Biochemica et Biophysica Acta, 507 (1978) 185—197

BBA 77924

CALORIMETRIC, ^{13}C NMR, AND ^{31}P NMR STUDIES ON THE INTERACTION OF SOME PHENOTHIAZINE DERIVATIVES WITH DIPALMITOYL PHOSPHATIDYLCHOLINE MODEL MEMBRANES

J. FRENZEL, K. ARNOLD and P. NUHN

Sektion Biowissenschaften und Sektion Physik der Karl-Marx-Universität, Leipzig (G.D.R.)

(Received August 5th, 1977)

Summary

1. The dipalmitoyl phosphatidylcholine/water system was employed to study the interaction of phenothiazines with model membranes. In particular, the effects of the drugs upon the lipid phase transition were examined using differential scanning calorimetry and NMR spectroscopy. The studied phenothiazines have peripheral (diethazine) or central (chlorpromazine) properties.

2. Both drugs were observed to lower the phase transition temperature of dipalmitoyl phosphatidylcholine. The molar activity of chlorpromazine is somewhat higher than that of diethazine. At low concentrations the drugs affect the dipalmitoyl phosphatidylcholine pretransition endotherm.

3. In the ^{13}C NMR spectra of the drug-containing samples the signal of the trimethylammonium group of dipalmitoyl phosphatidylcholine is broadened, whereas a narrowing of the signal of the fatty-acid chain methylene groups is observed. Further, addition of the phenothiazines causes higher values of the effective chemical shift anisotropy of the ^{31}P in the phosphate group, in comparison to the pure dipalmitoyl phosphatidylcholine sample.

4. The results obtained by three different techniques indicate a higher fluidity in the fatty-acid chain region and a mobility reduction of the polar headgroup of the dipalmitoyl phosphatidylcholine molecules in the presence of the phenothiazines. These phenomena can be well accounted for by a model for the incorporation of the phenothiazines in the dipalmitoyl phosphatidylcholine bilayer, in which the dialkylaminoalkyl chains are located near the polar headgroups and the ring system does not penetrate far beyond the glycerol backbone into the hydrocarbon phase.

Abbreviations: T_1, temperature of transition onset; T_{max}, phase transition maximum temperature; ΔT, transition width in temperature; $\Delta T_{1/2}$, half-height transition width in temperature; NMR, nuclear magnetic resonance; $\Delta\nu_{eff}$, effective chemical shift anisotropy.

Introduction

In the last few years numerous investigations have been carried out to gain insight into the interaction of drugs with biological membranes or with simple model systems. It has been found that some substances can interact directly with specific protein receptor sites, whereas others interact with membrane lipids or with hydrophobic regions of the membrane. Now there is evidence that some antidepressant-active drugs may induce a transition of phospholipid acyl chains from an ordered gel to a liquid crystalline state in a phospholipid model membrane [1—5]. This demonstrates that, in principle, membrane fluidity in a local region could be affected by such interactions and that in this way the function of various membrane-bound proteins may be changed.

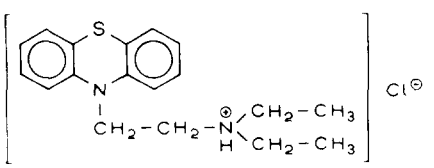
The purpose of the present study was to investigate the molecular mechanisms of the interaction of the drugs diethazine and chlorpromazine with model membranes composed of synthetic dipalmitoyl phosphatidylcholine by application of differential scanning calorimetry, ^{13}C and ^{31}P NMR spectroscopy and to correlate the results with the different pharmacological actions of both phenothiazine derivatives.

The advantage of the experimental methods chosen is that the same system can be used for all measurements and that the three techniques provide complementary information: differential scanning calorimetry shows the global behaviour of the "doped" dipalmitoyl phosphatidylcholine/water systems. On the other-hand, ^{13}C NMR spectroscopy gives some information about the mobility both in the polar headgroup region and in the fatty-acid chain region, whereas ^{31}P NMR spectroscopy reflects only the mobility in the headgroups; in particular, of the phosphate groups.

Materials and Methods

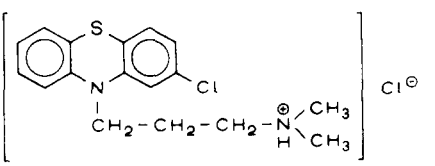
Chemicals

Dipalmitoyl phosphatidylcholine (1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine) was purchased from Ferak (Berlin) and used without further purification. The phenothiazine derivatives were obtained from Kombinat Arzneimittelwerk Dresden (AWD, G.D.R.) and were of AB 2/G.D.R. grade. They were used as hydrochlorides. The structures of the drugs are shown in Fig. 1. The water was twice distilled in an all-glass apparatus. ^2H_2O was from Isocommerz Berlin-Buch, G.D.R. (99.7 atom% ^2H). The chloroform was of analytical



Diethazine Hydrochloride

10-(2-Diethylaminoethyl)phenothiazine Hydrochloride



Chlorpromazine Hydrochloride

2-Chloro-10-(3-dimethylaminopropyl)phenothiazine Hydrochloride

Fig. 1. Structures of the studied phenothiazine derivates.

reagent grade and fractionally redistilled after purification from degradation products used before.

Differential scanning calorimetry

The drug/lipid mixtures were prepared as follows. In every case approx. 60 mg of the pure solid dipalmitoyl phosphatidylcholine and the correct amounts of the corresponding phenothiazine for the desired molar drug:lipid ratios were dissolved in a mixture of approx. 3 ml chloroform and a drop of water. Then the bulk solvent was eliminated in a rotary evaporator under an atmosphere of pure nitrogen. The last traces of the chloroform/water mixture were then expelled by placing the sample under vacuum in a vacuum desiccator over phosphorus pentoxide for at least 3 h.

The thin drug/lipid film was suspended in 1 ml of water by manual shaking. The suspension was carefully heated in a water bath to approx. 70°C. The aqueous dispersions of the drug/lipid mixtures were produced by mechanical agitation of the samples above the phase transition temperature of the lipid under nitrogen for 5 min. This step was repeated several times to ensure that the mixing of the different components was complete. This procedure appeared to provide more homogeneous aqueous phospholipid dispersions than may be obtained by shaking a mixture of dry phospholipid powder and the aqueous solution. Careful mixing was found to be necessary to obtain reproducible results. The dispersions of the drug/lipid mixtures in excess water had a bulk pH of approximately 3.

After this, the excess water was removed with a rotary evaporator under reduced pressure and nitrogen at 65°C. The final water content of the samples was adjusted to 50—80 weight% of water. The ready samples were sealed under nitrogen, briefly heated to approx. 60°C in the water bath and stored in the dark at room temperature overnight to ensure complete equilibration.

The phenothiazines which contain a sulphur atom in the tricyclic ring are very susceptible to decomposition by air oxygen or by light. Therefore, all solvents including water and ^2H_2O were deaerated with pure nitrogen. Thereafter all procedures were carried out under an atmosphere of pure nitrogen. Additionally, the samples were stored in a beaker which was wrapped and covered with aluminium foil to avoid exposure to light.

For calorimetric studies the samples were examined in sealed calorimeter pans with a differential scanning calorimeter (Perkin Elmer DSC-1B). Indium was used to calibrate the apparatus for quantitative heat determinations. All scans were obtained with a range setting of 4 and 2 mcal/s and a scanning rate of 4 K/min. Each drug/lipid mixture was run at least four times. All experiments were repeated using two separate preparations. The enthalpies of transition (ΔH) were calculated from the areas under the excess specific heat curves, which were determined by weighing the traces of the transition profiles.

Nuclear magnetic resonance spectroscopy

The NMR samples were prepared in a manner similar to that described for the calorimetric ones above. However, approx. 200 mg of the pure solid dipalmitoyl phosphatidylcholine were needed for one drug/lipid mixture. In all cases water was replaced by ^2H_2O. The final water content of the dispersions was

adjusted to 50 weight% of ^2H_2O as correctly as possible.

The ^{13}C Fourier transform and ^{31}P Fourier transform NMR spectra were obtained in a Bruker HX-90 spectrometer operating in a Fourier transform mode at 22.63 and 36.4 MHz, respectively. The instrument was equipped with temperature control, a deuterium "lock" and proton-decoupling facilities. In the case of the ^{13}C Fourier transform NMR spectra a pulse width of 20 μs was used and about 5000 free induction decays were accumulated. Subsequently, these data points were Fourier transformed to give a real frequency domain spectrum. For the ^{31}P Fourier transform NMR spectra up to 2000 free induction decays were accumulated employing a 0.1 s interpulse time. The measurements were made in the presence of strong broad band proton decoupling. Sufficient time was allowed for the tube and its content to come into thermal balance before the measurements were initiated.

Results

(1) Differential scanning calorimetry

The transition profile for a pure simple dipalmitoyl phosphatidylcholine/water dispersion without any drug (see Fig. 2a) showed, for the main transi-

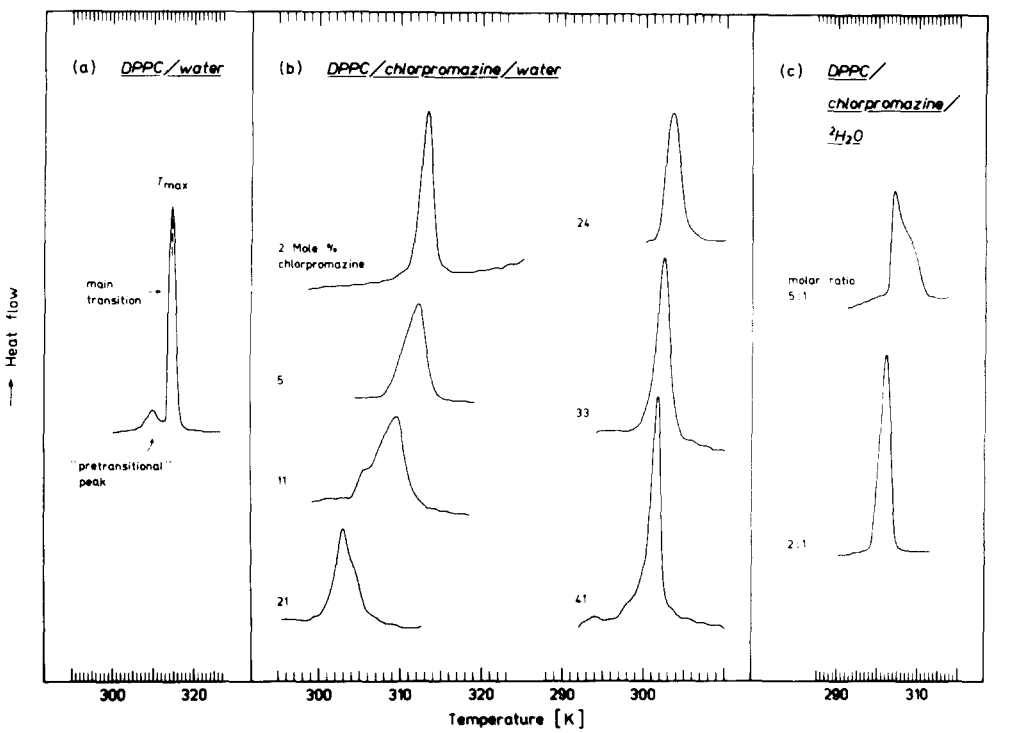


Fig. 2. Differential scanning calorimetry for dipalmitoyl phosphatidylcholine (DPPC)/chlorpromazine/water mixtures. Heating rate, 4 K/min. (a) Transition profile for a pure simple dipalmitoyl phosphatidylcholine/water dispersion without any drug. (b) Some thermograms of the dipalmitoyl phosphatidylcholine/water system at different drug concentrations (downward: 2, 5, 11, 21, 24, 33, and 41 mol% of chlorpromazine). (c) Thermograms of the dipalmitoyl phosphatidylcholine/chlorpromazine/^2H_2O dispersions which were used for the NMR studies. Molar ratios of dipalmitoyl phosphatidylcholine to chlorpromazine, 5 : 1 (above) and 2 : 1 (below). The given concentrations of chlorpromazine are the total concentrations of drug in the phase system dipalmitoyl phosphatidylcholine/water.

tion, an onset temperature and a maximum temperature of 313.1 K and of 314.6 K, respectively, and a width and half-height width for the transition of 4.4 K and 1.0 K, respectively. In the case of a sample which contained 50 weight% of water the calculation of the transition enthalpies (ΔH) yielded 1.5 kcal/mol for the "pretransitional" peak and 8.8 kcal/mol for the main endotherm. The main chain transition enthalpy from dipalmitoyl phosphatidylcholine is in good agreement with that found previously by Phillips et al. [6], Chapman et al. [7], Jacobson and Papahadjopoulos [8], Vaughan and Keough [9] and Jain et al. [3], but is lower than the value reported by Hinz and Sturtevant [10].

Below approximately 80 weight% of water the dipalmitoyl phosphatidylcholine/water dispersions containing the phenothiazine derivatives formed jelly-like masses. To avoid possible heterogenety in the samples by further evaporation of water a correspondingly higher water content was chosen for the drug-containing lipid/water dispersions. Pure dipalmitoyl phosphatidylcholine/water dispersions with so much water provide for the transition enthalpy of the main peak values which are about 1 kcal/mol lower ($\Delta H \approx 7.5$—7.9 kcal/mol). This discrepancy from the dispersions containing 50 weight% of water is unexpected because further water addition should only increase the proportion of the free water. In the simplest case the differences presumably result from the base line problem as outlined by Hinz and Sturtevant [10]. On the other hand, this discrepancy does not influence the relative effects produced by added phenothiazine derivatives because of the uniform base line determination for all studied samples. Moreover, the "zero point" was estimated under the same conditions.

The addition of the phenothiazines to the dipalmitoyl phosphatidylcholine/water system as a third component causes a shift in the phase transition temperature of the dipalmitoyl phosphatidylcholine to lower temperatures. Some

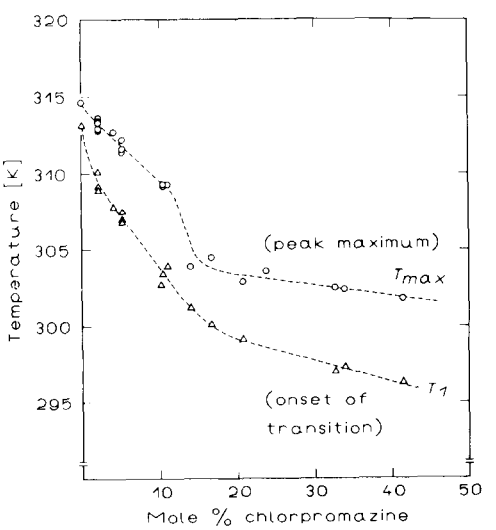


Fig. 3. Phase diagram, for chlorpromazine with dipalmitoyl phosphatidylcholine. △, T_1, Temperature of transition onset; ○, T_{max}, phase transition maximum temperature.

transition profiles for the example of chlorpromazine at different drug concentrations are shown in Fig. 2b. The shift increases as the concentration of the drug increases. Fig. 3 shows the phase diagram for chlorpromazine with dipalmitoyl phosphatidylcholine. The effect of chlorpromazine is larger than that of diethazine. The molar activities for a shift of the phase transition temperature by 1 K are 0.048 mol of diethazine/mol dipalmitoyl phosphatidylcholine and 0.033 mol of chlorpromazine/mol of dipalmitoyl phosphatidylcholine. An effect is already evident at such low concentrations as 2 mol% of drug. In the heating curves the "pre-transitional" peak can no longer be observed and the main peak becomes asymmetric.

The lowering of the phase transition temperature of dipalmitoyl phosphatidylcholine with increasing drug concentrations is accompanied by a broadening of the transition profile within the concentration range of 5—15 mol%. Therefore, the plots of width and half-height width for the transition as a function of the drug concentrations show a maximum near 10 mol% (9.5 K and 4.7 K, respectively, for chlorpromazine) within the concentration range examined (0—50 mol%). At higher concentrations the heating runs become narrow again. This peak broadening may have its cause in an incomplete miscibility of the components. For instance, in the sample there may be two portions, a smaller one containing a higher drug content than that added and a larger one with a lower amount of drug.

The enthalpies of transition (ΔH) of the drug/lipid mixtures are ploted as a function of the drug concentration in Fig. 4. It is apparent that for all these "doped" bilayers there were only little or no changes in the heat of transition at all concentrations tested. None of the examined drugs significantly lowered the enthalpy of transition of the lipid.

According to some empirical rules, postulated by Chapman et al. [2], a lowering of the phase transition temperature of dipalmitoyl phosphatidylcholine, without changes in the transition enthalpies, is an indication of an interaction of the added molecules with the polar headgroup region and a partial penetration into the hydrocarbon region.

The strong shift in the thermal transition of the phospholipid to lower temperatures is probably mainly related to the interaction of the phenothiazines

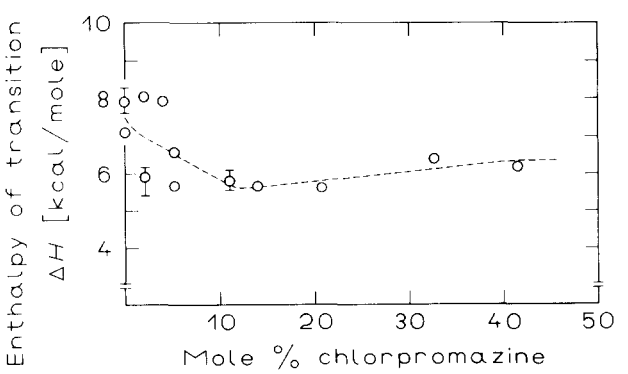


Fig. 4. Enthalpies of transition (ΔH) of the dipalmitoyl phosphatidylcholine/chlorpromazine/water system in dependence on drug concentration. Main transition heating run. The vertical bars on this graph are not error bars but represent the absolute values measured.

with the headgroup which results in a less efficient packing of the lipid fatty-acid chains.

In contrast, on addition of cholesterol the heat of transition was found to be significantly lowered [11]. This indicates that such a disruption of the lipid chain packing as occurs with cholesterol does not appear in the presence of phenothiazines.

In the heating curves the "pre-transitional" peak disappears at very low concentrations of chlorpromazine (Fig. 3). This "pre-transitional" peak is a feature of saturated long chain phosphatidylcholines [9]. Recent X-ray studies by Janiak et al. [12] show that the pretransition is associated with a structural transformation from a one-dimensional lammelar to a two-dimensional monoclinic lattice consisting of lipid lamellae distorted by a periodic ripple. The appearance of the pretransition may arise from specific interactions between the choline moiety of the polar headgroup and the structured water matrix sorrounding it.

Therefore, in agreement with our conclusion that chlorpromazine interacts with the polar region of phospholipids, pretransition is strongly affected by chlorpromazine.

Diethazine has the same effects upon the thermal behaviour of dipalmitoyl phosphatidylcholine/water dispersions, with the exception of a lower molar activity at high drug concentrations. In the limits of experimental error the calorimetric data are equal to those reported for chlorpromazine above.

(2) ^{31}P NMR measurements

The ^{31}P NMR spectra of unsonicated dipalmitoyl phosphatidylcholine/water dispersions in the presence of intense proton noise decoupling exhibit a solid state type of lineshape (see Fig. 5) which may be attributed to the chemical shift anisotropy of the phosphate phosphorus [13]. The individual spectra can be characterized by the aid of the effective chemical shift anisotropy $\Delta\nu_{eff}$ as indicated in Fig. 5.

The effective chemical shift anisotropy was measured in dependence on temperature for a pure dipalmitoyl phosphatidylcholine/^2H_2O dispersion as well as

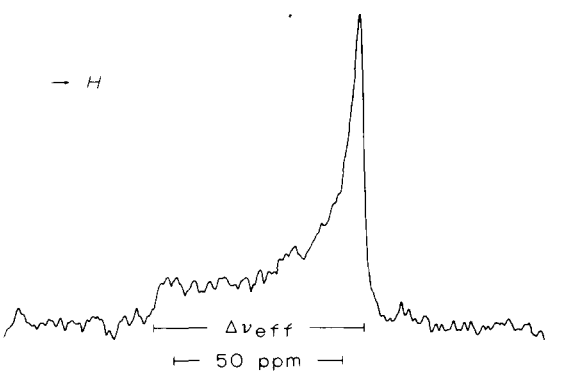


FIg. 5. 36.4 MHz ^{31}P NMR spectra of a dipalmitoyl phosphatidylcholine/chlorpromazine (2 : 1)/^2H_2O dispersion at 52°C in the presence of broad band decoupling.

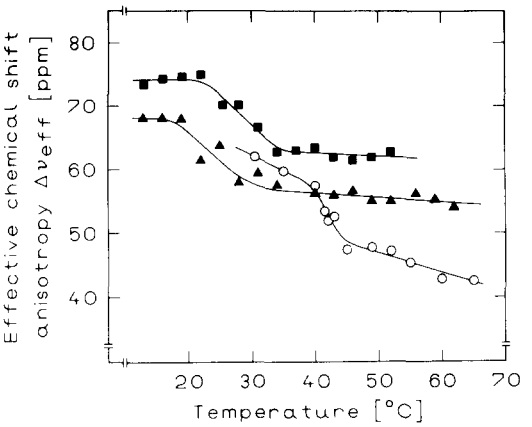


Fig. 6. Effective chemical shift anisotropy $\Delta\nu_{eff}$ as a function of temperature for the chlorpromazine-containing systems. ○, Pure dipalmitoyl phosphatidylcholine/^2H_2O dispersion; ▲, dipalmitoyl phosphatidylcholine/chlorpromazine (5 : 1)/^2H_2O dispersion; ■, dipalmitoyl phosphatidylcholine/chlorpromazine (2 : 1)/^2H_2O dispersion. For the drug-containing samples the following calorimetric data were obtained: in the case of the 5 : 1 mixture T_1 = 300.1 K and T_{max} = 304.5 K and for the 2 : 1 mixture T_1 = 297.3 K and T_{max} = 302.4 K.

in the presence of the phenothiazines for the molar ratios of dipalmitoyl phosphatidylcholine : phenothiazine derivative of 5 : 1 and 2 : 1.

Fig. 6 shows the graph of the observed values of $\Delta\nu_{eff}$ in dependence on the temperature for a pure dipalmitoyl phosphatidylcholine/^2H_2O sample and the dipalmitoyl phosphatidylcholine/chlorpromazine/^2H_2O samples. The experimental results for the dipalmitoyl phosphatidylcholine/diethazine/^2H_2O samples are very similar to the samples containing chlorpromazine.

The measurements of the pure dipalmitoyl phosphatidylcholine/^2H_2O sample are in agreement with those given by other authors [14,15]. A drastic change of $\Delta\nu_{eff}$ takes place in the range of thermal phase transition. In agreement with the results of the calorimetric measurements phase transition is shifted to lower temperatures in the presence of the phenothiazines. Further, in the range of temperatures above thermal phase transition a large change in $\Delta\nu_{eff}$ arises on addition of chlorpromazine to dipalmitoyl phosphatidylcholine, for which $\Delta\nu_{eff}$ is increased from about 45 ppm to approximately 55 ppm or 62 ppm for a dipalmitoyl phosphatidylcholine/chlorpromazine ratio of 5 : 1 and 2 : 1, respectively.

It was shown that the value of the effective anisotropy of the ^{31}P chemical shift may be directly related to the allowed motion in the phosphate group region of the polar headgroup [13—18]. The more strongly the motion of the phosphate group is restricted, the less effectively the ^{31}P chemical shielding tensor is averaged, and the larger the values of the effective chemical shift anisotropy.

Because in the presence of chlorpromazine or diethazine $\Delta\nu_{eff}$ is increased, it may be concluded that the motion of the phosphate is hindered by these drugs. In contrast, $\Delta\nu_{eff}$ is reduced to approximately 36 ppm on addition of equimolar concentrations of cholesterol to dipalmitoyl phosphatidylcholine [14]. This result was interpreted by the authros as being due to a higher mobil-

ity of the phosphate group in a dipalmitoyl phosphatidylcholine/cholesterol dispersion.

(3) ^{13}C NMR measurements

The normal ^{13}C Fourier transform spectra of unsonicated dipalmitoyl phosphatidylcholine/^2H_2O dispersions above and below the phase transition point are given in Fig. 7. Above the thermal phase transition temperature of about 42°C, the dispersions exhibit resonances for the carbons in the choline moiety ($-\overset{+}{N}(CH_3)_3$, $-CH_2\overset{+}{N}\lessdot$, $-O-CH_2-$) and in the $-CH_3$ groups of the fatty-acid chains which are resolved from the main methylene envelope. Below the thermal phase transition the chain resonances of dipalmitoyl phosphatidylcholine become even broader and lose intensity until they are barely detectable, whereas the resonance of the $-\overset{+}{N}(CH_3)_3$ groups in the choline moiety is also broadened but remains observable below the transition without loss of intensity. The ^{13}C Fourier transform spectra of unsonicated dipalmitoyl phosphatidylcholine/chlorpromazine/^2H_2O dispersions (2 : 1) at different temperatures are given in Fig. 7. The ^{13}C spectrum of chlorpromazine is also shown in the figure. In the dipalmitoyl phoshatidylcholine/chlorpromazine/^2H_2O spectrum ^{13}C resonances corresponding to chlorpromazine are detected for the $-N(CH_3)_2$ group and the methylene groups of the alkyl sidechains. No intense NMR resonances are detected for the carbon atoms of the tricyclic ring above the phase transition temperature and below it a broad band signal is observable. The $-N(CH_2)_2$ signal of chlorpromazine is not changed markedly on phase transition.

The ^{13}C linewidths of an unsonicated system are determined by chemical shift anisotropies and dipolar interactions [19]. In the gel phase, where the motion of the chain methylene groups is extremely strongly reduced, the linewidth is determined by approximately the full chemical shift anisotropy and ^1H-^{13}C dipolar interaction. In this case, a spectrum of the methylene groups of the chains can be obtained only by use of proton-enhanced ^{13}C NMR which removes dipolar broadening by high decoupling fields [19]. In our experiments the proton decoupling was insufficient and, therefore, a signal of the methylene groups was not observable in the gel state. In the liquid-cyrstalline phase the chemical shift anisotropy and dipolar interactions are partially averaged out by the molecular motion of the phospholipid molecule. As a consequence, a signal is observable. Because of the higher mobility of the methyl groups in the choline part a signal of these ^{13}C nuclei can be measured in the gel and liquid-crystalline phase.

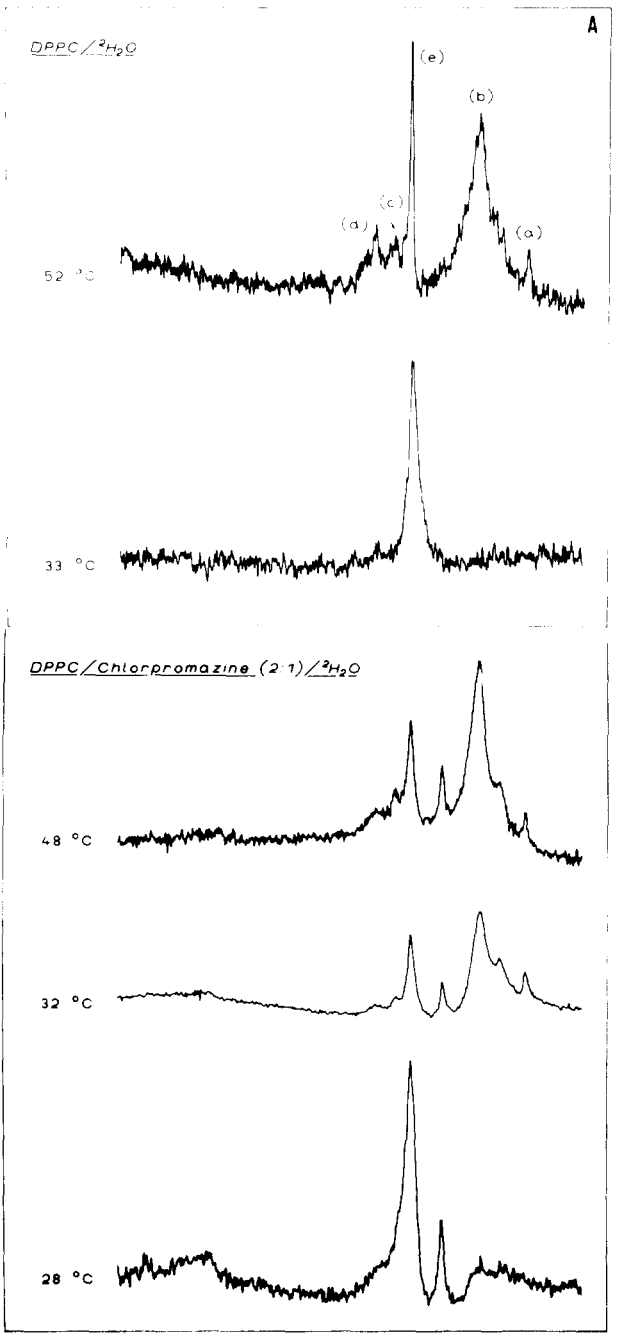
The ^{13}C linewidths of sonicated systems are smaller, and the same behaviour of spectral broadening in dependence on the phase state also exists [20—22]. But the disadvantage of these systems is that the linewidth is dependent on both molecular motion and vesicle size. However, differences in the ^{13}C linewidth of an unsonicated system may be assumed to reflect changes in the amplitude of the local motion only [19].

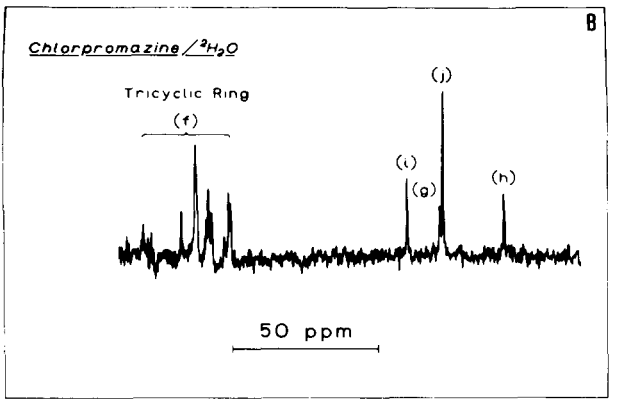
The following comparisons can be made between the two spectra of dipalmitoyl phosphatidylcholine/^2H_2O and dipalmitoyl phosphatidylcholine/chlorpromazine (2 : 1)/^2H_2O dispersions:

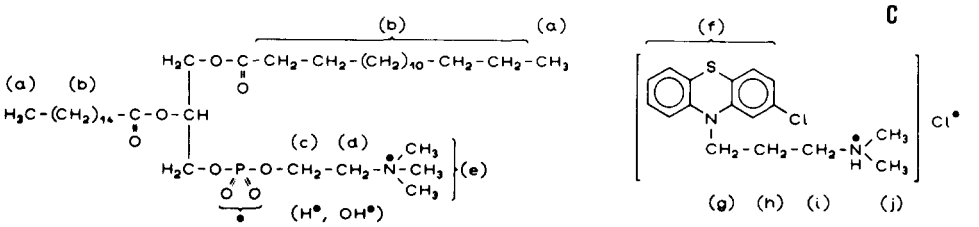
1. The resonance of the $-\overset{+}{N}(CH_3)_3$ groups in the choline moiety is broadened by a factor of 2 upon addition of chlorpromazine to dipalmitoyl phosphatidyl-

choline. This means that the motion of the trimethylammonium group is sterically hindered by chlorpromazine to a higher extent than in a pure dipalmitoyl phosphatidylcholine/^2H_2O/system.

2. The fatty-acid methylene resonances are markedly increased in amplitude upon addition of chlorpromazine to dipalmitoyl phosphatidylcholine. The







1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) Chlorpromazine Hydrochloride

Fig. 7. Normal ^{13}C Fourier transform NMR spectra of the unsonicated dipalmitoyl phosphatidylcholine (DPPC)/chlorpromazine (2 : 1)/^2H_2O system below and above the temperature of the phase transition of the lipid. For comparison, the spectra of a pure dipalmitoyl phosphatidylcholine (DPPC)/^2H_2O dispersion and of a pure chlorpromazine/^2H_2O solution are given.

intensity and linewidth of the signal is not changed down to temperatures as low as 32°C. At 28°C this peak is finally broadened indicating the phase transition of the fatty-acid chains. Therefore, we can conclude that the restriction of the motion of the methylene groups is lowered in the presence of chlorpromazine. The opposite effect on ^{13}C methylene group resonances was observed on addition of cholesterol. In agreement with the calorimetric studies it can be seen from the ^{13}C NMR spectrum that the fatty-acid chains are in the gel state up to about 30°C in the dipalmitoyl phosphatidylcholine/chlorpormazine (2 : 1) dispersion.

3. The ^{13}C NMR signals of the chlorpromazine methylene and $-N(CH_3)_2$ groups are observable at all temperatures studied indicating that these groups have a considerable mobility also in the crystalline state of dipalmitoyl phosphatidylcholine. But no intense ^{13}C NMR resonances corresponding to the tricyclic ring system are detected, which reflects the relative immobility of the ring system. Diethazine causes the same phenomena.

Discussion

All our experimental results can be interpreted on the basis of the following model of the interaction of the phenothiazine derivatives diethazine and chlorpromazine with a dipalmitoyl phosphatidylcholine model membrane: the

phenothiazines penetrate into the bilayer in such a way that the dialkylaminoalkyl chain is located near the polar headgroup of dipalmitoyl phosphatidylcholine and the phenothiazine ring is placed in the hydrophobic region of the membrane. By use of Dreiding models we can conclude that the phenothiazine molecules can penetrate into the membrane up to the second or third methylene group into the end of the chain.

Finally, the penetration of the tricyclic ring system into the bilayer and its immobilization is demonstrated by a strong broadening of the ^{13}C NMR resonances of the ring carbons. The basic alkyl chains have a considerable mobility, not only in the liquid crystalline state, but also in the crystalline state of the membrane, which is approximately equal to the mobility of the choline part of the phospholipids as can be seen from ^{13}C NMR spectra.

The measured shift of phase transition to lower temperatures and the small changes of transition enthalpies on addition of phenothiazines are in agreement with our model of interaction as discussed in Results above. Because the temperature of phase transition is connected with the melting of the fatty-acid chains we may conclude that the fluidity of the membrane is increased in the presence of the phenothiazines. In contrast, calorimetric measurements of Papahadjopoulos et al. [24] on vesicles prepared from phospholipids (dipalmitoyl phosphatidylcholine, bovine brain phosphatidylserine, dipalmitoyl phosphatidylglycerol) showed that local anesthetics such as dibucaine ($1 \cdot 10^{-4}$ M) cause a significant lowering of the gel-liquid crystalline transition temperature only with acidic phospholipids. At the same concentration dibucaine did not alter the phase transition temperature of neutral phospholipids (dipalmitoyl phosphatidylcholine). An appreciable increase in the fluidity of neutral phospholipid membranes occurred only at relatively high aqueous dibucaine concentrations ($2 \cdot 10^{-3}$ M).

By calorimetric measurements it was found that a finite miscibility occurs in the crystalline state of dipalmitoyl phosphatidylcholine for concentrations of phenothiazine higher than 10 mol%. In this case clusters of dipalmitoyl phosphatidylcholine molecules are formed. A clue to such a phase separation may be obtained by ^{13}C NMR also because the spectra of the "doped" dispersion and the pure dipalmitoyl phosphatidylcholine/^2H_2O dispersion are very similar in the crystalline state.

By calorimetric measurements of unsonicated systems and ESR, ^1H and ^{13}C NMR of sonicated systems Cater et al. [1] and Bermejo et al. [12] studied the effect of desipramine on dipalmitoyl phosphatidylcholine model membranes. Desipramine differs from chlorpromazine by the exchange of the sulphur atom in the tricyclic ring with a $-CH_2-CH_2-$ group. These authors suggested a model of interaction of desipramine with the dipalmitoyl phosphatidylcholine membrane which is very similar to the model discussed above. Therefore, we can conclude that the studied phenothiazines and desipramine interact in the same way with a dipalmitoyl phosphatidylcholine membrane.

The effect of cholesterol on a dipalmitoyl phosphatidylcholine membrane in the liquid crystalline state is opposite to that of the phenothiazines. It decreases the lipid chain flexibility [19] and the mobility of the phosphate group is increased [14]. This behaviour of cholesterol was described as a solidifying effect. Therefore, the behaviour of the phenothiazines which was discussed

above could be described as a reversed solidifying effect.

The two studied phenothiazines differ in the dialkylaminoalkyl part. Therefore their pharmacological activities are different.

The dialkylaminoethyl derivatives (diethazine) have mainly peripheral action and the dialkylamino-*n*-propyl derivatives (chlorpromazine) have markedly central action. Chlorpromazine is used as a neurolepticum.

Our experiments have demonstrated that both drugs cause the same changes of the properties of a dipalmitoyl phosphatidylcholine model membrane with the exception that the amount of substance which is necessary to get the same shift of phase transition is different at high drug concentrations. Therefore, the differences of the pharmacological action cannot be explained on the basis of a different interaction of the studied compounds with the dipalmitoyl phosphatidylcholine model membrane.

References

1 Cater, B.R., Chapman, D., Hawes, S.M. and Saville, J. (1974) Biochim. Biophys. Acta 363, 54—69
2 Bermejo, J., Barbadillo, A., Tato, F. and Chapman, D. (1975) FEBS Lett. 52, 69—72
3 Jain, M.K., Yen-Min Wu, N. and Wray, L.V. (1975) Nature 255, 494—495
4 Chapman, D. (1975) Chem. Ind. 1, 98—100
5 Kwant, W.O. and Seeman, P. (1969) Biochim. Biophys. Acta 193, 338—349
6 Phillips, M.C., Williams, R.M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234—244
7 Chapman, D., Urbina, J. and Keough, K.M. (1974) J. Biol. Chem. 249, 2512—2521
8 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152—161
9 Vaughan, D.J. and Keough, K.M. (1974) FEBS Lett. 47, 158—161
10 Hinz, H.-J. and Sturtevant, J.M. (1972) J. Biol. Chem. 19, 6071—6075
11 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333—340
12 Janiak, M.J., Small, D.M. and Shipley, G.G. (1976) Biochemistry 15, 4575—4580
13 McLaughlin, A.C., Cullis, P.R., Berden, J.A. and Richards, R.E. (1975) J. Magn. Resonance 20, 146—165
14 Cullis, P.R., de Kruyff, B. and Richards, R.E. (1976) Biochim. Biophys. Acta 426, 433—446
15 Niederberger, W. and Seelig, J. (1976) J. Am. Chem. Soc. 98, 3704—3706
16 Kohler, S.J. and Klein, M.P. (1976) Biochemistry 15, 967—973
17 Arnold, K. and Hofmann, A. (1976) Stud. Biophys. 59, 139—147
18 Gawrisch, K., Arnold, K., Rüger, H.-J., Kertscher, P. and Nuhn, P. (1977) Chem. Phys. Lipids, in the press
19 Urbina, J. and Waugh, J.S. (1974) Proc. Natl. Acad. Sci. U.S. 71, 5062—5067
20 Lee, A.G., Birdsall, J.M., Levine, Y.K. and Metcalfe, J.C. (1972) Biochim. Biophys. Acta 255, 43—56
21 Metcalfe, J.C., Birdsall, J.M., Feeney, J., Lee, A.G., Levine, Y.K. and Partington, P. (1971) Nature 233, 199—201
22 Levine, Y.K., Birdsall, J.M., Lee, A.G. and Metcalfe, J.C. (1972) Biochemistry 11, 1416—1421
23 Godici, P.E. and Landsberger, F.R. (1975) Biochemistry 14, 3927—3933
24 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504—519