrane or to an increased rate and extent of resealing of the membrane, since LPC did not protect erythrocytes from haemolysis in NaCl-sucrose mixtures where colloid osmotic haemolysis is prevented, but increased it (Fig. 5). Also LPC did not increase the area and volume of nystatin-treated cells (Table 2). The areas of nystatin-treated erythrocytes observed in the presence of 4 μmol·l^{-1} LPC in hypotonic media did not differ significantly from those without LPC, and the mean volume in a 81 mmol·l^{-1} NaCl solution with 4 μmol·l^{-1} LPC, which differed significantly from that without LPC, was smaller (Table 2). Also,

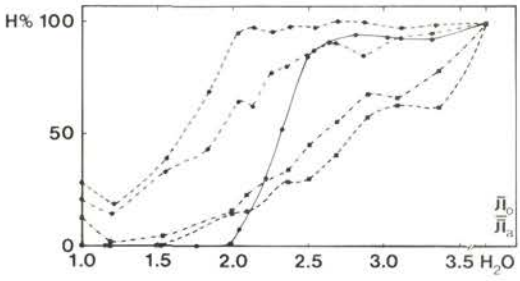

Fig. 5. Degree of haemolysis of untreated erythrocytes (●—●), nystatin-treated cells with (●-.-●) or without 4 μ mol·l^{-1} LPC (●- -●) after rapid swelling and 15 minutes' incubation in NaCl media, or nystatin-treated cells with (■-.-■) or without 4 μmol.l^{-1} LPC (■-■) in NaCl-sucrose mixtures (various concentrations of NaCl, 27 mmol·l^{-1} sucrose) as a function of the relative inverse ambient osmotic pressure of the media (π_o isotonic, π_h hypotonic osmotic pressure).

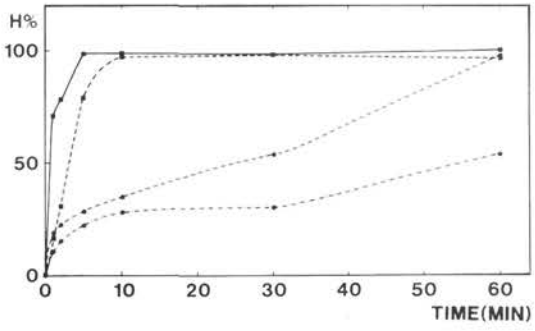

Fig. 6. Degree of haemolysis of untreated erythrocytes incubated in isotonic NH_4Cl solution at room temperature with (■—■) or without 4 μmol·l^{-1} LPC (■- -■), and degree of haemolysis of nystatin-treated erythrocyt›s incubated in isotonic NaCl solution at room temperature with (●-.-●) or without 4 μmol·l^{-1} LPC (●- -●) represented as a function of time.

differences between haemolysis with and without LPC increased with time (Fig. 6) instead of decreasing, as would be expected if the mechanism of protection were an increase in cell volume.

Table 2. Area and volume of nystatin-treated erythrocytes in experiments conducted with or without 4 $\mu mol \cdot ^{-1}$ LPC[a]

Medium ($mmol \cdot l^{-1}$ NaCl)	Number of cells scored	Area (μm^2)	Volume (μm^3)
80.9	94	139.4 ± 3.2	157.6 ± 5.5
80.9 (with 4 $\mu mol \cdot l^{-1}$ LPC	78	131.0 ± 2.7	142.8 ± 4.4[b]
60.8	72	160.1 ± 3.2	192.5 ± 5.6
60.8 (with 4 $\mu mol \cdot l^{-1}$ LPC)	65	156.3 ± 2.4	184.8 ± 4.2

[a] The cells were incubated at various osmotic pressures and fixed after 5 minutes of incubation. Values are means ± standard errors of the mean. Statistical analysis was carried out using the Kolmogorov test for normality and the Bartlett test for the equivalence of variances continued with Student's t test for difference between mean values.

[b] The values differ almost significantly from the corresponding values without LPC in the same medium ($p < 0.05$).

Contrary to colloid osmotic haemolysis caused by cation influx, LPC was not able to prevent colloid osmotic haemolysis caused by the influx of ammonia, but enhanced it (Fig. 6). Hence, LPC cannot prevent haemolysis due to permeation of molecules which do not use specific routes for influx (Jausel-Hüsken and Deuticke 1981).

Discussion

There is evidence that the dynamics of the haemolytic hole is determined by the lipids in the membrane bilayer, presumably balanced by electrostatic expansion of membrane components and by hydrophobic effects at the edge of the hole in the bilayer, with the cytoskeleton mainly passively preventing the membrane swelling or resealing (Makowski 1976; Lieber and Steck 1982; Minetti and Ceccarini 1982; Eskelinen et al. 1985). This is supported by the observation made in the present work that the behaviour of potassium release and haemolysis as a function of time was independent of the state of the cytoskeleton, and only the final degree of haemolysis in the particular medium was lower at higher temperatures when incubation of the heated cells was continued at room temperature (Figs. 1 and 4). Membrane components which participate in the resealing of ghosts to become

impermeable to dextran have been shown to be located at the inner layer of the membrane (Minetti and Ceccarini 1982). Lange et al. (1982) have shown that LPC, which usually penetrates very slowly into the inner lipid bilayer, was shifted during haemolysis to the inner lipid bilayer to a considerable extent, and they suggest the perimeter of the haemolytic hole to be the pathway for the transfer. Thus, the amphipathic LPC molecules shifted to the edges of the haemolytic hole and the inner lipid bilayer could decrease the energetic barrier between the water aggregate in the haemolytic hole and the hydrophobic lipid bilayer, and promote in this way membrane resealing. Some detergents have been already shown to promote the resealing properties of erythrocyte ghosts (Johnson 1982; Lieber and Steck 1982), and the rate constant of rubidium efflux from cells incubated in hypotonic media with LPC did not significantly differ from normal cells (Eskelinen and Bernhardt 1984). Thus, the erythrocytes can be protected against haemolysis by increasing their critical volume, but also by repairing the cell membrane injury. The increase of the critical volume by LPC may be the main mechanism of protection against haemolysis in sucrose media, and the greater differences between haemolysis with and without LPC in electrolyte media in comparison with sucrose media (Table 1) can be explained with the recovery of the membrane during leaking period and the suppression of salt fluxes by LPC.

In the case of colloid osmotic swelling caused by ammonia or salt influx the situation is different from hypotonic haemolysis: water flows in continuously, and an expanding of the membrane area or a resealing of the membrane injury once the osmotic equilibrium has been reached is insufficient to prevent lysis as in hypotonic haemolysis, but the primary cause to water influx must be abolished. LPC did not prevent haemolysis caused by ammonia influx and, thus, it could not prevent ammonia influx which takes place through the lipid bilayer. This is consistent with the observation of Deuticke et al. (1981) that exogenous LPC leaves the translipid permeation unaltered. In the case of colloid osmotic swelling caused by sodium influx through nystatin channels, the degree of haemolysis was lower with LPC than without it (Figs. 5—6). Thus, the influx of sodium must have been slowed down by LPC. Exogenous LPC has been already shown to inhibit the mediated transport processes of L-lactate, L-arabinose or oxalate (Deuticke et al. 1981). These authors suggested that the transport inhibition might either occur in the outer lipid layer by direct perturbation of the domain of the transport protein located there, or might be a consequence of a passive expansion of the membrane following the insertion of LPC into the outer leaf (Deuticke et al. 1981). Nystatin forms channels by associating with the membrane cholesterol (Cass and Dalmark 1973) and LPC has been also shown to form complexes with cholesterol and to influence the activity of membrane-bound enzymes (Chauhan et al. 1984). Thus, LPC may influence the properties of nystatin-channels, slow down sodium influx and protect in this way erythrocytes against colloid osmotic lysis.

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References

Bowdler A. J., Chan T. K. (1969): The time course of red cell lysis in hypotonic electrolyte solutions. J. Physiol (London) **201**, 437—452

Brandts J. F., Erickson L., Lysko K., Schwartz A. T., Taverna R. D. (1977): Calorimetric studies of the structural transitions of the human erythrocyte membrane. The involvement of spectrin in the A transition. Biochemistry **16**, 3450—3454

Cass, A., Dalmark M. (1973): Equilibrium dialysis of ions in nystatin-treated red cells. Nature **244**, 47—49

Chan T. K., La Celle P. L., Weed R. I. (1975): Slow phase hemolysis in hypotonic electrolyte solutions. J. Cell. Physiol. **85**, 47—57

Chauhan V. P. S., Ramsammy L. S., Brockerhoff H. (1984): Molecular interactions in the hydrogen belts of membranes. Glucose-6-phosphatase, lysophosphatidylcholine, and cholesterol. Biochim. Biophys. Acta **772**, 239—243

Coakley W. T., Nwafor A., Deeley J. O. T. (1983): Tetracaine modifies the fragmentation mode of heated human erythrocytes and can induce heated cell fusion. Biochim. Biophys. Acta **727**, 303—312

Deuticke B., Grunze M., Forst B., Luetkemeier P. (1981): Influence of enzymatic phospholipid cleavage on the permeability of the erythrocyte membrane: III. Discrimination between the causal role of split products and of lecithin removal. J. Membrane Biol. **59**, 45—55

Eskelinen S., Bernhardt I. (1984): The effect of an osmotic pressure gradient and lysophosphatidylcholine on the transient and constant potassium permeability properties of the erythrocyte membrane. Biomed. Biochim. Acta **43**, 947—953

Eskelinen S., Coakley W. T. (1986): Microscopic observation and video recording of cellular response to sudden changes in environment J. Microsc. **141**, 119—126

Eskelinen S., Saukko P. (1983): Effects of glutaraldehyde and critical point drying on the shape and size of erythrocytes in isotonic and hypotonic media. J. Microsc. **130**, 63—71

Eskelinen S., Saukko P. (1984): The hypotonic hemolysis and the protective action of lysophosphatidylcholine. Biorheology **21**, 363—377

Eskelinen, S., Coakley, W. T., Tilley, D. (1985): Thermal denaturation of the erythrocyte cytoskeleton alters the morphological changes associated with osmotic swelling. J. Therm. Biol. **10**, 187—190

Freedman J. C., Hoffman J. F. (1979): Ionic and osmotic equilibria of human red cells treated with nystatin. J. Gen. Physiol. **74**, 157—158

Jausel-Hüsken S., Deuticke B. (1981): General and transport properties of hypotonic and isotonic preparations of resealed erythrocyte ghosts. J. Membrane Biol. **63**, 61—70

Jay A. W. L. (1978): Hydraulic permeability coefficients of individual human erhtrocytes. Can. J. Physiol. Pharmacol. **56**, 458—464

Jay A. W. L., Rowlands S. (1975): The stages of osmotic haemolysis. J. Physiol. (London) **252**, 817—832

Johnson R. M. (1982): Promotion of membrane resealing by local anesthetics. Biochem. Pharmacol. **31**, 1784—1787

Lange Y., Gough A., Steck T. L. (1982): Role of the bilayer in the shape of the isolated erythrocyte membrane. J. Membrane Biol. **69**, 113—123

Lieber M. R., Steck T. L. (1982): Dynamics of the holes in human erythrocyte membrane ghosts. J. Biol. Chem. **257**, 11660—11666

Makowski L. (1976): A phase transition model for hemolysis. J. Theor. Biol. **61**, 47—53
Minetti M., Ceccarini M. (1982): Protein-dependent lipid lateral phase separation as a mechanism of human erythrocyte ghost resealing. J. Cell. Biochem. **19**, 59—75
Parpart A. K., Lorenz P. B., Parpart E. R., Gregg J. R., Chase A. M. (1947): The osmotic resistance (fragility) of human red cell. J. Clin. Invest. **26**, 636—640
Seeman P. (19/2): The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev. **24**, 583—655
Seeman P., Kwant W. O., Sauks T., Argent W. (1969): Membrane expansion of intact erythrocytes by anesthetics. Biochim. Biophys. Acta **183**, 490—498
Wollweber L., Stracke R., Gothe U. (1981): The use of a simple method to avoid cell shrinkage during SEM preparation. J. Microsc. **121**, 185—189

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