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Adsorption of the cationic antitumoral drug Celiptium to phosphatidylglycerol in membrane model systems. Effect on membrane electrical properties

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The binding of the cationic antitumoral drug Celiptium to the anionic phospholipid phosphatidylglycerol was studied by measuring surface potentials and surface pressures in monolayers, and by determination of electrophoretic mobility on liposomes. Surface potential and zeta potential data were interpreted in terms of the Gouy-Chapman-Stern theory of the diffuse electrical double layer. A unique drug-to-lipid adsorption constant K_a^D, could not be calculated. K_a^D was observed to increase rapidly from 10^4 M^{-1} to 10^6 M^{-1} with an increase in drug concentration from $5 \cdot 10^{-7}$ M to $7 \cdot 10^{-6}$ M. This was accompanied by a marked decrease (in absolute value) in the corresponding electrophoretic mobilities which, from negative at low drug concentrations, became positive at drug concentrations of 10^{-5} M and above. This indicates that the drug-to-lipid binding cannot be accounted for by a simple Langmuir adsorption isotherm, but corresponds to a more complex process, probably of a cooperative nature. Comparison of ΔV and zeta potential data shows that adsorption of Celiptium to phosphatidylglycerol not only lowers the electrical surface potential, ψ_0 (in absolute value) but also markedly reduces the polarization potential, ΔV_p. These observations suggest that Celiptium destabilizes the electrical properties of cell plasma membranes.

Introduction

Celiptium, or 2-*N*-methyl-9-hydroxyellipticinium (Fig. 1) is a cationic antitumoral drug currently used in cancer chemotherapy [1,2]. As for other ellipticine derivatives, its cytotoxic action is thought to be due to its interaction with DNA [3,4]. Nevertheless, doubt has been cast on this mechanism [5–7] and other possible targets have also been investigated [8]. Ellipticine and its derivatives are amphiphatic molecules, and we have shown that they interact strongly with acidic phospholipids, both in model [9,10] and natural membranes [11,12]. In contrast with the generally accepted view that substances with a permanent positive charge cannot easily permeate membranes, ellipticine and its quaternarized analogues rapidly diffuse through liposomes and biological membranes [12]. Moreover, it has recently been shown that Celiptium accumulates in bacterial [13,14] and eukaryotic [15] cells in response to the transmembrane electrical potential $\Delta\phi$ and that there is a relationship between drug accumulation and cytotoxicity [13,14]. Assuming that the free drug is the active form, the association constant of Celiptium with membrane components needs to be determined. The association constant with DNA has already been determined [3,16]. Celiptium bears two polar functions at the 2 and 9 positions (Fig. 1). The drug probably does not intercalate between phospholipids [12], but is more likely to be adsorbed onto the surface of the membrane

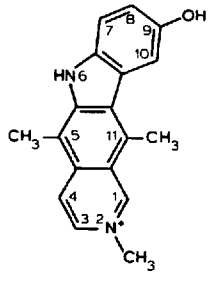

Fig. 1. Structure of the celiptium.

Abbreviation: PG, phosphatidylglycerol.

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[30]. This would lead to an alteration in the membrane surface potential.

In this study of surface potentials in monolayers and microelectrophoretic mobility in liposomes, we showed that Celiptium binds strongly to the acidic phospholipid phosphatidylglycerol. Both the polarization and electrostatic terms of the surface potential were found to be affected by binding of this drug.

Materials and Methods

Chemicals

The 2-*N*-methyl-9-hydroxyellipticinium, (Celiptium) was provided by Sanofi (France); its purity was assayed by high-performance liquid chromatography [17]. Phosphatidylglycerol, from the bacterium *Micrococcus luteus*, was prepared by a previously described method [18]. This lipid was shown to be substituted more than 90% by iso and anteiso methyl-branched myristic and palmitic acids [18]. Its purity was checked by thin-layer chromatography.

Monolayer experiments

Surface potential was measured with an apparatus using two americium electrodes which has been described elsewhere [19,20]. Surface pressure was also continuously recorded by means of a platinium plate connected to a torsion balance of our fabrication. The lipid was spread in the form of chloroform/methanol (5 : 1, v/v) solutions. Ultra-pure water from a Milli-Q apparatus (Millipore) was used to prepare buffers for the subphase.

Reproducibility of the π and ΔV determinations (carried out at 20 °C) were ± 0.2 mN $\cdot$ m^{-1} and ± 5 mV respectively. Celiptium–phosphatidylglycerol interactions were studied as follows: aliquots of a concentrated water solution (10^{-2} M, pH = 6.8) of the drug were injected stepwise under films of phosphatidylglycerol compressed at an initial surface pressure $\pi = 10$ mN $\cdot$ m^{-1}. π and ΔV were measured at equilibrium after 1 min stirring of the subphase at each step. In order to ensure a good ΔV recording [19], the subphase consisted of 10 mM NaCl (pH = 6.7). At this pH, Celiptium is protonated, whereas phosphatidylglycerol is fully ionized [21].

Phospholipid dispersions

Large multilamellar vesicles were prepared by direct suspension of phosphatidylglycerol in 10 mM NaCl (pH = 6.7) under gentle stirring, at 20 °C. At this temperature, the lipid was in the liquid phase [21]. All the experiments were carried out under nitrogen to avoid peroxidation of the lipid molecules. The lipid concentration was determined using Ames and Dubin's method [22]. The homogeneity and the mean size of the liposomes were determined by electron microscopy. The liposomal suspension appeared to be heterogeneous, the largest liposomes being around 1 μm in diameter. These large liposomes are the first to be observed in the light microscope of the microelectrophoresis apparatus, and thus a value of 1 μm would be used for calculation of the zeta potential from values of the electrophoretic mobility. Nevertheless, smaller liposomes can also be observed, and so calculations were also carried out based on a liposome diameter of 0.2 μm, the smallest that is clearly observable in our apparatus.

Electrophoretic mobility measurements

Experiments were carried out using a Rank Mark II (Rank Bros. Cambridge, U.K.) microelectrophoresis apparatus, with a planar cell maintained at 20 °C in a thermostated bath. Care was taken to focus on the stationary layer.

The phosphatidylglycerol concentration in the electrophoretic cell was $2.5 \cdot 10^{-5}$ M. Celiptium was added stepwise into the cell to reach a final drug concentration of 10^{-3} M. The pH of these suspensions ranged between 6.8 and 6.9 for drug concentrations between 10^{-7} M and 10^{-4} M. The migration rate of the particles was determined at least ten times in each direction. For a given liposome, it was systematically checked that the absolute value of the migration rate remained unchanged when the sign of the applied electric field was changed. Because of electrolytic phenomena, changes in the pH of the aqueous phase can sometimes be observed, especially when working around pH 7. We checked that the electrophoretic measurements did not significantly alter the pH of the liposomal suspensions.

Theory

The zeta potential was calculated using Henry's equation [34].

$$\zeta = (3/2)\eta\mu/\varepsilon_0\varepsilon_r \cdot f(\kappa a) \tag{1}$$

where μ is the electrophoretic mobility of the particle, ε_0 and ε_r are the permitivity of the free space and the aqueous phase respectively, κ is the reciprocal of the Debye screening distance (see Eqn. 3), and a is the radius of the diffusing particles. In our case, for a 10 mM NaCl solution, $f(\kappa a)$ was 1.37 and 1.49 for particle diameters of 0.2 μm and 1 μm, respectively. The data were interpreted in terms of the Gouy-Chapman-Stern theory [23,26] of the diffuse electrical double layer, which enables calculation of the electrostatic surface potential $\psi_{(x)}$ at any distance, x, from the interface plane for a given surface charge density σ

$$\psi_{(x)} = \frac{2kT}{e} \ln \frac{[1 + \alpha \cdot \exp(-\kappa x)]}{[1 + \alpha \cdot \exp(-\kappa x)]} \tag{2}$$

where

$$\kappa = \left[\frac{2e^2(C_b + D_b)N}{\varepsilon_r \varepsilon_0 kT} \right]^{1/2} \tag{3}$$

and

$$\alpha = \frac{\exp(e\psi_o/2kT)-1}{\exp(e\psi_o/2kT)+1} \quad (4)$$

and ψ_o is given by

$$\sinh(e\psi_o/2kT) = \sigma/(\sqrt{8N\varepsilon_o\varepsilon_r kT}\cdot\sqrt{C_b} \quad (5)$$

In these equations, k is the Boltzman constant, N is the Avogadro's constant, T is the absolute temperature, e is the electron charge, ε_o and ε_r are defined above, C_b is the uni-univalent ion (NaCl) concentration, and D_b is the drug concentration in the bulk aqueous phase, ψ_o is the electrostatic surface potential in the interface plane, at $x = 0$.

The surface charge density, σ, corresponds to the number of ionized lipid molecules per unit surface.

It has been shown that above pH 4 and for a 10 mM NaCl concentration in the bulk aqueous phase, the phosphate group of PG molecules is completely deprotonated. Since the experiments were carried out near pH 7, the protonated form of PG was ignored.

The surface charge density, σ, can be altered by adsorption of the monovalent cation M^+ and drug D^+ to the lipid. Assuming a 1 : 1 stoichiometric interaction between these cationic species and the anionic PG phosphate group PO^-, the equilibria are thus

$$PO^- + M^+ \rightleftarrows POM \quad (6)$$

with the association constant

$$K_a^M = \frac{[POM]}{[PO^-](M^+)_0} \quad (7)$$

and

$$PO^- + D^+ \rightleftarrows POD \quad (8)$$

with

$$K_a^D = \frac{[POD]}{[PO^-](D^+)_0} \quad (9)$$

Note that Eqns. 7 and 9 are in the form of a Langmuir adsorption isotherm after conversion of the surface concentrations into surface densities [26,27]. In these equations, square brackets refer to lipid surface concentrations while round brackets with subscripted zero indicate cation and drug volumic concentrations, in the interface plane, at $x = 0$.

These volumic concentrations are obtained from the Boltzman equation

$$(M^+)_0 = C_0 = C_b \exp(-e\psi_o/kT) \quad (10)$$

$$(D^+)_0 = D_0 = D_b \exp(-e\psi_o/kT) \quad (11)$$

Finally, to account for possible changes in drug concentration in the bulk aqueous phase by drug binding to lipid molecules, the following relationship was established

$$D_t = D_b + D_a \quad (12)$$

It assumes that the total drug concentration, D_t, is the sum of the concentrations, D_b and D_a, of those molecules which are still in solution in water (bulk) and bound to lipid molecules, respectively. D_a depends on both the drug-to-lipid adsorption constant K_a^D, and the number of PG molecules which are actually involved in the drug binding equilibrium.

Calculations were carried out in the following sequence:

First, in the absence of Celiptium, comparison of calculated and experimental zeta potentials gave an estimate of K_a^{Na}, the adsorption constant of Na^+ to PG. In these computations, the plane of shear [24] was fixed at a distance $x = 0.2$ nm from the interface plane, a value which Eisenberg et al. [26] considered reasonable.

Second, in the presence of Celiptium, the equilibrium [8] was also taken into account. Similar calculations led to an estimate of K_a^D, the drug-to-lipid adsorption constant. The plane of shear was still assumed to be 0.2 nm from the interface. Note that with the moderate salt concentration used (10 mM NaCl), the surface potential ψ_x only slowly decreases with the distance, x, from the interface. This means that small changes in x around 0.2 nm have little effect on the estimates of the zeta potential ψ_x and the adsorption constants K_a^{Na} and K_a^D.

The calculations were carried out using a modified form of a computer program kindly provided by S. McLaughlin.

Results

Monolayers

As shown in Fig. 2 and as previously reported [12], Celiptium, at concentrations up to 10^{-5} M in the subphase, did not alter the film surface pressure significantly. A slight film expansion from 10 to 23 $mN \cdot m^{-1}$ was observed when the drug concentration was increased from $3 \cdot 10^{-5}$ M to 10^{-3} M.

Addition of Celiptium in the subphase first led to an increase in ΔV from 220 to 300 mV up to a drug concentration of 10^{-5} M, indicating that the cationic drug molecules adsorb to the monolayer surface, and neutralize the negative charges on phospholipid molecules. With further increase in the drug concentrations, ΔV fell rapidly to a value of 40 mV for a final drug concentration of 10^{-3} M in the subphase.

Electrophoretic mobility

Fig. 3 shows the electrophoretic behavior of phosphatidylglycerol multilamellar vesicles in the presence

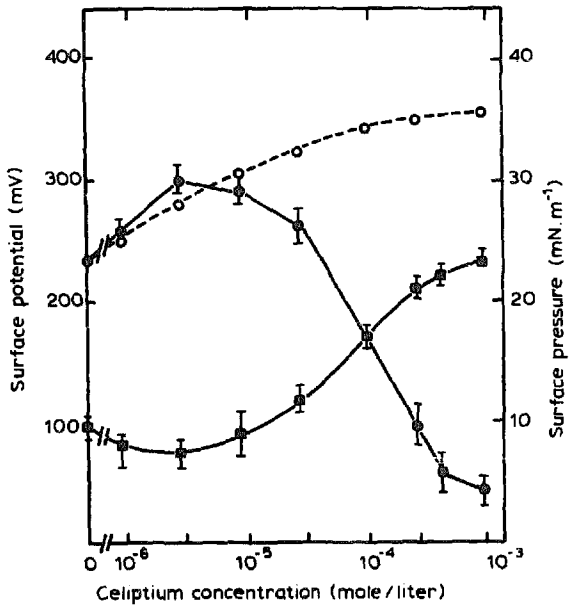

Fig. 2. Changes in surface potential (ΔV) and surface pressure (π) for phosphatidylglycerol monolayers as a function of Celiptium concentration in the subphase. PG was spread at an initial surface pressure of 10 mN·m^{-1}. The subphase was 50 mM NaCl (pH = 5.8). The dotted line corresponds to calculated ΔV values assuming a constant polarization potential, ΔV_p and sodium and Celiptium-to-PG adsorption constants, K_a^{Na} and K_a^D of 0.16 M^{-1} and 10^5 M^{-1}, respectively (see text).

of increasing Celiptium concentrations in the water phase. The measured mobilities and the corresponding zeta potentials (Eqn. 1) are shown in Table I. These calculations were carried out assuming an average vesicle diameter of 1 μm, which for an ionic strength of 10 mM, gave a value of 1.49 for the $f(\kappa a)$ function [25].

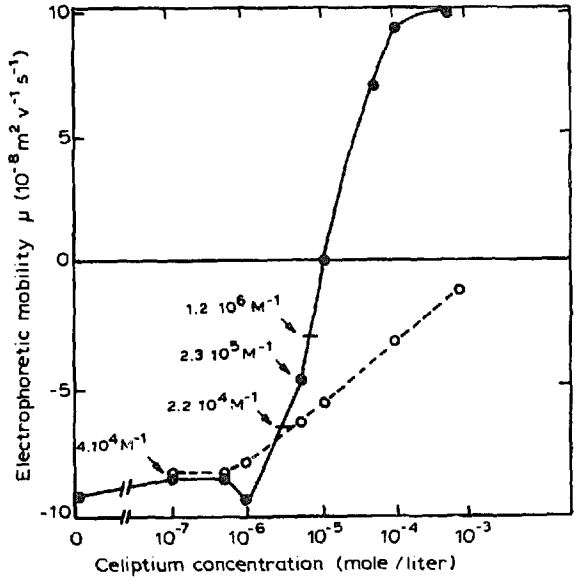

Fig. 3. Electrophoretic mobilities of phospahtidylglycerol vesicles as a function of Celiptium concentration in the aqueous phase. Numerical values (arrows) are the calculated drug-to-lipid association constants K_a^D (see Table II). The dotted line corresponds to the calculated electrophoretic mobilities assuming a constant K_a^D value of $4 \cdot 10^4$ M^{-1} and 1% available PG sites.

TABLE I

Electrophoretic mobilities, μ, and corresponding zeta potentials, ζ, for phosphatidylglycerol multilamellar vesicles in the presence of increasing Celiptium concentrations in the water phase

Celiptium concn. (M)	μ (10^{-8} m^2·V^{-1}·s^1)	ζ (mV)
0	−9.22	−119
10^{-7}	−8.68	−112
$5 \cdot 10^{-7}$	−8.82	−113
10^{-6}	−9.70	−125
$5 \cdot 10^{-6}$	−4.60	−59.3
10^{-5}	0	0
$5 \cdot 10^{-5}$	+7.06	+91
10^{-4}	+9.30	+120
$5 \cdot 10^{-4}$	+10	+129.6

Addition of Celiptium at concentrations of 10^{-7} M and $5 \cdot 10^{-7}$ M reduced (in absolute value) the electrophoretic mobility of PG liposomes, indicating that, even at these low concentrations, a significant number of the cationic drug molecules were already adsorbed to the surface of the vesicles, neutralizing the negative charges born by the phospholipid molecules.

Surprisingly, an increase in the mobilities of PG liposomes (in absolute value) was observed for drug concentrations around 10^{-6} M. In fact, this was accompanied by a large increase in the number of bright particles which could be observed in the microscope field, suggesting that Celiptium caused the large multilamellar PG vesicles to burst and redistribute in smaller vesicles.

On further addition of Celiptium to the water phase, the vesicles mobilities decreased rapidly. They were observed to be around zero for a drug concentration of 10^{-5} M, and to turn positive above this concentration. A positive upper-limit value of $10 \cdot 10^{-8}$ m$^2 \cdot$ V$^{-1} \cdot$ s^{-1} was reached for a final Celiptium concentration of $5 \cdot 10^{-4}$ M.

Celiptium-to-phosphatidylglycerol adsorption constant

It should be noted that the cation concentration in the bulk water phase was at least 1000-fold that of the lipid concentration. The changes in the concentration of cations which will result from their adsorption to lipid molecules can therefore be neglected in the calculation of their adsorption constant.

Making this assumption, a sodium-to-phosphatidylglycerol association constant of 0.16 M^{-1} was calculated from the electrophoretic mobility value of $-9.22 \cdot 10^{-8}$ m$^2 \cdot$ V$^{-1} \cdot$ s^{-1} found for PG vesicles in 10 mM NaCl, in the absence of celiptium. This is in reasonable agreement with the value of 0.4-0.6 M^{-1} obtained for the same lipid under different experimental conditions [26,28].

TABLE II

Computed values of the Celiptium-to-phosphatidylglycerol adsorption constant K_a^D and residual Celiptium bulk concentration D_b as a function of initial Celiptium bulk concentration D_t and the percentage of total PG anionic sites which are available to drug binding

Celiptium initial concn. (M)	% of PG anionic sites effectively available to drug binding			
	1%		10%	
	D_b (M)	K_a^D (M^{-1})	D_b (M)	K_a^D (M^{-1})
$5 \cdot 10^{-7}$	$3.37 \cdot 10^{-7}$	$4 \cdot 10^4$	0	n.d.
$3 \cdot 10^{-6}$ [a]	$2.8 \cdot 10^{-6}$	$2.2 \cdot 10^4$	10^{-6}	$6 \cdot 10^4$
$5 \cdot 10^{-6}$	$4.76 \cdot 10^{-6}$	$2.3 \cdot 10^5$	$2.7 \cdot 10^{-6}$	$4.1 \cdot 10^5$
$7 \cdot 10^{-6}$ [a]	$6.75 \cdot 10^{-6}$	$1.24 \cdot 10^6$	$4.6 \cdot 10^{-6}$	$1.8 \cdot 10^6$

[a] Interpolated values from Fig. 3.
n.d., not determined.

For drug molecules with a high adsorption constant, such as Celiptium, the situation is completely reversed. A PG bulk concentration of $2 \cdot 10^{-5}$ M was required for observation of enough lipid vesicles in the electrophoretic apparatus. This is 200-fold higher than the initial 10^{-7} M drug concentration we used. Under these conditions, the change in bulk drug concentration which will result from drug adsorption to lipid vesicles must be taken into account and Eqn. 13 needs to be incorporated in the calculation of K_a^D.

Moreover, in these calculations, the total number of lipid molecules does not have to be considered. For large multilamellar vesicles, the drug will only interact with the external leaflet of the outer bilayer, at least at low drug concentrations. As can be seen in Table II, the adsorption constant of Celiptium to PG was calculated on the assumption that 1% or 10% of the total PG anionic sites were available for drug interaction. A rough calculation shows that this would correspond to multilamellar vesicles consisting of about 5 and 50 lipid bilayers, respectively.

Calculations taking account of the total number of PG molecules ($2 \cdot 10^{-5}$ M PG concentration) led consistently to a negative residual drug concentration, D_b, for all K_a^D values able to account for the observed decreases in zeta potential.

As shown in Table II, for a $5 \cdot 10^{-7}$ M initial Celiptium concentration, calculations gave sensible results when only 1% of the total PG anionic sites (equivalent to a $2 \cdot 10^{-7}$ M PG concentration) were taken into account. This led to a value of K_a^D of $4 \cdot 10^4$ M^{-1}. For 10% available anionic sites, D_b was always negative and K_a^D could not be calculated. For a $3 \cdot 10^{-6}$ M Celiptium concentration, K_a^D values of the same order of magnitude were still calculated for 1% anionic sites ($K_a^D = 2.2 \cdot 10^4$ M^{-1}) and 10% anionic sites ($K_a^D = 6 \cdot 10^4$ M^{-1}). For higher drug concentrations ($5 \cdot 10^{-6}$ M and $7 \cdot 10^{-6}$ M), K_a^D was found to increase by one order of magnitude at each step.

As already mentioned, the vesicle diameter is one of the parameters which incorporated in the calculation of the zeta potential from the measured electrophoretic mobilities. PG vesicles were not homogeneous in size and their diameter was observed to decrease for Celiptium concentrations above 10^{-6} M. As can be seen in Table III, a decrease of vesicle diameter from 1 μm to 0.2 μm (the lowest observable vesicle diameter) significantly altered the calculated K_a^D value, but only at a drug concentration of $5 \cdot 10^{-7}$ M. For the higher Celiptium concentration of $5 \cdot 10^{-6}$ M, K_a^D values calculated for 1% and 10% available sites were comparable for the 0.2 μm and 1 μm diameter vesicles, with an average value of $2.6 \cdot 10^5$ M^{-1}.

Combination of surface potential and electrophoretic data

The surface potential, ΔV, in monolayers is the sum of two terms

$$\Delta V = \Delta V_p + \psi_o$$

ψ_o is the electrical surface potential in the interface plane as defined in Materials and Methods (Eqn. 5), while ΔV_p, often refered to as the polarization potential, is proportional to the projection on the normal to the interface plane of the overall dipole moment of lipid molecules. This includes water molecules in the lipid hydration shell [21]. Phospholipids are characterized by a large positive value of ΔV_p (around 400 mV). If one assumes that ΔV_p remains constant, the binding of Celiptium to PG in monolayers with increasing drug concentration in the subphase, would lead to a progressive decrease in ψ_o (in absolute value), and hence an increase in ΔV. This is illustrated by the dotted line in Fig. 2 which was obtained as follows. First, ψ_o was calculated for a 50 mM NaCl concentration in the subphase for a sodium-to-PG absorption constant of 0.16 M^{-1}. Subtracting this value (−115 mV) from ΔV (235 mV) gave ΔV_p (+350 mV). Second, ψ_o was calcu-

TABLE III

Influence of vesicle diameter on the calculation of the Celiptium-to-phosphatidylglycerol binding constant K_a^D

Vesicle diameter (μm)	% PG available sites	Celiptium initial concn. (M)	Zeta potential (ζ) (mV)	K_a^D (M^{-1})
0.2	1	$5 \cdot 10^{-7}$	−124	$7.5 \cdot 10^3$
	1	$5 \cdot 10^{-6}$	−65	$1.5 \cdot 10^5$
	10	$5 \cdot 10^{-6}$	−65	$2.7 \cdot 10^5$
1	1	$5 \cdot 10^{-7}$	−113	$4 \cdot 10^4$
	1	$5 \cdot 10^{-6}$	−59.3	$2.3 \cdot 10^5$
	10	$5 \cdot 10^{-6}$	−59.3	$4.1 \cdot 10^5$

lated for various Celiptium concentrations in the subphase, assuming a drug-to-lipid absorption constant of 10^5 M^{-1}. These values were added to ΔV_p to give the corresponding calculated ΔV values (dotted line). Experimental and calculated ΔV values were in good agreement for Celiptium concentrations below 10^{-5} M. Above this concentration, the marked decrease in the experimental ΔV seen in Fig. 2 indicates that, in fact, ΔV_p was not constant, but decreased with increasing drug concentrations. For example, at 10^{-4} M Celiptium in the subphase, comparison of experimental and calculated ΔV values produced a value of ΔV_p of only +170 mV.

Discussion

These results show that the cationic antitumoral drug Celiptium binds strongly to the anionic phospholipid, phosphatidylglycerol in two membrane model systems: monolayers and liposomes. Unfortunately, changes in electrophoretic mobility of PG liposomes with drug concentration cannot be accounted for by a unique drug-to-lipid adsorption constant K_a^D within the Gouy-Chapman-Stern theory of the electrical double layer. In fact, calculated K_a^D values are observed to increase rapidly with increasing drug concentrations in the aqueous phase.

By definition, an adsorption constant should not change with a change in drug concentration in the aqueous phase. For 1% availability of the total PG sites, Fig. 3 shows the expected changes in the electrophoretic mobility of liposomes for a Celiptium-to-PG adsorption constant, K_a^D, of $4 \cdot 10^4$ M^{-1}. It can be seen, discounting the error at 10^{-6} M, that this curve fits the experimental data up to a Celiptium concentration of $3 \cdot 10^{-6}$ M in the bulk aqueous phase. It deviates from the experimental curve above this concentration. This would indicate that Celiptium binding to PG can only be described by a Langmuir adsorption isotherm at low and moderate drug concentrations ($< 3 \cdot 10^{-6}$ M). For higher drug concentrations, this model is clearly not sufficient to account for the observed changes in electrophoretic mobility.

The shape of the experimental curve together with the fact that the calculated K_a^D values rapidly increase with drug concentration would tend to indicate a process of cooperative binding. Ellipticine and its derivatives are known to stack together forming *n*-mers of dimers above a drug concentration of $2 \cdot 10^{-4}$ M in the aqueous phase [29]. In fact, the interfacial drug concentration may be high (> 1 M) due to the electrical potential, ψ_o. A cooperative drug stacking process at the interface along with drug binding to lipid molecules might contribute to the formation of an increasingly densely packed Celiptium layer at the surface of lipid vesicle. This could reverse the sign of the electrical surface potential as is observed experimentally. This possibility is currently under investigation in our laboratory.

Assuming this latter possibility, the adsorption constant of between $2 \cdot 10^4$ and $4 \cdot 10^4$ M^{-1} found at low drug concentrations ($< 3 \cdot 10^{-6}$ M) can be considered to represent direct binding of Celiptium to PG. These values compare well with the apparent association constant of $3 \cdot 10^4$ M^{-1} found for the same molecules interacting in monolayers with cardiolipin [30]. Higher apparent association constants were found for 2-*N*-methylellipticinium interacting with cardiolipin ($2 \cdot 10^5$ M^{-1}) [30], or PG (10^6 M^{-1}) [12], and ellipticine interacting with various acidic phospholipids (around 10^6 M^{-1}) [11,12]. The differences probably stem from the fact that the dipolar Celiptium remains adsorbed at the membrane surface, whereas the amphiphilic molecules 2-*N*-methylellipticinium and ellipticine penetrate the lipid layer and intercalate between lipid molecules [12]. In any event, an association constant of $4 \cdot 10^4$ M^{-1} indicates a particularly high affinity of Celiptium for acidic phospholipids.

Others antitumoral drugs such as the anthracyclins have been shown to display high affinity for acidic phospholipids. It is interesting that these molecules, which are known to intercalate in DNA, also exhibit high binding constants for binding to phospholipids.

In monolayers, interaction of Celiptium with PG not only alters the electrical component ψ_o of the surface potential, but also the polarization term ΔV_p.

It should be noted that this decrease in ΔV_p was accompanied by an increase in film surface pressure, and that it occurred over the Celiptium concentration range of 10^{-5} M–10^{-3} M where the sign of the lipid vesicle surface charge was observed to reverse (Fig. 3). The dipolar structure of Celiptium may prevent it intercalating between lipid molecules. It probably remains at the interface in contact with lipid polar headgroups [12]. The formation of a positively charged Celiptium layer at the surface of the PG film, as suggested above for PG liposomes, might explain the observed increase in film surface pressure. The electrophoretic mobility measured for 10^{-4} M drug concentration in the water phase was identical in absolute value, to that measured for PG alone in the absence of Celiptium. At the 50 mM NaCl ionic strength used in the monolayer experiments, this would correspond to a positive electrical surface potential ψ_o of +115 mV, and to a ΔV_p value of 160–115 = 45 mV.

This effect of Celiptium on the surface potential of PG monolayers is reminiscent of that reported for the highly dipolar molecule Phloretin (μ = 5–6 D) which at $1.5 \cdot 10^{-4}$ M in the subphase typically reduces the surface potential of a monomolecular film of phosphatidylethanolamine by 200 mV [31]. The high positive polarization potential ΔV_p which characterizes phos-

pholipids is now recognized to be responsible for the very low permeability of lipid bilayers to cations [31,35]. Accordingly, it has been shown that 10^{-4} M Phloretin brings about a 10-fold increase and an increase of up to 10^3-fold in the conductance of phosphatidylethanolamine bimolecular membranes mediated by the K^+-valinomycin and the K^+-nonactin complexes, respectively [31]. Such an effect might explain the well-known influence of Phloretin on the transport of non-electrolytes and ions across various biological membranes [31,32].

In preliminary experiments, we observed that Celiptium led to a 5-fold increase in the K^+ conductance of bimolecular PG membranes treated with valinomycin (Sautereau, A.M. and Amblard, G., unpublished data).

A rather high Celiptium concentration is required to observe these effects on membrane surface potential. Nevertheless, we have recently shown that Celiptium accumulates inside living cells in response to the transmembrane electrical potential $\Delta\phi$ [14]. Typically, an accumulation of Celiptium of up to 150-fold could be detected inside the bacterium *Streptococcus pneumoniae* [14], for which the transmembrane potential is approximately +120 mV (inside negative) [14]. This drug has also been shown to accumulate in eukaryotic cells [15].

It is tempting to suggest that Celiptium, after accumulation inside its target cells, might alter the electrical properties of cell membranes and maybe their permeabilities to protons, anions and cations. In addition to its potent effect on DNA structure [8] and topoisomerase activity [33], this might contribute to the cytotoxicity of this molecule.

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