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EFFECTS OF PLASMENYLETHANOLAMINE ON THE DYNAMIC PROPERTIES OF THE HYDROCARBON REGION OF MIXED PHOSPHATIDYLCHOLINE-PHOSPHATIDYLETHANOLAMINE AQUEOUS DISPERSIONS

A SPIN LABEL STUDY

PAUL DEMEDIUK [a], DAVID L. COWAN [b] and EZIO A. MOSCATELLI [a,*]

[a] *Dalton Research Center and Departments of* [a] *Biochemistry and* [b] *Physics, University of Missouri-Columbia, M121 Health Sciences Center, Columbia, MO 65212 (U.S.A.)*

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Spin-labeled aqueous dispersions of total phospholipid extracts from whole brains of hibernating hamsters and rats chronically consuming ethanol were compared with dispersions from control animals. Order parameter values and approximate rotational correlation times for the nitroxide spin labels indicated that ethanol consumption results in an adaptive decrease in bilayer membrane fluidity, while hibernation produces increases in fluidity. Since it has been proposed that changes in plasmenylethanolamine such as those seen with hibernation play a role in the homeoviscous adaptation of brain membranes, electron spin resonance studies using aqueous phospholipid dispersions containing equimolar mixtures of rat brain phosphatidylethanolamine and phosphatidylcholine, or synthetic dioleylphosphatidylcholine and dioleylphosphatidylethanolamine, and brain plasmenylethanolamine were performed. The molar amount of plasmenylethanolamine was varied within the ethanolamineglycerophospholipid fraction of each dispersion. Order parameter values of spin labels in liposomes containing brain phosphatidylcholine and phosphatidylethanolamine increased in parallel with increases in plasmenylethanolamine concentrations, indicating that fluidity was decreasing. Liposomes composed of synthetic dioleyl phospholipids exhibited biphasic changes in order parameter (S) values as plasmenylethanolamine replaced the diacyl form. Below 30% (mol%) plasmenylethanolamine, S values decreased, while above 30%, S values were seen to increase; indicating an initial fluidization, followed by a decrease in fluidity.

Introduction

According to current dogma, 'normal' biological membranes are considered to be in a fluid state, and much attention is being directed at the dynamic aspects related to this concept [1,2]. Fluidity has been implicated as a physiologically important parameter in various membrane-related functions such as activity changes in membrane-bound enzymes [3,4], and transport of low molecular weight substances [5]. Conditions which perturb the optimal fluidity of biomembranes are therefore often functionally disruptive unless compensated for in some manner. Temperature

* To whom correspondence should be addressed.

Abbreviations: DOPC, dioleylphosphatidylcholine; DOPE, dioleylphosphatidylethanolamine; PlasE, plasmenylethanolamine; xNS, *N*-oxyl-4′,4′-dimethyloxazolidine derivative of x-ketostearic acid; CGP, cholineglycerophospholipid(s); EGP, ethanolamineglycerophospholipid(s).

fluctuations, high pressure [6], and exposure to ethanol [7] represent some examples of potential perturbants of membrane dynamics. A widely observed response to such influences is an alteration in the membrane lipid composition of both prokaryotic and eukaryotic organisms, termed homeoviscous adaptation [8]. Changes in esterified fatty acid unsaturation [9,10], ganglioside composition [11], and phospholipid class composition [12,14] have all been documented.

In our laboratory, studies on the central nervous system of hibernating hamsters and of rats chronically consuming ethanol have also demonstrated membrane lipid alterations [15,16]. One intriguing change which was observed was a significant difference in the levels of plasmenylethanolamines in whole brain, myelin and microsomes of both hibernators and ethanol-consuming rats. In hibernating hamsters (whose body temperatures fall to approx. 4°C during hibernation) a decrease in plasmenylethanolamine levels was found, while in alcoholic rats, an increase was seen. It is proposed that these changes represent one factor in the homeoviscous adaptation of CNS membranes in response to influences which rigidify (low temperature) and fluidize (ethanol) said membranes. The following experiments were undertaken to assess the validity of this hypothesis. The effect of changing levels of plasmenylethanolamine on the fluidity of phospholipid dispersions was examined using electron spin resonance spectroscopy.

Materials and Methods

Dioleylphosphatidylcholine (DOPC) and dioleylphosphatidylethanolamine (DOPE) were purchased from Sigma (St. Louis, MO). Bovine plasmenylethanolamine (PlasE) was purchased from Applied Science (State College, PA). The *N*-oxyl-4′,4′-dimethyloxazolidine derivatives of 5-, 12- and 6-ketostearic acid (5NS, 12NS, 16NS) were obtained through Syva (Palo Alto. CA). PlasE was determined to be approximately 98% pure by two-dimensional reactional TLC [15]. The acyl chain composition of the dioleyl phospholipids was greater than 98% oleic acid.

Whole brains from control and hibernating golden hamsters, *Mesocricetus auratus*, [15] and control and ethanol-consuming rats [16] were homogenized in water using a Potter-Elvejhem apparatus. Total phospholipids were extracted from the homogenates by the addition of 5 vol. chloroform/methanol (2 : 1) containing 0.01% butylated hydroxytoluene as an antioxidant. The lower phospholipid-containing phase of each sample was removed and the solvent was evaporated under a stream of N_2. The lipids were redissolved in chloroform and the solvent mixture was passed through a silicic acid column to separate the neutral and polar lipids. Adsorbed phospholipids were eluted with methanol and subsequently either used as they were for spin labeling experiments or further fractionated to isolate choline- and ethanolamineglycerophospholipids (CGP, EGP). Fractionation was performed by preparative TLC as follows: Magnesium acetate-silica gel H (Supelco, Bellefonte, PA)-coated plates (0.5 mm, 20 × 20 cm) were spotted using a TLC Streaker (Applied Science, State College, PA) and developed with chloroform/methanol/28% ammonia (130 : 50 : 10, v/v) [17]. Bands containing EGP and CGP, which comigrate, were visualized by spraying with water, scraped and extracted with chloroform/methanol (2 : 1) [15]. The two lipids were then separated on silica gel G (Supelco) plates (0.5 mm, 20 × 20 cm) using chloroform/methanol/acetone/acetic acid/water (100 : 20 : 40 : 20 : 10, v/v) as the developing solvent. After scraping and extraction, the purified CGP was stored under N_2 at −20°C while the EGP was subjected to mild acid hydrolysis which cleaves the vinyl ether linkage at the 1-position of PlasE, yielding a lyso phospholipid which can be separated from the remaining diacyl phospholipid by TLC [18]. In this procedure, the EGP fraction was evaporated to dryness under a stream of N_2. To the residue was added 1.5 ml chloroform, followed by 15.0 ml 0.1 M HCl in 95% methanol (0.3 ml concentrated HCl diluted to 30 ml with methanol). The tube was flushed with N_2, sealed, and incubated at 20°C for 40 min, after which 2.5 ml chloroform, 0.5 ml methanol and 1.5 ml distilled water were added. The tube was shaken and centrifuged, and the lower phase was removed and evaporated to 0.5 ml under N_2. The concentrated reaction extract was streaked onto a 20 × 20 cm silica gel G plate (0.5 mm) and developed with chloroform/ methanol/ water (140 : 60 : 8, v/v). The diacylPE and lysoPE bands

were visualized, scraped, and extracted as above.

All of the purified lipids used in the experiments were transmethylated according to the method of Brockerhoff [19] to produce fatty acid methyl esters and dimethylacetals for gas-liquid chromatographic analysis. The hexane extracts from the transmethylation were applied to silica gel G plates (0.25 mm) and developed with benzene [20]. Bands were visualized under ultraviolet light after spraying with 0.05% (w/v) Rhodamine G in 95% ethanol. The fatty acid methyl ester or dimethylacetal bands were then scraped into Pasteur pipets and eluted with 1.5 ml chloroform. The eluates were reduced under N_2 to 0.5 ml each and analyzed on a Hawlett Packard 7620A gas-liquid chromatograph equipped with a flame ionization detector and a 3385A microprocessor-based integrator. Fatty acid methyl esters and dimethylacetals were chromatographed at 170°C and 135°C, respectively, on a 3% EGSS-X column (Supelco) with an He flow rate of 50 ml/min.

The *N*-oxyl-4′,4′-dimethyloxazolidine derivatives of 5-, 12- and 16-ketostearic acid (5NS, 12NS, 16NS) were each dissolved in ethanol at a concentration of 1 mg/ml, and stored at −20°C under N_2. The synthetic dioleylphospholipids to be used in the experiments were dissolved together in chloroform/methanol (2 : 1, v/v) to give a molar ratio of 1 : 1 and a total concentration of 1 mg/ml. The phosphatidylcholine and phosphatidylethanolamine isolated from rat brains were also combined together at a molar phosphorus ratio of 1 : 1 with the concentration being determined by phosphorus analysis. The solution was adjusted to give a final concentration of 1 mg/ml. These two solutions were used as controls in the spin labeling protocols. In order to determine the effects of plasmalogen, the same types of solution were prepared, including varying proportions of bovine PlasE. The molar ratio of CGP to EGP was kept constant at 1 : 1 throughout, while the proportion of PlasE varied only in the EGP fraction.

Spin labeling was achieved by adding aliquots of spin label in ethanol solution to control and experimental phospholipid solutions such that the spin label to phospholipid molecular ratio was in the range of 1 : 100. After addition of the spin label, the lipid mixtures were coated on the inside of screw-cap test tubes by removing the solvent under a stream of N_2. To each sample, 0.5 ml of 0.01 M sodium phosphate buffer (pH 7.4) was added. The test tubes were then purged of air with N_2 and capped. Homogeneous suspensions were formed by alternately heating to 50°C and vigorously vortexing until all the visible lipid was removed from the glass and the suspensions were of a uniform pearly white translucence. All of the solutions used in the spin labeling were deoxygenated prior to use by bubbling N_2 gas through them for 5 min.

Spectral measurements were performed at 28°C in an aqueous flat cell (Varian, Palo Alto, CA) using a Varian V-4500 electron paramagnetic resonance spectrometer. Spectra were taken with a field sweep of 100 G, a modulation amplitude of 0.5 G, time constant of 0.3 s, and a sweep time of 10 min.

Apparent order parameters, S, were calculated from the spectra using conventional methods including corrections for polarity differences in the environment of the spin probes [21]. The splitting between the outer hyperfine peaks ($2T_{\|}$) and the splitting between the inner hyperfine peak ($2T_{\perp}$) were measured directly from the spectra for use in the calculations. With these values and literature values for the hyperfine splitting elements (T_{xx}, T_{zz}) of the static interaction tensor (T) parallel to the static Hamiltonian ($\mathcal{H}$) principal nuclear hyperfine x and z axes, S can be determined using Eqn. 1 below:

$$S = \frac{(T_{\|} - T_{\perp})[1/3\,(T_{zz} + 2T_{xx})]}{(T_{zz} - T_{xx})[1/3\,(T_{\|} + 2T_{\perp})]} \tag{1}$$

In the case of 16NS, the outer hyperfine splittings were not measureable, so an approximate expression for S using only the inner hyperfine splitting and no polarity correction was utilized [21]. This approximation, $S(T_{\perp})$, is calculated with Eqn. 2:

$$S(T_{\perp}) = \frac{3[(T_{zz} + T_{xx}) - 2T_{\perp}]}{2(T_{zz} + T_{xx})} - \frac{1}{2} \tag{2}$$

As a cross-check on the order parameter calculations, an approximation of the rotational correlation time, τ_c, for spin labels undergoing rapid, isotropic motion was also calculated from the

spectra [23] using Eqn. 3:

$$\tau_c = 6.5 \cdot 10^{-10}(W_0)\left[(h_0/h_{-1})^{1/2} - 1\right] \quad (3)$$

Here, W_0 is the width of the midfield line in gauss and h_0 and h_{-1} are the heights of the mid- and high-field lines, respectively. Although Eqn. 3 is derived for isotropic motion, τ_c is a useful empirical parameter for characterization of changes in spin label motion [21]. Since the τ_c formulation has been routinely used in the literature for 12NS, τ_c will be calculated only for this label. The single-crystal values for T_{zz} and T_{xx} used in Eqns. 1 and 2 are taken from Ref. 22. For 12NS, T_{zz} = 29.9 G, T_{xx} = 5.6 G, for 5NS T_{zz} = 32.4 G, T_{xx} = 6.1 G. For 16 NS, approximate values were extrapolated from data found in Ref. 23, with values of 28.6 and 5.4 being used.

Results

Differences in the order parameters of spin-labeled dispersions of total brain phospholipid extracts from normal rats, ethanol-consuming rats, and warm-adapted and hibernating hamsters were observed (Table I). The spin probes 5NS, 12NS and 16NS suspended in liposomes from 'alcoholic' rat brains each exhibited a statistically significant increase in S values when compared with controls. These same spin labels in aqueous phospholipid dispersions from hibernating hamsters were characterized by decreases in S vs. controls. The percent change in S for each label was very similar, being approx. 16% for hamsters and approx. 10% for rats.

TABLE II

EFFECT OF PlasE ON ORDER PARAMETER VALUES OF SPIN-LABELED RAT BRAIN PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE

'Percent PlasE in sample' is the percentage of PlasE in the EGP portion of a 1:1 mixture of EGP and CGP. Values (S) are presented ± S.E. (n = 4)

Percent PlasE in sample	5NS	12NS	16NS
0	0.57 ± 0.007	0.45 ± 0.004	0.19 ± 0.008
30	0.59 ± 0.005	0.46 ± 0.007	0.19 ± 0.006
40	0.61 ± 0.005	0.46 ± 0.006	0.19 ± 0.006
50	0.61 ± 0.007	0.47 ± 0.006	0.20 ± 0.004
60	0.63 ± 0.008	0.48 ± 0.008	0.20 ± 0.008
70	0.63 ± 0.004	0.48 ± 0.007	0.20 ± 0.005
100	0.65 ± 0.006	0.50 ± 0.004	0.21 ± 0.005

Aqueous phospholipid dispersions containing controlled ratios of naturally occurring choline- and ethanolamineglycerophospholipids with varying amounts of plasmenylethanolamine also demonstrated changes in order parameter values (Table II). While S values for all three spin probes showed constant increases when PlasE was introduced into the dispersions, the most striking differences were seen with the 5NS spin label. The S value for 5NS increased a total of 14%, with the 12NS and 16NS S values increasing 11% and 10%, respectively.

The more chemically defined mixture of synthetic dioleylphospholipids exhibited biphasic changes in S values as PlasE replaced the diacyl

TABLE I

ORDER PARAMETER VALUES OF SPIN-LABELED TOTAL PHOSPHOLIPID EXTRACTS

Values (S) are quoted ± S.E. (n = 4).

Brain sample (total phospholipids)	5NS	12NS	16NS
Control rat	0.51 ± 0.007	0.39 ± 0.007	0.02 ± 0.005
Alcohol-consuming rat	0.56 ± 0.009 [a]	0.43 ± 0.008 [a]	0.18 ± 0.005 [a]
Control hamster	0.48 ± 0.005	0.37 ± 0.008	0.18 ± 0.007
Hibernating hamster	0.42 ± 0.006 [a]	0.31 ± 0.005 [a]	0.15 ± 0.008 [a]

[a] $P < 0.05$ as determined by Student's t-test.

TABLE III

EFFECT OF PlasE ON ORDER PARAMETER VALUES OF SPIN LABELED MIXTURES OF DOPC AND DOPE

See Table II.

Percent PlasE in sample	5NS	12NS	16NS
0	0.66±0.006	0.50±0.005	0.22±0.005
20	0.64±0.005	0.47±0.007	0.21±0.005
30	0.60±0.004	0.44±0.004	0.20±0.006
40	0.62±0.007	0.45±0.005	0.19±0.008
50	0.63±0.005	0.46±0.008	0.19±0.004
60	0.64±0.007	0.46±0.006	0.20±0.007
70	0.64±0.004	0.47±0.004	0.20±0.005
80	0.65±0.008	0.47±0.003	0.21±0.004
100	0.67±0.005	0.48±0.007	0.21±0.008

form of the ethanolamine phospholipid (Table III). At 20% and 30% PlasE there was a decrease in S for 5NS and 12NS, while for 16NS the order parameter value decreased up to 40% PlasE. This was then followed by increases in S as the PlasE concentration was further increased.

The approximate rotational correlation times for dispersions labeled with 12NS corroborate the order parameter calculations (Table IV). τ_c decreases in liposomes from hibernators, and remains constant in cold-acclimated samples. A con-

TABLE IV

APPROXIMATE ROTATIONAL CORRELATION TIMES FOR 12NS

$n = 4$ for all determinations. Natural phospholipids were isolated from rat brain (see Materials and Methods). x% PlasE represents percentage of PlasE in the EGP portion of a 1:1 mixture of EGP and CGP. Synthetic phospholipids are DOPC and DOPE (see Materials and Methods).

Sample	τ_c (s) ($\times 10^{10}$)
Warm-acclimated extracts	44.31±0.03
Hibernator extracts	41.26±0.03
Control rat extracts	46.22±0.02
Alcohol-consuming rat extracts	48.91±0.03
Natural phospholipids	
0% PlasE	53.62±0.01
30% PlasE	55.22±0.03
40% PlasE	56.04±0.02
50% PlasE	56.72±0.04
60% PlasE	57.52±0.05
70% PlasE	58.31±0.01
100% PlasE	59.81±0.02
Synthetic phospholipids	
0% PlasE	62.13±0.03
20% PlasE	61.51±0.01
30% PlasE	61.03±0.01
40% PlasE	61.18±0.02
50% PlasE	61.45±0.04
60% PlasE	61.82±0.03
70% PlasE	62.02±0.02
80% PlasE	62.32±0.02
100% PlasE	62.71±0.01

TABLE V

FATTY ACID AND ALKENYL COMPOSITION OF SPIN-LABELED DISPERSIONS

$n = 4$ for all determinations. tr., trace; n.d. not determined.

Fatty acid	PC (rat brain)	PE (rat brain)	PlasE (2-position)	Fatty aldehyde	PlasE (1-position)
14:0	1.1±0.05	tr.	n.d.	16:0	23.6±0.1
16:0	37.9±0.1	9.1±0.05	1.8±0.08	17:0	1.2±0.06
16:1	1.0±0.05	1.5±0.06	n.d.	18:0	25.6±0.1
18:0	12.1±0.06	30.3±0.1	2.3±0.05	18:1	40.3±0.1
18:1	29.7±0.1	33.4±0.1	20.1±0.1		
18:2	1.1±0.05	1.6±0.05	n.d.		
20:0	tr.	tr.	n.d.		
20:1	1.5±0.06	3.3±0.05	9.5±0.05		
20:4	4.6±0.05	11.1±0.06	18.6±0.1		
22:5	3.3±0.06	3.5±0.05	16.3±0.06		
22:6	2.1±0.05	9.8±0.08	19.8±0.01		

stant increase is seen in τ_c in the 'natural' dispersions, while a biphasic response occurs in the synthetic dispersions.

The acyl and alkyl side-chain compositions of the natural phospholipids used here are given in Table V and are in agreement with typical compositions seen in the literature [23,24].

Discussion

The ESR spectra obtained were consistent with the interpretation that the spin probes were oriented in homogeneous environments such that rapid anisotropic motion about the long axis of each label occurred. This was true for all samples studied and strongly suggests that the labels sampled bulk motional properties of liquid-crystalline lipid. In this case, the order parameter, S, for each label reflects the membrane fluidity; or more accurately, the flexibility of the incorporated probes. An S value of zero indicates a freely moving probe, while an S value of 1 indicates complete immobilization about the long axis. Therefore, increases in S represent decreases in fluidity, and vice versa. The approximate rotational correlation times, τ_c, for 12NS are also useful for characterizations of changes in spin-label motion, with increases in τ_c being indicative of more restricted spin-label motion.

Observations of brain plasmenylethanolamine changes in hibernation [15] and chronic ethanol consumption [16] led to the hypothesis that this lipid class plays a role in the regulation of CNS membrane fluidity. As such, some indication that fluidity differences did occur in our experimental animals was deemed necessary. The results using spin-labeled total brain phospholipid liposomes from hamsters and rats demonstrated significant differences in the segmental motion of three distinct spin probes, and implied that membranes from ethanol-consuming rats are less fluid than controls, while membranes from hibernating hamsters are more fluid than controls. These results are consistent with the fluorescence polarization experiments of Goldman and Albers [25] on hamster brain microsomes, and the ESR experiments of Chin and Goldstein [26] on brain membranes of mice that had been chronically treated with ethanol. It is interesting to note that in these preparations the percentage change in S is approximately the same for all three labels. Since the nitroxide radical of each of the spin probes used resides at a different depth within the interior of the bilayer, this indicates that the observed fluidity changes are not regionalized in terms of depth, at least in bulk lipid.

The experiments in which the plasmalogen levels of aqueous dispersions of defined phosphatidylcholine/phosphatidylethanolamine content were varied, showed that changes in the plasmalogen levels of these model membrane systems produced changes in order parameter values and approximate rotational correlation times. Both the S values and τ_c values of spin-labeled liposomes containing natural phosphatidylcholine and phosphatidylethanolamine monotonically increased as diacylphosphatidylethanolamine was replaced by plasmenylethanolamine, indicating that the particular plasmenylethanolamine preparation used here caused a decrease in bilayer fluidity. In contrast to the results from total brain phospholipids, there is a differential percentage increase in S among the three probes incorporated into these compositionally less complex liposomes. 5NS shows the greatest increase in S, followed by 12NS and then 16NS. One possible interpretation of this difference is that, in contrast to the situation in total brain phospholipids, there is some localization of the effects of plasmenylethanolamine on bilayer dynamics in the relatively simple EGP and CGP systems.

The experiments in which the plasmalogen levels of mixed DOPC/DOPE dispersions were varied also showed changes in S and τ_c values. However, unlike the constant changes seen with natural PC and PE, S and τ_c of these synthetic lipids displayed biphasic behavior, with both values initially decreasing, indicating a decrease in fluidity. The initial fluidization could be signalling the onset of a phase change which is completed when some critical mix of natural and synthetic phospholipid is reached. Once the phase changes has been completed, a further increase in PlasE would then serve to decrease fluidity. Boggs et al. [27] and Cullis and De Kruijff [28] have examined phase transitions in a variety of ethanolamine phospholipids, and have shown that these lipids can undergo a lamellar to hexagonal phase transition

at temperatures above the gel-to-lamellar phase transition. Boggs et al. [27] have also demonstrated that plasmenylethanolamine destabilizes the lamellar phase of phosphatidylethanolamine and that the transition to the hexagonal phase causes an increase in fatty acid spin label motion. These findings are consistent with the results seen here.

As with the dispersions of natural lipids, the *S* values of the synthetic lipid dispersions displayed differential percent changes among the spin probes. Upon fluidization, the order parameter of 12NS increased to a greater degree than that of 5NS, indicating that the increase in motion and disorder is greater deep in the interior of the bilayer than near the polar head groups. Differences of this type have been seen by Boggs et al. [27] when comparing lamellar and hexagonal phases of ethanolamine phospholipids. The increases in order parameter values seen at PlasE levels above 30% are characterized by a larger percentage increase for 5NS than for 12NS. This behavior is similar to that seen with the natural PC/PE dispersions, suggesting that if a phase change occurred, the effect of PlasE on the fluidity of the 'new' phase is the same as that which occurs with the natural lipids.

From the above evidence, it is not possible to determine which structural features of the plasmalogen molecule are responsible for the spin label mobility variations reported here. The ether linkage at the 1-position, the double bond between carbon atoms 1 and 2 of the fatty aldehyde, the existence or lack of *cis* double bonds further down the fatty aldehyde chain, and the nature of the acyl substituents at the glyceryl 2-position may all potentially have an effect on bilayer dynamics. The larger percentage increases in the order parameter of 5NS vs. 12NS suggests that either the double bond between C1 and C2 of the alk-1-enyl chain or the ether linkage of this chain to the 1-position of glycerophosphoethanolamine (or both) contributes very strongly to the observed decrease in fluidity. Studies performed in other laboratories are somewhat confusing in this context. Vaughan and Keough [29] and Boggs et al. [27], using differential scanning calorimetry and synthetic ether analogues of PE, have shown that the ether linkage causes an increase in the transition temperature of the gel-to-lamellar phase change. It was speculated that the substitution of an ether linkage for a carboxyl-containing ester linkage may allow for close packing of lipids as well as an increase in the strength of intermolecular hydrogen bonding between polar headgroups. Goldfine et al. [30] have studied the phase behavior of plasmenylethanolamine isolated from *Clostridium butyricum* in which both the alk-1-enyl and the acyl chains were greater than 90% *trans*-9-18 : 1 (elaidate). The dielaidoyl PlasE was found to have a gel to lamellar phase transition temperature several degrees lower than the corresponding dielaidoyl PE, as determined by differential scanning calorimetry. From this datum it was hypothesized that the *cis* C1-C2 double bond caused a perturbation in intermolecular interactions in PE bilayers. Unfortunately, all of the above studies have examined single-component dispersion with calorimetric techniques and are therefore difficult to compare directly with the isothermal, spectroscopic results in this report. The several experiments in which spin labels have been used are also inconclusive in this regard. Boggs et al. [27] utilized spin labels as indicators of phase transitions in single component PE bilayers, while Schwartz and Paltauf [31] examined diacyl and diether phosphatidylcholine/cholesterol mixtures.

Without fatty acyl compositional and positional data on PC and PE for direct comparison with plasmenylethanolamine, it is not possible to draw conclusions regarding the absolute effects (if any) of the hydrocarbon chains of PlasE. It has recently been shown that changes in the structures of hydrocarbon chains in the 1- and 2-positions of glycerophospholipids can have nonequivalent effects on the packing of lipids in bilayers [32–34].

The studies into the effect of added plasmalogen on phospholipid bilayer fluidity reported on here indicate that changes in plasmalogen levels can play a role in homeoviscous adaptation, with increases in plasmalogen causing decreased fluidity and decreases in plasmalogen being associated with increased fluidity. This is not to say that plasmalogen changes are primary regulators of fluidity. The fluidity differences seen between control and experimental brain lipid dispersions are, in fact, expected to be due to a sum total of various lipid compositional changes, of which changes in plasmenylethanolamine levels are one

example. Based on the spin label evidence obtained in the experiments, and on the acyl and alkyl side-chain compositions of the lipids used, no conclusion can be drawn as to which structural features of the plasmenylethanolamine molecule are responsible for the resulting spin-label mobility differences. Further investigations using a variety of membrane and synthetic diacyl phospholipids and plasmenylphospholipids over a range of temperatures from that seen with hibernation to physiological, may serve to clarify this question.

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