Biochimica et Biophysica Acta, 471 (1977) 361—371

BBA 77872

INDUCTION OF ATP DEPLETION, INTRAMEMBRANE PARTICLE AGGREGATION AND EXPOSURE OF MEMBRANE PHOSPHOLIPIDS IN CHICKEN ERYTHROCYTES BY LOCAL ANESTHETICS AND TRANQUILIZERS *

YAIR GAZITT **, ABRAHAM LOYTER and ITZHAK OHAD

Department of Biological Chemistry, The Hebrew University of Jerusalem, 20 Mamilla Road, Jerusalem (Israel)

(Received May 9th, 1977)

Summary

Incubation of chicken erythrocytes with 1 mM tetracaine, 10 mM lidocaine and 0.24—0.48 mM chlorpromazine significantly reduced the ATP content of the cells, while procaine even at concentrations as high as 10 mM had only a slight effect. When chlorpromazine was used, it was found that the final level of the ATP was dependent on the drug concentration, which at 0.48 mM depletes the cells to about 10% of the initial ATP content. The ATP depletion of chicken erythrocytes was accompanied by dephosphorylation of certain membrane proteins which were identified by acrylamide gel electrophoresis as an 180000 dalton protein band and peptides with molecular weight of 60000—100000. Treatment of chicken and rat erythrocytes with 0.5 mM tetracaine and 1 mM lidocaine or with 0.48 mM chlorpromazine induced significant aggregation of intramembrane particles as revealed by the freeze-etching technique. Procaine (10 mM) had no effect. Incubation of chicken erythrocytes with the above-mentioned drugs induced also exposure of the masked membrane phospholipids to the action of phospholipase-C (*Bacillus cereus*) and to phospholipase A_2 (bee venom). Negligible amounts of phospholipids were hydrolyzed in the untreated cells, while about 40% of the membrane phosphatidylethanolamine and 50% of the phosphatidylcholine were hydrolyzed by phospholipase A_2 in chicken erythrocytes treated with 0.48 mM chlorpromazine.

Treatment of chicken and rat erythrocytes with 0.48 mM chlorpromazine resulted also in an increase in the amount of the phospholipid fraction which could be extracted by dry ether. About 41% and 60% of phospholipids were

---

* Preliminary results of this work were presented at the Annual Meeting of the Israel Biochemical Society, February, 1976 [23].

** Present address: College of Physicians and Surgeons, Columbia University, Cancer Research Center, 630 West 168th Street, New York, N.Y. 10032, U.S.A.

extracted from chlorpromazine-treated chicken and rat erythrocytes, respectively, as compared to 25% and 35% of phospholipids extracted from the same untreated cells.

Introduction

It has been reported that local anesthetics, besides their effects on excitable tissues, have a variety of additional effects such as displacement of membrane bound Ca^{2+} [1,2], decrease in osmotic fragility of erythrocytes [3] and inhibition of virus-induced cell fusion [4].

Recently it has been shown that dibucaine increases the fluidity of acidic phospholipid vesicles, as well as that of 3T3 cell membranes [5,6]. In addition, it was shown that treatment with dibucaine or tetracaine induced an increase in the aggregation of intermembrane particles in normal 3T3 cells. Intramembrane particle aggregation accompanied by exposure of phospholipids towards phospholipases [7—9] was also demonstrated after ATP depletion of rat [8] or chicken [9] erythrocytes. A correlation between dephosphorylation of certain plasma membrane proteins and aggregation of intramembrane particles was thus established. It was therefore of interest to check whether the effect of the above drugs on the distribution of the intramembrane particles were due also to their effect on the phosphorylation level of the plasma membrane proteins. This might be of further significance, since these drugs are known to act directly on the membrane.

We wish to report here that local anesthetics and tranquilizers such as chlorpromazine cause a dramatic decrease in the ATP content of the cells, which is accompanied by an increase in particle aggregation and exposure of membrane phospholipids.

Materials and Methods

The medium (A) for cell handling and enzymatic treatment contained: 135 mM KCl; 5.4 mM NaCl and 0.8 mM $MgSO_4$ in 30 mM Tris · HCl buffer (pH 7.4).

Chicken erythrocytes were collected from the necks of decapitated chickens and rat erythrocytes were obtained by heart punture using heparin (100 units/ml blood) as an anticoagulant. The cells were washed 3 times in medium A and the buffy coat was carefully removed.

For treatment with local anaesthetics and chlorpromazine, the cells were suspended in buffer (5%, v/v) and the drugs were added at the outlined concentrations. The incubation was carried out at 37°C for 60 min with gentle shaking and finally the cells were washed three times in medium A.

Restoration of the ATP level of the cells was carried out as previously described [10].

Treatment with phospholipase A_2 (bee venom) and phospholipid analysis

Cell suspensions of 5% (v/v) were treated with chlorpromazine at the outlined concentrations of the drug. The cells were then washed and resuspended

to 10% (v/v) in medium A. Phospholipase A_2 (Sigma, bee venom) was added at a final concentration of 4 units/ml, with the addition of $CaCl_2$ to a final concentration of 1 mM. Incubation was carried out for 1 h at 37°C with shaking. The reaction was stopped by diluting 5 times with cold medium A, followed by centrifugation at $10000 \times g$ for 10 min at 4°C. The degree of hemolysis was determined on the supernatant [10] and the pellet was resuspended in 3—6 vols. of medium A. The phospholipids were then extracted according to Burger et al. [11]. The extracted phospholipids were finally dissolved in 1 ml of chloroform/methanol (2 : 1 v/v) and the phospholipid phosphorus was determined according to Bartlett [12]. Aliquots of equal volumes were applied to thin layer chromatography plates and the phospholipids were separated using two-dimensional chromatography and analysed as previously described [7].

Treatment with phospholipase C and sphingomyelinase, and determination of hemolysis

Cell suspensions (5% v/v) were incubated in medium A for 1 h at 37°C with the addition of the various drugs at the concentrations metioned in the figure, and with or without the addition of sphingomyelinase (0.1 units/ml) and phospholipase C (1.0 units/ml). The cells were then pelleted and the degree of hemolysis was determined on the supernatant as previously described [10]. No hemolysis occurred in the absence of phospholipases.

Labelling of chicken erythrocytes with $^{32}P_i$

Labelling of erythrocytes with $^{32}P_i$ was carried out essentially as reported by Gazitt et al. [16]. Fresh erythrocytes (150 ml) suspended in medium A to 5% (v/v) were incubated at 37°C with the addition of 5 mM glucose and 0.01 mM (final concentration) potassium phosphate buffer. At zero time $^{32}P_i$ (carrier free) was added to give a specific radioactivity of $2.5 \cdot 10^3$ cpm/pmol. Aliquots of 1.5 ml were taken out at intervals of 30 min for ATP and membrane labelling determination. After 3 h incubation, 30 ml of the cell suspension were remoeved for cell fractionation and to the rest of the cells 0.24 mM chlorpromazine was added (ATP depletion period). Samples of 1.5 ml were taken at intervals of 2.5 min from the onset of the ATP depletion period and samples of cell suspension (30 ml) were removed at 7.5 and 15 min after the addition of the drug. The cells remaining after 15 min of treatment with chlorpromazine were washed 3 times in medium A and resuspended in a medium for ATP regeneration [10]. The specific radioactivity of $^{32}P_i$ was adjusted again to $2.5 \cdot 10^3$ cpm/pmol. Aliquots of 1.5 ml were taken out as above at intervals of 30 min from the onset of the ATP regeneration period. All the aliquots were washed 5 times in medium A buffered with 20 mM of potassium phosphate buffer (instead of Tris · HCl buffer) immediately after sampling. From each sample, 0.5 ml of cell suspension was hemolysed and washed with trichloroacetic acid/NaOH as previously described [9]. The remaining cell suspension (1 ml) was taken for ATP determination [10]. The 30-ml aliquots were washed 5 times in medium A, as above, and concentrated to 30% cells (v/v). The cells were homogenized and the plasma membrane was purified as previously described [9].

The radioactivity of the various fractions and samples was determined by the usual procedures [9]. The protein content of each fraction and sample was determined according to Lowry [14]. ATP was estimated by the luciferin/luciferase method as previously described [9].

Acrylamide gel electrophoresis

Acrylamide gels (7.5%) were prepared, electrophoresed and stained with Coomassie brilliant blue (R-250, Serva), according to Fairbanks [15]. Samples of 100 μg protein were applied to each gel. The gels were sliced and the radioactivity was determined [9].

Preparation of erythrocytes for freeze-etching and analysis of the results were done as described by Gazitt et al. [8]. Electron micrographs were taken with a Phillips EM 300 electron microscope, operated at 80 kV.

Ether extraction of ghosts prepared from fresh and drug-treated cells was done essentially as described before [16].

Phospholipase C (*B. cereus*) was obtained from Makor Chemicals, Israel. Sphingomyelinase-c (*Staphylococcus aureus*) was prepared as previoulsy described [8].

Results

Aggregation of intramembrane particles induced by treatment of erythrocytes with chlorpromazine and local anaesthetics

Treatment of intact chicken erythrocytes with low concentrations of chlorpromazine (0.48 mM) results in a significant aggregation of intramembrane particles revealed by freeze-fracturing of drug-treated intact cells (Fig. 1a, b). Incubation of chicken erythrocytes with 0.5 mM tetracaine and 10 mM lidocaine caused additionally agglutination of intramembrane particles, while 10 mM procaine had practically no effect (not shown). Aggregation of intramembrane particles is induced also in rat erythrocytes by treatment with chlorpromazine (Fig. 1d, c). The aggregation of particles is observed only on the fracture face designated as PF according to the recently proposed nomenclature [17]. This face contains about $3.5 \cdot 10^3$ particles per μm^2 in non-treated cells [8,13], and some decrease was observed in this value following treatment with chlorpromazine (Table I).

As a semi-quantitative estimation of the degree of aggregation one can measure the average linear distance between the particles or count isolated particles per unit linear distance obtained from densitometric tracings of negative prints [8]. When such an analysis was carried out on replicas of freeze fracture faces of chicken erythrocytes treated with increasing concentrations of chlorpromazine, it was found that the number of isolated particles decreased from 32 ± 2 to 16 ± 2 particles per μm length, and the average distance between particles increased from 120 ± 10 Å to 390 ± 45 Å when the chlorpromazine concentration was raised from 0 to 0.48 mM (Table I).

It has been reported previously that experimental conditions which induce aggregation of intramembrane particles accompanied by an increase in the smooth particle free area are associated with an increased in the pure lipid bilayer phase of the membrane [8,9]. It was also demonstrated recently that

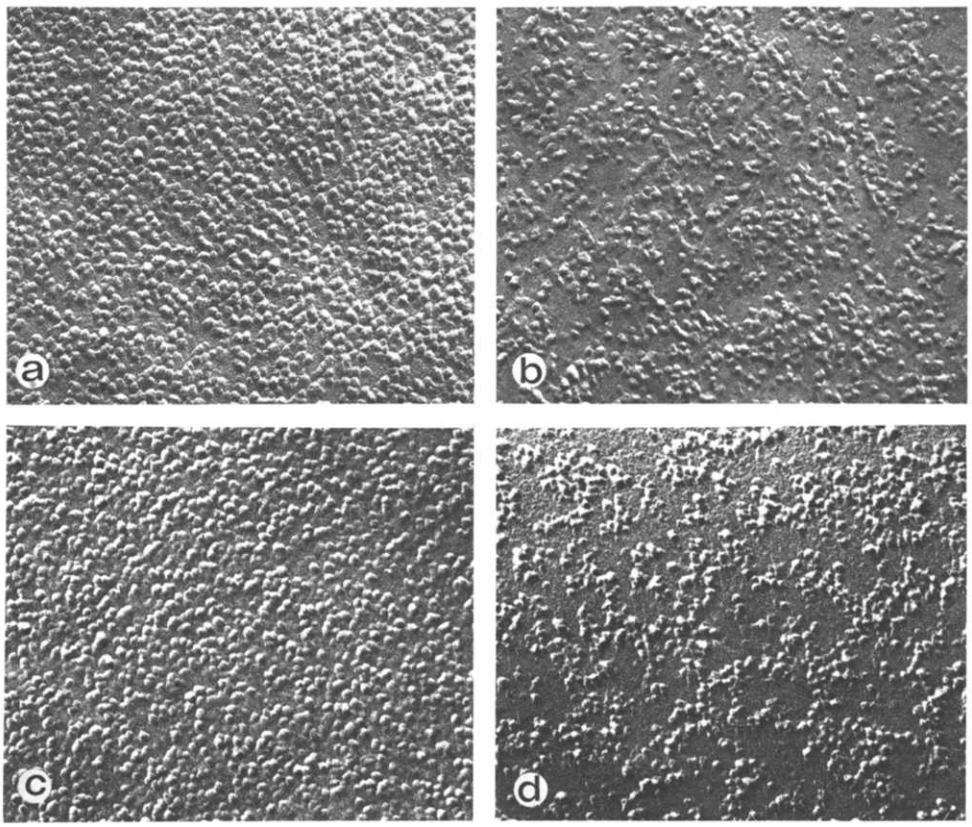


Fig. 1. Effect of chlorpromazine on intramembrane particle aggregation of fresh chicken and rat erythrocytes. (a) Non-treated chicken erythrocytes; (b) chicken erythrocytes treated with 0.48 mM chlorpromazine; (c) non-treated rat erythrocytes; (d) rat erythrocytes treated with 0.40 mM chlorpromazine. Notice the marked increase in intramembrane particle aggregation concomitant with an increase in the smooth area. The cells were prepared for freeze-etching as described previously [8]. Incubation of the cells with the drug was carried out as described in Materials and Methods. Magnification, × 114 000.

TABLE I

NUMBER AND DISTRIBUTION OF MEMBRANE PARTICLES SEEN IN THE FREEZE-FRACTURED MEMBRANE OF CHLORPROMAZINE-TREATED CHICKEN ERYTHROCYTES

Cell suspensions of 5% (v/v) were treated with chlorpromazine as described in Materials and Methods. Freeze-fracture and analysis of the distribution of membrane particles were carried out as described previously [7].

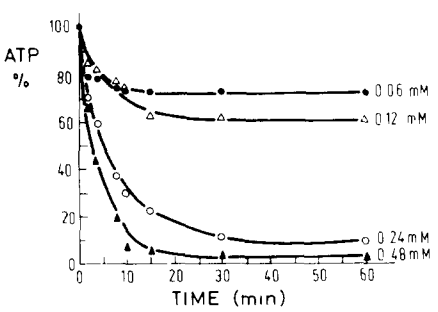
Chlorpromazine concentration (mM)	Particles/μm^2	Single particles/μm length	Average length of smooth area (Å)
0	3475 ± 350	32 ± 2	120 ± 10
0.06	3550 ± 340	25 ± 2	140 ± 15
0.12	3300 ± 295	27 ± 2	145 ± 15
0.24	3650 ± 245	18 ± 2	350 ± 40
0.48	2950 ± 355	16 ± 2	390 ± 46

this phase can be differentially extracted by treatment of ghosts obtained from such cells with dry ether [16]. When ghosts obtained from cells treated with 0.48 mM chlorpromazine were prepared and extracted as described [5,16], it was found that in both chicken and rat erythrocytes an increase in the dry ether extractable phospholipids resulted, as compared to the non-treated cells. The ether-extractable phospholipids were increased from 25% to 40% and from 35% to 60% in chicken and rat erythrocytes, respectively (not shown). The values obtained in this case are similar to those reported for the same cells following ATP depletion which was shown to induce aggregation of intramembrane particles [8,9,16].

Effect of treatment with local anaesthetics and chlorpromazine on ATP content of chicken erythrocytes

The increase in intramembrane particle aggregation and extractability of phospholipids with dry ether, induced by chlorpromazine, might be due to depletion of ATP similar to those obtained by metabolic inhibitors. Thus it was interesting to check the effect of these drugs on the ATP content of the cells.

Treatment of chicken erythrocytes with 10 mM lidocaine, 1 mM tetracaine, and 0.24 mM chlorpromazine resulted in a drastic decrease of the ATP content of the cells (from 0.46 to about 0.10 μmol ATP/ml packed cells), while procaine, which was shown to have no effect on the distribution of intramembrane particles, caused only a small reduction (18%) in the ATP content. The effect of treatment with chlorpromazine on the ATP content of the cells was very rapid. As shown in Fig. 2, the kinetics of ATP depletion are not influenced by the concentration of chlorpromazine, which has an effect only on the final ATP level and not on the time of response. Reduction in ATP content of chicken erythrocytes to less than 10% of the initial level is obtained within 10 min at a concentration of 0.48 mM chlorpromazine.



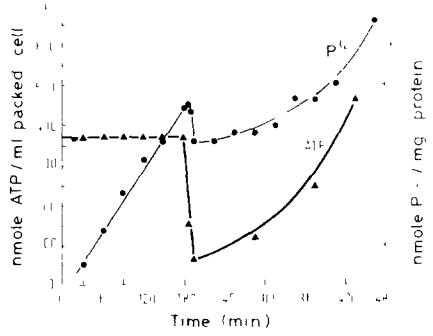


Fig. 2. Kinetics of chlorpromazine-induced ATP depletion of fresh chicken erythrocytes. Numbers represent chlorpromazine concentration. For experimental details see Materials and Methods.

Fig. 3. Kinetics of chlorpromazine-induced ATP depletion and dephosphorylation of erythrocyte membranes. Chicken erythrocytes were incubated for 3 h in medium A containing $^{32}P_i$ ($2.5 \cdot 10^3$ cpm/pmol) After 3 h, 0.24 mM chlorpromazine was added for 15 min. The cells were then washed 3 times and resuspended for 4 h in medium A, containing ATP-regenerating system [10]. Aliquots of 0.5 ml were taken for ATP determination and for $^{32}P_i$ determination, as described in Materials and Methods.

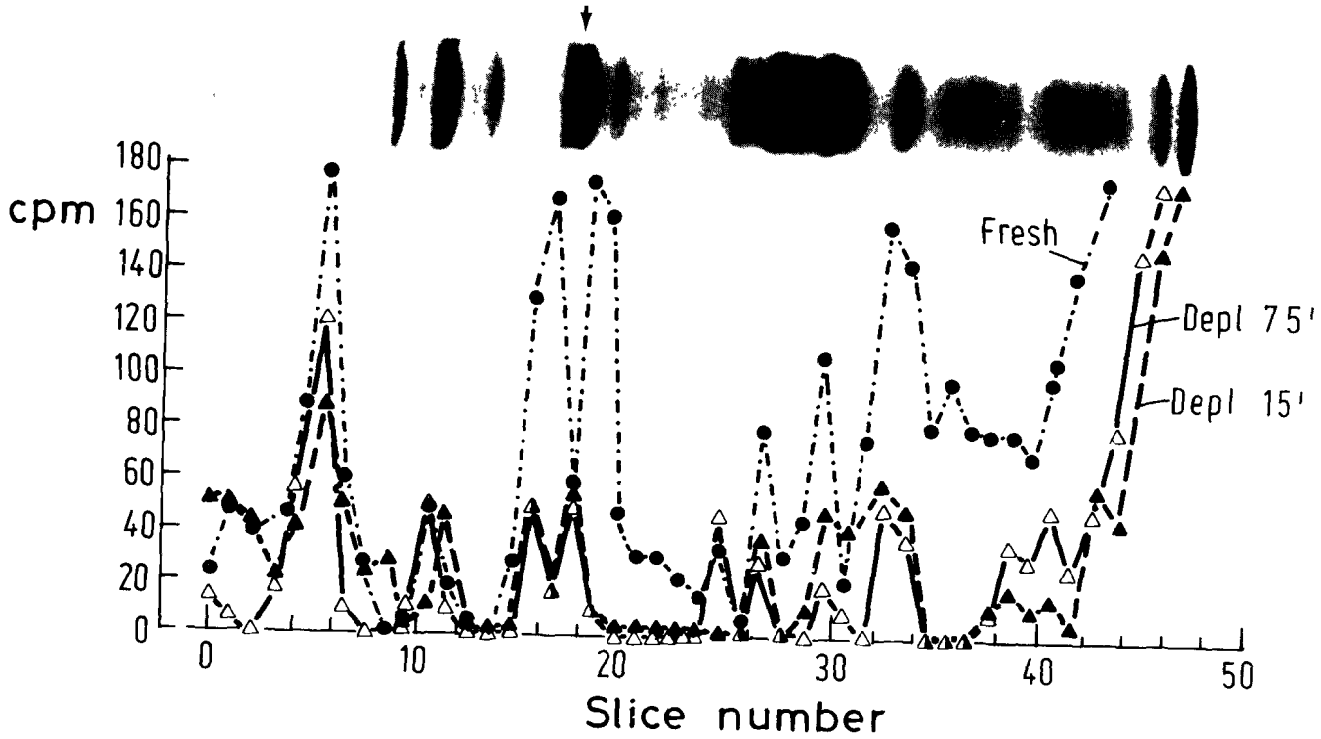


Fig. 4. Distribution of radioactive phosphate in various peptide bands of the plasma membrane of control and chlorpromazine-treated chicken erythrocytes. The gels (7.5%) were sliced from the top into 2-mm slices. Arrow indicates the 180 000 dalton peptides For experimental details see *Materials and Methods*.

Chlorpromazine-induced dephosphorylation of membrane proteins in chicken erythrocytes

The rapid depletion of ATP induced by chlorpromazine results in the loss of covalently-bound phosphate of membrane proteins. This effect is reversible, as demonstrated by washing off the drug and incubating the cells in a medium which allows restoration of ATP conetnt (Fig. 3). Electrophoretic analysis of ^{32}P-labelled proteins of purified plasma membrane shows that most of the radioactive phosphate is associated with a band of relative mobility corresponding to 180000 daltons and three other bands of electrophoretic mobility corresponding to 60000—100000 daltions. In addition, a highly radioactive peak is found in the region of 300000 daltons, which correponds to a faintly stained peptide band. The spectrin region is poorly labelled (Fig. 4). Incubation of the labelled cells for 7.5 min with 0.24 mM chlorpromazine is sufficient to cause a loss of over 70% of the ^{32}P contained in the 180000 dalton 60000–100000 dalton bands, while only small reductions are observed in the bands of 300000 dalton and the spectrin region. These results are practically unchanged after 15 min incubation (Fig. 4). The radioactivity found in the low molecular weight region is not affected by treatment with chlorpromazine. The significance of these radioactive bands is not yet clear.

Exposure of masked membrane phospholipids to phospholipase following treatment of erythrocytes with local anaesthetics and chlorpromazine

It has been shown before that exposure of phospholipids to phospholipase C, induced by ATP depletion of chicken erythrocytes, can be detected only after removal of sphingomyelin [7].

Treatment of chicken erythrocytes with anesthetics at concentrations which cause a reduction in the ATP content renders the cells susceptible to hemolysis by the combined effect of phospholipase C and sphingomyelinase (Table II). As expected, procaine, which has no effect on ATP content of the cells and does not induce intramembrane particle aggregation, does not sensitize the cells to

TABLE II

EFFECT OF LOCAL ANESTHETICS ON PHOSPHOLIPASE-INDUCED HEMOLYSIS OF FRESH CHICKEN ERYTHROCYTES

Hemolysis was induced by the combined action of phospholipase C and sphingomyelinase. For experimental details see Materials and Methods.

Additions	Hemolysis %
None	6
0.1 mM tetracaine	25
0.5 mM tetracaine	40
1.0 mM tetracaine	80
1.0 mM lidocaine	6
5 mM lidocaine	10
10 mM lidocaine	35
1 mM procaine	6
5 mM procaine	6
10 mM procaine	8

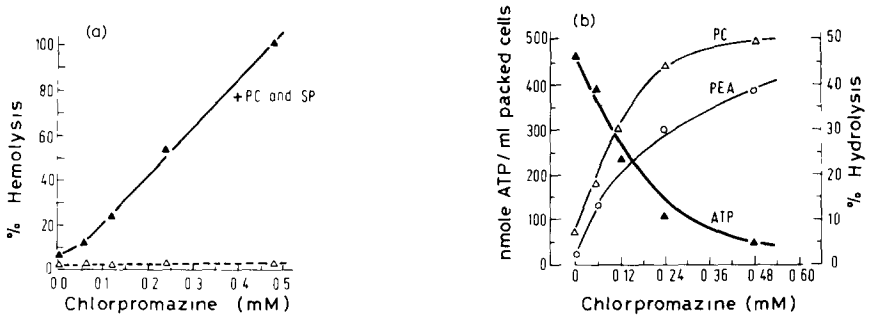


Fig. 5. Induction of hemolysis of chicken erythrocytes by phospholipase C (PC) and sphingomyelinase (SP), and hydrolysis of membrane phospholipids by phospholipase A_2 following treatment with chlorpromazine. (a) Hemolysis of phospholipase and sphingomyelinase; (b) hydrolysis of membrane phospholipids by phospholipase A_2. No hemolysis was observed following the treatment with the drug or with phospholipase A_2. For experimental details see Materials and Methods. PEA, phosphatidylethanolamine.

hemolysis by phospholipases. These results indicate that membrane phospholipids become exposed to phospholipases following a reduction in the ATP level of the cells induced by treatment with the above-mentioned drugs. A similar effect was observed when the cells were treated with chlorpromazine. The correlation between the ATP content, cell lysis induced by phospholipase C and sphingomyelinase at different concentrations of chlorpromazine and phosphatidylethanolamine, and phosphatidylcholine hydrolysis induced by phospholipase A_2, are shown in Figs. 5a and 5b, respectively.

Discussion

In a series of previous papers we have established the existence of a correlation between ATP depletion of erythrocytes (rat, chicken and toad) and exposure of masked phospholipids toward phospholipase C or phospholipase A_2 [7—9]. In addition, it was shown that ATP depletion induces aggregation of intramembrane particles in chicken and rat erythrocytes [8,9] and it was demonstrated that in chicken erythrocytes prolonged ATP depletion induces dephosphorylation of all the membrane proteins [9]. Based on this data it was suggested that the sequence of events occurring during ATP depletion includes dephosphorylation of membrane proteins which affects the aggregation of intramembrane particles by interfering with either lipid-protein interaction or protein-protein interaction, or both. This process induces an increase in the protein/free lipid bilayer phase of the membrane, which was reflected by increased susceptibility of membrane phospholipids to phospholipases as well as to other reagents acting on the outer surface of the cell [8,9]. This conclusion was supported also by the finding that differential extraction of lipids by dry ether exhibited an increase in the amount of extractable phospholipids in ATP-depleted cells [16].

The data reported in the present work demonstrate that rapid ATP depletion is induced by treatment of erythrocytes with lidocaine, tetracaine and chlorpromazine, which is accompanied by aggregation of intramembrane particles

and exposure of membrane phospholipids. Unlike the metabolic inhibitors used for induction of ATP depletion [7–10], the anesthetic drugs and chlorpromazine are known to interact specifically with the membrane components [4–6,18], and thus one should expect that the ATP-depletion induced by their utilization should disclose specific membrane phenomena.

The advantage of use of these drugs for induction of ATP depletion is evident from the fact that their action is extremely rapid and that they required only 10 min as compared to 4–8 h required for ATP depletion by metabolic inhibitors. The conclusion that dephosphorylation of membrane proteins is the cause of the other observed membrane phenomena in ATP-depleted cells is well supported by the present findings. Further evidence for this conclusion is found in the behaviour of procaine. This drug, which is known to have only a weak anaesthetic effect, has been shown in this work to be ineffective in the reduction of the ATP level of the cells as well as in the exposure of membrane phospholipids towards phospholipases and aggregation of intramembrane particles. Moreover, preliminary results of experiments showed that, unlike lidocaine and tetracaine which cause an increase in the amount of phospholipids extracted by dry ether from chicken erythrocytes, procaine has practically no effect (Gazitt, Y., unpublished results).

The phosphorylation of membrane proteins demonstrated in this and previous reports [9] implies a relatively rapid turnover of the phosphate bonds. The rapid depletion and dephosphorylation induced by the anaesthetics and chlorpromazine shown in this work, suggests that due to their interaction with membrane components these drugs accelerate dephosphorylation of proteins and thus ^{32}P turnover. In the absence of ATP regeneration this would lead to a rapid depletion of the ATP content of the cells.

The effect of the anaesthetic drugs and chlorpromazine can be due either to an activation of a membrane protein phosphatase such as that which was reported to be present in human erythrocytes [19] or to an alteration of the composition of protein · lipid complexes which might expose the phosphate bonds of the protein toward the active site of an otherwise unaffected phosphatase. Experiments devised to measure directly the activation of membrane phosphatase in isolated erythrocyte ghosts by addition of the above-mentioned drugs are now in progress in our laboratory.

The rapid depletion of ATP obtained by the use of chlorpromazine discloses differences in the dephosphorylation rate of different membrane proteins and eventually points towards the components whose dephosphorylation might play a major role in the changes induced in the membrane organization. According to the data shown here, the 180000 dalton band and peptides which can tentatively be identified as bands III, IV and V in human erythrocytes appear to be involved.

The possibility exists that the 180000 dalton band which was shown to be specific to chicken [9] and rat [20] erythrocytes but absent from human cells might play a role similar to that of spectrin in the control of distribution of the intramembrane particles. This is suggested by the fact that the 180000 dalton band in chicken cells is rapidly dephosphorylated and phosphorylated, as is the case with spectrin in human cells. Preliminary results also indicate that the 180000 dalton band is readily exposed to pronase digestion in ghosts but not

in intact cells (Gazitt, Y., unpublished results). Alternatively, it is possible that that alteration of membrane organization following dephosphorylation of membrane peptides is due to a change in the interaction between the chicken erythrocyte spectrin and other dephosphorylated peptides, among them band V. In human erythrocytes these proteins are considered to be the equivalent of actin whose interaction with spectrin was suggested to control the aggregation state of intramembrane particles [21].

On the basis of the above data it is not possible to decide whether the dephosphorylation of membrane proteins affects the aggregation of membrane particles by altering only the interaction between spectrin or spectrin-like peptides with intrinsic membrane peptides, or by altering the interaction between the latter and the adjacent lipids, or both. In this context it is interesting to note the suggestion made by Kury and MacConnel [22] that a steady state between free and bound spectrin may exist in human erythrocytes which is dependent on the state of its phosphorylation. Furthermore, it was suggested that detachment of spectrin from the membrane in the dephosphorylated state would increase the fluidity of membrane phospholipids. Thus it is tempting to speculate that detachment of dephosphorylated proteins may bring about an increase in the fluidity of membrane phospholipids resulting in a change of the lipid protein interaction. It should be mentioned that an increase in membrane fluidity has been observed after addition of dibucaine and tetracaine [5].

Acknowledgement

This work was aided by a grant from the United States—Israel Binational Science Foundation (BSF No. 158), Jerusalem, to A.L.

References

1 Seeman, P. (1972) Pharmacol. Rev. 24, 583—655
2 Chen, S.S. (1974) J. Physiol. 238, 313—328
3 Roth, S. and Seeman, P. (1971) Nature New Biol. 231, 284—285
4 Poste, G. and Reeve, P. (1972) Expt. Cell Res. 72, 556—560
5 Post, G., Papahadjopoulos, D., Jacobson, K. and Vail, W.J. (1975) Biochim. Biophys. Acta 394, 520—539
6 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504—519
7 Gazitt, Y., Ohad, I. and Loyter, A. (1975) Biochim. Biophys. Acta 382, 65—72
8 Gazitt, Y., Loyter, A., Reichler, Y. and Ohad, I. (1976) Biochim. Biophys. Acta 419, 479—495
9 Gazitt, Y., Ohad, I. and Loyter, A. (1976) Biochim. Biophys. Acta 436, 1—14
10 Frisch, A., Gazitt, Y. and Loyter, A. (1973) Biochim. Biophys. Acta 291, 690—700
11 Burger, S.P., Fujii, T. and Hanahan, D.J. (1968) Biochemistry 7, 3683—3700
12 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
13 Gazitt, Y., Loyter, A. and Chad, I. (1977) Israel J. Med. Sci., in the press
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, J.R. (1951) J. Biol. Chem. 193, 265—275
15 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606—2617
16 Gazitt, Y., Ohad, I. and Loyter, A. (1976) Biochem. Biophys. Res. Commun. 72, 1359—1366
17 Branton, D., Bullivant, S., Gilula, N.B., Karnovsky, M.J., Moor, H., Northcote, D.G., Packer, L., Satir, B., Satir, P., Speth, V., Staehlin, L.A., Steere, R.L. and Weinstein, R.S. (1975) Science 190, 54—56
18 Sheetz, M.P. and Singer, S.J. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4457—4461
19 Taborsky, G. (1974) Adv. Protein Chem. 28, 1—65
20 Lenard, J. (1970) Biochemistry 9, 5037—5039
21 Elgsaeter, A., Shotton, D.M. and Branton, D. (1976) Biochim. Biophys. Acta 426, 101—122
22 Kury, P.G. and MacConnell, H.M. (1975) Biochemistry 14, 2798—2803
23 Gazitt, Y., Loyter, A. and Chad, I. (1976) Israel J. Med. Sci. 12, 1326