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THE INFLUENCE OF PHOSPHOLIPIDOSIS INDUCING DRUGS ON THE PHASE TRANSITION OF LIPIDS

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Abstract

The interaction of four monovalent cationic amphiphilic drugs (phentermine, chlorphentermine, amitriptyline, 1-chloro-amitriptyline) with dipalmitoylphosphatidylcholine (DPPC) bi-layer vesicles was investigated by optical (fluorescence, 90° light-scattering) and calorimetric (DTA) techniques. From the obtained results - disappearance of pretransition, lowering of temperature of main transition, minor effect on heat of transition - it is deduced, that the drug - lipid interaction is based on two effects: hydrophobic interaction and ion-complex formation between drug and lipid.

Amphiphilic drugs show as well hydrophilic as lipophilic affinities, based on their capability of ionization by protonation and on having a large hydrophobic moiety, usually containing one or more aromatic rings. It is well established, that certain cationic amphiphilic drugs may cause an abnormal accumulation of phospholipids in organs with a high content and/or rapid turnover of phospholipids in animals and man /1, 5, 6/. Freeze-fracture studies of cytoplasmic inclusions

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in various cell types after in vivo treatment of test animals with different lipidosis inducing drugs revealed the accumulation of lamellated or crystalloid cytoplasmic inclusion bodies containing polar lipids /3/. From NMR-studies /11/ and from biochemical investigations /10/ it was deduced, that a direct interaction between the amphiphilic drugs and the polar lipids should be responsible for the impaired degradation of the lipids and the accumulation of the lipid-drug-complexes within lysosomes. From binding studies of a number of amphiphilic drugs to different polar lipids in vitro it was suggested, that the hydrophobic forces are mainly responsible for the binding of amphiphilic drugs to phosphatidylcholine /7/. Lüllmann et al. /5, 6/ proposed, that amphiphilic molecules can form ionpair complexes with phospholipids, the positively ionized basic group binding electrostatically with the negatively charged phosphate of the phospholipid, and the hydrocarbon portion of both molecules interacting through hydrophobic bonding.

In our studies dipalmitoylphosphatidylcholine (DPPC) was chosen as model lipid having a head group containing both positive and negative charges (zwitterionic) and being present in great quantities in biological membranes. The zwitterionic structure leads to pronounced electrostatic forces between the hydrophilic head groups which favour a rigid crystalline structure still allowing individual molecules to move within the plane of this two-dimensional crystal. The interaction between the monovalent cationic amphiphilic drugs and the polar lipids now can be expected to affect the crystalline

structure of the model bilayer membranes (vesicles) made from DPPC and with that the temperature and enthalpy of the gel to liquid crystal phase transition. At this transition the crystalline (gel) organization of the lipid chains is converted into a melted fluid state. Chapman et al. /4/ have shown that the characteristic transition temperature and the corresponding enthalpy of a lipid can be shifted to higher or lower values dependent upon interactions of the lipids with, for example, metal ions, polypeptides or proteins. Therefore, it seems reasonable to investigate a phase shift possibly caused by phospholipidosis inducing drugs.

In recent years sophisticated optical /for review see 8, 14/ and calorimetric /9/ methods have been developed for the measurement of this phase transition in biological and model membranes. In this paper, therefore, a straightforward physical approach is started applying as well the fluorescence technique as 90° light-scattering and differential thermal analysis (DTA) for the study of the influence of the amphiphilic drugs phentermine, chlorphentermine, amitriptyline, 1-chloroamitriptyline (for chemical structures see table 1) on the phase transition of bilayer vesicles made from DPPC.
From a combination of the results obtained from the optical and the calorimetric measurements statements on the influence of the drugs on the cooperativity of the lipid phase transition are made.

Materials and methods

Lipid material L-α-dipalmitoylphosphatidylcholine was purchased from Sigma Chemical Comp. and used without further purification. The drugs used were phentermine (Mirapront®, Mack, Illertissen, Germany), chlorphentermine (Pre-Sate®, Warner-Chilcott, USA), amitriptyline, 1-chloro-amitriptyline (Hoffmann-La Roche, Basel, Switzerland). All compounds existed as hydrochloric salts.

As fluorescent probe N-phenylnaphthylamine (NPN) obtained from Fluka, Buchs, Switzerland, was used.

For the preparation of the lipid vesicles the DPPC was first dissolved in a chloroform solution. The chloroform was then removed under a flow of nitrogen, the last traces were expelled under vacuum. The resulting lipid film was taken up in destilled water (usually $1.4 \cdot 10^{-3}$ M) and sonicated with a Branson sonifier for 6 min under a stream of nitrogen at 50 W and 315 K - that is above the phase transition temperature of DPPC. After a 10-fold dilution these samples were used directly for 90° light-scattering measurements (λ = 400 nm) which were performed - as were the fluorescence measurements - with an Aminco Bowman spectrophotofluorometer at increasing temperature which was continuously monitored via a thermocouple inserted directly into the sample cell.

For the fluorometric measurements methanolic solution (3 %) of NPN at final concentrations of $1 \cdot 10^{-5}$ M in water were added to the lipid dispersions (lipid concentration $1.4 \cdot 10^{-4}$ M).

The drugs were added in a concentration range of $8 \cdot 10^{-4}$ - $3 \cdot 10^{-2}$ M. All samples were subsequently incubated at 323 K for 20 min.

After incubation the samples were cooled to 285 K and the measurements were performed at heating rates of 1-2 K/min. The measurements were repeated several times and the transition temperature was determined as described by Träuble and Overath /13/.

The calorimetric measurements were performed using a DTA-device based on a microcalorimeter development described earlier /2/. The sensitivity of the instrument is 0.2 mJ/s and is achieved by optimal thermal insulation of the samples in high vacuum. For absolute determinations the transition enthalpies are determined by direct comparison with the melting enthalpies of undecan acid (T_m = 301.5 K, $\triangle H_m$ = 25.1 kJ/mol) and dodecan acid (T_m = 316.5 K, $\triangle H_m$ = 36.6 kJ/mol) from the areas of the endotherm peak. The heating rate was adjusted to 2.5 K/h. Each calorimeter run was repeated at least three times. The samples contained approximately 10 mg of sonicated DPPC-solution in 0.3 ml water, the reference sample instead of DPPC the same amount of one of the fatty acids. The drugs were added to give the same final concentrations as stated above.

The DTA-measurements were only performed with two of the drugs (chlorphentermine and 1-chloro-amitriptyline). The drug : lipid ratio had to be restricted to values below 50 : 1 due to the limited sensitivity of the DTA-device requiring a

high lipid concentration. On the other hand, the optical measurements were extended to drug : lipid ratios up to 200 : 1 allowing to study even small absolute effects of weakly interacting drug (e.g. phentermine).

As a measure of cooperativity of the phase transition the expression

$$\left(\frac{\Delta H_{cal}}{\Delta H_{van't\ Hoff}} \right)^2 = \sigma$$

can be used. H_{cal}, the true enthalpy is determined calorimetrically and the van't Hoff enthalpy is calculated from fluorescence intensity curves according to $\Delta H_{van't\ Hoff} = 4\ R \cdot T_m^2 \left(\frac{d\theta}{dT}\right)_{\theta=1/2}$ (R gas constant, T_m temperature of main transition, θ molar ratio of melted to non-melted lipid).

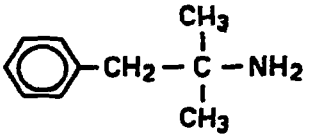
According to this definition $\sigma = 1$ would represent the case of vanishing whereas $\sigma \longrightarrow 0$ that of increasing cooperativity.

Results

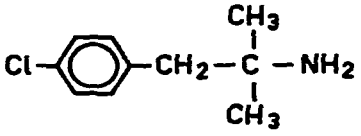
In table 1 the chemical structure of the four drugs of this study are compiled. In view of the quantitatively different interactions between the various drugs and the lipid demonstrated below it should be pointed out that on the one hand phentermine and chlorphentermine and on the other hand amitriptyline and 1-chloro-amitriptyline differ only in a chlorine atom at the aromatic ring system. The optical measurements were performed as well fluorometrically as by 90° light-scattering to elucidate a possible interaction of the fluorochrome with the

Table 1: Generic names and chemical structures of the compounds studied

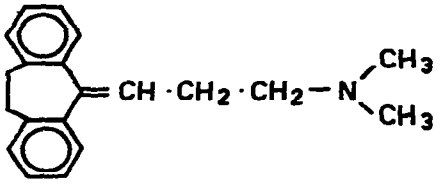
(a)



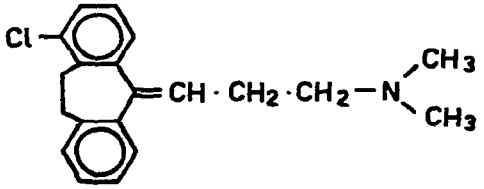
(b)



(c)



(d)



(a) phentermine, (b) chlorphentermine,
(c) amitriptyline, (d) 1-chloro-amitriptyline

drug or the lipid-water system. The dye itself was found to cause a slight shift in the transition temperature (0.5 - 1.0 K) as compared to the light-scattering data. This fact was accounted for by taking the transition temperature of the pure lipid-water system measured by 90° light-scattering ($T \approx 314$ K) as a reference.

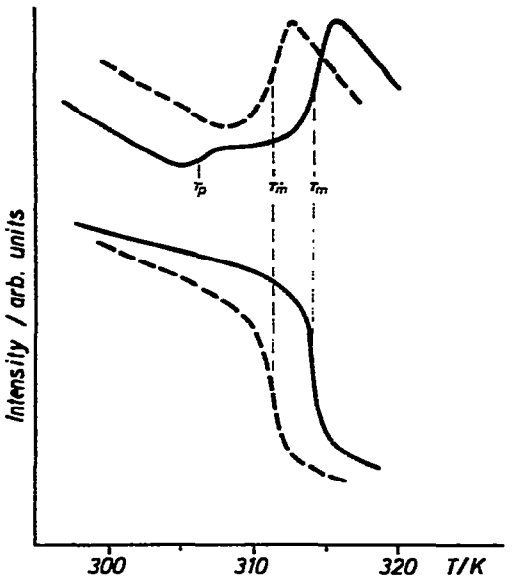


Fig. 1: Spectrometer scans for fluorometric (top) and light-scattering (bottom) measurements with the pure DPPC-water system (——) and chlorphentermine added at a drug : lipid molar ratio of 52 : 1 (- - -).
(T_p temperature of pretransition; T_m, T_m' temperatures of main transition of the two systems.)

Figure 1 shows examples for spectrometer scans from light-scattering and fluorometric measurements with pure lipid-water systems and with chlorphentermine added (drug : lipid molar ratio 52 : 1). In the fluorometric scan of the pure system an endothermic pretransition ($T_p \approx 307$ K) is indicated. This pretransition does not appear in the respective scan of the drug-lipid system. In fact, it vanishes already at the low drug concentration (52 : 1). At the pretransition the arrangement of the lipid hydrocarbon chains is transformed

from a tilted order to an order where the chains are perpendicular to the bilayer surface associated with an increase in the mobility (fluidity) of the polar head groups. With this increase in fluidity the temperature of the main transition is lowered which is also evidenced in figure 1. Here, the influence of the drug on the main transition expresses itself in a temperature shift of approximately ΔT_m = 2.5 K.

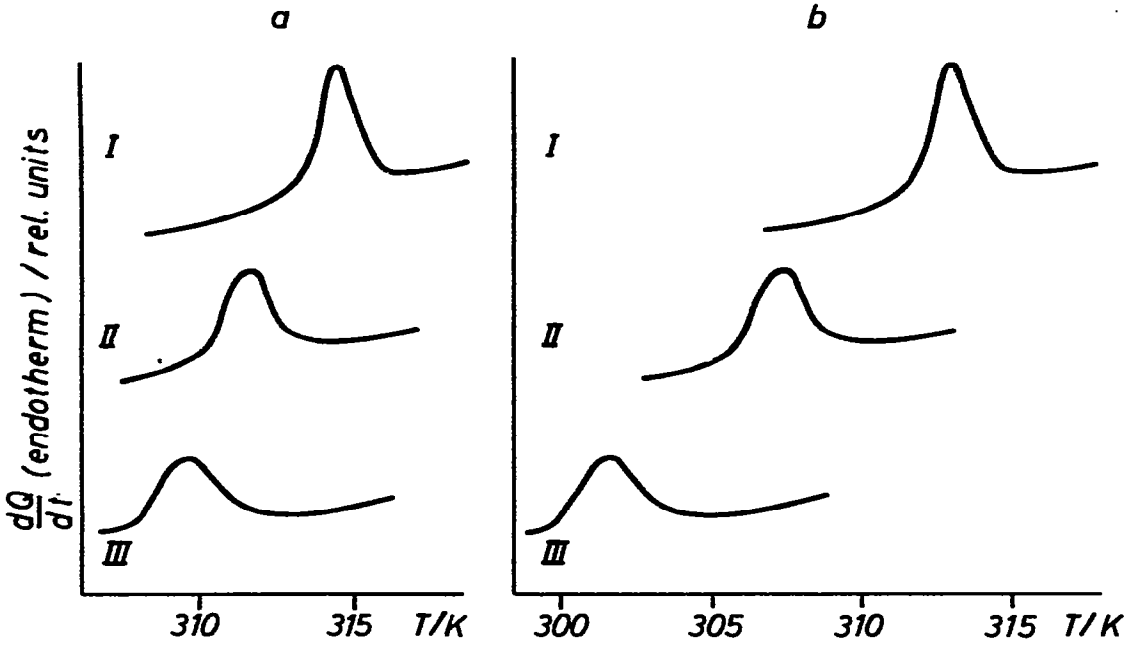


Fig. 2: DTA-heating curves of DPPC-water systems with varying amounts of (a) chlorphentermine and (b) 1-chloro-amitriptyline. Drug : lipid molar ratios: I) 0 : 1, II) 13 : 1, III) 40 : 1

In figure 2 typical DTA-curves are presented for the DPPC-water system with varying amounts of chlorphentermine (a) and 1-chloro-amitriptyline (b). Evidently only minor changes in the heat of transition are induced by the drug-lipid interaction. For example, the measured value for the pure DPPC-water system is ΔH_{cal} = 36 kJ/mole, whereas at a chlorphentermine : lipid ratio of 40 : 1 ΔH_{cal} is lowered to 30.5 kJ/mole.

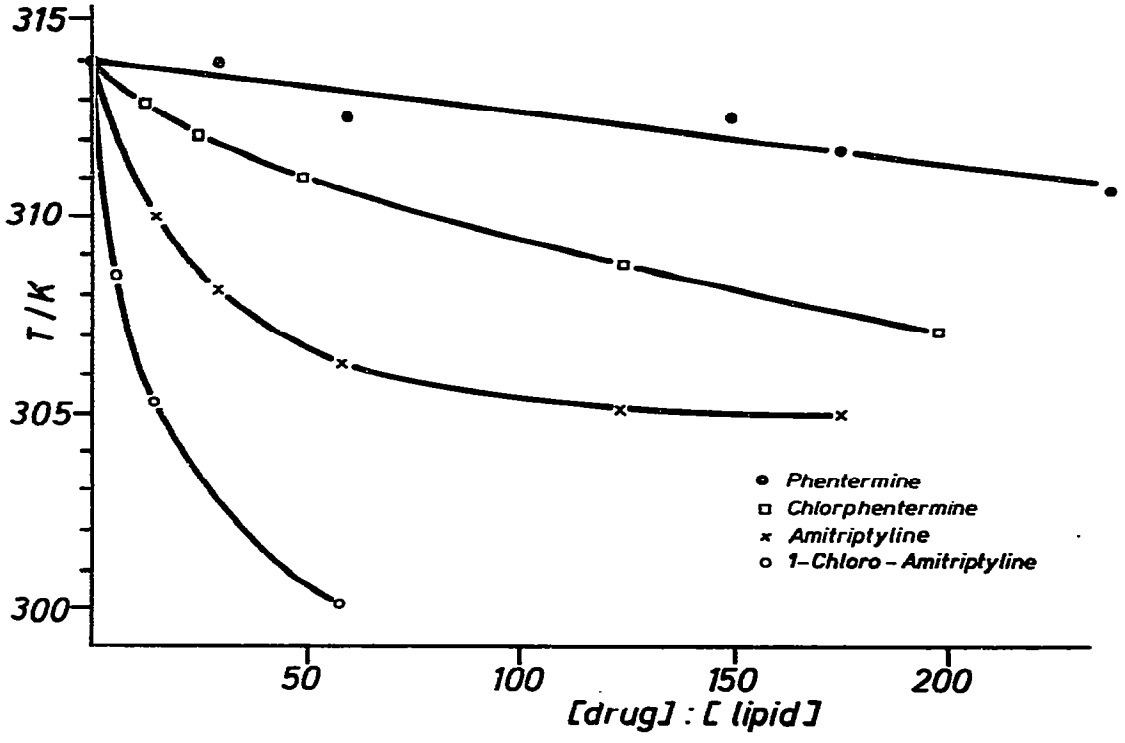


Fig. 3: Gel to liquid crystal phase transition temperatures for the DPPC-water system in dependence on the concentration (molar ratios) of the four investigated drugs

Evaluation of the corresponding fluorometric scans show that the van't Hoff-enthalpies $\Delta H_{van't\ Hoff}$ are also decreasing with increasing drug concentrations in a comparable order of magnitude relative to the pure DPPC-water system. Thus, within the margins of error of approximately 10 % involved in both measurements the cooperativity of the phase transition seems to be almost unaffected by the drug interaction.

The dependence of the shift in the transition temperature of the DPPC-water system on the concentration of the various drugs is plotted in figure 3. From this the quantitatively different effects of the four drugs become obvious. In fact, the various experimental techniques applied gave similar results for the decrease of the transition temperature T_m with increasing drug content.

Discussion

The results from the present investigations on the interaction between the four amphiphilic drugs and the DPPC may be summarized as follows: disappearance of the pretransition at relatively low drug concentrations, shift in the temperature T_m of the main transition, and minor effect on the corresponding heat of transition, ΔH_{cal}, and the van't Hoff-enthalpy, $\Delta H_{van't\ Hoff}$, leading to an almost unaffected cooperativity of the main transition.

From this it can be deduced that the interaction is mostly restricted to the polar head region of the lipids, because it is known /12/, that those compounds penetrating the interior of the lipid bilayer and disturbing the order of the hydrocarbon chains lead to a marked change in the heat of the main transition, whereas compounds interacting electrostatically with the polar head group do not - or to a minor extent - affect this transition.

This evidence is also backed by two other investigations by NMR which indicate that the drug molecules do neither interact with the alkyl chains of the phospholipids /1/ nor with nonpolar lipids /11/.

A quantitative comparison of the drug induced shift of the phase transition temperature by the various drugs at same drug : lipid molar ratios with the partition coefficients of the drugs as determined by Lüllmann and Wehling /7/ (amitriptyline was not included in their investigations) points to a strong correlation of the drug-lipid interaction with the corresponding appropiate partition coefficients into the lipid.

For the mode of interaction between the amphiphilic drugs and the polar lipid it is therefore supposed that the hydrophobicity of the drugs is the vehicle governing the quantity of the effect which is, however, based mainly on ionic interaction between the protonated aliphatic chain of the drugs and the negatively charged phosphate groups of the phospho-

lipid leading to complexation. This way a further penetration of the drugs into the region of the hydrocarbon chains and disturbation of the crystalline structure is largely prohibited.

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