INTERACTIONS BETWEEN ELLIPTICINE AND SOME DERIVATIVES AND PHOSPHOLIPIDS IN MODEL MEMBRANES

E. M. EL MASHAK, C. PAOLETTI[+] and J. F. TOCANNE

Centre de Recherche de Biochimie et de Génétique Cellulaires, CNRS, 118 route de Narbonne, 31077 Toulouse Cédex and
[+]*Laboratoire de Pharmacologie et de Toxicologie Fondamentales, CNRS, 205 route de Narbonne,*
31078 Toulouse Cédex, France

Received 3 August 1979
Revised version received 14 September 1979

1. Introduction

Ellipticine (5-11-dimethyl-[6H]-pyridino[4,3]-carbazole) and some analogues display an antitumoral activity against both experimental and human tumors as well as several other pharmacological properties [1].

The mechanism of the cytotoxic action of these drugs is supposedly related to their interaction with DNA [2,3]. However, before reaching the nucleus or other intracellular sites, these drugs have to interact with the cytoplasmic membrane and eventually to cross it. It can be observed (fig.1) that ellipticine and its 9-methoxy derivative have a net amphipathic character, a structural feature which might endow these substances with the ability to interact with membrane components, especially with phospholipids. Moreover, it has been postulated that the partition coefficient of ellipticine derivatives in an octanol–water system could be related to the pharmacological properties of these substances [4]. Finally, one can assume that the biodisposition and the pharmacokinetics of ellipticine derivatives of therapeutic interest depend on their lipophilicity.

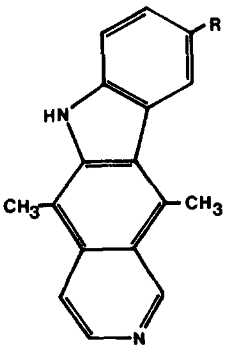

Fig.1. Ellipticine and its derivatives. R = : H, ellipticine; OH, 9-hydroxy-ellipticine; OCH_3, 9-methoxy-ellipticine; NH_2, 9-amino-ellipticine.

We therefore have undertaken an in vitro study of the interactions of four different ellipticine derivatives with phospholipids by means of the monolayer technique at the air–water interface.

Through surface pressure (π) and surface potential (Δv) measurements, it is shown that ellipticine and 9-methoxyellipticine interact with acid phospholipids but not with the zwitterions phosphatidylcholine and phosphatidylethanolamine. No interaction was detected with the dipolar 9-hydroxy-ellipticine whereas the dibasic 9-amino-ellipticine is postulated to adsorb at water–acidic lipid interfaces.

2. Experimental

2.1. *Chemicals*

Ellipticine (NSC 71795), 9-hydroxy- (NSC 210717), 9-methoxy- and 9-amino-ellipticine were synthesized by Drs N. Dat Xuong and P. Lecointe. The purity of these compounds was checked by high pressure liquid chromatography [5]. The p*K* values of these drugs are: 6.8, 6.6, 6.8 and 5.0, respectively (J. B. Le Pecq, personal communication). Didodecanoylphosphatidylglycerol was of synthetic origin [6]. Ditetradecanoylphosphatidylcholine and phosphatidylethanolamine (from *Escherichia coli*) were purchased from Sigma (USA). Bovine Brain phosphatidylserine was obtained from Calbiochem (USA), and phosphatidic (from egg lecithin) from Lipid Products (England). Phosphatidylinositol and cardiolipin were purified from the membrane lipids of *Micrococcus luteus* [7], as well as a sample of phosphatidylglycerol. All these compounds were pure as indicated by thin-layer chromatography.

2.2. *Methods*

The surface potential was determined with the apparatus described in [8,9]. Owing to the basicity of the drugs, the film surface pressure was measured by means of a floating barrier [9]. Experimental conditions were identical to those in [8,9]. The lipids were spread from chloroform/methanol 5/1 (v/v) solutions. Reproducibility of π and $\Delta\nu$ determinations (carried out at 20°C) were $\pm$ 0.2 mN .m^{-1} and $\pm$ 10 mV respectively.

2.3. *Procedure*

Unless otherwise stated, drug–lipid interactions were studied as follows: concentrated water solutions (10^{-3} M, pH 5) of the drugs were injected stepwise under films of phospholipids compressed at initial π = 5 mN .m^{-1}, π and $\Delta\nu$ being measured at equilibrium, after 1 min stirring of the subphase at each step. In order to ensure a good $\Delta\nu$ recording [8], the subphase was 10 mM NaCl at pH 6. At this pH and ionic strength, phosphatidylcholine and phosphatidylethanolamine on one hand, acidic lipids on the other hand can be regarded as zwitterions [10] and anions [7,10,11], respectively. Ellipticine and its 9-hydroxy- and 9-methoxy-derivatives are nearly totally protonated. As shown below, NaCl at 10 mM does not prevent the drug–lipid interactions when they occur.

3. Results

3.1. *Influence of the lipid structure*

It should be noted first that the drugs alone are slightly surface active. The presence of ellipticine at 10^{-5} M in the trough decreased the water surface tension by 0.5 mM .m^{-1} and increased $\Delta\nu$ by 75 mV (insert, fig.2). Nevertheless, no film could be obtained by spreading the drug at the water surface.

Injecting ellipticine under films of phosphatidylcholine or phosphatidylethanolamine to reach 10^{-5} M resulted in small π increases of 1 and 4 mN .m^{-1} and in $\Delta\nu$ increases of 75 mV (from 210–285 mV) and 50 mV (from 330–380 mV), respectively (fig.2). Modifying the initial film pressure or the subphase ionic strength did not significantly alter these results.

A completely new picture was observed with the acidic lipids tested. As can be seen in fig.2, adding ellipticine to the subphase resulted in a considerable $\Delta\nu$ jump. It is of note that at saturation, each system stabilized at the same potential of 480 $\pm$ 5 mV. A large π increase was observed, depending on the nature of the lipid (fig.2). The most important effect was found for phosphatidic acid and cardiolipin ($\Delta\pi \sim$ 21 mN .m^{-1}), two lipids which potentially bear two negative charges. Tests carried out with phosphatidylglycerol but at pH 7.2 (phosphate buffer) gave identical results. Control experiments carried out with the same lipid compressed at initial surface pressures of 10, 17 and 26 mN .m^{-1} gave similar results, namely large π increases of $\Delta\pi$ = 26, 19 and 10 mN .m^{-1}, respectively and $\Delta\nu$ jumps of about 300 mV, to reach the same final value of 480 mV.

From π and $\Delta\nu$ data, the drug concentration required to obtain the halfeffect appeared to be $<$ 10^{-6} M, a result which indicates a very high affinity of the drug for acidic lipids.

3.2. *Influence of the drug structure*

Since the interaction of ellipticine with acidic lipids appeared to be non-specific, additional tests were carried out with phosphatidylglycerol and phosphatidylinositol only. 9-Methoxy-ellipticine interacting with didodecanoylphosphatidylglycerol gave results nearly identical to those obtained with ellipticine, in terms of both π and $\Delta\nu$, while the 9-hydroxyellipticine, at 10^{-5} M, raised π and $\Delta\nu$ by 1 mN .m^{-1} and 120 mV, respectively. These values are not very different from those measured for the ellipticine–phosphatidylcholine system (fig.2). Testing 9-amino-ellipticine against phosphatidylinositol gave rise to a small π increase of 1 mN .m^{-1} but to a $\Delta\nu$ jump of 180 mV (from 130–310 mV). With phosphatidylglycerol from *M. luteus*, a similar $\Delta\nu$ jump of 170 mV was detected, but in this case π decreased by 3 mN .m^{-1}.

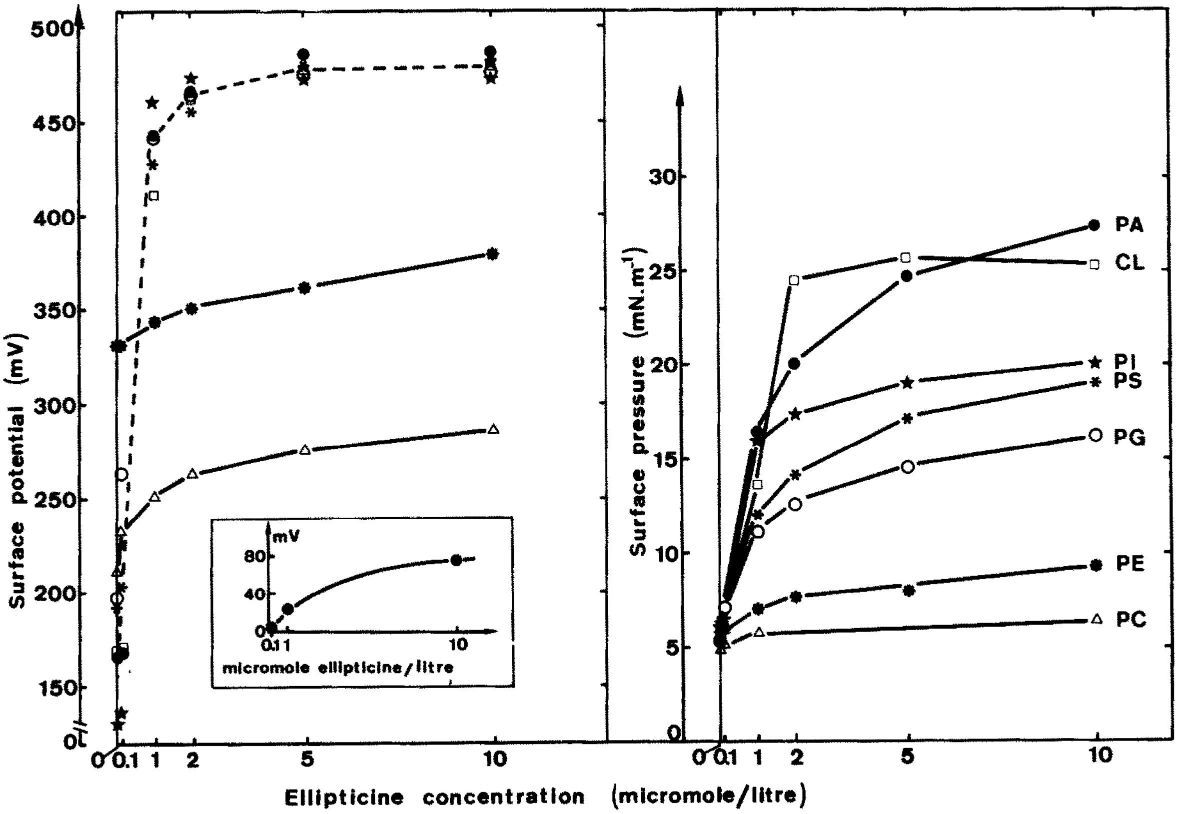

Fig.2. Changes in π and $\Delta\nu$ for films of various lipids compressed at initial π = 5 mN .m^{-1} on a 10^{-2} M NaCl subphase (pH 6), as a function of the concentration of ellipticine in the subphase. Phosphatidylcholine (△) (PC); phosphatidylethanolamine (∗) (PE); phosphatidylglycerol (○) (PG); phosphatidylserine (∗) (PS); phosphatidylinositol (★) (PI); cardiolipin (□) (CL); phosphatidic acid (●) (PA).

3.3. *Competition between ellipticine and cations*

Assays were carried out by stepwise injection of concentrated salt solutions (1 M, pH 6) under films of didodecanoylphosphatidylglycerol compressed at an initial 10 mN .m^{-1}, ellipticine remaining constant at 10^{-5} M. Results are presented in fig.3 together with π and $\Delta\nu$ data obtained for the same lipid molecular area but without drug in the subphase. As can be seen, Na$^+$ had no effect on the drug–lipid interactions. π and $\Delta\nu$ values remained unchanged upon addition of NaCl and different from those observed in the absence of drug. The same was found with Ca^{2+} up to 10^{-2} M. At 10^{-1} M, $\Delta\nu$ dropped to 280 mV, similar to that observed in the absence of drug (270 mV). However, the π remained nearly constant at 12 mN .m^{-1}, well above the 1 mN .m^{-1} found for the lipid without drug in the subphase. While $\Delta\nu$ points to a competition between the drug and Ca^{2+} for the phosphate groups, π still accounts for the presence of the drug within the film. This complex system deserves a more detailed study to be fully elucidated.

4. Discussion

Ellipticine and its 9-methoxy derivative display a very high affinity for acidic phospholipids. The π increases which result from the drug–lipid interactions are too large to be simply due to an adsorption of the drugs at the water–lipid interface. More likely, it is suggested that such pressure increases originate from an intercalation of the drugs between the lipid molecules, the NH$^+$ group interacting with the phos-

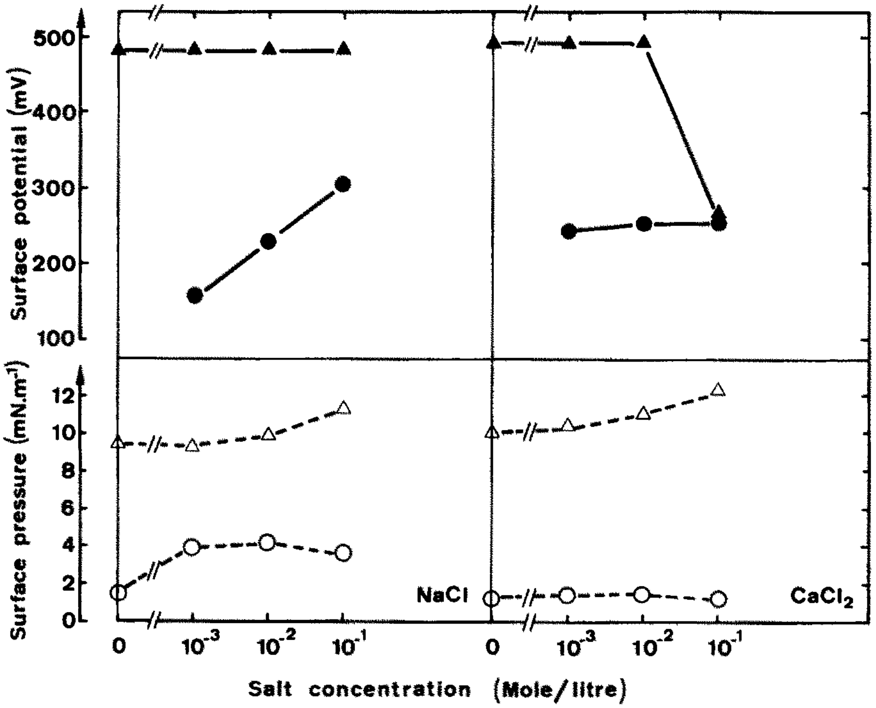

Fig.3. Changes in surface potential (full symbols) and surface pressure (open symbols) measured for films of didodecanoylphosphatidylglycerol as a function of the concentration of NaCl and $CaCl_2$ in the subphase, in the presence of ellipticine at 10^{-5} M (▲,△) or in its absence (●,○).

phate function and the aromatic moiety being in contact with the lipid acyl chains. Accordingly, the very high and constant surface potential observed at saturation for each lipid might account for a strong PO^- ... NH^+ dipole, similarly oriented in each system.

In contrast, the zwitterions phosphatidylcholine and phosphatidylethanolamine appear to be very poor ligands, at least at the pH 5–6 considered. π and $\Delta\nu$ data measured for these two lipids are similar to those recorded in the absence of lipid at the water surface and they could simply originate from the presence of the drug in the proximity of the water–lipid interface. Nevertheless while some penetration of the drug within films of these lipids cannot be completely excluded, it should be limited to a few drug molecules only, owing to the strong repulsive forces and therefore to the energy increase which would rapidly generate at the interface [12]. Likewise, π and $\Delta\nu$ data for dipolar 9-hydroxy-ellipticine do not allow us to conclude any significant interaction with phosphatidylglycerol. The lack of interaction in this case can be related to the presence of the hydroxyl group, a polar function which is very difficult to bring into a hydrophobic lipid environment [13].

π and $\Delta\nu$ data measured for dibasic 9-amino-ellipticine are similar to those recorded for the methyl ester of lysine, a molecule which has been shown to adsorb strongly at phosphatidylglycerol–water interfaces [9]. Thereby, the hypothesis of an adsorption of the drug at water–acidic lipid interfaces can be envisaged, the molecule interacting with two phosphate groups in the interface plane.

Another interesting finding is the high Ca^{2+} concentration which is required for destabilizing the ellipticine–lipid interactions. This cation is known to have a very high affinity for the phosphate group of acidic phospholipids [14] and to play a very important role in stabilizing the membrane structure [15]. Our data, suggest that ellipticine could compete with Ca^{2+} for the phosphate lipid sites in membranes.

The interaction of ellipticine with acid phospholipids could be one factor among several which are responsible for its complex pharmacological action, since the drug concentration at which the lipid layer is saturated ($\sim 10^{-6}$ M) is close to those necessary for saturating DNA [1] or microsomes [16], two other biological structures displaying a strong affinity for ellipticine. The binding of the drug to microsomal lipids which contain ~2.5–5% of acid phospholipids (phosphatidylserine and phosphatidylinositol) [17] is probably of great pharmacological significance: the metabolic transformation of the drug is carried out in the microsomal machinery [18] and ellipticine is the most potent inhibitor of the cytochrome *P*-450 reduction [16,19]. It has been shown [20] that the removal of the lipids from the rat liver microsomes decreases the apparent affinity of ellipticine for cytochrome *P*-450 by a factor of ~10.

The biological action of ellipticine and 9-methoxyellipticine might derive from perturbations in the organization and the fluidity of the lipid matrix in membranes. Such perturbations might either be directly responsible for the loss of some essential cell function or lead to the secondary disentanglement of reactive membrane sites and their subsequent exposure to extrinsic agents.

At last, these results suggest that some lipids can serve as a reserve binding pool for ellipticine and some of its hydrophobic derivatives. This observation must be taken into account for explaining some pharmacokinetics data [21,22].

References

[1] Juret, P., Tanguy, A., Le Talaer, J. Y., Abbatucci, J. S., Dat-Xuong, N., Le Pecq, J. B. and Paoletti, C. (1979) La Nouv. Presse Méd. 8, 1495–1498.

[2] Festy, B., Poisson, J. and Paoletti, C. (1971) FEBS Lett. 17, 321–323.

[3] Le Pecq, J. B., Dat-Xuong, N., Gosse, Ch. and Paoletti, C. (1974) Proc. Natl. Acad. Sci. USA 71, 5078–5082.

[4] Hansch, C. (1972) Cancer Chemother. Rep. 56, 433–440.

[5] Muzard, G. and Le Pecq, J. B. (1979) J. Chromatog. 169, 446–452.

[6] Tocanne, J. F., Verheij, H. M., Op den Kamp, J. A. F. and Van Deenen, L. L. M. (1974) Chem. Phys. Lipids 13, 389–403.

[7] Sacré, M. M. and Tocanne, J. F. (1977) Chem. Phys. Lipids 18, 334–354.

[8] Sacré, M. M., El Mashak, E. M. and Tocanne, J. F. (1977) Chem. Phys. Lipids 20, 305–318.

[9] El Mashak, E. M. and Tocanne, J. F. (1979) J. Colloid Interface Sci. 70, 56–66.

[10] Papahadjopoulos, D. (1968) Biochim. Biophys. Acta 163, 240–254.

[11] Shah, D. O. and Schulman, J. H. (1965) J. Lipid Res. 6, 341–349.

[12] McLaughlin, S. and Harary, H. (1976) Biochemistry 15, 1941–1948.

[13] Kellner, B. M. J. and Cadenhead, D. A. (1979) Chem. Phys. Lipids 23, 41–48.

[14] Williams, R. J. P. (1970) Quart. Rev. 24, 331–365.

[15] Razin, S. (1972) Biochim. Biophys. Acta 265, 241–296.

[16] Lesca, P., Lecointe, P., Paoletti, C. and Mansuy, D. (1978) Biochem. Pharmacol. 27, 1203–1209.

[17] Yang, C. S. (1977) Life Sci. 21, 1047–1058.

[18] Lesca, P., Lecointe, P., Paoletti, C. and Mansuy, D. (1977) Biochem. Pharmacol. 26, 2169–2173.

[19] Guenthner, T. M., Kahl, G. F. and Nebert, D. W. (1979) Biochem. Pharmacol., in press.

[20] Sautereau, A. M. and Lesca, P., Chem. Biol. Interactions, in press.

[21] Chadwick, M., Silveira, D. M., Platz, B. B. and Hayes, D. (1978) Drug Metab. Dispos. 6, 528–541.

[22] Van-Bac, N., Moisand, C., Gouyette, A., Muzard, G., Dat Xuong, N., Le Pecq, J. B. and Paoletti, C. (1979) submitted.