Binding of the calcium antagonist flunarizine to phosphatidylcholine bilayers: charge effects and thermodynamics

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We have examined the partitioning/transfer of the Ca^{2+} antagonist flunarizine from the aqueous phase into phospholipid bilayers. We show that the binding of the cationic amphiphilic drug flunarizine to phospholipid bilayers displays traditional linear concentration-dependent characteristics once unmasked of electrostatic effects. The coefficient for the binding/partitioning of flunarizine to phosphatidylcholine was found to be 28700 M^{-1}, supporting the notion that this drug may be particularly membrane-active. The thermodynamics of the partitioning/transfer process have also been studied using high-sensitivity titration calorimetry. Binding was found to be predominantly enthalpy-driven with only a small entropic contribution; $\Delta H = -22.1$ kJ·mol^{-1} (-5.3 kcal·mol^{-1}) at 27 °C. This is in conflict with established ideas of entropy-driven partitioning of drugs into phospholipid membranes as a result of the 'hydrophobic effect'. The strong enthalpic nature of binding is interpreted as being indicative of strong lipophilic interactions between the drug and the phospholipid phase.

INTRODUCTION

The interaction of cationic amphiphilic drugs with membranes has long been of interest to the membrane biochemist. The pharmacological activity of such molecules usually takes place at the plasma-membrane level and frequently involves an initial interaction of the drug with the membrane phospholipids even if these phospholipids are not the final 'target' of the drug molecule.

It is generally thought that amphiphilic drugs are intercalated into the phospholipid bilayer with the hydrophobic moieties inserted into the acyl chain region and their charged groups positioned in the vicinity of the glycerol backbone/phosphate region. By insertion into the phospholipid bilayer, amphiphilic drugs are able to exert a significant influence upon the phase behaviour of phospholipids. Differential scanning calorimetry (d.s.c.) has been used to assess the influence of a variety of drugs on the phase behaviour of phospholipids in a wide range of studies [see references 11–17 in Thomas and Verkleij (1990)]. We have recently used d.s.c. to demonstrate the strong influence of the Ca^{2+} antagonist flunarizine (Figure 1) on the phase behaviour of different phospholipid classes and molecular species (Thomas and Verkleij, 1990).

The (specific) Ca^{2+} antagonists have been subdivided into four classes, flunarizine being a class IV Ca^{2+} antagonist [see Godfraind (1987) and Vanhouette and Paoletti (1987) for definitions]. Classes I, II and III, represented by verapamil, diltiazem and nifedipine respectively, have each been shown to have a specific binding site on the α_1-polypeptide of the L-type voltage-gated Ca^{2+} channel found in the plasma membrane of 'excitable' cells (Hosey and Lazdunsky, 1988). It is thought that these three classes of Ca^{2+} antagonist exert their 'medically beneficial' effects by their regulation of the activity of this ion channel. Class IV Ca^{2+} antagonists (flunarizine and related molecules) have no proven specific binding with the voltage-gated Ca^{2+} slow channel. It is thought that this class of Ca^{2+} antagonist is protective by its ability to prevent the damage resulting from elevated intracellular Ca^{2+} concentrations (Todd and Benfield, 1989). This is in contrast with the three other classes of Ca^{2+} antagonist which are thought to prevent the occurrence of elevated Ca^{2+} concentrations. At this moment, ideas about the precise mode of action of flunarizine remain purely speculative. It is, perhaps, interesting to note that flunarizine has proven itself to be particularly useful in the treatment of certain neurological conditions where its ability to successfully negotiate the blood–brain barrier may be essential to its pharmacological activity.

Owing to the greatly differing chemical structures of the Ca^{2+} antagonists, it is inevitable that they have quite different physicochemical properties. Unlike many Ca^{2+} antagonists, flunarizine is very hydrophobic, even in its charged state. In order to gain a better understanding of the interaction of flunarizine with phospholipids we have investigated the binding of this drug to phosphatidylcholine (PtdCho) bilayers using centrifugation assays and ζ-potential measurements. We have then analysed the data by applying the Gouy–Chapman theory, which has been successfully used previously to examine the binding of many amphiphilic molecules to phospholipid bilayers [see McLaughlin (1989) for a recent review].

We have also examined the thermodynamic parameters of the flunarizine–PtdCho binding/partitioning process. Using a high-sensitivity titration calorimeter, we have measured the enthalpy of this process. We have been able to calculate the Gibbs energy from the experimentally derived partition coefficient and thereby also able to deduce the entropic contribution to the transfer.

MATERIALS AND METHODS

Materials

The experiments were carried out using 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, which was purchased from Avanti Polar Lipids (Birmingham, AL, U.S.A.) in dry form. Flunarizine was a gift from Janssen Pharmaceutica (Beerse, Belgium). The molar absorption coefficient of flunarizine in water was determined under experimental conditions (pH 5.0) to be $\epsilon_{252} = 20740$ (± 460) $M^{-1}\cdot cm^{-1}$ (average of six measurements).

Abbreviations used: d.s.c., differential scanning calorimetry; PtdCho, phosphatidylcholine.

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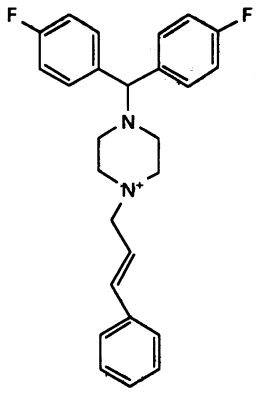

Figure 1 Structure of the univalent cationic form of flunarizine {1-[bis-(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl)piperazine}

Binding studies

ζ-potential measurements

Lipid–drug dispersions were prepared as follows. Briefly, 4 mg of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was suspended by simple mechanical dispersion in 5 cm^3 of a series of different concentrations of flunarizine (50–100 μM) in 100 mM NaCl, 30 mM citric acid/disodium hydrogen phosphate buffer (pH 5.0). Experiments were carried out at pH 5.0 to ensure that flunarizine (pK_{a1} = 7.7, Janssen Pharmaceutica, Analytical Department) was completely in its univalent charged form (pK_{a2} for flunarizine is 2.4 and is not of relevance to the studies presented here). ζ-potential measurements of the large multilamellar lipid dispersions were carried out at 25 °C using a Rank Brothers Mark II microelectrophoresis apparatus (Cambridge, U.K.). Care was taken to ensure that observations were made at the stationary level of the cell where there is effectively no net solvent flow. The average of 16 measurements in both directions was used to estimate the electrophoretic mobility, u. The ζ-potential was calculated from the Helmholz–Smoluchowski equation:

$$\zeta = \eta u/\epsilon_r\epsilon_0 \quad (1)$$

where η is the viscosity, ϵ_r the dielectric constant and ϵ_0 the permittivity of free space (McLaughlin, 1977). The ζ-potential is defined as the surface potential at a distance of 0.2 nm from the bilayer surface since the plane of hydrodynamic shear has been estimated to be at this distance from the membrane surface (Eisenberg et al., 1979). Drug concentration at equilibrium was estimated as is described for the centrifugation binding assays below.

Centrifugation binding assays

Large multilamellar dispersions were prepared as described above. The drug–lipid dispersions were centrifuged at 20 °C for 2 h at 340000 *g* giving a clear supernatant. After careful removal of the supernatant, the equilibrium concentration of flunarizine in solution ($c_{eq.}$) was determined spectrophotometrically (see under Materials). The mole fraction of bound drug, X_b, is defined as

$$X_b = n_D/n_L \quad (2)$$

where n_D drug molecules are bound to a total of n_L lipid molecules at a given drug concentration.

Drug–lipid dispersions were also prepared by freeze–thaw and extrusion methods. No differences were found in the extent of drug binding to PtdCho using three different methods, indicating that flunarizine is able to reach its partition equilibrium rapidly. The results presented in this paper for binding and electrophoretic mobility studies were all obtained from simple dispersion of the phospholipid in drug-containing buffer.

Calorimetry

Sample preparation

Large unilamellar vesicles were prepared by the extrusion method (Hope et al., 1985); 20 mg of dry phospholipid was dissolved in a small volume of chloroform in a small round-bottomed flask and evaporated to dryness on a rotary evaporator to produce a thin lipid film which was further dried overnight under high vacuum. The lipid was dispersed in 10 cm^3 of 100 mM NaCl, 30 mM citric acid/disodium hydrogen phosphate buffer (pH 5.0) and subjected to five freeze–thaw cycles. Unilamellar vesicles (approx. 100 nm in diameter) were formed by extrusion of the phospholipid dispersion ten times through 0.1 μm polycarbonate filters (Nuclepore Corporation, Pleasanton, CA, U.S.A.).

Measurement of binding/partition enthalpies

Reaction enthalpies were determined using a MicroCal MC-2 high-sensitivity titration calorimeter (MicroCal, Northampton, MA, U.S.A.) as described by Wiseman et al. (1989). The sample cell (1.278 cm^3) was filled with the extruded vesicles (2.5 mM PtdCho). Injections (10, 25 and 75 mm^3) of a flunarizine solution (approx 75 μM flunarizine in pH 5.0 buffer) were made from an injection syringe, rotating at a speed of 400 rev./min., coupled to a digital stepping motor. Measurements were made at 27 and 35 °C (±0.2 °C). The drug/phospholipid ratios for the different injection regimes, i.e. the ratio after the final injection, were as follows: 1:440 for 10 × 10 mm^3, 1:220 for 8 × 25 mm^3 and 1:200 for 3 × 75 mm^3 injections. The enthalpy value derived from the last injection was never significantly different from that derived from the first for each of the injection schedules used, indicating that the phospholipid remained in excess.

RESULTS

Drug binding to phospholipids

We have examined the binding/partitioning of flunarizine with 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine bilayers at pH 5.0. The results of centrifugation binding assays are shown in Figure 2. The binding displays non-linear characteristics indicating that less drug is taken up at higher concentrations than might be expected according to a simple partitioning where

$$X_b = K_p c_{eq.} \quad (3)$$

This deviation away from linear concentration-dependent binding can be explained by consideration of the influence of the drug's positive charge. Incorporation of flunarizine into PtdCho bilayers under conditions of low pH where it possesses a positive charge (electrical charge, $z = +1$) will cause a charging-up of the phospholipid bilayer.

We have studied the phenomenon of the charging-up of the PtdCho bilayers using microelectrophoresis techniques. The velocity of movement of PtdCho vesicles dispersed in a series of buffers of different flunarizine concentration has been measured. It is then possible to derive the ζ-potential of the vesicles from their velocities of movement by application of eqn (1). Figure 3 shows the relationship between ζ-potential and the bulk equilibrium concentration of flunarizine and clearly demonstrates

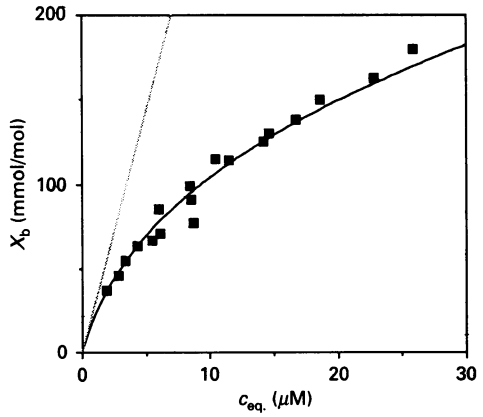

Figure 2 Binding of charged flunarizine ($z = +1$) to PtdCho bilayers studied by centrifugation binding assay

The extent of binding (X_b, mmol of flunarizine/mol of phospholipid) is plotted against the equilibrium concentration of flunarizine in the bulk solution ($c_{eq.}$). The dashed line represents an equilibrium concentration-dependent partitioning, $X_b = K_p c_{eq.}$ where $K_p = 28700\ M^{-1}$. The solid line represents the predicted binding curve calculated using the Gouy–Chapman theory in order to account for electrostatic effects.

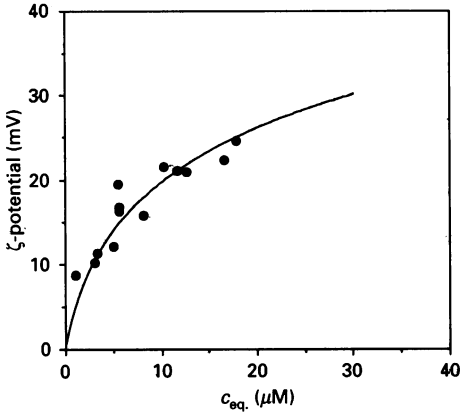

Figure 3 ζ-potential measurements of PtdCho multilamellar vesicles dispersed in buffer containing various concentrations of flunarizine

The solid line represents theoretical values of ζ-potential calculated using $K_p = 28700\ M^{-1}$ and $z = +1$, taking electrostatic effects into account by means of the Gouy–Chapman theory.

that the phospholipid bilayers do, indeed, become progressively more positively charged as drug is taken up from the aqueous phase. The charge will repel the like-charged drug molecules in the aqueous phase close to the phospholipid bilayer giving rise to a concentration gradient of the drug, with the surface concentration of the drug being lower than that some distance away ($\geqslant 2$ nm) in the bulk solution. The Gouy–Chapman theory can be used to describe such an exponential reduction in the concentration of ions in free solution approaching a charged surface of like charge (Aveyard and Haydon, 1973; McLaughlin, 1977).

In order to be able to use the Gouy–Chapman theory, we must first calculate the surface charge density, σ, from the experimentally derived degree of binding, X_b. The intercalation of n_D drug molecules into a phospholipid bilayer of n_L lipid molecules results in an increase in the surface area (Seelig, 1987). The total surface area, A_T, can be defined as

$$A_T = n_D A_D + n_L A_L \quad (4)$$

Table 1 Parameters for the binding of flunarizine to PtdCho bilayers

Membrane surface charge density (σ) was calculated using eqn. (7). Surface potential (Ψ_0) was calculated by application of the Gouy–Chapman equation [eqn. (8)]. c_m was calculated using the Boltzmann equation [eqn. (9)].

$c_{eq.}$ (μM)	X_b (mmol/mol)	σ (mC/m^2)	Ψ_0 (mV)	c_m (μM)	X_b/c_m (M^{-1})
1.93	37.5	8.45	10.1	1.30	28908
2.65	46.5	10.4	12.4	1.63	28526
2.84	47.0	10.5	12.5	1.74	27035
3.37	55.6	12.3	14.6	1.90	29256
3.65	55.8	12.3	14.6	2.05	27160
4.25	65.5	14.3	16.9	2.19	29942
4.34	65.3	14.3	16.8	2.24	29179
5.25	72.4	15.7	18.5	2.54	28498
5.49	67.9	14.8	17.4	2.76	24554
5.98	87.5	18.6	21.8	2.54	34418
6.12	72.3	15.7	18.4	2.96	24391
7.49	80.2	17.2	20.2	3.39	23680
8.04	88.9	18.9	22.1	3.38	26313
8.34	101	21.2	24.5	3.18	31785
8.48	93.0	19.7	22.9	3.45	26993
8.68	78.7	16.9	19.9	3.98	19794
10.4	118	24.3	27.9	3.48	33909
11.4	117	24.1	27.7	3.84	30447
14.2	128	26.1	29.7	4.42	28963
14.6	133	27.0	30.6	4.39	30316
16.7	141	28.3	32.0	4.75	29681
18.6	154	30.5	34.2	4.86	31702
22.8	157	32.7	36.2	5.49	30417
25.8	184	35.4	38.8	5.62	32736

where A_D and A_L are the effective surface areas of flunarizine and PtdCho respectively. These effective surface areas are experimentally accessible. The surface area for 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine has been determined by a number of different methods ($A_L = 0.68$ nm^2, Altenbach and Seelig, 1984; Evans et al., 1987). The molecular area occupied by the drug at a surface has recently been determined using monolayer techniques ($A_D = 0.83$ nm^2; P. G. Thomas and A. Seelig, unpublished work) by measuring its surface activity and applying the Gibbs adsorption isotherm equation (Gaines, 1966). This value represents a 'minimum' area for the drug molecule and was determined at pH 5.0. The binding of n drug molecules with a charge of ze_0 to a neutral phospholipid bilayer gives rise to a surface charge, Q, where

$$Q = n_D z e_0 \quad (5)$$

leading to a surface charge density of

$$\sigma = Q/A_T = n_D z e_0/(n_D A_D + n_L A_L) \quad (6)$$

which can be transformed, with the help of eqn. (2), to

$$\sigma = z e_0 X_b/(A_L + A_D X_b) \quad (7)$$

Analysis of the centrifugation binding assay data easily leads to the determination of σ for each of the experimental samples. These values are given in Table 1. Values for surface potential, Ψ_0, can be derived by computer 'fitting' procedures, matching experimentally derived values of σ with those predicted by the Gouy–Chapman theory according to

$$\sigma^2 = 2000\epsilon_0\epsilon_r RT\Sigma_i c_{i,eq.}[(\exp -z_i F_0\Psi_0/RT) - 1] \quad (8)$$

where $c_{i,eq.}$ is the concentration of species i (i.e. Na^+) in the bulk aqueous phase, z_i is the signed valency of species i and F_0 the Faraday constant. The calculated surface potentials are also given in Table 1. The concentration of flunarizine in solution at the membrane surface (denoted c_m) can then be determined with

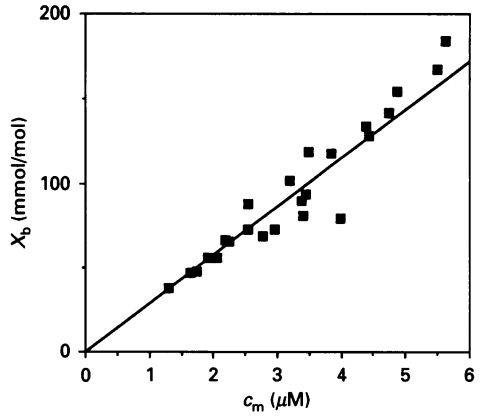

Figure 4 Flunarizine binding to PtdCho bilayers correcting for the influence of the drug's charge ($z = +1$) on its aqueous concentration at the bilayer surface

The extent of flunarizine binding (X_b, mmol of flunarizine/mol of phospholipid) is plotted against the interfacial concentration of flunarizine (c_m). The solid line corresponds to $X_b = K_p c_m$ where $K_p = 28700\ M^{-1}$.

the knowledge of the surface potential using the Boltzmann equation

$$c_m = c_{eq.} \exp\left(\frac{-z_i F_0 \Psi_0}{RT}\right) \quad (9)$$

Values for c_m are also presented in Table 1 and clearly show the remarkable effect that the partitioning of charged flunarizine molecules into the phospholipid bilayer has on the surface concentration of the drug, this being only 25% of the bulk concentration at the higher concentrations studied. Table 1 also shows the values of X_b/c_m. It is immediately apparent that the value X_b/c_m is constant and that this value will correspond to the partition coefficient for the transfer of flunarizine from the aqueous to the phospholipid phase. Plotting the interfacial concentration at the membrane surface, c_m, instead of the equilibrium concentration in bulk solution (Figure 4) generates a linear relationship between extent of drug binding and drug concentration in solution where

$$X_b = K_p c_m \quad (10)$$

The 'true' partition coefficient can then be easily calculated and is found to be $K_p = 28700 \pm 3350\ M^{-1}$.

Binding thermodynamics

In order to derive the thermodynamic parameters of the transfer process we have measured the reaction enthalpy for the association of flunarizine with PtdCho bilayers by using a high-sensitivity titration calorimeter (Wiseman et al., 1989). Figure 5(a) shows an example of one such experiment. In this experiment, a series of eight injections of 25 mm^3 of a 68.3 μM flunarizine solution were made, each injection lasting 25 s with a 5 min interval between consecutive injections. The flunarizine-binding reaction is exothermic and is constant for each injection, indicating that the phospholipid is in sufficiently large excess over added drug. The reaction enthalpies are shown in Figure 5(b), the average being $\Delta h = -41.71$ (± 0.84) μJ per injection. The heat of dilution was determined by injecting the same drug solution into the reaction cell containing only pH 5.0 buffer and was found to be $\Delta h_{dil.} = -5.06$ (± 1.51) μJ per injection. This gives a corrected value for the reaction enthalpy of $\Delta h = -36.65$ μJ (-8.76 μcal) per injection. It can be calculated that approximately 97% of the injected flunarizine binds

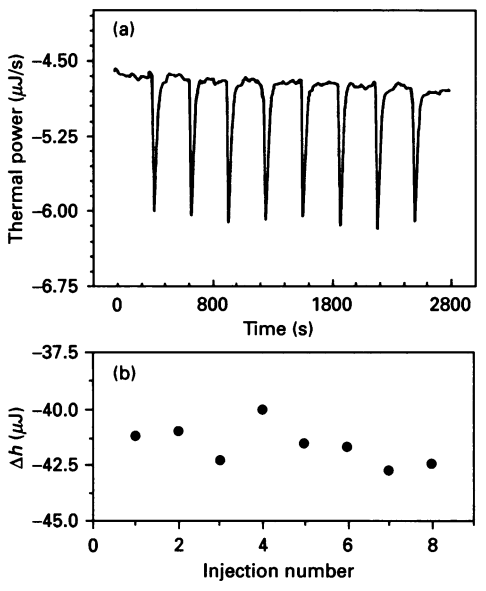

Figure 5 Measurement of the reaction enthalpy for the transfer of flunarizine from the aqueous phase to PtdCho bilayers

(**a**) Representation of the raw data acquired from eight injections of 25 mm^3 of a 68.3 μM flunarizine solution into a large unilamellar vesicle dispersion of PtdCho at a temperature of 27 °C. (**b**) Plot of the reaction enthalpies obtained by integration of the data presented in (**a**).

Table 2 Heats of reaction for the transfer of flunarizine from the aqueous phase to PtdCho bilayers

Temperature (°C)	Enthalpy ($kJ \cdot mol^{-1}$)	No. and volume of injections
27	−22.01	10 × 10 cm^3
	−22.22	8 × 25 cm^3
	−22.18	3 × 75 cm^3
35	−24.10	10 × 10 cm^3
	−24.98	8 × 25 cm^3
	−24.56	3 × 75 cm^3

to the phospholipids under the present experimental conditions. Results have been corrected accordingly for this factor.

Because of the limited solubility of flunarizine in water it was not feasible to vary the flunarizine concentration. However, we can assess the reaction enthalpy under different experimental conditions by using the same solution with different injection schedules. It can be seen from Table 2 that it has been possible to get highly reproducible results by injecting different volumes. The reaction enthalpy has been determined at both 27 and 35 °C allowing us to estimate the specific heat capacity for the transfer process.

DISCUSSION

In this study we have examined the binding of a cationic amphiphilic drug, flunarizine, to phospholipid bilayers. This binding can be successfully described as a simple charge-related phenomenon using standard surface chemistry theory coupled with ideas derived from monolayer studies (Seelig et al., 1988). We can check the theory and assumptions made in the calculations by a back-calculation using the Gouy–Chapman theory to generate synthetic data for ζ-potentials and X_b at specified free drug concentrations. Figures 2 and 3 show that there is excellent

agreement between the theoretically (solid lines) and the experimentally derived data. This supports the assumptions made in the calculations presented here, namely that the plane of hydrodynamic shear is approx. 0.2 nm from the membrane surface, that 0.83 nm^3 is a good approximation for the molecular dimensions of flunarizine and that the drug possesses a charge of $z = +1$ under our experimental conditions. Drug binding at the membrane interface has been shown to cause a shift in the pK_a of that drug molecule (Miyazaki et al., 1992); however, it is unlikely that such a shift would influence our results at almost 3 pH units below the pK_a of flunarizine in water.

The derived partition coefficient of $K_p = 28700\ M^{-1}$ can therefore be seen as the 'true' partition coefficient which has been unmasked of electrostatic influences. This is a particularly large value, especially when one considers that flunarizine is in its charged water-soluble form under the experimental conditions used in this study. It can be seen as being indicative of a strong interaction of flunarizine with phospholipids. Indeed, more than 97% of the drug has partitioned into the bilayers at the lower concentrations tested. These results support the notion that flunarizine is a particularly membrane-active drug which can penetrate and cross phospholipid bilayers and suggest that this strong interaction of the drug with phospholipids will have an important bearing on its mechanism of action.

We have also experimentally measured the enthalpy of the transfer of flunarizine from the aqueous phase to PtdCho bilayers at two different temperatures (Table 2). It is possible to derive the Gibbs function for this process from the partition coefficient using the relationship

$$-\Delta G = RT\ln(55.6K_p) \quad (11)$$

where the factor 55.6 corrects for the cratic contribution (the water molar concentration) to the free energy change (Cantor and Schimmel, 1980). Knowledge of the enthalpic and free-energy changes for the reaction permits the calculation of the entropy change via the relationship

$$\Delta G = \Delta H - T\Delta S \quad (12)$$

The calculated values are presented in Table 3. The free-energy function for the process is large and negative, indicating that the transfer from water to phospholipid is strongly favoured energetically, in agreement with results previously presented by Huang and Charlton (1972), Seelig and Ganz (1991) and Bäuerle and Seelig (1991) in their studies of the transfer of toluidinylnaphthalene sulphonate and amlodipine from the aqueous to a phospholipid phase. It is also clear from the values in Table 3 that the transfer of flunarizine from the aqueous to the phospholipid phase, like the transfer of amphiphilic molecules in the above-mentioned studies, is an enthalpy-dominated process. This is in conflict with the generally accepted view that the transfer of amphiphilic molecules from the aqueous to the membranous phase is governed by the so-called 'hydrophobic effect', that is to say that it is an entropy-driven process (Tanford, 1973). On the other hand, we can calculate, from the values presented in Table 3, that the specific heat capacity for the transfer process, $\Delta C_p = 301\ J \cdot mol^{-1} \cdot K^{-1}$ (72 $cal \cdot mol^{-1} \cdot K^{-1}$). A large negative heat capacity as seen here for this reaction is one of the basic indicators of the 'hydrophobic effect'

Table 3 Thermodynamic parameters for the partitioning/transfer of flunarizine from the aqueous phase into PtdCho bilayers

Temperature (°C)	ΔG [kJ/mol (kcal/mol)]	ΔH^* [kJ/mol (kcal/mol)]	$T\Delta S$ [kJ/mol (kcal/mol)]
27	−35.77 (−8.55)	−22.13 (−5.29)	+13.64 (+3.26)
35	−36.61 (−8.75)	−24.56 (−5.87)	+12.05 (+2.88)

* Values corrected for 97% drug binding under experimental conditions.

Our experimental observations can, perhaps, be best explained by considering that the entropy-driven thermodynamic event of the hydrophobic drug leaving the aqueous phase may be masked by a second (or more) enthalpy-driven event(s). A good candidate for such an event would be the interaction of the drug with the lipid phase. This would be expected to be an energetically favourable and strongly enthalpic interaction like the thermodynamically similar processes of transfer of apolar aromatic molecules between aqueous and pure liquid environments (Amidon and Anik, 1980) and the formation of inclusion complexes by benzene derivatives with cyclophane ring systems (Smithrud et al., 1991). In both of the above-mentioned cases, the enthalpic nature of the transfer processes has been attributed to non-bonding interactions between the apolar molecules involved. We can envisage that such interactions will also play an important role in the thermodynamics of the binding of flunarizine to phospholipid bilayers.

In recent years the general term 'hydrophobic effect' has been reconsidered and has been subdivided into the two clearly distinct concepts of hydrophobic hydration and hydrophobic interactions (Franks, 1975; Huot and Jolicoeur, 1985). The enthalpic nature of the transfer process presented here indicates that it cannot be thought of thermodynamically solely in terms of the changes occurring in the aqueous phase, i.e. only in terms of hydrophobic hydration (entropy) as has often previously been the case. Hydrophobic, or perhaps better expressed, lipophilic interactions between the transferred molecule and its new apolar environment should be considered too. That is to say, the energy of transfer will be a summation of several thermodynamic events including entropy-driven hydrophobic repulsion of the drug out of the water phase and enthalpy-driven hydrophobic attraction between drug and phospholipid.

Hydrophobic/lipophilic interactions have previously been considered to be thermodynamically insignificant. However, it is becoming increasingly apparent that forces such as van der Waals interactions are thermodynamically very important in processes such as protein folding and receptor–ligand interactions (Ross and Subramanian, 1981). Similarly, we can envisage the importance of these interactions to the process examined in this paper.

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