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Plasma membrane lipid composition modulates action of anesthetics

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The effect of LM fibroblast plasma membrane phospholipid composition on the selectivity of charged amphipathic anesthetics for exofacial or cytofacial leaflets was examined. Because preference of charged amphipaths for one of the plasma membrane bilayer leaflets may be conferred by net changes in the polar headgroup composition, LM fibroblasts were cultured in the presence of choline or N-demethylated analogs in order to change this polar headgroup composition. These altered bases were incorporated into plasma membrane phospholipids. A significant difference in 1,6-diphenyl-1,3,5-hexatriene (DPH) limiting anisotropy was observed between plasma membrane leaflets of phosphatidylcholine-, but not phosphatidylethanolamine-enriched cells. Phenobarbital, which preferentially decreased the limiting anisotropy of 1,6-diphenyl-1,3,5-hexatriene in the exofacial leaflet, had little or no preferential effect in phosphatidyl-*N*,*N*-dimethylethanolamine-enriched membranes. Prilocaine preferentially reduced the limiting anisotropy of 1,6-diphenyl-1,3,5-hexatriene in the exofacial leaflet in phosphatidyl-*N*-methylethanolamine-enriched membranes, exactly opposite to its effect in phosphatidylcholine-enriched membranes. In contrast, prilocaine had no selective effect in phosphatidylethanolamine-enriched membranes. In summary, the phospholipid polar headgroup composition can dramatically affect the selectivity of charged amphipathic anesthetics in altering the limiting anisotropy, a measure of restriction to motion of 1,6-diphenyl-1,3,5-hexatriene, in individual monolayers.

Introduction

Over a decade ago, Sheetz and Singer [1] first proposed that the asymmetry in net charge at the surface of the two leaflets of cell plasma membranes could establish an asymmetric transbilayer distribution of charged amphipaths (e.g., charged anesthetics) intercalating into the monolayers. Such a preferential insertion would thereby selectively fluidize that monolayer and elicit dramatic functional consequences as measured by alterations in cell shape [1] and activity of asymmetrically disposed membrane-bound enzymes [2–6]. Indeed, the two leaflets of red blood cell and LM fibroblast plasma membranes differ dramatically in fluidity (reviewed in Refs. 7 and 8). However, the role of membrane phospholipid composition on the selectivity of charged amphipaths has heretofore not been examined. Extrapolation of findings with amphipath specificity in red blood cells plasma membranes to other membranes may therefore be inappropriate. It is well known that

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Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; r_∞, limiting anisotropy; $(6R)^{-1}$, rotational relaxation time in ns; r, steady-state anisotropy; r_0, anisotropy in the absence of rotational motion; τ, fluorescence lifetime.

plasma membrane phospholipid composition varies dramatically between species, organs, subcellular fractions, etc. [9]. In fact, the composition of major phospholipids in a single cell type may vary widely across species, e.g., the content of phosphatidylcholine in mammalian erythrocyte whole membranes varies from undetected to 47.5%, sphingomyelin content varies 3-fold, while phosphatidylethanolamine and phosphatidylserine vary less than 2-fold (reviewed in Ref. 9). Within any cell type and species, nutritional, endocrine, sex, age, drug, and pathological condition are among variables which may also alter phospholipid headgroup composition [10–13]. Even within a single cell type, the cell surface plasma membrane may be subfractionated into basolateral, brush-border, or canalicular subfractions in liver [14], kidney [15,16] and intestinal mucosa [17]. Plasma membrane subfractions differ markedly in phospholipid composition and fluidity [14–17]. Herein, the effect of altering the LM plasma membrane phospholipid polar head group composition on the selectivity of the charged anesthetics for exofacial vs. cytofacial leaflets of the LM cell plasma membrane was examined. The limiting anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) in the whole plasma from cells fed choline or one of its N-demethylated analogs, which are incorporated into the membrane, was constant. However, both the ratio of cytofacial to exofacial limiting anisotropy and the selectivity of the charged anesthetics for individual leaflets of the plasma membrane were highly dependent on the phospholipid polar head group composition, breaking down completely in plasma membranes from cells cultured with ethanolamine.

Materials and Methods

Chemicals

Prilocaine was the generous gift of Astra Pharmaceuticals (Södertälje, Sweden and Worcester, MA, U.S.A.). Phenobarbital and choline were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *N,N*-Dimethylethanolamine, *N*-methylethanolamine and ethanolamine were purchased from Eastman Organics, Inc. (Rochester, NY, U.S.A.). Bovine serum albumin was acquired from Miles Laboratories (Elkhart, IN, U.S.A.). 1,6-Diphenyl-1,3,5-hexatriene was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Other reagents were of highest commercially available quality.

Cell culture

LM cells, a transformed mouse fibroblast line obtained from the American Type Culture Collection (CCL 1.2), were cultured in suspension in a lipid-, protein-, and serum-free medium as described [10]. Choline or its demethylated analogs, ethanolamine, *N*-methylethanolamine and *N,N*-dimethylethanolamine, were added to the medium at 40 μg/ml [10]. Cells were routinely cultured in choline-containing media. For culture with choline analogs, log phase cells were washed twice with medium containing the desired analog before resuspending at $(0.8–1.0) \cdot 10^6$ cells/ml. Cells cultured with choline were treated in parallel. The cells were harvested three days later. Supplementation of LM cells with choline analogs under these conditions did not alter cell growth. The cultures were routinely tested for mycoplasmal, bacterial and fungal contamination.

Isolation of plasma membranes and cell trinitrophenylation

To determine fluorescence parameters of probe molecules in each membrane leaflet, techniques previously established in our laboratory [18,19] and reviewed extensively elsewhere [7,8], were used. Briefly, membranes were either prepared without covalently linked fluorescence quenching agent or with quencher linked to the outer leaflet of the plasma membranes as follows: One-half of each culture was treated with trinitrobenzenesulfonic acid under nonpenetrating conditions (4°C, 4 mM) as described earlier [20,21]. Excess reagent was removed and plasma membranes were isolated by a modification [22] of a published procedure [10]. Microsomes and mitochondria were also routinely isolated in order to determine if any of the trinitrobenzenesulfonic acid penetrated the cell according to previously published procedures [20,21]. Analysis of microsomal and mitochondrial phospholipids revealed that less than 2%of the phosphatidylethanolamine in these intracellular organelle membranes was trinitrophenylated. When the trinitrobenzenesulfonic

acid treatment procedure was performed under penetrating conditions (37°C), 60–80% of phosphatidylethanolamine in microsomes and mitochondria was trinitrophenylated. If isolated microsomes or mitochondria were treated with trinitrobenzenesulfonic acid at 4°C, 53 and 63% of the phosphatidylethanolamine was trinitrophenylated, respectively. Treatment of isolated microsomes and mitochondria at 37°C, resulted in 66 and 79%, respectively, of phosphatidylethanolamine trinitrophenylated. Thus, if trinitrobenzenesulfonic acid had penetrated the intact cell during the treatment procedure, then the intracellular membrane phosphatidylethanolamine would have been extensively trinitrophenylated. As noted above, this was not the case. Transbilayer flip-flop of trinitrophenylated phosphatidylethanolamine was insignificant under the conditions used for fluorescence measurement herein, no additional plasma membrane phosphatidylethanolamine became available for trinitrophenylation. Membrane protein concentration was determined by the procedure of Lowry et al. [23].

Fluorescence spectroscopy

All fluorescence measurements were made at 37°C. Steady-state fluorescence polarization, P, and fluorescence lifetime, τ, were obtained with a T format SLM4800 subnanosecond spectrofluorometer (SLM Instruments, Inc., Champaign-Urbana, IL, U.S.A.) at 30, 18, and 6 MHz essentially as described [18]. The lifetime was simultaneously measured relative to a reference solution of dimethyl-*p*-bis[2-(phenyloxazolyl)]benzene in absolute ethanol [24]. Lifetime data were subjected to heterogeneity analysis by the method of Weber [25] as described [11]. The DPH lifetime in LM plasma membranes was best described as a single component. The fluorescent probe DPH was incorporated at a concentration of 1 μg per 100 μg membrane protein as described [26]. The excitation wavelength for DPH in LM plasma membranes was 362 nm and fluorescence emission was monitored above 424 nm using cutoff filters.

Differential polarized phase fluorometry, as described by Weber [27] and extended by Lakowicz et al. [28], was used to obtain the rotational rate, R, in radians/s, and the limiting anisotropy, r_∞, of membrane-bound DPH. The limiting anisotropy reflects restriction to probe motion and can be converted to an order parameter, S, since $S = (r_\infty/r_o)^{1/2}$ where r_o, the anisotropy in the absence of motion [29], is equal to 0.390 for DPH [28]. This order parameter facilitates comparison to data obtained with electron spin resonance or nuclear magnetic resonance techniques. The rotational rate can be converted to a rotational relaxation time in ns according to the expression $(6R)^{-1}$.

Treatment with charged anesthetics

Anesthetics were prepared fresh daily in deionized water, pH 10. The drugs were added directly to membranes resuspended in phosphate buffered saline pH 7.2 in the fluorometer cuvette. The pH of the buffered sample was not changed significantly by addition of the anesthetic solution. Measurements commenced usually within 1 min after addition. No effect of longer incubation times was noted.

Results

Substitution of choline with choline analogs alters LM plasma membrane lipid composition

Substitution of variously demethylated congeners of choline for choline in the culture medium of LM fibroblasts results in their substitution for choline in the plasma membrane phospholipids: the percent content of choline/*N*,*N*-dimethylethanolamine/*N*-methylethanolamine/ethanolamine in plasma membrane phospholipids containing these compounds was 56.1 : 0 : 0 : 43.9, 15.8 : 52.7 : 0 : 31.5, 21.7 : 0 : 56.3 : 21.9 and 28.3 : 0 : 0 : 71.7 for choline, *N*,*N*-dimethylethanolamine, *N*-methylethanolamine and ethanolamine fed cells, respectively.

Effect of phospholipid composition on dynamic and static properties of DPH in LM plasma membranes

The limiting anisotropy and rotational relaxation time (ns) of DPH in LM plasma membranes and individual monolayers were determined from measurements of fluorescence polarization, lifetime, and differential polarized phase fluorescence as described in Materials and Methods. Neither the fluorescence polarization, 0.228 ± 0.002, nor the lifetime, 9.71 ± 0.13 ns (a single component) at 37°C was significantly altered by choline analog

substitution in the LM plasma membrane.

Steady-state fluorescence polarization has often been used as a measure of membrane fluidity. However, it is comprised of both static and dynamic terms: the limiting anisotropy, a static parameter dependent on membrane lipid order or restriction to the range of probe motion, and the rotational relaxation time, a dynamic term that reflects the rate of molecular rotation. In the whole LM plasma membrane, which represents an intensity weighted average of DPH properties in the exofacial and cytofacial leaflets, the values for these parameters are 0.137 ± 0.007 and 1.20 ± 0.01 ns, respectively. The range of motion of the DPH probe is considerably restricted in LM plasma membranes at 37°C, yet the probe moves rapidly. Choline analog substitution in the LM plasma membrane dramatically alters neither of these parameters in the whole membrane, an illustration of homeoviscous adaptation. In contrast, when the limiting anisotropy and rotational relaxation time of DPH are resolved in each leaflet of the membrane from choline supplemented cells (three methyl groups), in the inner monolayer the DPH moves less rapidly (longer rotational relaxation time of 1.28 ± 0.03 ns) and has a greater restriction to motion (limiting anisotropy, 0.155 ± 0.003) than in the outer monolayer, 1.12 ± 0.03 ns and 0.119 ± 0.003, respectively. Plasma membrane phospholipid methylation alters the static component, limiting anisotropy, without significant change in the dynamic component, rotational relaxation time, of DPH motion in the individual monolayers. This effect is best expressed in Fig. 1 where the ratio of inner/outer monolayer limiting anisotropy and of inner/outer monolayer order parameter are expressed as a function of phospholipid methylation. These ratios are significantly greater than 1.0 in plasma membranes from choline but not ethanolamine supplemented cells. In summary, the polarization, lifetime, limiting anisotropy and rotational relaxation time of DPH in the whole membrane are not altered with alterations in plasma membrane phospholipid polar headgroup. In contrast, the individual leaflets are affected dramatically. This demonstrates that, despite the maintenance of fluidity in the whole membrane ('homeoviscous adaptation'), the difference in fluidity between the leaflets ('vertical gradient') is reduced with decreasing methylation, to essentially zero in the ethanolamine-fed cells.

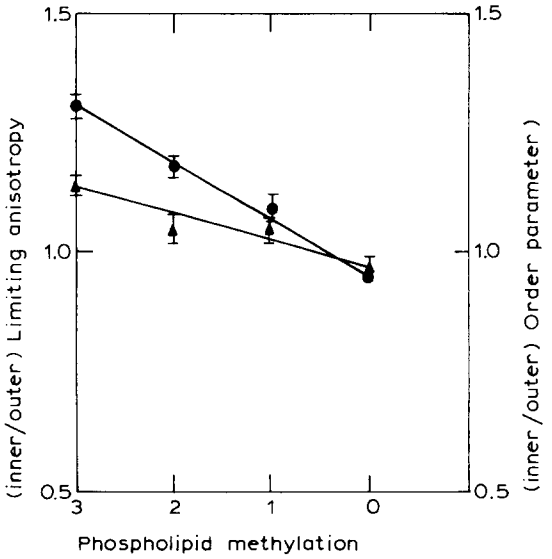

Fig. 1. Effect of phospholipid methylation on the ratio of inner monolayer to outer monolayer limiting anisotropy and order parameter. Limiting anisotropy (●, upper line) and order parameter (▲, lower line) were determined at 37°C as described in Methods. Values represent the mean ± S.E. ($n = 3$).

Substitution of choline with choline analogs alters selectivity of charged anesthetics

As shown in Fig. 2, phenobarbital significantly ($P < 0.05$) decreases the limiting anisotropy of DPH in the exofacial leaflet (lower curves) of LM

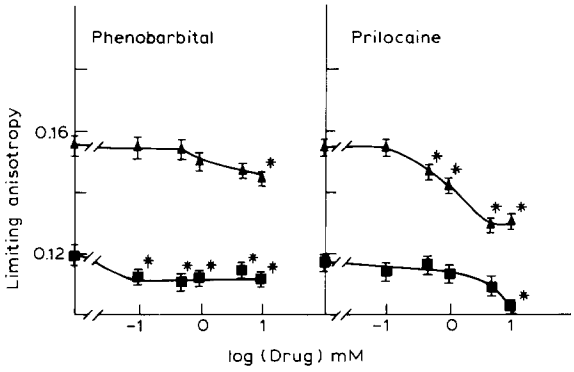

Fig. 2. Effect of phenobarbital and prilocaine on limiting anisotropy of DPH in choline-fed LM cell plasma membranes. Triangles (upper curve) and squares (lower curve) refer to inner (cytofacial) monolayer and outer (exofacial) leaflet, respectively. Values represent the mean ± S.E., $n = 3$. An asterisk signifies $P < 0.05$ (Student's t-test) as compared to no drug.

plasma membranes at a much lower dose than in the inner monolayer. At doses lower than 0.1 mM (data not shown) phenobarbital does not significantly decrease the limiting anisotropy. The opposite behavior is observed with prilocaine. At high concentration, both charged anesthetics lower the limiting anisotropy of DPH in both leaflets of the membrane. Thus, at certain concentrations phenobarbital and prilocaine can selectively fluidize the exofacial and cytofacial leaflets of the plasma membrane, respectively.

Because the net charges on the polar head groups may be the principal mechanism conferring preference of charged amphipaths for one of the leaflets of the plasma membrane, changes in the polar headgroup for the leaflets, e.g., replacement of choline with N-demethylated analogs, could change the preferential effects on limiting anisotropy. Phenobarbital and prilocaine do not interact directly with DPH to produce quenching artifacts (Fig. 3). Absorbance corrected fluorescence and fluorescence lifetime (data not shown) were unaltered. It is apparent that phenobarbital decreases the limiting anisotropy of DPH in plasma membranes from LM cells fed *N*,*N*-dimethylethanolamine (Fig. 4). However, phenobarbital, which preferentially decreases the limiting anisotropy of the exofacial leaflet of choline-fed cells (Fig. 2), has little or no preferential effect in *N*,*N*-dimethylethanolamine-fed cells (Fig. 4). Ethanolamine-fed LM cells have no significant gradient of limiting anisotropy between the leaflets as compared to that in plasma membranes from LM cells fed choline (Fig. 1). Phenobarbital exhibits a slight preferential decrease in the limiting anisotropy of the exofacial leaflet in the ethanolamine-fed cells (Fig. 4). Thus, phenobarbital effects preferential decreases in limiting anisotropy of DPH in exofacial leaflet of plasma membranes from choline-fed and perhaps ethanolamine-fed cells but not from *N*,*N*-dimethylethanolamine fed cells. Prilocaine preferentially reduces the limiting anisotropy of the exofacial leaflet in *N*-methylethanolamine-fed cells (Fig. 5), exactly opposite to its effect in choline-fed cells (Fig. 2). In ethanolamine-fed cells, prilocaine is equally effective in decreasing limiting anisotropy in both of the leaflets (Fig. 5). Thus, prilocaine appears cytofacially selective in plasma membranes from choline-fed cells, exofacially elective

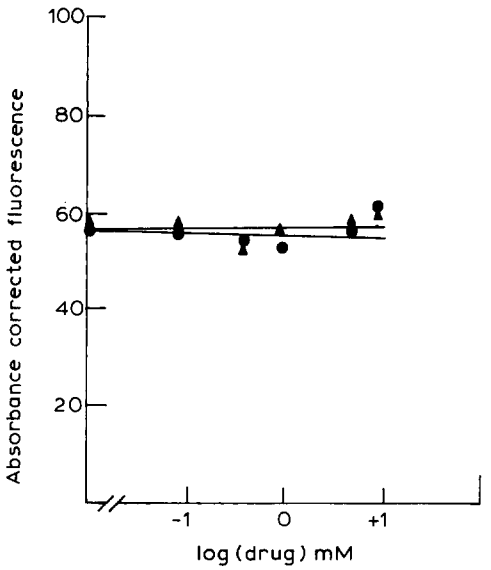

Fig. 3. Effect of phenobarbital and prilocaine on fluorescence intensity of 1,6-diphenyl-1,3,5-hexatriene in LM cell plasma membranes. All conditions were as described in the legend to Fig. 2, except that absorbance corrected fluorescence intensity was determined in the presence of increasing concentrations of phenobarbital (●) or prilocaine (▲).

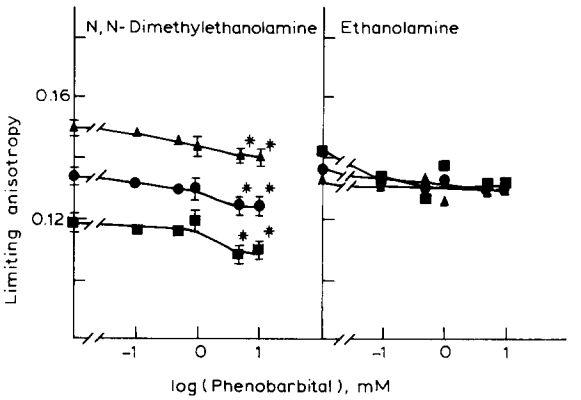

Fig. 4. Effect of phenobarbital on limiting anisotropy of DPH in LM cell plasma membranes. (Left panel) Effect of various phenobarbital concentrations on limiting anisotropy of the whole membrane (●), cytofacial leaflet (▲) and exofacial leaflet (■) in plasma membrane of LM cells fed three days with *N*,*N*-dimethylethanolamine replacing choline. (Right panel) Effect of various concentrations of phenobarbital on the limiting anisotropy in plasma membrane of LM cells fed three days with ethanolamine replacing choline. The error bars refer to the mean of three determinations. An asterisk refers to $P < 0.05$ as compared to no drug.

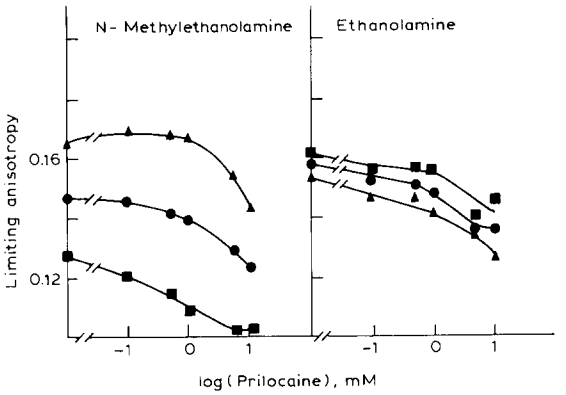

Fig. 5. Effect of prilocaine on limiting anisotropy of DPH in LM cell plasma membranes. Symbols are the same as in Fig. 4. (Left panel) Plasma membranes from cells cultured with *N*-methylethanolamine. (Right panel) Cells cultured with ethanolamine.

in plasma membranes from *N*-methylethanolamine-fed cells and non-selective in plasma membranes from ethanolamine-fed cells.

Effect of choline analogs and charged anesthetics on temperature dependencies of DPH in LM plasma membranes

The effect of anesthetics on the characteristic temperatures ('breakpoints') of Arrhenius plots of some probe molecules (e.g., fluorescence, NMR, ESR) or enzyme activities have been commonly employed as measures of changes in fluidity. Arrhenius plots of 1,6-diphenyl-1,3,5-hexatriene

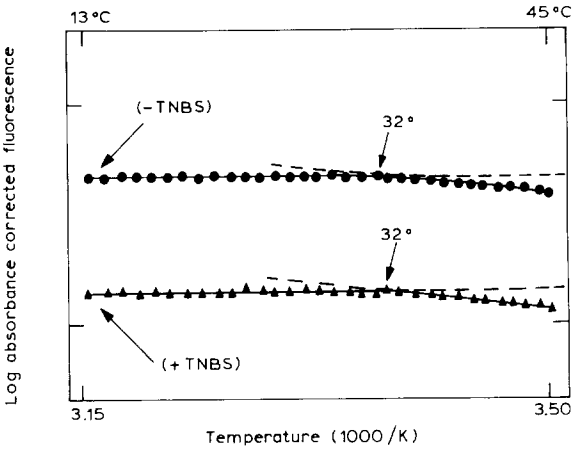

Fig. 6. Arrhenius plots of 1,6-diphenyl-1,3,5-hexatriene fluorescence in LM plasma membranes. LM fibroblasts were cultured and treated with (▲) and without (●) trinitrobenzenesulfonic acid (TNBS) under nonpenetrating conditions (4°C) as described in Materials and Methods. Plasma membranes were isolated, 1,6-diphenyl-1,3,5-hexatriene was incorporated, and Arrhenius plots of absorbance corrected fluorescence intensity were determined.

absorbance corrected fluorescence in plasma membranes from LM cells labelled without and with trinitrobenzenesulfonic acid under nonpenetrating conditions both showed breakpoints near 32°C (Fig. 6). The precision of this determination was ±1 Cdeg. Arrhenius plots of DPH absorbance corrected fluorescence in the absence or presence of exofacial trinitrophenyl groups and in the absence or presence of 10 mM

TABLE I

EFFECT OF ANESTHETICS ON CHARACTERISTIC TEMPERATURES IN ARRHENIUS PLOTS OF 1,6-DIPHENYL-1,3,5-HEXATRIENE IN LM FIBROBLAST PLASMA MEMBRANE

LM fibroblasts were cultured with and without choline or analogs and treated with trinitrobenzenesulfonic acid under nonpenetrating conditions (4°C) as described in Materials and Methods. Plasma membranes were isolated, 1,6-diphenyl-1,3,5-hexatriene was incorporated, exposed to drug (10 mM), and Arrhenius plots of absorbance corrected fluorescence intensity were determined.

Drug	Trinitrobenzenesulfonic acid	Characteristic temperature (°C)			
		Choline	*N*,*N*-dimethylethanolamine	*N*-monomethylethanolamine	ethanolamine
None	–	32	31	30	32
None	+	32	33	32	34
Phenobarbital	–	32	32	31	31
Phenobarbital	+	31	30	32	33
Prilocaine	–	None	None	None	None
Prilocaine	+	None	None	None	None

phenobarbital or prilocaine with plasma membranes from choline- or analog-fed cells are shown in Table I. Characteristic temperatures are found in these plots. With neither drug nor trinitrobenzenesulfonate treatment, a characteristic temperature is shown in the range 30–32°C in plasma membranes from cells fed choline or analogs. In the presence of exofacial trinitrophenyl quenching groups and absence of anesthetic, the same characteristic temperature is present unaltered. This result indicates that the breakpoint is associated with the cytofacial leaflet, or that both leaflets, irrespective of the nutritional supplementation, have a very similar characteristic temperature. Phenobarbital has no effect on the characteristic temperature, either in intact or trinitrophenylated membranes from cells with choline or choline analog supplementation. Prilocaine, however, abolishes the breakpoint resulting in linear Arrhenius plots within the temperature range examined (13–45°C) for intact or trinitrophenylated membranes from choline- and analog-fed cells. Thus, the characteristic temperature in Arrhenius plots appears to be located in the cytofacial leaflet of the LM plasma membranes. In addition, the presence of a breakpoint in the inner but not outer monolayer of the plasma membranes is also consistent with, without offering definitive proof, the outer monolayer being more fluid than the inner monolayer.

Discussion

The present investigation resolves the static and dynamic aspects of DPH motion in LM plasma membranes. The data reported for effect of choline analog supplementation on the limiting anisotropy of DPH at 37°C is consistent with earlier work (using another fluorescent problem, *trans*-parinaric acid, and steady-state anisotropy) showing the maintenance of whole LM plasma membrane fluidity at 24°C with changes in lipid polar headgroups [26]. The difference in steady-state polarization of *trans*-parinaric acid between the LM plasma membrane leaflets at 24°C indicated that the inner monolayer was more rigid for choline- and ethanolamine-fed cells. The inner monolayer was slightly, but not significantly, more rigid in *N,N*-dimethylethanolamine- and *N*-methylethanolamine-fed cells. In the studies using DPH at 37°C reported here, the factors comprising steady-state anisotropy (polarization) have been resolved and therefore lead to a clearer understanding of the changes in fluidity brought about by changes in polar head groups. In choline-, *N,N*-dimethylethanolamine- and *N*-methylethanolamine-fed cells, the limiting anisotropy indicated that the cytofacial leaflet was more rigid than the exofacial leaflet, although the gradient tended to decrease. In ethanolamine-fed cells, the difference between the limiting anisotropy values of the leaflets was no longer statistically significant. Since the average limiting anisotropy of the intact membrane (inner and outer monolayer) did not differ among the various nutritional regimens, homeoviscous isothermal adaptation into decreasing phospholipid N-methylation [26] may, in part, be due to dimunition and/or abolition of the vertical gradient in fluidity. As reviewed elsewhere [8], abolition of such a transbilayer fluidity gradient could have considerable functional significance.

It has been proposed that predominance of anionic phospholipid headgroups on the cytofacial leaflet will preferentially associated with and be fluidized by cationic amphipaths while anionic amphipaths will be excluded and thereby preferentially associate with and fluidize the zwitterionic neutral phospholipid headgroups on the exofacial leaflet [1]. This concept has been tested by shape changes in erythrocytes [1] and by observing changes in activity of asymmetrically disposed membrane-bound enzymes [2–6]. Even though the phase transition temperature may or may not correlate with viscosity (fluidity) of the liquid state, such characteristic temperatures in membranes have been taken as a fluidity index [2,3,5]. In contrast, the data presented here offer direct evidence in support of the above hypothesis. It is evident from an examination of the data that direct fluorescence quenching of DPH fluorescence by phenobarbital or prilocaine, a potential artifact that drastically affects both limiting anisotropy and rotational relaxation time measurements, did not occur. Such direct quenching by some anesthetics, e.g., tetracaine and procaine, has been reported with 12-(9-anthroyl)stearic acid in model membranes [30]. The results presented

herein are consistent with the interpretation that there is preferential fluidization (i.e., decreased fluorescence anisotropy) in the predicted direction for phenobarbital and prilocaine. This single leaflet preference of these drugs in the plasma membranes from choline-fed LM cells cannot be extrapolated to LM plasma membranes with altered phospholipid polar headgroup composition. Because the selectivity is postulated to be due to electrostatic interactions, this is perhaps to be expected, since the charge on the choline group as compared to the various demethylated derivatives is expected to differ substantially [31]. The selectivity of monolayer changes in fluidity for one drug in plasma membranes with different choline analog headgroups or for the four drugs in plasma membranes within a single headgroup followed no evident pattern. For example, phenobarbital preferentially fluidized the exofacial leaflet in choline- and *N*-methylethanolamine- and perhaps ethanolamine- but not in dimethylethanolamine-fed cells. Prilocaine, on the other hand, preferentially fluidized the cytofacial leaflet in choline- and *N*,*N*-dimethylethanolamine-fed cells, preferentially fluidized to exofacial leaflet in plasma membranes from *N*-methylethanolamine-fed cells, and showed no preference in ethanolamine-fed cells. Therefore, it may be concluded that the transbilayer distribution of charged amphipaths in plasma membranes cannot be predicted solely on the basis of the amphipath charge. Furthermore, extrapolation to other membranes is also filled with uncertainty.

As summarized in the introduction, the composition of major phospholipids in a single cell type may vary widely across species, e.g., the content of phosphatidylcholine in mammalian erythrocyte whole membranes varies from undetected to 47.5% [9]. Thus, it might be predicted that the selectivity of charged amphiphilic drugs or other compounds in cell plasma membranes will be highly variable and cannot be simply extrapolated from shape changes or other parameter alterations in a single membrane type from a single species. Data consistent with this possibility have also been reported in liver. In rat liver plasma membranes, there is a characteristic temperature at 28°C in Arrhenius plots of ESR order parameter [32] and 5′-nucleotidase activity [4]. The characteristic temperature at 28°C was assigned to the exofacial leaflet and the 5′-nucleotidase activity was responsive only to agents that perturbed this characteristic temperature. In contrast, Arrhenius plots of hamster liver plasma membrane 5′-nucleotidase activity and 4-anilinonaphthalene-1-sulfonic acid fluorescence displayed two characteristic temperatures [3]. One characteristic temperature was assigned to each leaflet. Thus, either the enzyme activity was affected by fluidity in both leaflets or perhaps the enzyme resided in different domains in one leaflet. In either case, specificity of charged amphipaths demonstrated by breakpoints of Arrhenius plots of 5′-nucleotidase activity would not be demonstrated in the same way in rat vs. hamster liver plasma membranes.

Acknowledgments

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References

1 Sheetz, M.P. and Singer, S.J. (1974) Proc. Natl. Acad. Sci. USA 71, 4457–4461
2 Houslay, M.D., Dipple, I. and Gordon, L.M. (1981) Biochem. J. 197, 675–681
3 Houslay, M.D. and Palmer, R.W. (1978) Biochem. J. 174, 909–919
4 Dipple, I., Gordon, L.M. and Houslay, M.D. (1982) J. Biol. Chem. 257, 1811–1815
5 Houslay, M.D., Dipple, I., Rawal, S., Sauerheber, R.D., Esgate, J.A. and Gordon, L.M. (1980) Biochem. J. 190, 131–137
6 Salesse, R., Garnier, J., Leterrier, F., Daveloose, D. and Viret, J. (1982) Biochemistry 21, 1581–1586
7 Schroeder, F. (1984) Progr. Lipid Res. 23, 97–113
8 Schroeder, F. (1985) in Subcellular Biochemistry, Vol. 11 (Roodyn, D.B., ed.), pp. 51–101, Plenum Publishing Co., New York
9 White, D.A. (1973) in Form and Function of Phospholipid (Ansell, B.G., Hawthorne, J.N. and Dawson, R.M.C., eds.), pp. 441–482, Elsevier, New York
10 Schroeder, F., Perlmutter, J.F., Glaser, M. and Vagelos, P.R. (1976) J. Biol. Chem. 251, 5015–5026
11 Schroeder, F., Goetz, I.W. and Roberts, E. (1984) Eur. J. Biochem. 142, 183–191
12 Schroeder, F., Fontaine, R.N., Feller, D.J. and Weston, K.G. (1981) Biochim. Biophys. Acta 643, 76–88

13 Schroeder, F., Goetz, I. and Roberts, E. (1984) J. Neurochem. 43, 526–539
14 Kremmer, T., Wisher, M.H. and Evans, W.H. (1976) Biochim. Biophys. Acta 455, 655–664
15 Hise, M.K., Mantulin, W.W. and Weinman, E.V. (1984) Am. J. Physiol. 247, F434–F439
16 Le Grimellec, C., Gioicondi, M.-C., Carriere, B., Carriere, S. and Cardinal, J. (1982) Am. J. Physiol. 242, F246–F253
17 Chapelle, S. and Gilles-Baillien, M. (1983) Biochim. Biophys. Acta 753, 269–271
18 Hale, J.E. and Schroeder, F. (1982) Eur. J. Biochem. 122, 649–661
19 Schroeder, F. (1980) Eur. J. Biochem. 112, 293–307
20 Fontaine, R.N. and Schroeder, F. (1979) Biochim. Biophys. Acta 558, 1–12
21 Schroeder, F. (1980) Biochim. Biophys. Acta 599, 254–270
22 Schroeder, F. and Gardiner, J.M. (1984) Cancer Res. 44, 3262–3269
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
24 Lakowicz, J.R., Cherek, H. and Balter, A. (1981) J. Biochem. Biophys. Methods 5, 131–146
25 Weber, G. (1981) J. Phys. Chem. 85, 949–953
26 Schroeder, F. (1978) Biochim. Biophys. Acta 511, 356–376
27 Weber, G. (1978) Acta Phys. Pol. A54, 859–865
28 Lakowicz, J.R., Prendergast, F.G. and Hogen, D. (1979) Biochemistry 18, 508–519
29 Heyn, M.P. (1979) FEBS Lett. 108, 359–364
30 Surewicz, W.R. and Leyko, W. (1982) J. Pharm. Pharmacol. 34, 359–363
31 Hayashi, M. (1979) Membrane (J. Membrane Soc. Jap.) 4, 121–131
32 Gordon, L.M., Sauerheber, R.D., Esgate, J.A.., Dipple, I., Marchmont, R.J. and Houslay, M.D. (1980) J. Biol. Chem. 255, 4519–4527