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Interactions of a Phenothiazine Tranquilizer with Phosphatidylcholine and Phosphatidylcholine/Cholesterol Membranes†

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ABSTRACT: The interaction of the phenothiazine type tranquilizer methochlorpromazine (MCP) with phosphatidylcholine and phosphatidylcholine/cholesterol bilayer membranes has been studied by ^2H and ^{31}P nuclear magnetic resonance. The effect on bilayer order was monitored with the use of both deuterium-labeled dipalmitoylphosphatidylcholine and dioleoylphosphatidylcholine. In the absence of cholesterol, MCP is largely intercalated into the upper region of the fatty acyl chains and effectively disorders only the lower portion of the bilayer near the center of the membrane. Cholesterol affects the distribution of the drug among the two regions of phospholipid binding, expelling it to the membrane surface. Cholesterol opposes the disordering of the membrane, as well as expelling the drug, and therefore, the order of the membrane depends on the relative concentrations of the stabilizer, cholesterol, and the destabilizer, methochlorpromazine.

Chlorpromazine is a major tranquilizer as well as, in a broader sense, a general anesthetic (Seeman, 1972). It has been suggested that the pharmacological mode of action of the phenothiazine type drugs is related to their presence in or at biological membranes (Guth & Spirtes, 1964; Bhise et al., 1983). In particular, the reduction in membrane permeability to dopamine has been cited as the origin of their antipsychotic function (Bhise et al., 1983; Creese et al., 1976). The specific interactions responsible may be quite complex and involve disordering of the lipid (Pang et al., 1980), disruption of ion channels (Lee, 1976), interaction with membrane protein (LaBella, 1981), or modification of the membrane surface (Bhise et al., 1983; Zografi & Munshi, 1970; Seeman & Bialy, 1963). Some evidence has been found for the incorporation of chlorpromazine into the hydrophobic region of model membranes (Frenzel et al., 1978; Kitamura et al., 1981), and we have recently reported the presence of two lipid binding sites for methochlorpromazine (MCP)[1] (Forrest & Mattai, 1983). The phenothiazine is displaced from one of these sites by cholesterol. The effect of these agents on membrane organization has been the subject of a number of electron spin resonance studies, some of which explored the effect of cholesterol addition or depletion (Neal et al., 1976; Pang & Miller, 1978; Ogiso et al., 1981). In light of the inability of nitroxide-labeled fatty acid probes to even qualitatively monitor the effect of cholesterol on fluid bilayer systems (Taylor & Smith, 1980), the effect of MCP on the membrane was studied by the nonperturbing methods of ^2H and ^{31}P NMR using both saturated and unsaturated labeled phospholipids. The effects on the membrane components of the presence of cholesterol

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[1] Abbreviations: MCP, methochlorpromazine (2-chloro-10-[3-(trimethylammonio)propyl]phenothiazine); NMR, nuclear magnetic resonance; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; PC, phosphatidylcholine; CHOL, cholesterol; CSA, chemical shift anisotropy; ESR, electron spin resonance; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine.

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and its possible regulation of the action of the tranquilizing agent have also been investigated.

Materials and Methods

Chlorpromazine hydrochloride purchased from Sigma Chemical Co. was used to prepare methochlorpromazine-d_3 as reported (Huang et al., 1970). DPPC-d_{62} and DPPC-*2,2,2′,2′*-d_4 were synthesized by the acylation of glycerophosphocholine (Chadha, 1970; Oldfield et al., 1978) with the appropriately deuterium-labeled fatty acid anhydrides (Reeves et al., 1978; Selinger & Lapidot, 1966; Cubero Robles & Van den Berg, 1969; Oldfield et al., 1978). DPPC-d_9 was prepared by methylation of DPPE with CD_3I (Boulanger et al., 1980). Oleic-*2,2*-d_2 acid was prepared by base exchange with D_2O (Reeves et al., 1979) while oleic-*9,10*-d_2 acid was prepared from stearolic acid by reduction with deuterium gas over a Lindlar catalyst (Adkins & Burks, 1955; Lindlar & Dubius, 1973; Seelig & Waespe-Sarcevic, 1978). DOPC-*2,2,2′,2′*-d_4 and DOPC-*9,9′,10,10′*-d_4 were synthesized by the method of Warner & Benson (1977). Cholesterol-*3α*-d_1 was prepared as has been described (Fieser, 1963; Rosenfeld et al., 1954). Egg yolk PC was extracted according to the method of Singleton et al. (1965). Sample components were codissolved in chloroform. After solvent removal, the samples were placed under high vacuum overnight and subsequently dispersed in excess deuterium-depleted water by vortex mixing and numerous freeze–thaw cycles (Forrest, 1978; Westman et al., 1982). NMR spectra were recorded on a Nicolet 360 NB spectrometer operating at 55.4 MHz for ^2H and at 146.2 MHz for ^{31}P.

^2H spectra were recorded by using a single pulse sequence employing 60° pulses of approximately 35-μs duration. The spectra were sampled at rates of 2–5 times per s depending on estimates of the T_1 relaxation times for the various probe molecules (Davis, 1983). Under these conditions, some saturation of the terminal methyl groups of DPPC-d_{62} may occur. Also, due to limited pulse power, intensities far from the carrier frequency are diminished, resulting in distortion from the true line shapes. In most cases, 10000–20000 transients were averaged. ^{31}P spectra were recorded with broad-band proton decoupling using a two-level decoupling sequence. Relaxation times were measured by using an inversion–recovery sequence in which the repetition rate was much greater than $5T_1$.

Results

Saturated Acyl Chains. Small quantities (<10%) of DPPC-d_{62} were incorporated into egg PC liposomes. At this concentration, the lipids are completely miscible in the liquid-crystalline phase (Davis et al., 1980). The ^2H NMR spectra of DPPC-d_{62} in this fluid bilayer are shown in Figure 1 for increasing quantities of added drug. The spectra are a superposition of powder patterns for all the positions labeled. There exists considerable intensity in the outer spectral extremes, characteristic of a plateau of high order for the first several segments of the acyl chains (Seelig & Seelig, 1974). Increased concentrations of MCP result in a general shift in the intensity toward the center of the spectra, indicating decreased average order. However, the overlap of many of the resonances makes their assignment difficult. However, the quadrupole splittings for the terminal methyl group, the methylene adjacent to it, and the plateau region may be readily extracted. The changes brought about by the incorporation of the drug are shown in Figure 2. For example, the splitting of the terminal methyl groups is decreased by up to 45% whereas that of the plateau region is reduced by less than 15%. To partially circumvent the overlap of the DPPC-d_{62} resonances, similar experiments were performed with DPPC-*2,2,2′2′*-d_4. In the absence of MCP, the spectra consist of three resonances with quadrupole splittings of approximately 30 kHz for the two equivalent deuterons of the *sn*-1 chain and splittings of approximately 12 and 19 kHz for the two nonequivalent deuterons of the *sn*-2 chain (Seelig & Seelig, 1974; Haberkorn et al., 1977). These values change relatively little (<10%) upon addition of methochlorpromazine, indicating that the average orientation of these C–D bond axes is not influenced significantly. The most noticeable changes upon drug incorporation were a broadening and loss of intensity of the resonance for both the *sn*-1 and *sn*-2 chain deuterons. This may be the result of a "chain freezing effect" (Oldfield et al., 1978) or an exchange broadening between lipid molecules experiencing different motional restrictions (Tamm & Seelig, 1983). It is clear from the foregoing that the acyl chains in the region of the ester linkages are affected quite differently by the drug than are the segments near the membrane core.

Unsaturated Acyl Chain PC. First of all, the C-2 methylenes again give rise to three resonances when dispersed in an egg PC matrix, giving rise to splittings of approximately 26.4

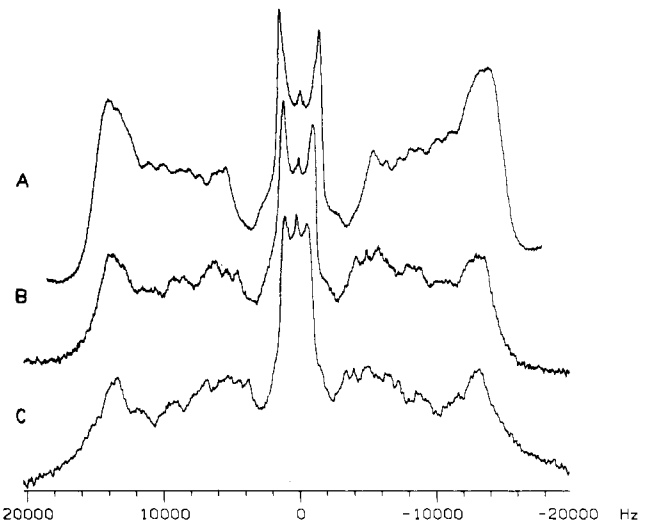

FIGURE 1: ^2H NMR spectra of DPPC-d_{62} in 450 mM PC multilamellar liposomes containing (A) no MCP, (B) 13 mol % MCP, and (C) 23 mol % MCP.

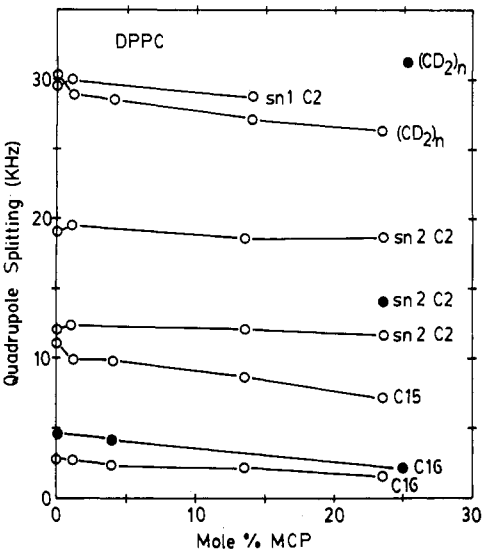

FIGURE 2: ^2H NMR quadrupole splittings vs. concentration of MCP for various positions along the fatty acyl chain of DPPC. The solid circles correspond to a cholesterol/PC mixed bilayer where 25 mol % of the lipid present is cholesterol.

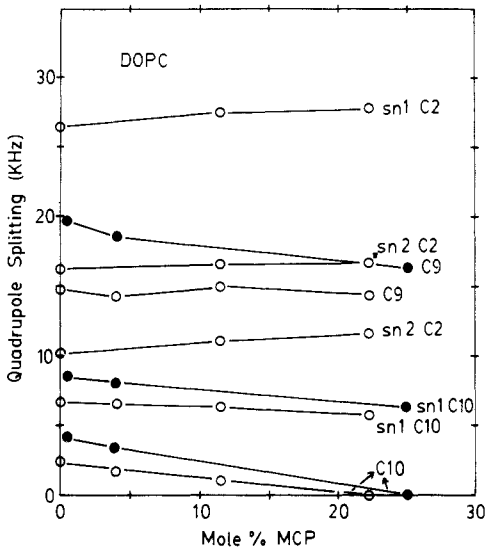

FIGURE 3: ^2H NMR quadrupole splittings vs. concentration of MCP for C-2, C-9, and C-10 of deuterium-labeled DOPC. The solid circles correspond to PC/CHOL mixed bilayers where 25 mol % of the lipid present is cholesterol.

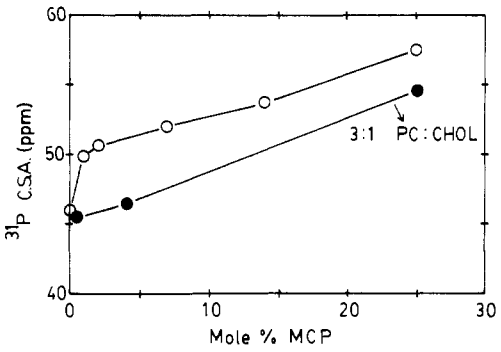

FIGURE 4: ^{31}P chemical shift anisotropy for liposomes containing increasing quantities of MCP: (○) PC bilayers; (●) bilayers containing 25 mol % CHOL.

kHz for the *sn*-1 chain and of 16.1 and 10.2 kHz for the *sn*-2 chain. Figure 3 shows small increases in order (<15%) as the tranquilizer concentration is increased. The DOPC which is deuterium labeled at the double bonds gives rise to three quadrupole splittings of approximately 15.0, 6.5, and 2.5 kHz which are assigned to deuterons at the 9-position of both chains, the deuteron at the 10-position of the *sn*-1 chain, and the deuteron at the 10-position of the *sn*-2 chain, respectively (Seelig et al., 1981; Tamm & Seelig, 1983); i.e., the two deuterons at position 10 are motionally inequivalent. As shown in Figure 3, the C-9 deuterons are relatively unaffected by the binding of MCP; the quadrupole splitting for the C-10 *sn*-1 deuteron decreases approximately 15%, while the quadrupole splitting for the C-10 *sn*-2 deuteron collapses to a singlet. Line-width measurements indicate the possibility of an unresolved splitting of approximately 25% of the unperturbed value. These effects at the position of the double bond are indicative of changes in the tilt angle of the cis double bond (Seelig & Waespe-Sarcevic, 1978).

Head-Group Region. The quadrupole splittings of DPPC-d_9 present in the lipid bilayer remained constant at 1200 ± 50 Hz in the absence of any added drug or in its presence at PC:MCP mole ratios of 115:1 to 6:1. Figure 4 shows that as the concentration of MCP is increased, the chemical shift anisotropy of the PC phosphate groups is increased substantially. The maximum residual CSA observed was approximately 58 ppm. At an equimolar concentration of PC and

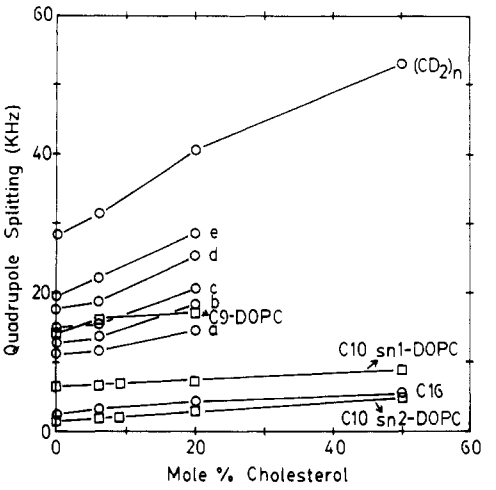

FIGURE 5: ^2H NMR quadrupole splittings for multilamellar liposomes with a constant total lipid:MCP ratio of 25:1 vs. increasing cholesterol concentration: (○) DPPC; (□) DOPC.

MCP, the ^{31}P spectrum showed the presence of a considerable quantity of an isotropic phase whose line width was approximately 300 Hz, as well as the presence of the bilayer phase. The isotropic phase results from the formation of small rapidly tumbling particles since the ^2H signal of MCP-d_3 also collapses to a relatively narrow singlet at this concentration. The increased chemical shift anisotropy suggests an immobilization of the phosphate group, as does a decrease of 25% in the ^{31}P T_1 in the presence of 23 mol % of the drug.

Methochlorpromazine-d_3. We have previously reported the interaction of this drug with PC liposomes in terms of two binding sites (Forrest & Mattai, 1983). As the ratio of MCP:PC is increased from approximately 100:1 to approximately 3:1, the line width of the spectral singlet increased from about 130 to 600 Hz in a roughly linear fashion. In addition, at 21 °C, the ^2H T_1 relaxation time decreased from 23 to 19 ms. When the temperature was raised to 45 °C, this difference became larger (55 vs. 38 ms). In contrast, extremely small amounts of MCP-d_3 which could be solubilized in water yielded a much longer T_1 of 84 ms in the absence of the membrane at 21 °C.

Effect of Cholesterol Addition. Figure 5 illustrates the effect of increasing cholesterol content on the order of the acyl chain region of DPPC and DOPC bilayers in which the total lipid:MCP ratio was kept constant at 25:1. The peaks for some of the DPPC-d_{62} resonances have been designated alphabetically due to some uncertainty of the absolute assignments of the perdeuterated chains. Whereas the presence of low quantities of the drug decreased the quadrupole splittings of many resonances near the membrane center, the presence of cholesterol counteracted the disordering of MCP. At this drug concentration, the overall order of the hydrophobic region is dominated by the condensing effect of cholesterol. For example, the quadrupole splittings of the terminal methyl group and the main methylene envelope increase by 80–90% in the presence of an equimolar amount of cholesterol; i.e., the effect of cholesterol is the same as has been seen previously for various liquid-crystalline phosphatidylcholine systems (Oldfield et al., 1978; Jacobs & Oldfield, 1979). Essentially linear increases in order were also observed for DOPC deuterium labeled at the 9- and 10-positions of the double bond as has been reported for pure DOPC in the liquid-crystalline phase (Tamm & Seelig, 1983). In addition, broadening of the spectra resulted in the disappearance of the signal for position-9 of both the *sn*-1 and *sn*-2 chains at cholesterol concentrations above 20 mol %. Figure 6 shows an increase in the quadrupole

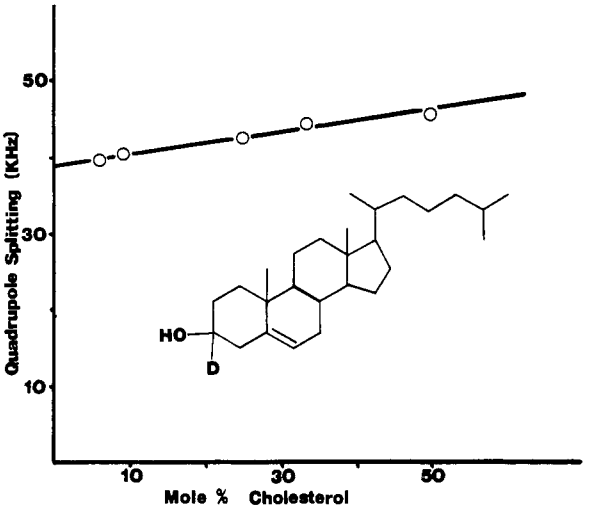

FIGURE 6: Quadrupole splitting of cholesterol-3α-d_1 for multilamellar liposomes with a lipid:MCP ratio of 25:1 as a function of increasing CHOL concentration.

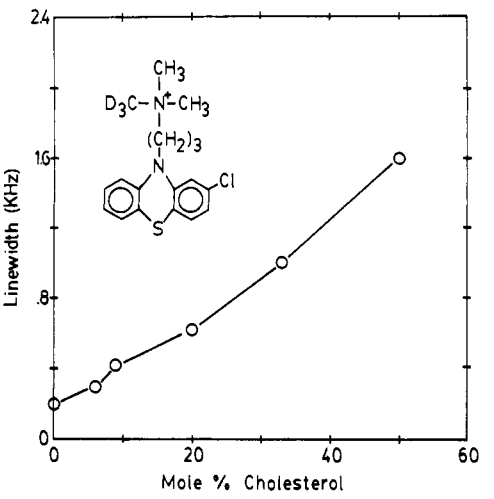

FIGURE 7: Line width of MCP-d_3 in bilayer membranes at a total lipid:MCP ratio of 25:1 as a function of increasing cholesterol concentration.

splitting of cholesterol-3α-d_1 as cholesterol is added in the presence of 4 mol % MCP. Again, the dominant effect is that of the ordering of cholesterol which has previously been reported for egg PC/cholesterol bilayers in the absence of any added drug (Taylor & Smith, 1981).

The addition of cholesterol to the 25:1 PC:MCP membrane decreased the PC phosphorus chemical shift anisotropy in a gradual fashion from approximately 51 ppm at 0% sterol to approximately 47 ppm at 50 mol % cholesterol. Cholesterol addition itself has little effect on the ^{31}P CSA of liquid-crystalline PC bilayers (Brown & Seelig, 1978), and the value of 47 ppm observed at high cholesterol content is very near to that found in the pure PC bilayer in the absence of MCP. Figure 7 shows that the line width of the MCP-d_3 signal of a 25:1 lipid:MCP membrane increases as cholesterol is added. At the highest cholesterol content, the resonance is split into a powder pattern with a quadrupole splitting of approximately 1150 Hz; i.e., the average order sensed by the drug is much higher in the presence of cholesterol. Associated with the pure PC membrane are two binding sites for MCP with fast exchange between them. Cholesterol displaces MCP from one of these sites, leading to more complete occupation of the site of higher order. The variation of the amount of added labeled drug to a 1:1 cholesterol:PC membrane verifies this fact. Throughout the range of lipid:MCP ratios of 100:1 through 3:1, a single quadrupole doublet with a splitting of 1150 ± 50 Hz and a half-width of approximately 1600 Hz was observed. The drug is still bound to the membrane, but only one site of constant order is available. The quadrupole splitting of 1150 Hz for the trimethylammonium group of MCP-d_3 is very similar to that found for the trimethylammonium group of the phospholipid and suggests that both of these species are present in the same environment at the membrane surface.

Phenothiazine Addition to a 3:1 PC:CHOL Membrane. As shown in the preceding sections, MCP orders the PC phosphate group, and disorders the central membrane region of pure PC bilayers, and low concentrations of the drug have little effect on the ordering effect of large quantities of cholesterol. The relative ordering and disordering abilities of cholesterol and the tranquilizer are of interest since it is apparent that the overall membrane properties will largely be reflected by its composition. The deuterium NMR quadrupole splittings for a number of positions along the chain of DOPC or DPPC present in a 3:1 PC:CHOL membrane vs. MCP concentration are shown in Figures 2 and 3. The quadrupole splittings for the 9- and 10-positions of DOPC all decrease as the concentration of MCP is increased. A similar effect is seen for the terminal methyl group of DPPC. Also, for the relatively cholesterol-rich membrane, a quadrupole splitting of approximately 31 kHz is found for the plateau region at a liquid:MCP ratio of 3:1 whereas 45–50 kHz would be observed in the absence of the drug.

The ^{31}P CSA was also examined as a function of MCP concentration for this mixed membrane. The results shown in Figure 4 are still indicative of decreased head-group mobility, but the degree of this immobilization is less than occurs in the cholesterol-free system.

Discussion

There is no simple relationship between the ordering of motion and the "fluidity" within phospholipid bilayer membranes. Fluidity has been defined as referring solely to the rate of motion and not to the ordering of a molecular system (Seelig, 1977). Deuterium quadrupolar splittings as measured here are directly proportional to the order parameter of the labeled segment, and as such are indicators of the flexibility of the hydrocarbon chains and the average number of gauche conformations therein. A large proportion of these gauche conformations are associated with kinks and jogs in the hydrophobic region, and their presence reduces the effective bilayer thickness (Seelig & Seelig, 1974).

In the absence of cholesterol, a large portion of the drug intercalates into the hydrophobic region of the fluid membrane, causing increases in the amplitude of the angular excursions of the lower portion of the fatty acyl chains without significantly destabilizing the upper portion near the ester linkages. This finding is essentially the same as has been found for the interaction of chlorpromazine with human erythrocyte ghosts as reported by fatty acid spin-labels (Ogiso et al., 1981). Also, the proximity of the C-2 methylene protons to the aromatic ring system of chlorpromazine has been reported to be the cause of an upfield ^{1}H chemical shift (Katamura et al., 1981). The insertion of the drug creates an increased free volume in the central portion of the membrane due to a mismatch of its length and the effective length of the acyl chains. The positively charged trimethylammonium group interacts with the PC phosphate groups, increasing the residual chemical shift anisotropy (Frenzel et al., 1978) as well as decreasing the ^{31}P T_1 relaxation time. However, when the membrane is composed of 50 mol % cholesterol, the drug affects the head-group region differently, even small concentrations increasing the apparent

mobility of the phosphate group, while the flexibility of the hydrophobic region is dominated by the ordering effect of cholesterol. Cholesterol, whose order parameter is largely determined by rigid body motions, does not sense the presence of low quantities of MCP. These results as well as a concentration-independent MCP-d_3 line width in the 1:1 PC:CHOL membrane indicate that the condensing effect of cholesterol tends to exclude the drug from the membrane interior, leading to a high concentration at the interface. Ultimately, this may lead to a layer of drug molecules completely covering the interface, which according to the theory of Kesting (Kesting et al., 1968; Bhise et al., 1983) alters the permeability properties of the membrane. Therefore, the lipid composition of the membrane governs the disordering effect of the tranquilizer by dictating its distribution between the two lipid binding sites at different levels in the bilayer.

An examination of the results for the 3:1 PC:CHOL membrane shows that the membrane-intercalated MCP is a more efficient structural destabilizer of the lower chain region than cholesterol is a stabilizer. However, even at the high drug concentration of 3:1 lipid:MCP, the overall order is greater when cholesterol is present since it affects the distribution of MCP between the more hydrophobic and interface sites. The ^{31}P CSA measurements indicate a decrease in phosphate group mobility which is less than for the cholesterol-free membrane, reflecting the drug site distribution; i.e., opposing effects are at work, head-group ordering produced by one site and head-group disordering produced by the other.

However, in the presence or absence of cholesterol and at no drug concentration is there any evidence of a stabilizing effect (i.e., an increase in order) produced in the hydrophobic region of DPPC. This is in contrast to ESR studies of chlorpromazine which indicated a slight ordering effect of low concentrations in the absence of cholesterol (Pang & Miller, 1978; Neal et al., 1976). The results presented here show that, at most, MCP has little effect on the order of segments very near the surface or disorders them, albeit less than it disorders segments further down the acyl chains.

In the case of DOPC, in the absence of cholesterol, the deuterons at the C-2 position of both the *sn*-1 and *sn*-2 chains show slight increases in quadrupole splittings through the range from 1 mol % MCP to 23 mol % MCP. The small ordering effect is much less than can be induced by cholesterol and may be a reflection of differential interaction with unsaturated PC's since small decreases in order were seen for similarly labeled DPPC.

As we have previously pointed out, the principle of the effect of variation in lipid composition on the binding of molecules to membranes may explain discrepancies between membrane/water partition coefficients as measured by centrifugation and the filtration technique of hygroscopic desorption (Forrest & Mattai, 1983; Gaffney et al., 1983; Conrad & Singer, 1981). Cholesterol-rich membranes may become coated with a layer of the drug in question, or the membrane may serve as a site of hemimicelle formation at lower concentrations (Conrad & Singer, 1981). The surface drug may be stripped from the membrane during filtration processes but would be detected as membrane bound in centrifugation studies. We have found no evidence for comicellization of MCP and PC until they are present in equimolar proportions. Both the MCP-d_3 signal and the ^{31}P PC signal collapse to relatively sharp singlets at this point.

Conclusions

Overall, it can be concluded that the order of the hydrophobic membrane region is dependent on the lipid composition. MCP has little effect on the average angular excursions of the upper region of the saturated fatty acyl chains but increases the flexibility of the lower portions. Order is restored by cholesterol addition as a consequence of its condensing effect and its expulsion of the added drug to the water/membrane interface. In the absence of cholesterol, the drug slightly increases the order of C-2 deuterons of the unsaturated phospholipid, DOPC. While the ordering is small (<15%), it is opposite to the slight disordering of the same position of DPPC, suggesting that the more subtle variations in chain unsaturation may also be important in the ordering or disordering efficacy of the drug. However, cholesterol is a much more effective ordering agent. As a general conclusion, it can be stated that membranes containing less than equimolar quantities of cholesterol will be disordered by intercalated phenothiazines, resulting in decreased thickness of the hydrophobic region. In high cholesterol concentration membranes, the hydrophobic region is highly ordered, and alteration of permeability properties may involve interface effects. It must be restated at this point that changes in the measured order parameters are indicative of structural changes only and do not reflect the dynamic aspects of chain mobility. Alterations in bilayer fluidity are manifested by changes in rates of motion and as such await determination by relaxation time measurements.

Acknowledgments

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Registry No. Methochlorpromazine, 19077-31-7; cholesterol, 57-88-5.

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An Electron–Electron Double-Resonance Study of Interactions between [^{14}N]- and [^{15}N]Stearic Acid Spin-Label Pairs: Lateral Diffusion and Vertical Fluctuations in Dimyristoylphosphatidylcholine†

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ABSTRACT: Vertical fluctuations of the terminal methyl groups of stearic acid acyl chains toward the surface of dimyristoylphosphatidylcholine (DMPC) bilayers have been investigated by using spin-label electron–electron double-resonance (ELDOR) methodology. Spin-label pairs consisting of two populations of stearic acid spin-labels were employed, each at 0.25 mol % concentration, where the nitroxides of the first population were ^{15}N substituted and the nitroxides of the second contained ^{14}N. Various combinations of labels with the nitroxide moieties located at carbons 5, 12, or 16 (C5, C12, C16) were used. ELDOR permits measurement of collision frequencies between the two constituents of the pair, for example, between ^{15}N spin-labels at C5 and ^{14}N labels at C16. Intramolecular contributions to the ELDOR effect including nitrogen nuclear relaxation are eliminated by the use of spin-label pairs. Above the main phase transition temperature, bimolecular collisions between C5 and C16 occur with about half the frequency of C16:C16 collisions. It is concluded that vertical fluctuations are very pronounced. A dependence of these fluctuations on temperature and pH has been observed. Lateral diffusion constants calculated from the bimolecular collision frequencies of C16:C16 pairs are 4.56×10^{-8}, 5.77×10^{-8}, and 8.09×10^{-8} cm^2/s at 27, 37, and 47 °C. These values are in good agreement with previous measurements of lipid diffusion in DMPC.

Current investigations of the structure of biological membranes often concern the extent to which various constituents can diffuse laterally and interact. A number of biophysical methods have been developed to examine the lateral mobility of integral membrane components, including photobleaching recovery and redistribution techniques, pulsed gradient nuclear magnetic resonance, eximer formation, electron paramagnetic resonance (EPR)[1] techniques employing line-width analysis, and, more recently, observation of electron–electron double resonance (ELDOR). Of these, only eximer formation and the EPR methods are rigorously capable of examining mo-

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; ELDOR, electron–electron double resonance; END, electron–nuclear dipolar; EPR, electron paramagnetic resonance; Hex, Heisenberg spin exchange; psEED, pseudosecular electron–electron dipolar; C5, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidineoxyl; C12, 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidineoxyl; C16, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidineoxyl; tanone, 4-oxy-2,2,6,6-tetramethyl-1-piperidineoxyl.

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