EFFECTS OF ANESTHETIC TETRADECENOLS ON PHOSPHATIDYLCHOLINE PHASE TRANSITIONS

Implications for the Mechanism of the Bilayer Pretransition

TIMOTHY J. O'LEARY,* PHILIP D. ROSS,‡ AND IRA W. LEVIN*
*Laboratory of Chemical Physics and ‡Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT The effects of *cis*- and *trans*-9,10-tetradecenols on the phase transitions of dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholines were investigated using high sensitivity scanning calorimetry and Raman spectroscopy. Both alcohols lowered the gel to liquid crystalline phase transition temperatures for all three phosphatidylcholines, with *cis*-tetradecenol showing a considerably greater effect than *trans*-tetradecenol in each case. While both alcohols increased the temperature of the dimyristoylphosphatidylcholine pretransition, and decreased the temperature of the distearoylphosphatidylcholine pretransition, *cis*-tetradecenol lowered the temperature of the dipalmitoylphosphatidylcholine pretransition, while *trans*-tetradecenol dramatically raised the pretransition temperature. These results are interpreted in terms of the reduction in gel (L_β) phase chain tilt and changes in the ease of acyl chain *trans-gauche* isomerization which are introduced by the alcohols, and the consequent effects of these changes on the pretransition and the gel to liquid crystalline phase transition. The data clearly show that caution is necessary in applying information on lipid-anesthetic interactions obtained from model membranes to the problem of clinical anesthesia, since qualitatively different results may be obtained when lipids of differing acyl chain lengths are employed. Superficial interpretation of such data might lead to erroneous conclusions.

INTRODUCTION

General anesthesia is widely believed to result from drug-induced perturbations of excitable membranes (1–3). Common features of many theories are the supposition that anesthetic-lipid interactions, as opposed to anesthetic-protein interactions, dominate the membrane effect, and that all general anesthetics affect the lipid membranes in essentially the same way. As a result, many investigators have examined lipid-anesthetic interactions using a variety of experimental methods and anesthetics. Although early studies suggest that there might be a correlation between the physiologic effects of general anesthetics and their effects on phospholipid gel to liquid crystalline phase transitions, more recent studies on nonvolatile anesthetic alcohols and steroids have demonstrated no correlation between anesthetic potency and the effects on the gel to liquid crystalline phase transition (4–6). A correlation between potency and changes in both the midpoint and width of the dipalmitoylphosphatidylcholine pretransition was demonstrated for the anesthetic steroids, however (4). This correlation, if general, would suggest that perturbation of acyl chain packing, rather than direct perturbation of *trans-gauche* isomerization, results in the clinical effects of general anesthetics. Although effects of anesthetics on the bilayer pretransition have been noted for several volatile anesthetics (7), no systematic attempts to correlate these effects with clinical potency have been made for nonsteroidal general anesthetics.

The anesthetic alcohols, *cis*- and *trans*-9,10-tetradecenol, provide an interesting pair of compounds with which to test the generality of the observations that the effects of anesthetic steroids on the pretransition correlate with anesthetic potency. These alcohols are equipotent as anesthetics (5, 6), but are conformationally rather different, in contrast to the steroids, which exhibited wide variations in potency associated with only small changes in conformation. In addition, the alcohols are essentially insoluble in water, assuring that relatively little partitioning into the aqueous phase will occur. We have studied the effects of *cis*- and *trans*-9,10-tetradecenols on both the pretransition and the gel to liquid crystalline phase transition of dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholine liposomes using Raman spectroscopy and high sensitivity scanning calorimetry. Together, these techniques allow us to determine accurately the effects of perturbing molecules on the phase transitions and to assess their effects on *trans-gauche* isomerization and lateral chain–chain inter-

Dr. O'Leary's present address is Laboratory of Cell and Molecular Biology, Division of Biochemistry and Biophysics, Center for Drugs and Biologics, FDA, Bldg. 29, Room 500, Bethesda, Maryland 20892.

actions in the lipid bilayer (4, 8). The effect of changes in the acyl chain length on the thermodynamics of the pretransition was further explored by calorimetric studies of the interactions of *cis*- and *trans*-9,10-hexadecenols with dipalmitoylphosphatidylcholine bilayers. The results of these studies are useful in clarifying our understanding of the lipid pretransition and the role of model systems in studying the lipid effects of general anesthetics.

EXPERIMENTAL

Dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholines (DMPC, DPPC, and DSPC) were obtained from Sigma Chemical Co., St. Louis, MD; *cis*- and *trans*-9,10-tetradecenols and 9,10-hexadecenols were purchased from Nu-Chek Prep, Inc., Elysian, MN. Multilayers containing ~10–40 mol% alcohol were prepared by first dropping warm (liquid) alcohol onto anhydrous lipid, allowing the alcohol to soak in, and then hydrating the mixture to form a 1:10 (wt/wt) lipid+alcohol/water dispersion. The dispersion was cycled through its phase transition temperature 10 times, allowed to remain 20°C above the transition temperature of the pure lipid for 1–2 h, and then held below the gel to liquid crystalline phase transition temperature for at least 48 h before making either calorimetric or spectroscopic measurements. Highly concentrated dispersions were used to minimize the possibility of significant amounts of alcohol partitioning into the aqueous phase and to permit the spectroscopic and calorimetric measurements to be made under identical concentration conditions.

Raman spectra in the carbon–hydrogen (CH) stretching mode region were obtained with a previously described spectrometer (9). Spectra were signal-averaged for 4–6 scans at a scan rate of 1 cm^{-1}/s and at an instrumental resolution of 5 cm^{-1}. Temperature profiles in the CH stretching mode region were constructed using the I_{2850}/I_{2880} and $I_{2,935}/I_{2,880}$ peak height ratios, which reflect acyl chain packing characteristics and *trans-gauche* isomerization (8). No attempt was made to subtract the contributions of the alcohols to the Raman spectra. Hence, both the original spectra and the peak height ratios contain contributions from both phosphatidylcholine and alcohol hydrocarbon chains.

The heat flow scanning calorimeter described by Ross and Goldberg (10) was used in the work. In a typical experiment, ~60 mg of the liposome preparation were loaded into the calorimetric cell, with the tare cell unfilled, and heated at 14.8 K per h. In several experiments the scan rate was varied from 7–35 K per h to determine the scan rate dependence of the calorimetric measurements on both pure phosphatidylcholine and phosphatidylcholine-alcohol dispersions. The solvent contribution to the heat capacity was determined in a separate loading of the sample cell and subtracted from the phospholipid scan. The data were corrected for instrumental response using the method described by O'Leary et al. (4). The gel-to-liquid crystalline phase transition endpoint was defined as the temperature at which there was no discernible difference between the excess heat capacity curve and the baseline (approximately twice the baseline root mean square noise). We were able to reproducibly determine the midpoint of the gel-to-liquid crystalline phase transition to within 0.03 K, and the midpoint of the pretransition to within 0.1 K.

RESULTS

The Raman spectra of both the gel and liquid crystalline phase samples are virtually identical to those for pure dipalmitoylphosphatidylcholine, as illustrated in Fig. 1 for gel and liquid crystalline phase DPPC-*cis*-tetradecenol dispersions. This similarity between the CH stretching mode region spectra for pure and alcohol-containing dispersions is further illustrated in Fig. 2, in which temperature profiled derived from the $I_{2,935}/I_{2,880}$ Raman spectral

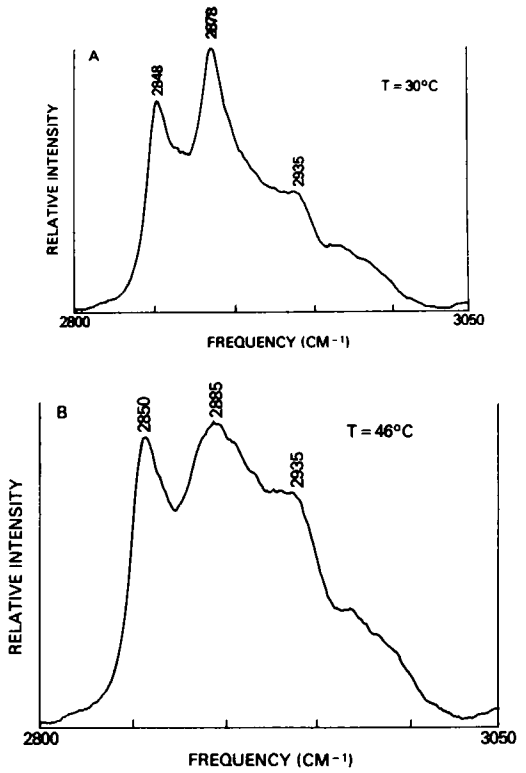

FIGURE 1 Raman spectra of (*A*) gel and (*B*) liquid crystalline phase 20 mol% *cis*-tetradecenol-DPPC dispersions in the 2,800–3,100 cm^{-1} CH stretching mode region. These spectra are essentially identical to those of 20 mol% *trans*-tetradecenol or pure DPPC dispersions at the same temperatures.

intensity ratios are presented. The profiles derived for pure dipalmitoylphosphatidylcholine and for alcohol-DPPC mixtures are essentially identical both above and below the gel to liquid crystalline phase transition. Both tetradecenols lowered the temperature of the gel-to-liquid crystalline phase transition; the *cis*-isomer had a greater effect than the *trans*-isomer in all three lipid systems.

Calorimetric results for the pure phosphatidylcholines and phosphatidylcholine-tetradecenol dispersions are summarized in Table I and illustrated in Figs. 3 and 4. With all three phosphatidylcholine species both tetradecenols lowered the gel to liquid crystalline phase transition temperature; for DPPC this lowering was approximately linear with alcohol concentration in DPPC (Fig. 4). In all cases, the *cis*-tetradecenol exhibited a greater lowering of the phase transition temperature than did *trans*-tetradecenol. In addition to lowering the gel-to-liquid crystalline phase transition temperature, both alcohols caused a significant broadening of this transition and a decrease in the maximum excess heat capacity. In all cases, *cis*-tetradecenol caused considerably greater broadening of the gel-to-liquid crystalline transition than did the *trans*-alcohol. Detailed determinations of the absolute enthalpies of the pretransition and gel-to-liquid crystalline phase transitions were not carried out; the data indicate, however, that the effect of

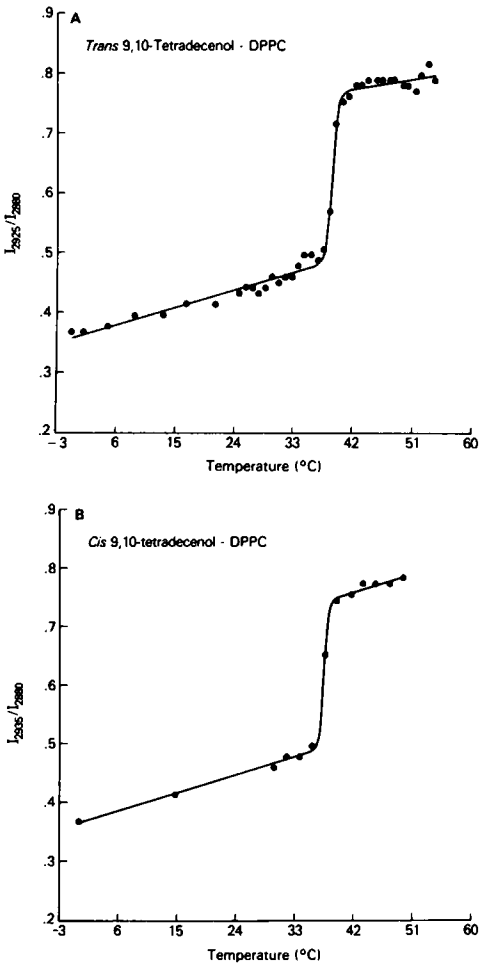

FIGURE 2 Temperature profiles constructed using the $I_{2,935}/I_{2,280}$ spectral ratios for (*A*) DPPC-*trans*-tetradecenol and (*B*) DPPC-*cis*-tetradecenol.

these alcohols on the enthalpy of the gel to liquid crystalline phase transition, if any, is small.

The hexadecenols both lowered the maximum excess heat capacity of the DPPC gel-to-liquid crystalline phase transition. Up to 20 mol% *cis*-hexadecenol had no effect on the transition temperature; similar concentrations of *trans*-hexadecenol raised the gel-to-liquid crystalline phase transition tempearture (Table I). Broadening of the transition appeared as a low temperature shoulder when *cis*-tetradecenol was added to the bilayer. A corresponding shoulder appeared on the high temperature side of the transition when *trans*-tetradecenol was added to the bilayer.

Both tetradecenols lowered the DSPC pretransition temperature; the lowering was significantly greater for the *cis*-alcohol than for the *trans*. In contrast, both tetradecenols raised the DMPC pretransition temperature; in this case the *cis*-alcohol demonstrated a slightly larger effect. Finally, *cis*-tetradecenol slightly lowered the DPPC pretransition temperature while *trans*-tetradecenol, in contrast, markedly increased the pretransition temperature. As was the case for the gel to liquid crystalline phase

TABLE I
PRETRANSITION TEMPERATURE (T_p), GEL TO LIQUID CRYSTALLINE PHASE TRANSITION TEMPERATURE (T_m), AND PRETRANSITION RELATIVE ENTHALPY ΔH_{Rel} (PRETRANSITION ENTHALPY/MAIN TRANSITION ENTHALPY) FOR PURE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLCHOLINE-ALCOHOL DISPERSIONS

Lipid	Alcohol isomer	Alcohol mole fraction	T_p	T_m	ΔH_{Rel}
			°C	°C	
DMPC	—	0.00	15.1	24.09	0.180
	trans C_{14}	0.18	16.9	24.04	0.117
	cis C_{14}	0.22	17.6	22.16	0.095
DPPC	—	0.00	34.9	41.37	0.139
	trans C_{14}	0.10	35.6	41.26	0.127
	trans C_{14}	0.19	37.1	40.83	0.096
	trans C_{14}	0.30	38.6	40.77	—
	cis C_{14}	0.09	34.7	40.85	0.130
	cis C_{14}	0.18	34.3	39.80	0.085
	cis C_{14}	0.27	34.4	39.30	0.072
	cis C_{14}	0.41	34.2	37.79	—
	trans C_{16}	0.08	34.9	41.44	0.116
	trans C_{16}	0.20	35.7	41.79	0.035
	cis C_{16}	0.07	34.9	41.34	0.106
	cis C_{16}	0.20	35.2	41.34	0.085
DSPC	—	0.00	50.9	54.86	0.130
	trans C_{14}	0.18	49.2	54.21	0.054
	cis C_{14}	0.22	41.2	52.81	0.008

transition, these temperature changes were monotonic with increasing alcohol concentration (Fig. 4) and were accompanied by a decrease in the enthalpy of the pretransition relative to that of the gel-to-liquid crystalline transition (Table I). Calorimetric data obtained at scan rates between 7 and 35 K per h showed no kinetic effects, as manifested by changes in thermogram shape or characteristic temperatures, in either the pretransition or the gel-to-liquid crystalline phase transition for tetradecenol-containing dispersions, although there was a slight scan rate dependence of the pretransition characteristics of pure DPPC liposomes.

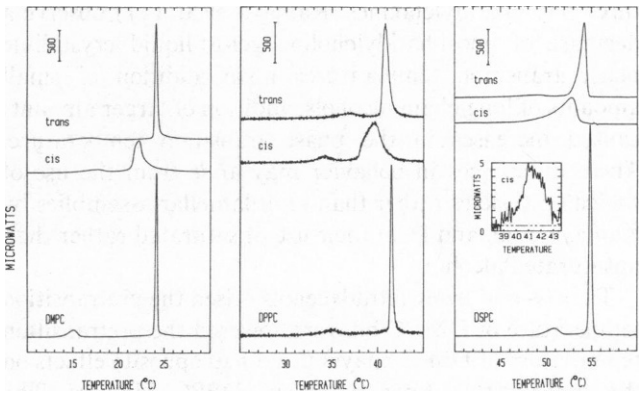

FIGURE 3 Calorimetric traces for pure phosphatidylcholine and phosphatidylcholine-alcohol dispersions. The alcohol-lipid dispersions contain ~20 mol% alcohol (see Table I for precise concentrations). *Inset* shows the pretransition for the *cis*-tetradecenol-DSPC dispersion.

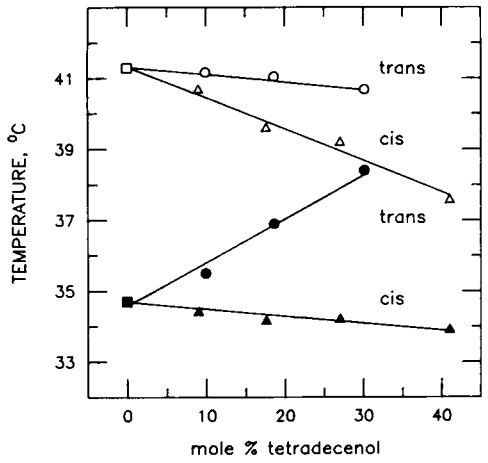

FIGURE 4 Midpoint temperatures for the (*A*) pretransition (T_p, closed figures) and the (*B*) gel-to-liquid crystalline transition (T_m, open figures) of DPPC as a function of alcohol concentration. Triangles denote dispersions containing *cis*-tetradecenol. Circles denote dispersions containing *trans*-tetradecenol.

Both *cis*- and *trans*-hexadecenol increased the temperature of the DPPC pretransition. Although *trans*-hexadecenol caused a greater increase in this temperature than did the *cis*-isomer, it was nevertheless smaller than for *trans*-tetradecenol incorporated into the bilayer at comparable concentrations.

DISCUSSION

The similarities in the $I_{2,935}/I_{2,880}$ and the $I_{2,850}/I_{2,880}$ ratios for dispersions either of *cis*- or *trans*-tetradecenols (Figs. 1 and 2) and those for pure dipalmitoylphosphatidylcholines indicate that there are only small differences in the number of *gauche* isomers and in the "tightness" of acyl chain packing in either the gel or the liquid crystalline phase lipids (8). The small differences in phase transition temperatures provide evidence for small alteration of the lipid bilayer by both alcohols. Both tetradecenols lower the gel to liquid crystalline phase transition temperatures of all three phosphatidylcholines. Kamaya et al. (11) observe a decrease of phosphatidylcholine gel-to-liquid crystalline phase transition temperatures upon addition of small amounts of long chain alcohols; addition of larger amounts caused increases in the phase transition temperature. These differences in behavior may arise from the use of sonicated vesicles rather than multilamellar assemblies by Kamaya et al. and from their use of saturated rather than unsaturated alcohols.

The *cis*- and *trans*-tetradecenols raised the pretransition temperature of DMPC bilayers, lowered the pretransition temperature of DSPC bilayers, and had opposite effects on the pretransition temperature of DPPC bilayers. The qualitatively different perturbations of the phase transition temperatures of the different phosphatidylcholines by the two tetradecenols have an important implication for studies on anesthetic-model membrane interactions. Although these anesthetics have qualitatively different effects on lipid bilayers constituted from DMPC, DPPC, and DSPC, they are equipotent as general anesthetics (5). We conclude, therefore, that because a study finds a correlation between anesthetic effect and lipid perturbation in a single lipid system, it does not follow that such a correlation may be expected in every lipid system or with all types of general anesthetics. In particular, we conclude that the correlation between the potencies of steroid anesthetics and their effects on the DPPC pretransition (4) is not a universal property of general anesthetics.

Lowering of the gel-to-lipid crystalline phase transition temperatures by the tetradecenols can be readily explained by noting that these molecules increase the free volume, that is, the average volume accessible to a single lipid molecule, in the gel state bilayer. For the *trans*-compound this results from a slight lateral displacement of the acyl chain axis below the double bond (Fig. 5); for the *cis*-alcohol this displacement, and hence the additional free volume, is considerably greater than for *trans*-tetradecenol because the double bond introduces a bend into the acyl chain. Theoretical models of the lipid bilayer gel-to-liquid crystalline phase transition predict a decrease in the gel-to-liquid crystalline phase transition temperature, T_m, upon introduction of free volume into the bilayer (12–14). The decrease in T_m results from the tendency of the bilayer to maximize its entropy by filling the void volume by means of *trans-gauche* isomerization; this effect is increased as the void volume is increased. The relative magnitudes of the changes that we have found result from introducing alcohols with *cis*- or *trans*-double bonds in the

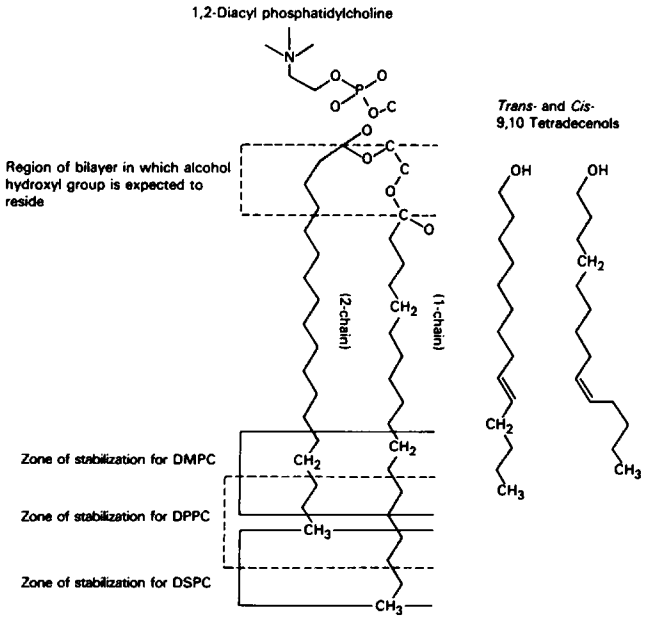

FIGURE 5 Structural formulae of a symmetric diacylphosphatidylcholine (DSPC) and the *trans*- and *cis*-tetradecenols, showing the possible zones of stabilization within which penetration of the alcohol terminal methyl is expected to stabilize the planar phase.

hydrocarbon chains are similar to those that result from replacing saturated with unsaturated acyl chains in pure lipid systems (14). When hexadecenols are incorporated into the bilayer these free volume effects, which would be expected to decrease the gel-to-liquid crystalline phase transition temperature, apparently are offset by increases in the van-der-Waals attraction resulting from the longer chain lengths.

Changes in the transition temperatures caused by the presence of alcohols were accompanied by broadening of the calorimetric transition profiles, which in some cases appeared to be composed of multiple components. This may indicate the presence of spatial inhomogeneities or phase separation in the samples. The identical endpoint temperatures for the phosphatidylcholine-tetradecenol and phosphatidylcholine *cis*-hexadecenol gel-to-liquid crystalline phase transitions and for pure phosphatidylcholines strongly suggest separation into alcohol-rich and alcohol-poor regions.

Changes in the pretransition temperature similar to those observed upon introduction of *cis*- and *trans*-unsaturated alcohols into the lipid bilayer have been observed previously. Reductions have been observed upon introduction of several different perturbants, including inhalation anesthetics (7) and steroids (4) into phosphatidylcholine bilayers; increases in the pretransition temperature have been reported upon incorporation of small amounts of myristic, palmitic, or stearic acid (15) into DPPC multilayers. The observed temperature changes and decreases in the enthalpy relative to that of the gel to liquid crystalline phase transition (Table I) may be understood in terms of the effects of the alcohols on three factors: chain tilt in the gel ($L_{\beta'}$) phase, the effects of acyl chain length and asymmetry on chain packing, and the effects of acyl chain length and asymmetry on *trans-gauche* isomerication in the gel ($L_{\beta'}$) and ripple (P_{β}) phases.

Below the pretransition temperature, gel ($L_{\beta'}$) phase phosphatidylcholine acyl chains tilt significantly (~30° for DPPC) with respect to the bilayer normal (16). Tilting allows the lipid acyl chains to pack relatively tightly even though the phosphatidylcholine headgroup area is ~15% larger than the cross-sectional area of the chains. In the ripple (P_{β}) phase, headgroup/acyl chain mismatch is accommodated by vertical displacement of the headgroups with respect to one another (17, 18). Lipids such as DPPE, which have smaller headgroups, and hence no headgroup/chain mismatch, demonstrate no chain tilt, no ripple phase, and no distinguishable pretransition. Although Chowdry et al. (19) have observed complex behavior of the excess heat capacity around the phosphatidylethanolamine gel-to-liquid crystalline phase transition, no structural data corroborate the assertion that this corresponds to the phosphatidylcholine pretransition. Since the surface projections of the polar "headgroups" of the long-chain alcohols and carboxylic acids do not differ significantly from that of their hydrocarbon chains, incorporation of such compounds is expected to reduce the overall headgroup/acyl chain mismatch. The expected result is reduction of acyl chain tilt in the gel ($L_{\beta'}$) phase, as has been observed experimentally for phosphatidylcholines into which long-chain alkanes have been incorporated (20). Reducing the chain tilt by introduction of perturbants, such as the alcohols, reduces in turn the structural differences between the gel and ripple phases, leading to a reduced enthalpy and entropy of the pretransition, as observed experimentally (Table I). The observed lack of scan rate dependence in the pretransition thermograms of the tetradecenol-phosphatidylcholine dispersions indicates that the activation energy of this transition is very small. The reduction of the activation energy for the pretransition, in contrast to that of pure phosphatidylcholines, indicates that the structural difference between the gel ($L_{\beta'}$) and the ripple (P_{β}) phases has been reduced by the incorporation of alcohol. This result is consistent with the conclusion that the alcohols reduce chain tilt in the gel phase, as demonstrated by x-ray diffraction measurements for long-chain alkanes (see above; and 20).

In addition to affecting the amount of chain tilt, the structural details of the lipid hydrocarbon chains themselves may be expected to influence the ease of *trans-gauche* isomerization at the pretransition. One consequence of chain tilting in the phosphatidylcholines is a reduction in the relative displacement of a lipid molecule's acyl chain termini from the ~2–3 CH_2 units expected in a non-tilted lipid, to ~1–2 CH_2 units. In the ripple phase, the 2–3 CH_2 unit relative displacement is presumably restored. Were the acyl chains to remain in nearly all *trans* configurations, the result would be an increase in the apparent "void volume" near the bilayer center; this void is filled via *trans-gauche* isomerization of the acyl chains (21, 22). The amount of additional void volume introduced by rippling is greatest for systems having the largest chain tilt. Lengthening the *sn*-2 chain in comparison with the *sn*-1 chain should decrease the additional void volume created by bilayer rippling, thus reducing the tendency to form the rippled phase, and increasing the pretransition temperature. In contrast, lengthening the *sn*-1 chain relative to the *sn*-2 chain is expected to decrease the pretransition temperature. Data from Chen and Sturtevant (23) on the pretransition temperatures of mixed-chain phosphatidylcholines confirm these expectations (Table II). For example, lengthening the *sn*-2 chain of DMPC by 2 CH_2 units increases the pretransition temperature by 6.4°C; lengthening the *sn*-1 chain by 2 CH_2 units decreases the pretransition temperature by 3.6°C. This type of analysis of chain length asymmetry has been used previously (24–26) to analyze the temperatures and entropies of the gel-to-liquid crystalline phase transition of asymmetric phospholipids.

Because the intercalated hydrocarbon chains of the long-chain alcohols may be expected to participate in the pretransition in the same way as the lipid acyl chains, it is reasonable to incorporate their packing characteristics into

TABLE II
PRETRANSITION TEMPERATURES (T_p) FOR SYMMETRIC AND MIXED CHAIN PHOSPHATIDYLCHOLINES*

Lipid	*sn*-1 chain length	*sn*-2 chain length	T_p
			°C
PMPC	16	14	10.8
DMPC	14	14	14.4
MPPC	14	16	22.8
SPPC	18	16	30.8
DPPC	16	16	34.8
PSPC	16	18	39.9
DSPC	18	18	50.4

*From Chen and Sturtevant, reference 23.

the above model. We postulate, therefore, that if the effective length of an alcohol incorporated into the lipid bilayer is such that there is less variation in the effective hydrocarbon chain lengths at the bilayer center, thus reducing the void volume available to accommodate *trans-gauche* isomerization in the ripple phase, the pretransition temperature will be increased. In contrast, if the alcohol causes increased variation in the effective penetration of hydrocarbon chains, the pretransition temperature will be decreased, unless this variation is so large that it promotes chain interdigitation (27).

To determine the effective penetration depth of the alcohol hydrocarbon chains into the bilayer, one must first determine the location of the hydroxyl group. Three possible regions of the lipid bilayer would provide a polar environment in which this group could reasonably be expected to reside, namely, the headgroup-water interface, the phosphate region (possibly with hydrogen bonding between the alcohol and the phosphate oxygen atoms), and the glycerol backbone (with possible hydrogen bonding to either of the ester oxygen atoms). Of these, the glycerol backbone region seems most likely, since the amount of hydrophobic contract between hydrocarbon chains is maximized, and the amount of contact between alcohol hydrocarbon chain and polar regions of the lipid bilayer is minimized. The *sn*-2 chain C(1) carbon atom would appear a-priori to be a reasonable upper limit for the placement of the alcohol oxygen, while the *sn*-1 chain C(1) carbon atom would appear to provide a reasonable lower limit. These limits are consistent with conclusions drawn for the saturated long-chain alcohols on the basis of nuclear magnetic resonance experiments (28–30) and conformational analysis (31).

Fig. 5 schematically illustrates a diacylphosphatidylcholine molecule, together with very approximate zones of stabilization within which, according to the model presented above, penetration of the alcohol terminal methyl group would be expected to increase the pretransition temperature. The precise limits of these zones are expected to depend on the amount of chain tilt (and hence, indirectly, on the alcohol concentration). Penetration of the terminal methyl group on either side of this zone is expected to decrease the phase transition temperature or, in some cases, to promote formation of an interdigitated bilayer (27). If we assume the alcohol oxygen to lie about halfway between the limits described above (Fig. 5), both tetradecenols would be expected to increase the DMPC pretransition temperature, because their terminal methyl groups fall within the zone of stabilization for DMPC. *Trans*-tetradecenol, whose terminal methyl group falls within the zone of stabilization for DPPC, would be expected to increase the DPPC pretransition temperature while *cis*-tetradecenol, whose methyl group does not fall within this zone, would be expected to lower it. Since the terminal methyl groups of both tetradecenols fall outside the zone of stabilization for DSPC, both would be expected to lower the DSPC pretransition temperature. Incorporation of *cis*- or *trans*-hexadecenol into DPPC bilayers should cause an increase in the pretransition temperature, since both fall within the zone of stabilization for DPPC. This model thus correctly predicts the directions, and appears to predict the relative magnitudes, of the pretransition temperature shifts caused by these alcohols. Thus, the incorporation of unsaturated alcohols into phosphatidylcholine bilayers perturbs the pretransition by two somewhat independent mechanisms: the reduction of chain tilt by reduction of the headgroup/acyl chain mismatch (independent of the depth of penetration into the bilayer) and promotion or hindrance of acyl chain *trans-gauche* isomerization, depending on the depth to which the alcohol penetrates the bilayer.

The success of this qualitative model in predicting both the effects of acyl chain length asymmetry and incorporation of unsaturated alcohols on the lipid bilayer pretransition suggests that *trans-gauche* isomerization plays an important role in determining the thermodynamic characteristics of the pretransition just as it does in the gel-to-liquid crystalline phase transition (32). The importance of *trans-gauche* isomerization is implicit (though not formally incorporated) in the theoretical models formulated by McConnell et al. (33, 34), and is not recognized at all in other models of the pretransition (35–38).

In summary, we have determined the effects of *cis*- and *trans*-9,10-tetradecenols and hexadecenols on the phase transitions of several phosphatidylcholines using high sensitivity scanning calorimetry and Raman spectroscopy. These data demonstrate the need for caution in interpreting studies on lipid-anesthetic interactions in simple liposome systems. They show the exquisite sensitivity of the pretransition temperature to small changes in molecular dimensions, and in particular, show that variation in the depth of penetration of acyl chains into the lipid bilayer plans an important role in determining the temperature of the lipid pretransition. Finally, they demonstrate that reduction of acyl chain tilt resulting from incorporation of anesthetic alcohols substantially decreases the magnitude of the pretransition.

We would like to thank Dr. V. A. Parsegian and C.-H. Huang for several valuable discussions.

Received for publication 21 March 1986 and in final form 28 July 1986.

REFERENCES

1. Seeman, P. 1972. The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* 24:583–655.
2. Roth, S. H. 1979. Physical mechanisms of anesthesia. *Annu. Rev. Pharmacol. Toxicol.* 19:159–178.
3. Trudell, J. R. 1977. A unitary theory of anesthesia based on lateral phase separations in nerve membranes. *Anesthesiology.* 46:5–10.
4. O'Leary, T. J., P. D. Ross, and I. W. Levin. 1984. Effects of anesthetic and nonanesthetic steroids on dipalmitoylphosphatidylcholine liposomes: a calorimetric and Raman spectroscopic investigation. *Biochemistry.* 23:4636–4641.
5. Pringle, M. J., and K. W. Miller. 1978. Structural isomers of tetradecenol discriminate between the lipid fluidity and phase transition theories of anesthesia. *Biochem. Biophys. Res. Commun.* 85:1192–1198.
6. Miller, K. W. 1983. Anesthetized liposomes. *In* Liposome Letters. A. D. Bangham, editor. Academic Press, Inc., New York. 251–259.
7. Mountcastle, D. B., R. L. Biltonen, and M. J. Halsey. 1978. *Proc. Natl. Acad. Sci. USA.* 75:4906–4910.
8. Levin, I. W. 1984. Vibrational spectroscopy of membrane assemblies. *Adv. Infrared Raman Spectrosc.* 11:1–48.
9. O'Leary, T. J., and I. W. Levin. 1984. Melting behavior of anhydrous dipalmitoylphosphatidylcholine bilayers. *J. Phys. Chem.* 88:1790–1796.
10. Ross, P. D., and R. N. Goldberg. 1974. A scanning microcalorimeter for thermally induced transitions in solution. *Thermochim. Acta.* 10:143–151.
11. Kamaya, H., N. Matubayasi, and I. Ueda. 1984. Biphasic effect of long-chain n-alkanols on the main phase transition of phospholipid vesicle membranes. *J. Phys. Chem.* 88:797–800.
12. O'Leary, T. J. 1982. Effects of small nonpolar molecules on membrane compressibility and permeability. A theoretical study of the effect of anesthetic gases. *Biophys. Chem.* 15:299–310.
13. O'Leary, T. J. 1983. A simple theoretical model for the effects of cholesterol and polypeptides on lipid membranes. *Biochim. Biophys. Acta.* 731:47–53.
14. Berde, C. B., H. C. Anderson, and B. S. Hudson. 1980. A theory of the effects of head-group structure and chain unsaturation on the chain melting transition of phospholipid dispersions. *Biochemistry.* 19:4279–4293.
15. Schullery, S. E., T. A. Seder, D. A. Weinstein, and D. A. Bryant. 1981. Differential thermal analysis of dipalmitoylphosphatidylcholine-fatty acid mixtures. *Biochemistry.* 20:6818–6824.
16. Rand, R. P., D. Chapman, and K. Larsson. 1975. Tilted hydrocarbon chains of dipalmitoyl lecithin become perpendicular to the bilayer before melting. *Biophys. J.* 15:1117–1124.
17. Stamatoff, J., B. Feuer, H. J. Guggenheim, G. Tellez, and T. Yamane. 1982. Amplitude of rippling in the PB phase of dipalmitoylphosphatidylcholine bilayers. *Biophys. J.* 38:217–226.
18. McIntosh, T. J. 1980. Differences in hydrocarbon chain tilt between hydrated phosphatidylethanolamine and phosphatidylcholine bilayers: a molecular packing model. *Biophys. J.* 29:237–246.
19. Chowdhry, B. Z., G. Lipka, A. W. Dalziel and J. M. Sturtevant. 1984. Multicomponent phase transitions of diacylphosphatidylethanolamine dispersions. *Biophys. J.* 45:901–904.
20. McIntosh, T. J., S. A. Simon, and R. L. MacDonald. 1980. The organization of n-alkanes in lipid bilayers. *Biochim. Biophys. Acta.* 597:445–463.
21. Yellin, N., and I. W. Levin. 1977. Hydrocarbon chain disorder in lipid bilayers: temperature dependent Raman spectra of 1,2, diacylphosphatidylcholine-water gels. *Biochim. Biophys. Acta.* 468:490–494.
22. Levin, I. W., and S. F. Bush. 1981. Evidence for acyl chain *trans-gauche* isomerization during the thermal pretransition of dipalmitoylphosphatidylcholine bilayer dispersions. *Biochim. Biophys. Acta.* 640:760–766.
23. Chen, S. C., and J. M. Sturtevant. 1981. Thermotropic behavior of bilayers formed from mixed-chain phosphatidylcholines. *Biochemistry.* 20:713–718.
24. Mason, J. T., C.-H. Huang, and R. L. Biltonen. 1981. Calorimetric investigations of saturated mixed-chain phosphatidylcholine bilayer dispersions. *Biochemistry.* 20:6086–6092.
25. Mason, J. T., and C.-H. Huang. 1981. Chain length dependent thermodynamics of saturated symmetric chain phosphatidylcholine bilayers. *Lipids.* 16:604–608.
26. Davis, P. J., and K. M. W. Keough. 1986. Chain arrangements in the gel state and the transition temperatures of phosphatidylcholines. *Biophys. J.* 48:915–198.
27. Rowe, E. S. 1985. Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines. *Biochim. Biophys. Acta.* 813:321–330.
28. Pope, J. M., L. W. Walker, and D. Dubro. 1984. On the ordering of n-alkane and n-alcanol solutes in phospholipid bilayer model membrane systems. *Chem. Physics. Lipids.* 34:259–277.
29. Thewalt, J. L., S. R. Wassall, H. Gorrissen, and R. S. Cushley. 1985. Deuterium NMR study of the effect of n-alkanol anesthetics on a model membrane system. *Biochim. Biophys. Acta.* 817:355–365.
30. Thewalt, J. L., A. P. Tulloch, and R. S. Cushley. 1986. A deuterium NMR study of labeled n-alkanol anesthetics in a model membrane. *Chem. Physics Lipids.* 39:93–107.
31. Brasseur, R., P. Chatelain, E. Goormaghtigh, and J. M. Ruysschaert. 1985. A semiempirical conformational analysis of the interaction of n-alkanols with dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta.* 814:227–236.
32. Nagle, J. F. 1980. Theory of the main lipid bilayer phase transition. *Annu. Rev. Phys. Chem.* 31:157–195.
33. Marder, H., H. L. Frisch, J. S. Langer, and H. M. McConnell. 1984. Theory of the intermediate rippled phase of phospholipid bilayers. *Proc. Natl. Acad. Sci. USA.* 81:6559–6561.
34. Falkovitz, M. S., M. Seul, H. L. Frisch, and H. M. McConnell. 1982. Theory of periodic structures in lipid bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 79:3918–3921.
35. Doniach, S. 1979. A thermodynamic model for the monoclinic (ripple) phase of hydrated phospholipid bilayers. *J. Chem. Phys.* 70:4587–4596.
36. Pearce, P. A., and H. L. Scott. 1983. Statistical mechanics of the ripple phase in lipid bilayers. *J. Chem. Phys.* 77:951–958.
37. Chan, W. K., and W. W. Webb. 1981. Possible martensitic transformation in hydrated phospholipid liquid crystals. *Phys. Rev. Letters* 46:39–42.
38. Cevc, G., B. Zeks, and R. Podgornik. 1981. The undulations of hydrated phospholipid multilayers may be due to water-mediated bilayer-bilayer interactions. *Chem. Phys. Letters.* 84:209–212.