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LIPID PHASE TRANSITIONS AND DRUG INTERACTIONS

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SUMMARY

1. The lecithin–water model biomembrane system has been used to study the interaction of a number of drug molecules. In particular the effects of the drugs upon the lipid endothermic phase transitions were studied using differential scanning calorimetry, microscope studies and NMR and ESR spectroscopy.

2. The drug molecules studied were of two classes, designated A and B. The A series consists of a set of structurally related morphine compounds whilst the B series consists of a set of antidepressant molecules similar to desipramine.

3. At low concentrations the drugs were observed to affect the lecithin pretransition endotherm, showing that they were altering the tilting of the lipid chains as well as decreasing the area per lipid polar group at the lipid–water interface.

4. Both sets of drugs were observed to affect the phase transition temperature of the lecithin studied (dipalmitoyl- and dimyristoyllecithin), some drugs more markedly than others. The shifts of transition temperature caused by the morphine drugs appeared to be quite sensitive to their precise structure.

INTRODUCTION

An important question much studied in recent years is why the lipids of cell membranes contain a wide range of different fatty acids [1]. Information to answer this question originated from observations made on simple lipid systems. Thus, when a soap is heated to a certain transition temperature (sometimes hundreds of degrees below the final melting point) the hydrocarbon chain portion 'melts'. The all planar *trans* configuration breaks up and the chains now contain gauche isomers [2]. The phospholipids of some cell membranes also exhibit this same property [3] and show an endothermic phase transition from crystalline gel to liquid crystal at a temperature dependent upon chain length and unsaturation [4]. Above the transition temperature the lipid chains are melted and fluid, whereas below this temperature the chains are organised in a crystalline manner [5].

Abbreviation: Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl.

A related question is whether it is possible for some molecules to trigger local lipid-phase changes in a membrane system. Recent experiments with model biomembrane systems [6] show that the characteristic transition temperature of a lipid can be shifted to higher or lower temperatures dependent upon interactions of the lipid with metal ions, polypeptides or proteins. This shows that in principle membrane fluidity in a local region could also be affected by such interactions. This in turn will change permeability characteristics, diffusional characteristics and enzyme activity in that region. If the interaction is such as to shift a portion of membrane from a gel to fluid condition, the change in membrane properties may be dramatic in character.

In this paper we examine the effects of a number of central nervous system drugs on lecithin–water (model biomembrane) systems to ascertain whether these molecules can cause marked changes in lipid transition temperature, and pose the question whether similar changes could be in any way related to their biological or pharmacological action.

MATERIALS AND METHODS

Lipid materials 1,2-dimyristoyllecithin and 1,2-dipalmitoyllecithin were obtained from Koch Light and used without further purification. The drugs were obtained from Reckitt and Colman Ltd. The series A drugs (morphine derivatives) were used as the free base whereas the series B drugs (tricyclic antidepressants) were used as the hydrochloride salt. The structures of the drugs are shown in Figs 1 and 2.

All drug:lipid mixtures were made by first mixing the two components in a chloroform solution. The chloroform was then removed under a flow of nitrogen, the last traces being expelled by placing the samples under vacuum for at least 3 h. The samples were then made up in excess water (1 : 1 or 2 : 1 for calorimetric studies and 4 : 1 for electron spin resonance studies). The lipid:drug mixtures were dispersed by heating the samples above the transition temperature of the lipid and then mixing them on a bench vibrator. Careful mixing was found to be critical to attain reproducible results.

For calorimetric studies samples were examined using a Perkin Elmer DSC-2 differential scanning calorimeter. (The morphine derivatives were also run on a DSC-1B instrument. No significant difference was found with the scans obtained with the two calorimeters). All the scans shown were obtained using the DSC-2 instrument with a heating rate of 5 °K/min and a range setting of 2 mcal/s. Each drug: lipid sample was run at least three to four times. The heats of transition were calculated from the area under the curves which was determined by use of a fixed arm planimeter.

For ESR studies the samples were run on a Varian E3 ESR Spectrometer. Tempo (2,2,6,6-tetramethylpiperidine-1-oxyl) in water was added to the drug:lipid mixtures before final mixing to give a final concentration of $2 \cdot 10^{-4}$ M. The methyl stearate spin label methyl-12-nitroxide stearate was added in methanol to the chloroform solution of the drug:lipid mixtures. These samples were then prepared as described above. The final concentration of the methyl stearate label was $5 \cdot 10^{-4}$ M.

NMR spectra were obtained using a Varian HA 100 Spectrometer.

Microscope studies were carried out on lipid–water–drug mixtures to deter-

mine whether excess drug was visible, using a heated stage microscope. Except when otherwise stated the drug:lipid mixtures represented a homogeneous system.

RESULTS

The morphine derivative M IV and desipramine are representatives of two distinct drug series, the structures of which are shown in Figs 1–2. Within each series the compounds are closely related in structure. The first, series A, is a series of morphine derivatives, the structures of which are shown in Fig. 1. The structure of morphine is also shown.

The second series, series B, are the tricyclic antidepressant molecules whose structures are shown in Fig. 2. (This series of drugs may be classified in two ways. Firstly, the classification can be based on those drugs which contain a nitrogen atom

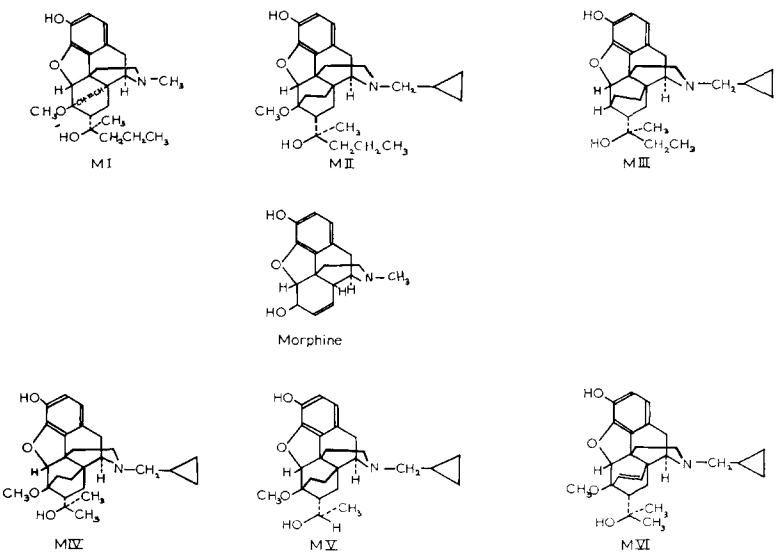

Fig. 1. The structures of the morphine drugs (series A).

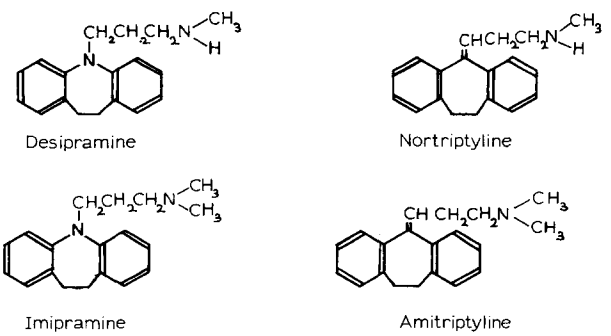

Fig. 2. The structures of the tricyclic antidepressant drugs (series B).

in their ring structure (desipramine and imipramine) as compared to those which do not (amitriptyline and nortriptyline). Alternatively, they may be classified on the basis of the degree of substitution on the amino group in the aliphatic side chain. Desipramine and nortriptyline contain a secondary amino group whereas imipramine and amitriptyline contain a tertiary amino group.)

The interaction of M IV and desipramine with a lecithin–water model biomembrane system was studied using calorimetry and NMR and ESR spectroscopy. The effects of these drugs on the transition temperature of the lipid were determined within a concentration range of 2–60 moles %. The heating scans obtained at 50 mole % were then directly compared with similar scans obtained from the other drugs within each series.

The calorimetric curves for the lipid as a result of interaction with each drug are shown in the various figures. The NMR and ESR spectroscopic studies of the desipramine or M IV drug:lipid mixtures are also presented.

(1) Differential scanning calorimetry

All heating runs were started at 250 °K (−23 °C) so that ice melting endotherms could be observed. This ensured that excess water was present. The water peaks are not shown in the diagrams.

Figs 3 and 4 show the heating curves for a concentration range of the dipalmitoyl lecithin–water system with the drug M IV and desipramine respectively. It is apparent that for both drugs a shift in the phase transition temperature of the lecithin occurs. The shift increases as the concentration of the drugs increases. An effect is evident at a concentration as low as 2 mole %. (It should also be noted that at equimolar concentrations equally dramatic shifts are evident with dimyristoyllecithin.) The phase diagrams for desipramine and M IV with dipalmitoyllecithin are shown in Fig. 5.

The heats of transition of lipid:drug mixtures are plotted as a function of the drug concentrations in Fig. 6. It is apparent that for both desipramine and M IV there are only very minor changes in the heat of transition at all concentrations tested.

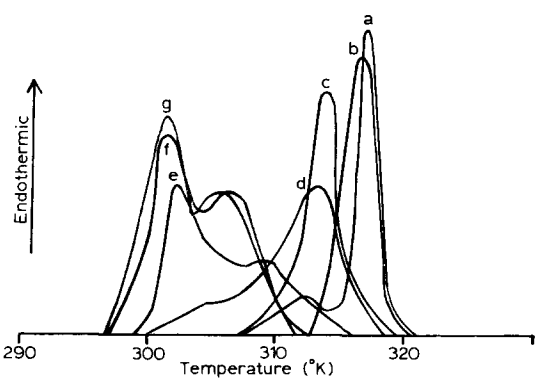

Fig. 3. Differential scanning calorimetric heating curves of the 1,2 dipalmitoyllecithin–water system with varying amounts of the morphine drug M IV. Lipid: drug molar ratios were as follows: a, 100:0; b, 50:1; c, 25:1; d, 90:10; e, 75:25; f, 50:50; g, 40:60. (Samples prepared and run as described in the text.)

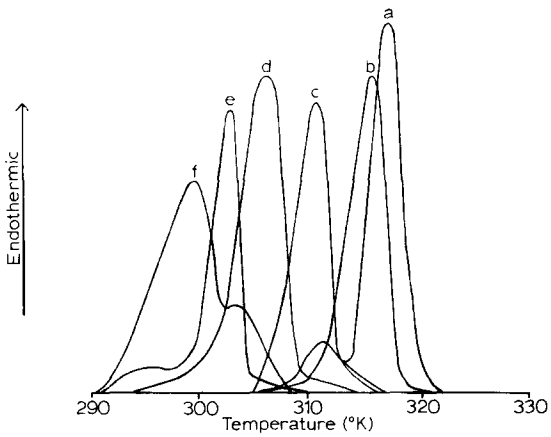

Fig. 4. Differential scanning calorimetric heating curves of the 1,2 dipalmitoyllecithin–water system with varying amounts of the tricyclic antidepressant drug Desipramine. Lipid: drug molar ratios were as follows: a, 100:0; b, 50:1; c, 90:10; d, 75:25; e, 50:50; f, 40:60. (Samples prepared and run as described in the text.)

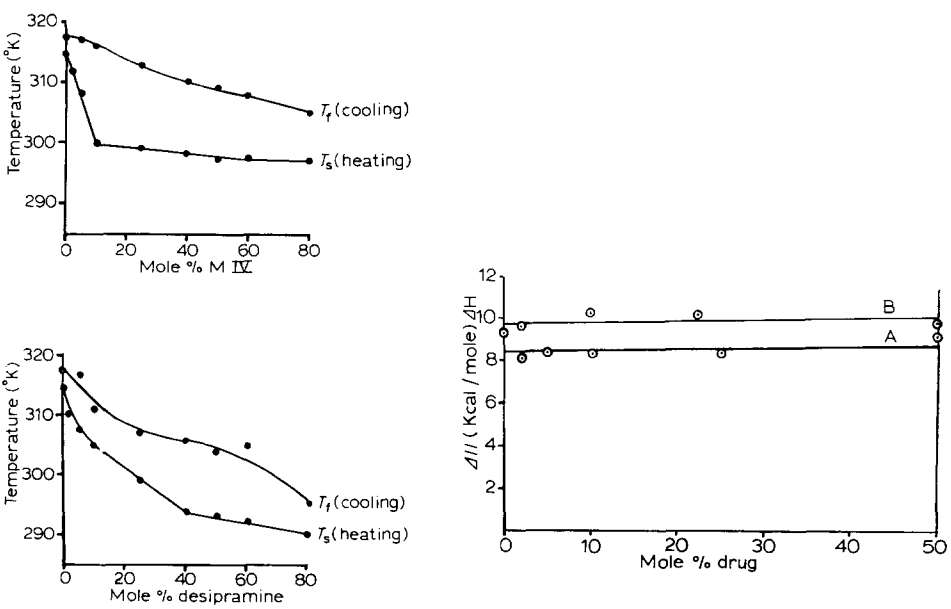

Fig. 5. The phase diagrams for the dipalmitoyllecithin–water system with varying concentrations of morphine compound M IV and desipramine. (Samples prepared and run as described in the text.)

Fig. 6. Heats of transition of the dipalmitoyllecithin–water system with A. morphine compound M IV and B desipramine. (The area under the differential scanning calorimetric heating curve was measured using a fixed arm planimeter.)

Figs 7 and 8 show the effect of the series A drugs when mixed in equimolar concentrations with dipalmitoyllecithin. Fig. 7 shows a comparison of the four morphine ethano compounds M II–M V. The structural differences in the drugs are confined to the alkyl side chain on the alcohol residue present on C_7 of the morphine core. Apart from the minor modifications in this alkyl side chain, involving the sequential removal of methyl residues, each of the drugs is of identical structure. It can be observed that these minor structural modifications are reflected by quite marked differences in the transition temperature of the lecithin-water system. i.e. as the length of the alkyl side chain is decreased in the order M II ($-CH_2CH_2CH_3$) > M III ($-CH_2CH_3$) > M IV ($-CH_3$) > M V ($-H$) the phase transition is shifted to lower temperatures. Therefore, the shorter the length of alkyl side chain of the morphine molecule, the lower becomes the phase transition temperature of the lipid.

The effects on the lipid phase transition of the drugs M IV and M VI are shown in Fig. 8. These two drugs are structurally identical except that in M VI a double bond replaces the single bond between C_6 and C_{14} which is present in M IV. A marked difference occurs. (It should be noted that in this case the microscope

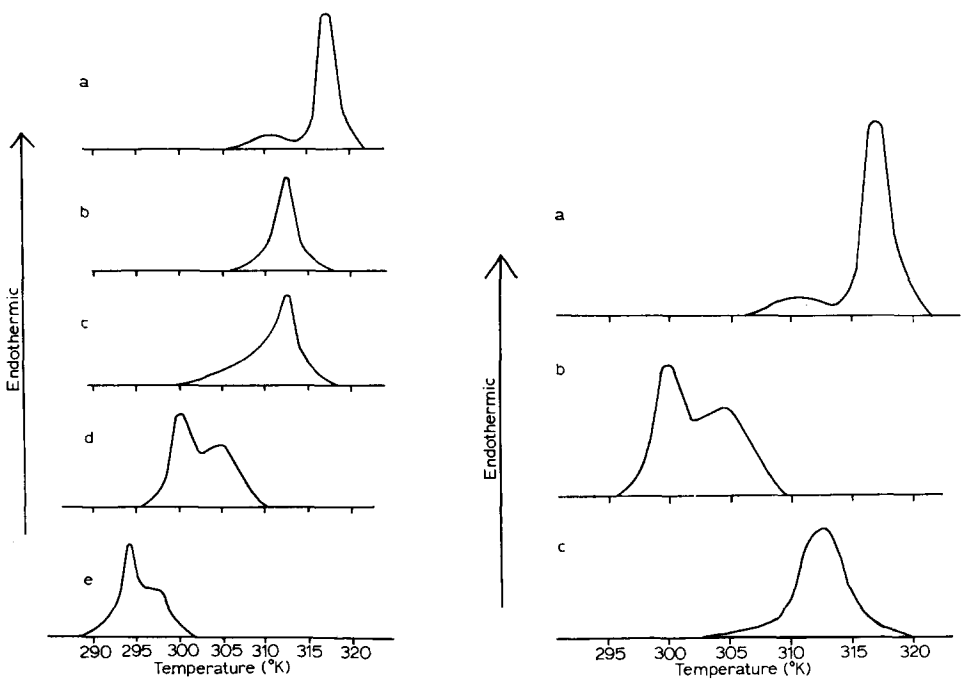

Fig. 7. Differential scanning calorimetric heating curves of the dipalmitoyllecithin–water system with various series A drugs. a, dipalmitoyllecithin; b, dipalmitoyllecithin/M II; c, dipalmitoyllecithin/M III; d, dipalmitoyllecithin/M IV; e, dipalmitoyllecithin/M V. (50:50 molar ratio lipid:drug). (Samples prepared and run as described in the text.)

Fig. 8. Differential scanning calorimetric heating curves of the dipalmitoyllecithin–water system with various series A drugs. a, dipalmitoyllecithin; b, dipalmitoyllecithin/M IV; c, dipalmitoyllecithin/M VI. (50:50 molar ratio lipid:drug). (Samples prepared and run as described in the text.)

examination of the final M VI:DPL mixture revealed that some crystalline drug was still apparent. This was the only case where this was apparent.)

The results of the calorimetric scans of equimolar mixtures of each of the four tricyclic antidepressants (series B) and dipalmitoyllecithin are shown in Fig. 9. In contrast to the morphine derivatives minor changes in the structure of the series B drugs were not accompanied by marked changes in the phase transition temperature. Each of the drugs shifted the transition temperature approximately to the same extent while small differences were apparent in the form of differing minor peaks of differing intensities. In terms of these differences the drugs fell into two classes, namely desipramine:nortriptyline and imipramine:amitriptyline. Accordingly, it is apparent that the degree of substitution of the amino group in the aliphatic side chain of the drug was having a greater effect than the presence or absence of a nitrogen atom in the ring structure.

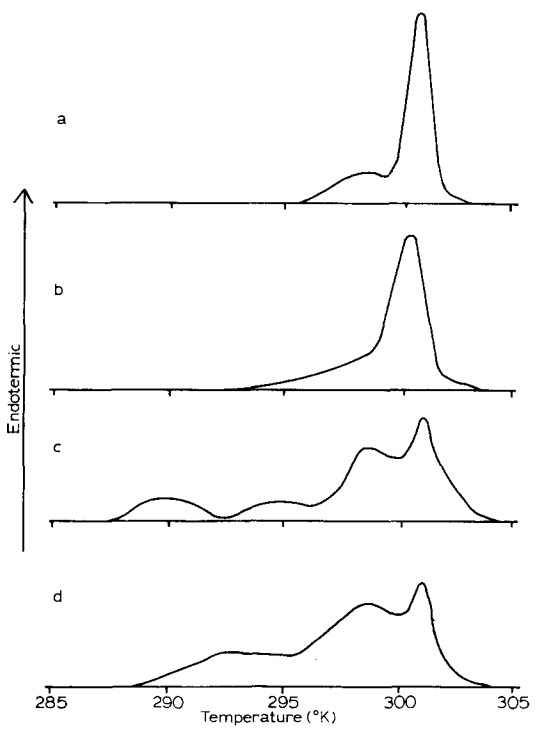

Fig. 9. Differential scanning calorimetric heating curves of the dipalmitoyllecithin–water system with the series B drugs. a, Desipramine; b, Nortriptyline; c, Imipramine; d, Amitriptyline. (50:50 molar ratio lipid:drug). (Samples prepared and run as described in the text.)

The importance of the aliphatic side chain of the antidepressants was further emphasized when the effect of iminodibenzyl was examined. This compound which possesses a ring structure identical to desipramine and imipramine but does not have an aliphatic side chain, had a negligible effect upon the transition temperature of the lipid. It therefore appears that the aliphatic side chain is essential for the effect of

these compounds on the transition temperature of the lipid in these model systems.

None of the drugs tested significantly lowered the heat of transition of the lipid.

(2) Electron spin resonance (ESR)

The ESR studies were confined to the drugs M IV and desipramine using the spin label Tempo. The transition temperature of the dipalmitoyllecithin–water system with and without the morphine drug M IV was ascertained by determining and plotting the Tempo spectral parameter (f) as described by Shimshick and McConnell [7]. This is determined by measuring amplitudes, H and P (Fig. 10) of the high-field nitroxide hyperfine lines. To a first approximation, H is proportional to the amount dissolved in the lipid bilayer. The change in the relative values of H and P as a function of temperature for the lipid reflects the fact that a transition of the

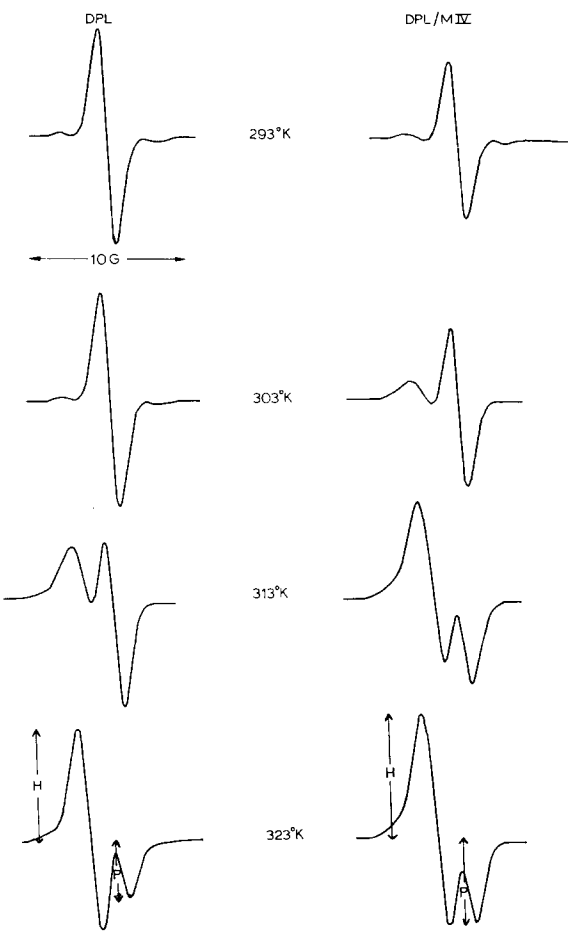

Fig. 10. ESR spectra of the dipalmitoyllecithin–water system with the drug M IV (50:50 molar ratio) using the nitroxide spin label Tempo. H and P are the distances measured in order to calculate the spectral parameter f (see Fig. 15). DPL, dipalmitoyllecithin.

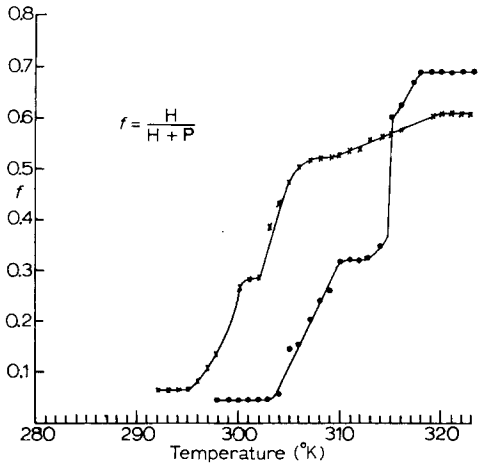

Fig. 11. The Tempo spectral parameter f vs temperature for aqueous dispersions of dipalmitoyllecithin and with the drug M IV (50:50 molar ratio).

TABLE I

HEATS OF LIPID PHASE TRANSITIONS OF 50 MOLE % DRUG: LIPID MIXTURES

Figures calculated from areas below curve of differential scanning calorimetric heating tracings. Areas obtained by the use of a planimeter.

Dipalmitoyl lecithin	H (kcal/mole dipalmitoyllecithin)
No additions	9.4
+ M II	8.8
+ M III	8.8
+ M IV	10.0
+ M V	9.0
+ M VI	8.6
+ Desipramine	9.9
+ Imipramine	9.7
+ Nortriptyline	9.4
+ Amitriptyline	9.9

TABLE II

TRANSITION TEMPERATURES OF DIPALMITOYLLECITHIN: M IV MIXTURE (1:1 MOLAR RATIO) BY DIFFERENTIAL SCANNING CALORIMETRY AND ESR

Method of measurement	Transition temperature (°K)	
	Dipalmitoyllecithin	Dipalmitoyllecithin: M IV
Differential scanning calorimetry	315	298, 302
ESR	315	298.5, 302

lipid from the 'gel state' to the 'liquid crystalline state' has occurred. The method is obviously dependent upon the ability to resolve the peaks of the high-field nitroxide lines which represent the hydrophobic and hydrophilic distribution of the spin label. (Unfortunately, this was not possible with the desipramine : dipalmitoyllecithin mixture). The results are shown in Fig. 11. The transition temperatures so determined are in good agreement with those obtained by calorimetry (Table II).

The results of the studies using the 12-nitroxide methylstearate spin label are shown in Fig. 12. It is evident that at a concentration of 50 moles % at 300 °K both desipramine and M IV result in a marked increase in the motion of the label, thus indicating that the hydrocarbon chains are in a fluid state. At 10 mole % increased motion is evident at 309 °K.

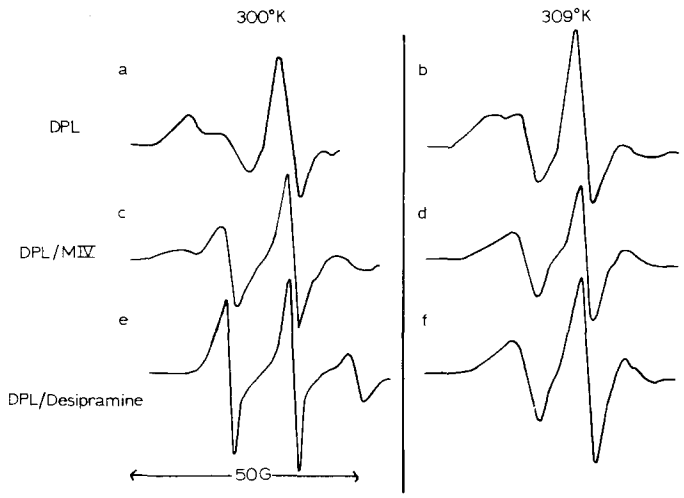

Fig. 12. ESR of the dipalmitoyllecithin–water system with mixtures of the drug M IV and with desipramine using the nitroxide spin label methyl-12-nitroxide stearate. a, dipalmitoyllecithin (DPL) at 300 °K; b, dipalmitoyllecithin at 309 °K; c, dipalmitoyllecithin/M IV 50:50 molar ratio at 300 °K; d, dipalmitoyllecithin/M IV 90:10 molar ratio at 309 °K; e, dipalmitoyllecithin/desipramine 50:50 molar ratio at 300 °K; f, dipalmitoyllecithin/desipramine 90:10 molar ratio at 309 °K.

(3) *Nuclear magnetic resonance (NMR)*

NMR spectra were studied with unsonicated samples of pure dipalmitoyl lecithin and equimolar mixtures with M IV and also desipramine. The mixture with desipramine gave rise to a detectable spectrum whereas no distinguishable signals were evident with the other samples.

DISCUSSION

An understanding of phospholipid interactions at the molecular level is a fundamental problem in membrane biology. Support for the concept that a membrane is comprised of protein regions separated by areas of lipid bilayer is increasingly evident. In such a membrane model the 'fluidity' of the lipid bilayer regions could be a critical factor in determining many functions of the membrane. It is, accordingly, of

great interest that it has now been demonstrated that not all the areas of lipid bilayer of a cell membrane are necessarily in the fluid state, but that areas of both lipid 'fluidity' and also 'rigidity' can coexist [8]. It is in the light of such developments that the study of the effects of triggering molecules on lipid phase transitions takes on added importance.

The investigation reported herein has been confined to the study of the effects of the drug molecules on the fully saturated phospholipid 1,2-dipalmitoyllecithin-water systems (and to a lesser extent the 1,2-dimyristoyllecithin–water system). The reason for the choice of this lipid was two-fold. Firstly, the hydrated dipalmitoyl-lecithin system is well characterised and is known to exist in the form of a bilayer [4]. Secondly, the lecithin class of phospholipids is often the most predominant in animal membranes [1] and thus provides the logical starting point to a study of this nature.

A feature of the lecithins is that they exhibit a 'pretransitional' endotherm as well as the main endotherm corresponding to the primary phase change [4]. This pretransitional endotherm has shown to be associated with an increase in the mobility of the polar groups [9]. Recent X-ray studies* have shown that this transition also corresponds to the lipid chains changing from a tilted condition (β'-conformation) to an orientation with chains perpendicular to the plane of the lamellae (β-conformation) [10]. The area per polar group at the lipid–water interface also decreases. In both forms the hydrocarbon chains are packed with rotational disorder in a two-dimensional quasi-hexagonal lattice.

At low concentrations (2 mole %) both the morphine compound M IV and desipramine remove the 'pretransitional' endotherm observed with pure dipalmitoyl lecithin. This shows that their presence at quite low concentrations causes the lipid to alter the tilt of the hydrocarbon chains (58°) to the vertical chain situation ($\beta' \rightarrow \beta$ modification) and also decreases the area per polar group at the lipid–water interface. A similar effect has been observed with small amounts of cholesterol [11] ($\approx$ 7 mole %) and of the antibiotic gramicidin A[6] and also it is reported that 7 % of decane can cause a similar effect [23].

Apparently a heterogeneous distribution of lipid chains or 'impurity' molecules can be more easily accommodated in the lamellae by having vertical lipid chains. Thus small amounts of drug molecules can affect the long range organisation in bilayer structure of both the lipid chains and also their polar groups – at least with the synthetic homogeneous lipids. The phase transition temperature can be affected by the presence of 'impurity' molecules in an analogous way to that exhibited by water or liquid when an added solute lowers the freezing point. It can be predicted that with this lipid a depression of 1 °K in the lipid phase transition would be brought about by the presence of 0.048 moles drug/mole dipalmitoyllecithin. In our experiments for desipramine and M IV we find values of approximately 0.005 and 0.01 moles drug/mole lipid**, respectively, for a one degree shift. This approach to the lowering of the transition temperature is perhaps too simplistic and additional factors may be involved in this case consisting of specific interactions of the drug with the lipid dependent upon the detailed molecular structure of the drug. (Note:

* Rand, P. and Larsson, K., unpublished studies.

** Hill (unpublished observation) found for the two simple alcohols *n*-octanol and *n*-nonanol the predicted value proved valid.

It is also possible under certain circumstances for the lipid phase transition temperature to be increased by the presence of additional molecules. Chapman et al.* have observed this with stearyl alcohol added to dipalmitoyllecithin. In this system a complex is observed to occur between the lipid and the long chain alcohol at 85 % alcohol and 15 % lipid.)

The phase diagrams for the effect of desipramine and M IV on dipalmitoyllecithin are shown in Fig. 5. Previous studies of mixtures of lipids of different chain lengths and different lipid classes have been made using calorimetry and phase diagrams obtained. In some systems a continuous series of solid solutions exist and the solidus curve has a continuously varying slope. In other systems the solidus curve shows a discontinuity in slope indicated a limited solid-phase miscibility in which the area below the solidus curve contains only solid solutions or only solid heterogeneous mixtures[6].

Shimshick and McConnell [7] have obtained phase diagrams by plotting the Tempo spectral parameter as a function of $1/T$ for binary mixtures of phospholipids. They observed that abrupt changes in slope occurred at the onset and completion of phase separation at definite temperatures, T_F and T_S, for a specific phospholipid composition, on the 'fluidus' and 'solidus' curves, respectively, of an equilibrium phase diagram. It was assumed that the S (solid) phase was of uniform composition and was in equilibrium with the coexisting F (fluid) phase unless there was more than a single S-phase present due to limited miscibility. Such 'immiscibility' was evident when a discontinuity in the slope of the 'solidus' curve of a phase diagram was apparent. Equilibrium between the F-phase and S-phase was seen to depend not only on rapid lateral diffusion in the F phase [13] but also sufficiently rapid lateral diffusion in the S-phase. It is thus necessary that the phospholipids are not fixed in the so-called solid phase but that they too are capable of lateral diffusion. Such a phenomenon has indeed now been demonstrated in this laboratory [14].

In this study we have obtained 'solidus' and 'fluidus' curves by plotting the onset temperatures gained from cooling and heating runs on the differential scanning calorimeter. In each case the points plotted correspond to the onset temperature of the melting or freezing of the lipid:drug mixture even when this is the starting temperature of a minor peak. It should be emphasized that the resultant phase transition temperature in this system is essentially that of the lipid component and is not an average of two extreme temperatures as is often the case when two similar lipids are mixed [6].

In the case of M IV an obvious discontinuity is evident in the 'solidus' curve. This then strongly suggests that the drug:lipid mixtures do not form a complete range of S-phase solutions but instead have a limited S-phase miscibility. This immiscibility starts to become apparent at a drug concentration as low as 10 moles %. This concentration corresponds to the appearance by differential scanning calorimetry of a broad shoulder imposed on the main endothermic transition which at higher concentrations gives rise to a second transition. Therefore although in the present study the phase boundaries below the 'solidus' curve must remain essentially undetermined it is evident that this is at least a two component system. Even at a drug

* Chapman, D. and Ladbrooke, B. D. and Williams, R. M., unpublished observations.

concentration of 80 moles % the double transition was still apparent by differential scanning calorimetry.

The desipramine:dipalmitoyllecithin phase diagram does not display any discontinuities as clearly apparent as that with the M IV:dipalmitoyllecithin mixtures. However a less abrupt change in slope is evident at a drug concentration of 40 moles %. This corresponds to the appearance of a small minor peak in the differential scanning calorimetry scan which on increasing the drug concentrations becomes the major and finally the sole transition evident. Therefore below the drug concentration of 40 moles % no immiscibility is apparent in the S-phase. Above this drug concentration the mixture temporarily becomes a two-component system and finally above the drug concentration of 80 moles % once again returns to a one component system. The change in the slope of the 'solidus' curve may suggest that at a drug concentration of 40 moles % a small concentration of an immiscible drug: lipid complex becomes apparent which eventually at higher drug concentrations becomes the sole component of the system. However, as in the case of the M IV: dipalmitoyllecithin system, a more detailed study is required to define-precisely the phase boundaries evident in the S-phase. Microscopic examination demonstrated that above the 'fluidus' curve a liquid-crystalline phase became apparent.

The interesting feature of all the drugs examined in the present study is that even at molar : molar ratios the heat of transition is not markedly reduced. Previous studies by Chapman and co-workers [6, 15–19] have shown that those compounds which penetrate the interior of the lipid bilayer and disrupt the chain packing result in a lowering of the heat of the lipid phase transition, whereas those which remain at the surface of the bilayer and interact electrostatically with the polar headgroups of the lipid primarily affect the transition temperature. This indicates that a major disruption of the lipid chain packing by interdigitation of the drug as occurs with cholesterol is not occurring although superficial penetration within the area of the polar headgroup of the lipid would still be possible.

The sensitivity of the shift of the lipid phase transition to small variations in the molecular structure of the morphine molecules is of particular interest. (Morphine itself has no marked effect upon the phase transition.) These effects are somewhat similar to those where small modifications to the cholesterol structure strongly affect the degree of incorporation and interaction with the lipid [20, 21].

The influence of the morphine drugs increases as the aliphatic side chain is decreased. In fact, the shortening of the aliphatic side chain can be seen to increase the hydrophilic nature of the alcohol residue on C_7 and thus perhaps potentiate its interaction with the polar headgroups of the lipid. In this respect it would be interesting to see whether there was any direct correlation between the partition coefficients of the drugs and their ability to lower the lipid transition temperature.

It is of interest to note that when two drugs, one with agonist and the other with antagonist properties, were codispersed with the lipid the resultant shift in the transition temperature was similar to that obtained with the antagonist alone. Accordingly in the case examined the antagonists influence is dominant over that of the agonist.

Of the compounds tested, all those known to be primarily agonists (i.e. morphine, M I and M II) had comparatively small or negligible effects on the lipid phase transition temperature. The antagonist drugs M IV and M V had marked

effects on the lipid phase transition temperature, but the other two, namely M III and M VI had considerably smaller effects. Therefore, a strict rule on the basis of this lipid interaction separating agonists and antagonists is not evident from these results. The compound M IV is reported to have some antidepressant properties and it is molecules with these pharmacological properties which appear to be more dramatic in shifting the lipid phase transition. The mode of action of both desipramine and the morphine molecule M IV may be similar, but is not identical. The NMR data suggest that the desipramine is at least partially micellizing the lipid and even with unsonicated lipid gives rise to high resolution narrow signals whereas this does not occur with the morphine M IV compound. Furthermore, on sonication of the two drugs with dipalmitoyllecithin desipramine readily disperses the lipid giving rise to an opalescent solution, whereas M IV gives rise to a 'chalky' solution which readily settles out on standing.

A question of interest and importance to molecular biology and pharmacology is that pertaining to the basic interaction of drugs with cell membranes. In this respect it is relevant to consider how the central nervous system recognises the wide variety of drugs which are thought to act upon it and how this initial interaction between the central nervous system and the drug then potentiates the effect of the drug [22]. Although numerous modes of 'drug selectivity' have been proposed [23], a better understanding of the phenomenon of drug recognition and action would seem to hinge upon a basic understanding of the biochemical and biophysical nature of drug: membrane interactions.

Many drugs have supposed sites of action on the cell membrane. In addition, there is reason to believe that drugs can cause relatively major changes in the properties of cell membranes. In this respect it is essential to note that current research strongly suggests that anaesthetics act by disordering or fluidising the lipid regions of membranes. For example, Hubbel and McConnell [24, 25], have found that anaesthetics cause a melting or disordering of the membrane as demonstrated by spin label studies. Trudell et al. [26] have found that inhalation anaesthetics decrease the order of lipid within a bilayer and that this effect could be reversed by increased pressure.

It is in the light of such studies that results reported herein take on added interest. At concentrations as low as 2 mole % both desipramine and the morphine derivative M IV were effective in lowering the lipid transition temperature and therefore by implication increasing the fluidity of the lipid. These shifts were then of a far greater magnitude when the drugs were present at higher molar ratios. For ease of comparison of the effect of one drug with another related drug we have compared their effects at molar:molar ratios. We do not necessarily imply by this that such high relative concentrations will occur in the in vivo situation. It should be pointed out however that for the opiate drugs, the receptors are concentrated only in membrane fractions of nerve tissue [26] and, in turn, in specific areas of the brain [28]. Accordingly some of these drugs may be concentrated at certain membrane surfaces.

The ability of certain drugs to increase the fluidity of a lipid region in a membrane may also affect the metabolism of that drug. Recently, Stier and Sackman [29] have suggested that the cytochrome P_{450} system, which is the drug metabolising system situated in the microsomal fraction of the liver, may be enclosed in a 'rather rigid phospholipid halo'. This phospholipid is said to be in a quasi-crystalline structure

below 32 °C and to undergo a phase transition at 32 °C. A critical factor in the metabolism of a substrate by the cytochrome P_{450} system would seem to be its lipid solubility. However, the rate of metabolism of a drug could be determined not by its lipophilic nature but by its ability to fluidise the phospholipid halo surrounding the cytochrome P_{450} complex.

The fluidising effect of some of these drugs might under certain circumstances encourage cell fusion to occur. It has been suggested that surface active agents such as lysolecithin are fusogens [30].

Recently a technique involving the entrapment of drugs in liposomes (incorporating dipalmitoyllecithin) has been proposed which could lead to a more efficient uptake of the drug. It was suggested that this entrapment may overcome the problems of unwanted immunological reactions and drug resistance and that it may be possible to direct the liposomes specifically to their therapeutic site of action. From our studies it is apparent that a factor which must be considered prior to drug entrapment is the effect of the drug on the lipid. We have seen that a drug may drastically fluidise the lipid. This could be important in affecting the stability and diffusional characteristics of the carrier liposome. High relative concentrations of drug to lipid may sometimes be used in these liposome systems.

Whilst the present studies have used saturated lecithins for ease of experiments, similar shifts of phase transition temperature should occur with unsaturated lipids as well. Accordingly, the drugs could potentiate a dramatic change in fluidity, should some membrane lipids be present in a gel state. However, even where the membrane lipids are all in a completely fluid state (i.e. above their transition temperature), the drugs will shift the phase boundaries of the lipids and thus still affect the fluidity of the membrane.

In the present study, a range of drug concentrations were tested for only two drugs. The remaining drug molecules were examined at drug : lipid molar ratios of 1 : 1. Although phase diagrams were presented for the interaction of the morphine compound M IV and of desipramine with dipalmitoyllecithin, a more detailed study using ternary phase diagrams would be desirable. This would involve an investigation of the particular phases existing in specific regions of the phase diagram [10]. Such a study was considered to be beyond the scope of the present investigation but is important for future studies.

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