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Influence of chlorpromazine on the transverse mobility of phospholipids in the human erythrocyte membrane: relation to shape changes

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The influence of chlorpromazine (CPZ) on the transverse mobility of spin-labeled phospholipids incorporated into human erythrocytes was investigated by electron spin resonance. The very slow transverse diffusion of phosphatidylcholine, as well as the absence of transverse mobility of sphingomyelin were not modified even by sublytic concentrations (≈ 1 mM) of CPZ. On the other hand, the rapid outside-inside translocation of the aminophospholipids (Seigneuret and Devaux (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755), was slightly hindered in CPZ containing membranes. If the spin-labeled aminolipids were incorporated in erythrocytes and allowed to flip to the inner monolayer before CPZ addition, a fraction of the spin labels (10–15%) flipped back instantaneously from the inner to the outer leaflet, upon incubation with CPZ. Similar experiments carried out with spin-labeled phosphatidylcholine and spin-labeled sphingomyelin showed that a fraction of the spin-labeled choline derivatives flip instantaneously to the inner leaflet if CPZ was added after the spin labels. Addition of lysophosphatidylcholine had no effect on the spin-labeled phospholipid redistribution nor on their transmembrane mobility. We interpret the immediate effect of CPZ addition as being due to a reorganization of the bilayer accompanying the rapid CPZ membrane penetration, phenomenon which is independent of the CPZ effect on the steady-state activity of the 'aminophospholipid translocase', the latter effect being probably a direct CPZ-protein interaction. By comparison of the time course of phosphatidylserine transverse diffusion in control discocyte cells and in CPZ-induced stomatocytes, we infer that the difference in cell shape is not a major factor in the regulation of the active inward transport of aminophospholipids in human erythrocytes.

Introduction

Several laboratories have shown that the passage of aminophospholipids from the outer to the inner monolayer of the human erythrocyte membrane is a protein mediated phenomenon, which requires intracellular ATP [1–3]. The relocation of lipids can be accompanied by important erythro-

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Abbreviations: (0,2)PC, 1-palmitoyl-2-(4-doxylpentanoyl)phosphatidylcholine; (0,2)PS, 1-palmitoyl-2-(4-doxylpentanoyl)-phosphatidylserine; (0,2)PE, 1-palmitoyl-2-(4-doxylpentanoyl)phosphatidylethanolamine; (0,2)SM, *N*-(4-doxylpentanoyl)-*trans*-sphingenyl-1-phosphocholine; (0,2) refers to the general nomenclature of spin-labeled chains, 0 and 2 being, respectively, the number of methylene groups after and before the labeled position on the acyl chain; CPZ, chlorpromazine; EDTA, ethylene diaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; lysoPC, lysophosphatidylcholine.

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cyte shape changes. In particular, the addition of a few % of exogenous lipids on the outer or inner leaflets is sufficient to provoke morphological modifications, respectively, from discocytes to echinocytes or from discocytes to stomatocytes [2,4]. In the present article, we examine the influence of the amphiphilic drug chlorpromazine (CPZ) on phospholipid translocation in human erythrocytes. The motivations for such investigation carried out by the spin-label technique are threefold:

(i) CPZ has been shown to affect lipid fluidity of erythrocyte membranes [5–7] as well as to bind to the hydrophobic surface of membrane proteins [8,9]. Therefore it may affect the activity of a transmembrane protein such as the aminophospholipid translocase.

(ii) CPZ is rapidly accumulated on the inner monolayer of erythrocytes [4]. This very rapid diffusion through the lipid bilayer brings about a transbilayer redistribution of the phospholipids. Several authors have reported such CPZ induced alteration of endogenous lipid asymmetry in erythrocytes [10,11]. Other drugs, such as tetracaine [12], produce similar effects. Surprisingly phosphatidylserine appears to resist this drug induced redistribution [10,12]. This difference could be due to kinetic effects which cannot be assayed by the classical phospholipase A_2 technique. The spin-label technique on the other hand should allow us to follow any temporary transmembrane redistribution.

(iii) CPZ induces fast stomatocytosis, due to its spontaneous accumulation on the inner leaflet, thus it is interesting to investigate whether subsequent aminophospholipid translocation is prevented or reduced in such cells. In other words, does shape transformation influence phospholipid translocation or only phospholipid translocation influence cell shape?

The spin-labeled phospholipids employed are shown in Fig. 1.

A significant advantage of these molecules is a partial water solubility, which facilitates their rapid incorporation in biological membranes. Such incorporation takes place within a minute after mixing the spin labels with a membrane suspension. Secondly the paramagnetic group can be chemically destroyed by a water soluble non permeant

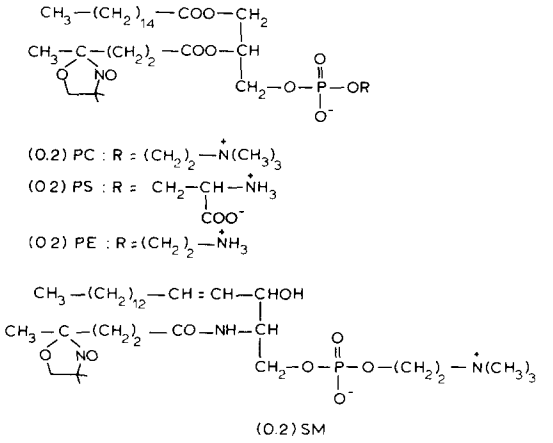

Fig. 1. Structures of the spin-labeled phospholipids (0,2)PC, (0,2)PS, (0,2)PE, (0,2)SM.

reducing agent such as ascorbate, provided the spin label is on the monolayer exposed to the reducing agent [1]. Note, however, that the nitroxide is not on the polar head group and, thus, does not perturb the head group specificity. Experiments with fresh erythrocytes or ATP loaded ghosts have shown that these spin-label analogues distribute themselves spontaneously between inner and outer leaflets as naturally occurring lipids do. Namely, at equilibrium the distribution of (0,2)PS, (0,2)PE, (0,2)PC and (0,2)SM on the inner monolayer is respectively $> 90\%$, $\approx 65\%$, $\approx 30\%$ and $< 5\%$ [1,13]. These results suggest that the above spin-labeled lipids can be used to report on drug induced modifications of the phospholipid asymmetry in red blood cells.

Here, spin-labeled lipids have been used to determine the kinetics of phospholipid outside-inside reorientation in human erythrocytes incubated with or without CPZ. Two types of experiments have been carried out with CPZ: in a first series of experiments, the membranes were equilibrated with CPZ, then spin-labels were added and subsequently the kinetics of their reorientation monitored by ESR. In a second series of experiments, spin-labeled lipids were added to the membranes in the absence of CPZ, their transmembrane reorientation followed by ESR during a certain interval, after which CPZ was added and the kinetics of flip-flop resumed. In parallel experiments, we have examined the morphological changes associated with the addition of CPZ and

exogenous phospholipids. For comparison, the effects of the addition of lysophosphatidylcholine on the aminophospholipid translocation was also examined.

Materials and Methods

Erythrocytes. Fresh human blood was obtained from healthy volunteers or from a local blood bank (Hopital Cochin, Paris). Blood collected on citrate/phosphate/dextrose was washed in 3 volumes of buffer A (10 mM Hepes, 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM glucose (pH 7.4)) by three 5 min centrifugations at $1000 \times g$, at 4°C. For Ca^{2+} depletion, erythrocytes were first washed in buffer B (10 mM Hepes, 70 mM NaCl, 70 mM KCl, 1 mM EGTA (pH 7.4)) then resuspended at 10% hematocrit in buffer B and ionophore A23187 was added at a concentration of 10 μM. After 15 min incubation at 37°C the cells were collected by centrifugation at $1000 \times g$ for 5 min, at a temperature of 4°C.

Spin-labeling. The spin-labeled phospholipids were synthesized in the laboratory following the procedures described previously [1,13]. Membrane labeling was carried out as described in Ref. 1. All samples contained 5 mM of di-isopropylfluorophosphate to minimize the hydrolysis of the spin-labeled phospholipids [14]. The ESR spectra were recorded with a Varian E109 spectrometer. The transverse orientation of the spin-labeled phospholipids was assayed at 4°C by ascorbate reduction of the labels exposed on the outer leaflet according to the method initially introduced by Kornberg and McConnell [15].

Chlorpromazine incorporation and hemolysis assay. CPZ (Specia) was dissolved in buffer A at a concentration corresponding to twice that of the final expected concentration of CPZ. Due to this precaution the local concentration of CPZ never exceeded twice the average final concentration. The fraction of lysed cells upon CPZ incubation was determined as follows: after 15 min incubation at 4°C, with various concentrations of CPZ added as described above, the supernatant was collected and its absorbance measured at 540 nm.

Scanning electron microscopy. Erythrocyte morphologies and changes of morphologies were observed with a scanning electron microscope. Control and treated fresh red cells were fixed in 1% glutaraldehyde in sample buffer, incubated on ice for 1 hour, postfixed in 1% osmic acid, sedimented on poly(L-lysine hydrobromide) (Sigma) pretreated glass coverslips [16], dehydrated in a graded series of alcohol and dried (critical point). After coating with gold, they were examined using a Jeol JSM 844 A scanning electron microscope. Cells were indexed on the basis of the grading scale of Fuji et al. [17]. Crenated cells (echinocytes) were given scores of +1 to +5 [18], discocytes were given a score of 0 and cupped or invaginated cells (stomatocytes) were given scores of −1 to −4: the average score of approximately 400 cells distributed in five fields selected at random was taken as the morphological index.

Results

Hemolysis test

The hemolysis test indicated that below 1 mM CPZ the percentage of lysed cells was lower than 5%. A similar test was carried out with lysophosphatidylcholine and gave 0.5 mM as the sublytic concentration.

Absence of effect of CPZ on the ESR spectra

ESR spectra were recorded immediately after incorporation in erythrocytes or after several hour incubation. In the case of (0,2)PS and (0,2)PE this delay allows a transmembrane reorientation from the outer to the inner monolayer. The spectra recorded in the presence or absence of CPZ were identical to those already published [19], i.e. no significant spectral modifications accompanied the incorporation of CPZ within the membranes.

Kinetics of phospholipid outside-inside translocation in the presence of CPZ

The outside-inside translocation of the various spin-labeled phospholipids was measured at 4°C in erythrocytes which had been incubated prior to spin-label addition, with respectively 0, 0.5 or 1 mM CPZ. Figs. 2A and 2B show the results obtained with the two extreme concentrations (0 and 1 mM) for the four spin-labeled phospholipids. As apparent in this figure, CPZ has no influence on PC and SM translocation, while a $\approx$ 30% decrease of initial rate and a $\approx$ 15% decrease of plateau levels can be observed with the two aminophospholipids. The intermediate concentration of CPZ

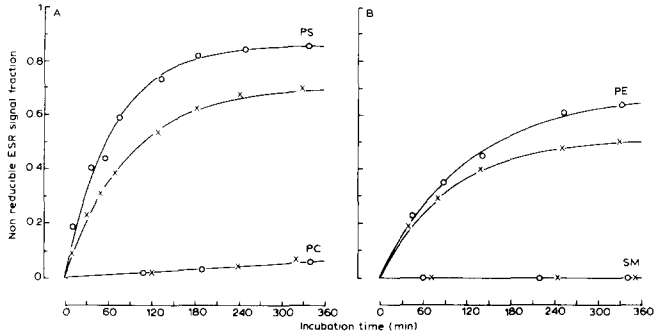

Fig. 2. Non reducible spin-label fraction as a function of the incubation time in erythrocytes at 4° C. Each data point corresponds to the level of the plateau of signal intensity reached within a few minutes after addition of 10 mM ascorbate. The incubation time is the time elapsed between addition of the label and addition of ascorbate. (A): (0,2)PS and (0,2)PC alone (○) or in the presence of 1 mM CPZ (*), added prior to spin-labeling. (B): (0,2)PE and (0,2)SM alone (○) or in the presence of 1 mM CPZ (*). Each spin-labeled lipid represents approx. 1% of the total amount of membrane phospholipids.

gave intermediate results and have not been represented in this figure. Experiments have been carried out also with Ca^{2+} depleted cells, the data were identical to those of Fig. 2, indicating that Ca^{2+}-CPZ competition was not responsible for the effect of CPZ on the aminophospholipid translocation.

Transitory effects of CPZ on phospholipid transmembrane distribution

In the following experiments the spin-labeled lipids were added to the membranes prior to CPZ addition. The data shown in Fig. 3A and 3B were obtained with (0,2)PS for two different concentrations of CPZ added after the spin label (arrow). For comparison the data obtained with CPZ added before the spin-labeled lipid are reproduced in the same figures (dotted curve). After a sudden passage of a fraction of (0,2)PS from the inner to the outer monolayer immediately after CPZ addition, the kinetics of phosphatidylserine translocation seems to resume so as to reach the plateau corresponding to that obtained when the same amount of CPZ was added before the spin-labeled

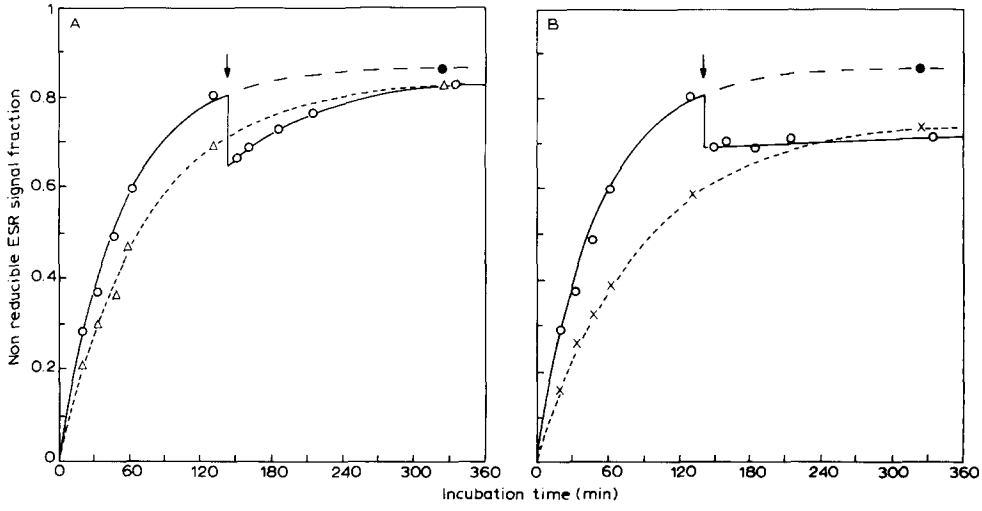

Fig. 3. The full line curves which join the open circles (○), in (A) and (B) correspond to the non reducible (0,2)PS fractions as a function of the incubation time at 4° C. After 150 min (indicated by an arrow), 0.5 mM of CPZ (A) or 1 mM of CPZ (B) were added. The closed symbols (●) are the data point obtained with a control sample which received no CPZ. The dashed curves (— — —) correspond to the non reducible (0,2)PS fractions as a function of incubation time with 0.5 mM CPZ (△, in A) or 1 mM CPZ (*, in B) added before the spin labels.

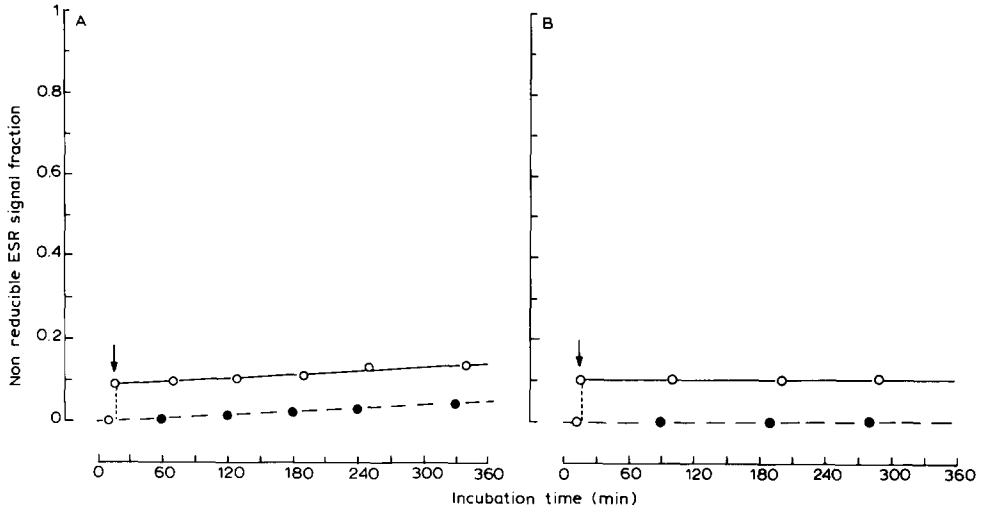

Fig. 4. Same experiments as described in Fig. 3. The spin labels are, respectively: A, (0,2)PC; B, (0,2)SM. The delay between spin-label incorporation and CPZ addition (indicated by an arrow) is 15 min.

lipids. The amplitude of the discontinuity in the kinetics curve and its sign depend on the duration of (0,2)PS incubation before CPZ addition. In particular if CPZ is added 15 min after (0,2)PS, the net effect of the phenothiazine incorporation is to provoke an immediate passage of a fraction of the spin labels from the outer to the inner monolayer as revealed by a sudden increase of the non reducible fraction immediately after the addition of CPZ (not shown). If the waiting time is ≈ 30 min, then CPZ seems to have no immediate effect on the transmembrane asymmetry, probably because of a compensation between redistributions between both layers. Finally the longer the incubation time, the larger is the negative discontinuity. The actual amplitude varies slightly from one experiment to another. On the average, the discontinuity is an increasing function of CPZ concentration. The same experiments were performed with the phosphatidylethanolamine derivative, (0,2)PE, and gave qualitatively the same results; the amplitude of the discontinuity was generally smaller and the waiting time before CPZ addition had to be longer, in order to obtain a discontinuity of about 10% of the signal (data not shown). Fig. 4A and 4B correspond to the experiments carried out with (0,2)PC and (0,2)SM. In such cases the discontinuity is independent of the waiting time and the kinetics of spin-label transmembrane redistribution after the discontinuity is parallel to that obtained when CPZ is added before the spin labels.

For a comparison, we have carried out experiments with lysophosphatidylcholine added instead of CPZ. The lysoPC had in fact practically no effect on (0,2)PS translocation whether added to the erythrocyte suspension before or after the spin-label (Fig. 5).

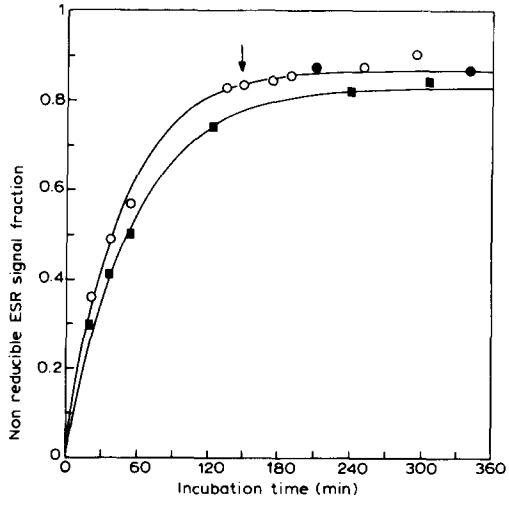

Fig. 5. Same experiment as described in Fig. 3, using (0,2)PS as a probe. The unlabeled compound added after 150 min is 0.5 mM lysoPC (sublytic concentration).

Kinetics of the shape transformations induced by CPZ in the absence or presence of spin-labeled lipids

When a sufficient amount of the spin-label derivative (0,2)PS is added to an erythrocyte suspension the average cell shape is rapidly transformed from discocyte to echinocyte. As indicated already in Ref. 1, the latter shape is not stable, discocytes and eventually stomatocytes are formed afterwards with a time constant of approximately one hour, due to outside-inside translocation of the exogenous phosphatidylserine. On the other hand, the addition of CPZ to an erythrocyte suspension provokes immediate and stable stomatocyte formation (Fig. 6A). If CPZ and (0,2)PS are added sequentially, the cells become stomatocytes but they slowly evolve from slightly cup shaped to completely invaginated cells (Figs. 6B, 6C and 6D). Fig. 7 is a comparison of the time course of shape transformation of erythrocytes due to (0,2)PS in the absence (top trace) and in the presence (bottom trace) of CPZ. This figure corresponds to the result of a statistical evaluation of the cell shapes at different intervals using a morphological index as defined by Ferrell and Huestis [18]. This figure shows that the time course of PS

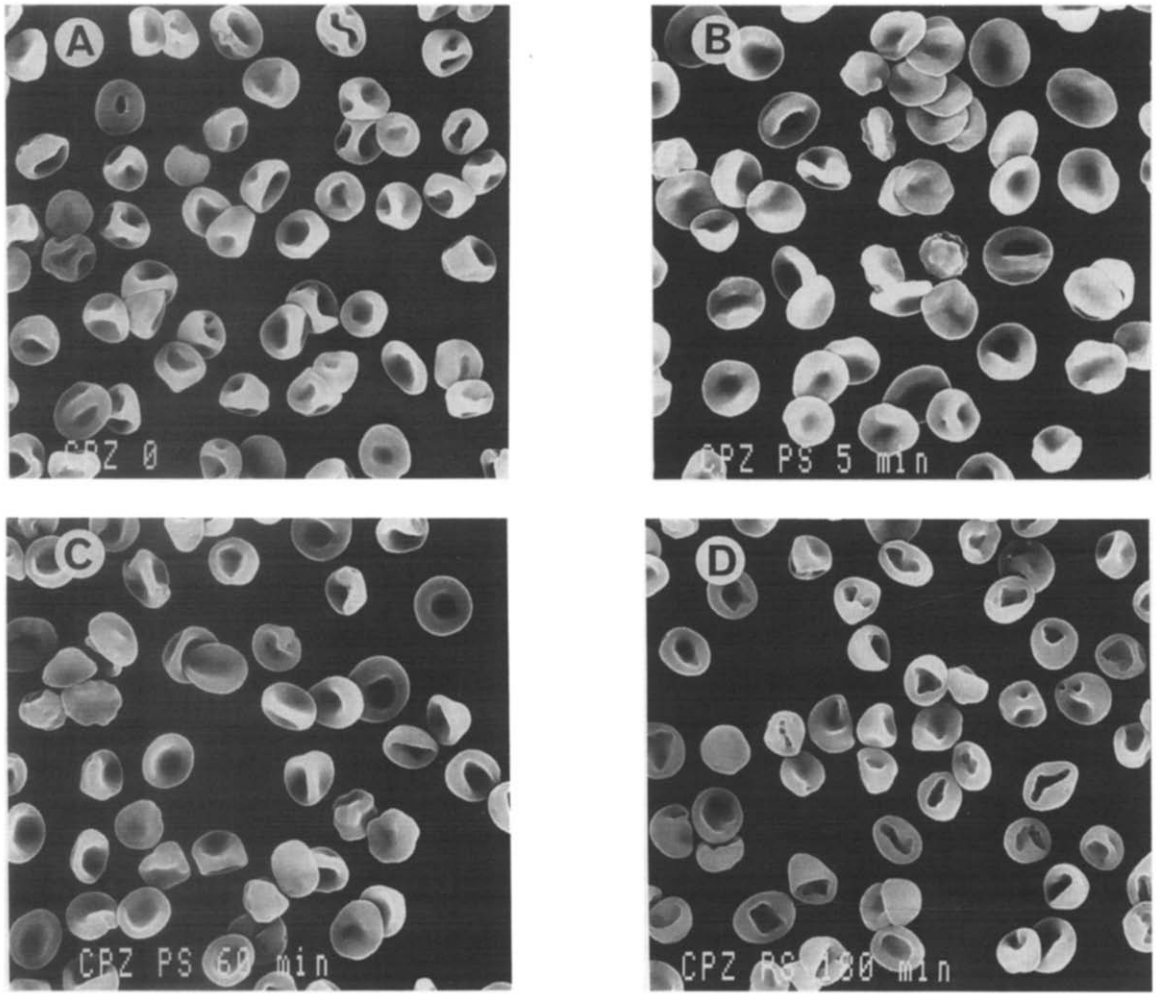

Fig. 6. Effect of the incorporation of exogenous PS on erythrocytes morphology for cell first incubated with sublytic concentration of CPZ. All cells were incubated with 1 mM CPZ. Typical morphologies are shown: (A) before addition of (0,2)PS; (B) 5 min after addition of (0,2)PS; (C) 60 min after addition of (0,2)PS; (D) 180 min after addition of (0,2)PS.

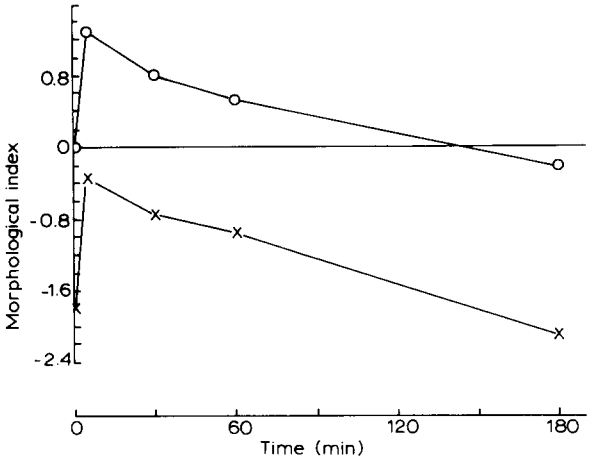

Fig. 7. Morphological indexes obtained with erythrocytes undergoing shape transformations due to (0,2)PS outside-inside translocation in the absence (○) and in the presence (*) of CPZ. The actual number of cells counted to determine each point was approx. 400.

translocation is not influenced by the actual shape of the cells.

Discussion

We have found a slight but significant inhibitory effect of CPZ on red cell aminophospholipid translocase activity, inhibition which results in: (i) a decrease of the rates of outside-inside transport of PS and PE analogues and (ii) a modified transmembrane equilibrium distribution of these spin labels. On the other hand the transverse mobility of phosphatidylcholine and sphingomyelin is CPZ independent which confirms the difference in the transmembrane reorientation mechanisms of the two classes of lipids: only the aminophospholipid translocation is driven by a membrane protein.

How does CPZ influence this protein activity? In model systems, the drug displays a dual behavior, either fluidizing or rigidifying depending on the cholesterol to phospholipid ratio [20,21]. There are slight discrepancies in the literature concerning the effect of low doses ($\approx 100\ \mu M$) of CPZ on erythrocyte fluidity (see Refs. 7 and 8). On the other hand erythrocytes treated with sublytic concentrations of CPZ (0.5–1 mM) showed in general an increase of motional freedom of stearic acid spin labels [5–7]. The fact that we have not detected a significant effect with (0,2)phospholipids, even at 1 mM CPZ, is perhaps an indication that the latter probes are not well adapted to detect a localized membrane perturbation. The modification of membrane fluidity by CPZ does not automatically imply a direct effect of CPZ on the lipids. In fact Minetti and Di Stasi [7] have provided evidence to show that the control of stearic acid spin-label freedom of motion is mediated by the spectrin-actin network and by the proteins that link the skeletal network to the membrane. This, together with the fact that CPZ has no effect on the passive transmembrane diffusion of phosphatidylcholine and sphingomyelin, suggests that, rather than by an overall modification of the properties of the lipid matrix in which the aminophospholipid translocase is embedded, most likely CPZ acts by a direct contact with the protein, like a local anaesthetic or an amphiphilic ligand [9].

An additional proof of the protein mediated effect of CPZ is provided by some recent data from our laboratory showing that sublytic concentrations of lidocaine, aspirine, imipramine, procaine, and decanol, which are amphiphilic drugs like CPZ, have no measurable influence on the aminophospholipid translocation in human erythrocytes (Leboulch, V., Farge, E. and Bitbol, M., unpublished data).

The influence of CPZ on the initial rate of outside-inside aminolipid translocation demonstrates a direct effect of CPZ on the establishment of lipid asymmetry and hence on the aminophospholipid translocase activity. The lower efficiency of the carrier protein is sufficient to explain as well a reduced lipid asymmetry at equilibrium. However the effect of CPZ on spectrin, reported by Minetti and Di Stasi [7], could also be partially responsible for the change in aminophospholipid transmembrane stationary distribution as suggested several years ago by Haest and collaborators [22]. Our present experiments cannot rule out the latter hypothesis.

Most likely the phenomenon observed in Figs. 3 and 4, namely the sudden redistribution of spin labels immediately after CPZ addition proceeds by a different mechanism. Rather than involving CPZ-protein interactions, it is probably a transient bilayer perturbation which accompanies the

rapid passage of CPZ through the membrane. This rapid passage is demonstrated by the very rapid erythrocyte stomatocyte formation upon CPZ addition. Isomaa et al. [11] have already suggested that amphiphiles like CPZ can trigger a rapid formation of nonbilayer phases which bring about a transbilayer redistribution of bilayer lipids.

According to our results a small fraction ($\approx$ 10%) of all lipids is redistributed after addition of 0.5–1 mM CPZ. If, as assumed throughout this paper, spin-labeled lipids are faithful reporters of endogenous lipids behavior, one would expect similar redistribution of naturally occurring lipids to take place after CPZ addition. Schrier et al. [10] have reported that this range of CPZ concentration produces indeed a measurable redistribution of PC, SM and PE but not of PS, as indicated by the phospholipase A_2 technique. However, since the phospholipase A_2 technique requires at least one hour incubation at 37 °C, it is not possible to analyze a transient redistribution by the latter method. Thus a redistribution of phosphatidylserine which is rapidly pumped back to the inner leaflet by the translocase, should be most difficult to see. On the other hand a redistribution of PE, because this latter molecule is less efficiently transported from the outer to the inner leaflet, may be detectable by the phospholipase A_2 technique. Finally SM and PC redistribution should be easy to observe because of the transmembrane stability of these lipids. Thus, we suggest that the reason why Schrier et al. observed a redistribution of all lipids but PS is due to the slow time course of the determination of lipid asymmetry. Similar remark applies probably to the findings of Schneider et al. [12] who reported that in the presence of tetracaine the endofacial PE seems to become accessible to phospholipase A_2, but not PS, in human erythrocytes.

As an alternative explanation of the mode of action of CPZ on aminophospholipid transport, one might speculate that cell shapes influence lipid transverse diffusion. For example the stomatocyte morphology might partially hinder the translocase. Such hypothetical inhibition would be physiologically useful, since the stomatocyte shape, according to Sheetz and Singer's bilayer couple hypothesis [4], is due to an excess of lipids on the inner leaflet: further accumulation of lipids on this leaflet should destabilize the membrane and result for example in endocytic vesicles [10,23,24]. However, we have indicated that for relatively low concentrations of CPZ (0.1 mM) most cells are stomatocytes and yet no inhibition of the translocase can be detected. Furthermore the addition of lysophosphatidylcholine which results in echinocyte formation has no significant effect on PS translocation either. Finally, Fig. 7 shows that the time course of PS outside-inside translocation, which can be estimated from the time course of cell shape modification, is identical in the presence or absence of CPZ.

In conclusion, experiments with CPZ prove that the erythrocyte cell shape is not an important regulating factor of the aminophospholipid translocase activity. On the other hand these experiments show that the putative protein will correct any accidental redistribution of aminophospholipids: this could be an important physiological function.

Acknowledgements

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References

1 Seigneuret, M. and Devaux, P.F. (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755.
2 Daleke, D.L. and Huestis, W.H. (1985) Biochemistry 24, 5406–5416.
3 Tilley, L., Cribier, S., Roelofsen, B., Op den Kamp, J.A.F. and Van Deenen, L.L.M. (1986) FEBS Lett. 194, 21–27.
4 Sheetz, M.P. and Singer, S.J. (1974) Proc. Natl. Acad. Sci. USA 71, 4457–4461.
5 Salesse, R., Garnier, J. Leterrier, F., Daveloose, D. and Viret, J. (1982) Biochemistry 21, 1581–1586.
6 Suda, T., Shimizu, D., Maeda, N. and Shiga, T. (1981) Biochem. Pharmacol. 30, 2057–2064.
7 Minetti, M. and Di Stasi, A.M.M. (1987) Biochemistry 26, 8133–8137.
8 Leterrier, F., Mendyk, A. and Viret, J. (1976) Biochem. Pharmacol. 25, 2469–2474.
9 Oswald, R. and Changeux, J.P. (1981) Proc. Natl. Acad. Sci. USA 78, 3925–3929.

10 Schrier, S.L., Chiu, D.T.-Y., Yee, M., Sizer, K. and Lubin, B. (1983) J. Clin. Invest. 72, 1698–1705.
11 Isomaa, B., Hägerstrand, H. and Paatero, G. (1987) Biochim. Biophys. Acta 899, 93–103.
12 Schneider, E., Haest, C.W.M., Plasa, G. and Deuticke, B. (1986) Biochim. Biophys. Acta 855, 325–336.
13 Zachowski, A., Fellmann, P. and Devaux, P.F. (1985) Biochim. Biophys. Acta 815, 510–514.
14 Zachowski, A., Hermann, A., Paraf, A. and Devaux, P.F. (1987) Biochim. Biophys. Acta 897, 197–200.
15 Kornberg, R.D. and Mc Connell, H.M. (1971) Biochemistry 10, 1111–1120.
16 Sanders, S.K., Alexander, E.L. and Braylan, R.C. (1975) J. Cell Biol. 67, 476–480.
17 Fuji, T., Sato, T., Tamura, A., Watkatsuki, M. and Kanaha, Y. (1979) Biochem. Pharmacol. 28, 613–620.
18 Ferell, J.E. and Huestis, W.H. (1984) J. Cell Biol. 98, 1992–1998.
19 Seigneuret, M., Zachowski, A., Hermann, A. and Devaux, P.F. (1984) Biochemistry 23, 4271–4275.
20 Neal, M.J., Butler, K.W., Polnaszek, C.F. and Smith, I.C.P. (1976) Mol. Pharmacol. 12, 144–155.
21 Pang, K.-Y. and Miller, K.W. (1978) Biochim. Biophys. Acta 511, 1–9.
22 Haest, C.W.M., Plasa, G., Kamp, D. and Deuticke, B. (1978) Biochim. Biophys. Acta 509, 21–32.
23 Ben-Bassat, I., Bensch, K.G. and Schrier, S.L. (1972) J. Clin. Invest. 51, 1833–1844.
24 Birchmeier, W., Lanz, J.H., Winterhalter, K.H. and Conrad, M.J. (1979) J. Biol. Chem. 254, 9298–9304.