Short communication

The Influence of External Surface Potential and Transmembrane Potential on the Passive Transbilayer Movement of Phospholipids in the Red Blood Cell Membrane

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Abstract. The passive transbilayer movement of spin-labelled analogues of phosphatidyl-choline (PC) phosphatidyl-ethanolamine (PE) and phosphatidyl-serine (PS) in red blood cell membranes was investigated at physiological and low ionic strength of the extracellular solution Passive transbilayer movement of aminophospholipids PS and PE was measured in ATP-depleted cells To discriminate between a possible surface potential and a transmembrane potential effect NaCl in physiological ionic strength solution was replaced either by sucrose or by Na-tartrate (constant osmolarity) Neither in sucrose (low ionic strength) nor in Na tartrate media a significant change of the translocation rate of the phospholipids was observed From these results it can be concluded that changes of the external surface potential as well as of the transmembrane potential do not affect the passive transbilayer movement of phospholipids in human red blood cells

Key words: Red blood cell membrane Erythrocyte Phospholipid transbilayer movement — Ionic strength — Membrane potential

It is now well established that phospholipids are asymmetrically distributed in a large variety of biological membranes e g red blood cell membranes (Op den Kamp 1979 Zachowski 1993) The existence of an aminophospholipid-translocase is thought to be responsible for the maintenance of this asymmetric distribution in eukaryotic plasma membranes (for review see Devaux 1991 Zachowski 1993) The active translocation of phosphatidylserine (PS) and phosphatidylethanolamine (PE) by such an ATP-dependent translocase was first described for the plasma membrane of human erythrocytes (Seigneuret and Devaux 1984) Other phospho-

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lipids like phosphatidylcholine (PC) and sphingomyelin (SM) are not recognized by this enzyme and traverse the plasma membrane only relatively slowly via passive diffusion (Seigneuret and Devaux 1984, Zachowski et al. 1985, Middelkoop et al. 1986).

The regulation of transverse phospholipid redistribution by physico-chemical factors (e.g. by the membrane electric field) is not well understood. In the present study we have investigated whether a change of the transmembrane potential and/or the external surface potential of the erythrocyte membrane do influence the passive movement of phospholipids across the membrane. It is well known that a change of the electrostatics can result in changes of the physico-chemical characteristics of biological membranes (Cevc 1990). In particular, an important motivation to this study was to investigate the hypothesis whether the significant increase of the leak fluxes of monovalent cations across the erythrocyte membrane caused by a decrease of the ionic strength of the extracellular solution (see e.g. Bernhardt et al. 1991) is associated or even based on an enhanced passive transbilayer movement of phospholipids. This hypothesis assumes a joint transport of an ion-phospholipid-complex across the membrane after binding of ions to the phospholipid head groups. The 'leak' transport for monovalent cations is defined as transport where all known specific transport pathways for these ions are inhibited.

Stored bank blood from healthy donors was used for the experiments. Red blood cells were separated by centrifugation for 8 min at 1500 × *g* at room temperature. Plasma and buffy coat were aspirated, and the cells were washed 3 times with physiological (high ionic strength, HIS) solution containing (mmol/l): NaCl 145, KCl 7.5, glucose 10, NaH_2PO_4/Na_2HPO_4 5.8, pH 7.4 at room temperature. For experiments carried out in low ionic strength (LIS) medium, in the final wash the cells were suspended in a solution of the following composition (mmol/l): sucrose 250, KCl 7.5, glucose 10, NaH_2PO_4/Na_2HPO_4 5.8, pH 7.4 at room temperature. In one series of experiments, 145 mmol/l NaCl of HIS solution was replaced by 55 mmol/l Na-tartrate plus 120 mmol/l sucrose (same ionic strength as the HIS solution). Also in this case, the final wash was carried out with the corresponding solution. All media had the same osmolarity (300 mosmol/l, measured with a vapour pressure osmometer). ATP depletion of erythrocytes was carried out according to Henseleit et al. (1990). The ATP concentration was determined with a luciferin-luciferase assay (Colora, Germany). Spin-labelled phospholipid analogues (1-palmitoyl-2-(4-doxylpentanoyl)-phosphatidylcholine (SL-PC), -phosphatidylserine (SL-PS) or -ethanolamine (SL-PE), kindly provided by P. F. Devaux (Paris) were added at time $t = 0$ to red blood cell suspensions in the corresponding solutions at 37°C (PC: 15% final label concentration corresponded to 1 mol% of endogenous cell phospholipids). It has been shown that these analogues incorporate in less than 1 min into the outer membrane leaflet at this temperature (Seigneuret et al. 1984). The amount of the label in the outer monolayer

was measured using the back exchange method (with 2% bovine serum albumin) as described previously (Morrot et al 1989) After reoxidation of reduced labels with ferricyanide (10 mmol/l) EPR spectra were recorded with a Bruker ECS 106 spectrometer Each experiment was repeated at least 3 times with blood from different donors The results are presented as mean ± S E M In order to compare the passive translocation of different phospholipids across the membrane (and also in different solutions) the rate constants of the phospholipid transbilayer movement were estimated by linear regression analysis of the curves representing the dependence of the phospholipid redistribution on time although it should be noted that such a procedure does not necessarily reflect the true mechanism

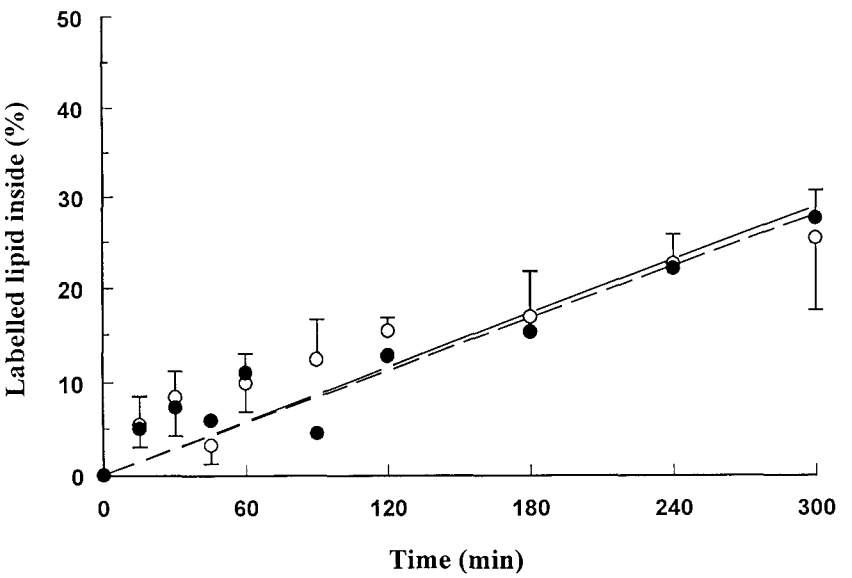

Figure 1. Transmembrane redistribution (inward movement) of a spin-labelled phosphatidylcholine in human red blood cells suspended in physiological ionic strength (open symbols) or low ionic strength (closed symbols) solutions at 37 °C Results (mean ± S E M) from 3 independent experiments

Fig 1 shows the transmembrane redistribution of SL-PC in non-ATP depleted human red blood cells suspended in HIS or LIS solution (also see Table 1) No significant change in the kinetics of traverse redistribution could be observed between both solutions In addition the influence of inhibitors of specific transport pathways for monovalent cations (used in experiments to measure the leak K^+ transport) on the SL PC redistribution was investigated The addition of 0 1 mmol/l ouabain

Table 1. Rate constant (k) of the membrane transbilayer inward movement of spin labelled phosphatidylcholine (PC) in control (non-ATP-depleted) and phosphatidylethanolamine (PE) as well as phosphatidylserine (PS) in ATP-depleted human red blood cells suspended in physiological ionic strength (HIS) or low ionic strength (LIS) solutions at 37°C. Results (mean ± S.E.M.) from 3 independent experiments

Solution	k (min^{-1})		
	PC	PE	PS
HIS	0.096 ± 0.007	0.11 ± 0.02	0.15 ± 0.02
LIS	0.093 ± 0.007	0.13 ± 0.02	0.12 ± 0.02

bumetanide and EGTA did not have a significant effect in both HIS or LIS media (data not shown).

For measuring passive transbilayer movement of SL-PE and SL-PS the redistribution experiments had to be carried out with ATP-depleted red blood cells to inhibit the ATP-dependent aminophospholipid-translocase (Seigneuret and Devaux 1984). SL-PC redistribution in control and ATP-depleted red blood cells did not show significant changes (data not shown) as already reported (Calvez et al. 1988).

The remaining ATP concentration determined in the depleted red blood cells used for the experiments (0.01 ± 0.01 mmol/l) was less than 1% of the control cells. The rate constants for the redistribution kinetics in HIS and LIS solution are presented in Table 1. Similar to SL-PC, no significant change of the passive redistribution rate for SL-PE and SL-PS could be observed in LIS in comparison to HIS solution. In addition, one can see from Table 1 that the rate constants of the passive transbilayer movement of all the three phospholipid analogues tested (and in both solutions) are in the same order. The slightly higher rate constants for SL-PS and SL-PE in comparison to SL-PC could be due to the residual ATP concentration in the depleted cells. Furthermore, one has to take into consideration that ATP may be inhomogeneously distributed in the cell population.

The replacement of NaCl by sucrose results (1) in a decrease of the ionic strength of the extracellular solution (i.e. an increase of the absolute value of the negative external surface potential) and (2) in changes of the transmembrane potential of the cells (from about −8 mV to about +15 mV (Glaser 1979)). To exclude a possible compensating effect of the transmembrane potential and the surface potential change experiments were carried out with NaCl of the HIS solution replaced by Na-tartrate plus sucrose (see above). Under these conditions the extracellular ionic strength and thus the external surface potential remains constant whereas the transmembrane potential changes in the same way as in the

sucrose-containing LIS solution (Glaser 1979 Halperin et al 1989) However also in the Na-tartrate (plus sucrose) solution for all the three phospholipid analogues no significant changes of the rate constant of translocation could be observed (data not shown)

From the obtained results one can conclude that neither the transmembrane potential nor the external surface potential does affect the passive transbilayer movement of the phospholipids in the red blood cell membrane On the other hand since the leak transport of monovalent cations significantly increases when the ionic strength of the external solution is reduced (e g Bernhardt et al 1991) a possible direct participation of the passive transbilayer movement of phospholipids in this effect is not supported by our data

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