Biochimica et Biophysica Acta, 983 (1989) 205–211
Elsevier

BBAMEM 74491

Effects of non-electrolyte molecules with anesthetic activity on the physical properties of DMPC multilammelar liposomes

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(Received 21 February 1989)

Key words: Anesthesia; Ethanol; Membrane; Fluorescence; Temperature

The effects of 13 non-electrolytes with moderate anesthetic potency on the order of DMPC liposomes were examined. Changes in order were monitored by steady-state fluorescence polarization techniques using 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH). At 30°C, all of the compounds tested decreased the DPH steady-state anisotropy (r_s), with potencies highly correlated to their oil/water partition coefficients. However, only the most hydrophobic anesthetics decreased TMA-DPH r_s. Some of the most hydrophilic compounds, including ethanol and urethane, actually increased TMA-DPH r_s, suggestive of an increase in membrane order. The concept of selectivity was borrowed from partitioning theory and used to explain some effects on anesthetic potency of decreasing temperature to 18°C. In the gel as opposed to the liquid crystalline phase, selectivity for decreasing membrane order (as monitored by DPH) markedly increased, suggesting that anesthetic partitioning and/or the site of anesthetic action was occurring in a more hydrophobic domain. The solute-independent difference (or capacity) between two membranes for perturbation was defined as membrane sensitivity. Sensitivity appeared to also decrease with decreasing temperature, despite the decrease in membrane partitioning. This effect is thought to result from the selective delivery of the anesthetic solute to the membrane interior and away from more hydrophilic domains where anesthetics may order membrane structure.

Introduction

Our understanding of the mechanisms of general anesthetic action are largely derived from the following observations. (a) The potency of molecules of diverse structure to induce anesthesia is significantly correlated with the partitioning of such molecules into organic solvents, particularly amphophilic solvents (reviewed in Ref. 1). (b) Anesthetics perturb membrane lipid structure as detected by a variety of techniques, including ESR, NMR and FPZ [2–5]. Such perturbations are significantly correlated with octanol/water partition coefficients [6]. (c) Anesthesia can be reversed by high pressure [7,8]. (d) The activity of some proteins, in particular the luciferases, are inhibited by pharmacologically relevant concentrations of anesthetics in the absence of lipid [9–11]. For inhibition of firefly luciferase, anesthetic potency parallels the octanol/water partition coefficients [10]. Overall, these core observations have been interpreted to support either a lipid 'first' [1] or a protein 'first' site [9–11] of anesthetic action.

Work in our laboratory has focused on the hypothesis that anesthetics act by interacting with specific nerve membrane lipid domains. For example, it has been shown that gangliosides, but not other sphingolipids, sensitize PC multilammelar liposomes to the disordering effects of ethanol [12] and a wide variety of anesthetic molecules including halothane and methoxyflurane [13]. Such data may suggest that the ganglioside-PC-rich exofacial half of the nerve membrane leaflet is particularly sensitive to anesthetic effects. ^{1}H-NMR has been used to simultaneously examine the disordering effects of ethanol on various lipid domains of synaptic plasma membranes [4]. Confirming earlier work with ESR and FPZ techniques [3,5], the ^{1}H-NMR data have demonstrated a significant ethanol-induced disordering of the membrane core, particularly in the region of the fatty acid terminal methyl groups. However, on the mem-

Abbreviations: FPZ, fluorescence polarization; PC, phosphatidylcholine; DMPC, dimyristoyl PC; ED_{A01}, concentration required to decrease anisotropy by 0.01; r_s, steady-state anisotropy; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; PBS, phosphate-buffered saline.

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brane surface in the region of the choline methyl groups, ethanol significantly ordered membrane structure. With increasing temperature, the surface ordering effect increased disproportionately to the interior disordering effect, due to the greater enthalpy of the surface binding.

The present study extends our observations of the membrane surface-ordering/interior-disordering effects of ethanol and related molecules. FPZ techniques were used to examine the effects of 13 different non-electrolyte anesthetic-like compounds on the order of DMPC multilamellar liposomes. The anesthetic compounds examined were chosen from three perspectives. One, for a number of the compounds, the DMPC/buffer partition coefficients are known [14]. Two, the compounds chosen covered a wide range of partition coefficients. Three, many of the compounds have been tested in the alcohol-sensitive and -insensitive strains of mice [15]; some, but not all, of the compounds discriminated between the strains [16]. The fluorescent probes used were DPH, which reports from the membrane interior [17] and TMA-DPH, which reports from a more superficial domain [18]. DMPC liposomes were chosen, since our previous data demonstrated the ordering phenomenon in the region of the choline methyl moiety of synaptic membrane PC. Finally, the anesthetic effects of the *n*-alcohols were examined both above and below the main phase transition temperature (T_m). ESR data [19] suggest that solutes, like ethanol, are primarily concentrated in the center of the bilayer at temperatures below T_m; however, above T_m more superficial domains become available for partitioning. Thus, it was predicted that the ordering effects of ethanol, which appear to be localized to more superficial domains, would be greatly attenuated in the gel phase. Finally, it should be noted that in the remainder of this article, the 13 compounds tested will be generally referred to as anesthetics, rather than non-electrolyte solutes. The use of the term anesthetic is one of convenience but is clearly trivial, since most of the compounds tested have no practical use as clinical anesthetic agents.

Methods

Materials

DMPC, the *n*-alcohols and urethane were obtained from Sigma Chemical, St. Louis, MO. Diethyl ether was obtained from Merck, St. Louis, MO. Chloroform, ethyl acetate and acetone were obtained from Fischer Chemical, Fair Lawn, NJ. The chlorinated ethanols were obtained from Kodak, Rochester, NY. DPH and TMA-DPH were obtained from Molecular Probes, Junction City, OR.

Liposome preparation

Aliquots of a stock DMPC solution were added to 13×100 cm testtubes and the volume was brought to 0.3 ml with chloroform. The sample was then dried as a thin film using a dry N_2 stream and hand rotation. Samples were then placed in a vacuum oven and dried for 1–3 h at room temperature. Samples dried for up to 24 h did not differ in terms of measured physical properties from those dried for only 1 h. The amount of residual solvent present after drying was not determined. Multilamellar liposomes were prepared by adding 2 ml of PBS (pH = 7.8) warmed to approx. 10 C° above the main transition temperature, coupled with intermittent shaking on a vortex mixer and then followed by a brief (10 s) sonication in a low-power sonicating water bath. Samples were used immediately after this step. Typically, each sample contained 100 μg of DMPC, suspended in a final vol. of 3.5 ml PBS. After blanking the fluorometer for light scattering, 0.025 μg of DPH or 0.100 μg of TMA-DPH dissolved in 2.5 μl of tetrahydrofuran was added. The sample was allowed to equilibrate until a constant total fluorescence was obtained. Probe incorporation was always performed at 30° C.

Fluorescence measurements

An HH-2 T-format spectrofluorometer (BHL, Burlingame, CA) with fixed excitation and emission polarization filters was used to measure fluorescence intensity parallel ($I_\parallel$) and perpendicular ($I_\perp$) to the polarization phase of the exciting light. Steady-state anisotropy (r_s) of fluorescence $((I_\parallel - I_\perp)/(I_\parallel + 2I_\perp))$ was calculated by an on-line microprocessor. The excitation wavelength was 355 nm (uncorrected) and a U-330 filter was used in the excitation beam and L-42 filters (Hoya Instrument) were used for the emitting light. The cuvette temperature was maintained by a circulating water bath and monitored continuously by a thermocouple inserted into the cuvette to a level just above the light beam.

The steady-state anisotropy is known to be comprised of two components such that

$$r_s = r_\alpha + r^f$$

where r_α is the hindered component while r^f is the component most closely related to viscosity or fluidity [17,20]. In general, it is considered that r_s is primarily a measure of membrane order and viscosity.

Upon addition of some of the compounds tested, there were significant volume changes. A PBS volume control was used to correct for the effect of such dilution on trivial depolarization.

To compare the effects of the various anesthetics on membrane order the $ED_{0.001}$ calculation was used. A minimum of four and usually six concentrations of anesthetic were used to define the range over which r_s decreased from a minimum of -0.002 to a maximum of -0.020. Such data were analyzed by linear regression

analysis and from the line equation obtained, the concentration of anesthetic necessary to decrease r_s by 0.01 was determined. Dose–response experiments as described above, were performed four to eight times for each compound; within each experiment, the effects of each concentration were examined in triplicate. The Student's *t*-test for unpaired data was used to compare the effects of two different anesthetics.

Results

Effects of n-alcohols on DPH and TMA-DPH anisotropy

The data in Fig. 1 illustrate the effects of C_2 to C_6 *n*-alcohols on DPH r_s at 30 °C. As expected, the $ED_{0.01}$ decreased with increasing chainlength (Table I). On average, each additional carbon increased potency 3.64-fold. At 18 °C or 6 C° below T_m, there was a sharp drop in the potency of ethanol and propanol as detected by the large increases in the $ED_{0.01}$. For butanol and hexanol, the $ED_{0.01}$ was only marginally affected (Fig. 2). In contrast to the ethanol and propanol data, the $ED_{0.01}$ for hexanol increased at 18 °C. On average, at 18 °C, each additional carbon increased the potency 5.59-fold; this value was significantly different from the 3.64-fold increase per carbon seen at 30 °C ($P < 0.05$).

At 30 °C, neither ethanol nor propanol, over a wide range of concentrations, were able to decrease TMA-DPH r_s by 0.01. The data obtained are shown in Fig. 3. Ethanol had no significant effect up to concentrations of 1 M, at which point a small but significant increase in r_s was observed. The effects of propanol were biphasic. The maximum decrease (-0.004) was observed at 660 mM propanol; at 1.4 M propanol there was no significant change in r_s. At 18 °C, ethanol had no

TABLE I

Summary of anesthetic effects on DPH fluorescence anisotropy in DMPC liposomes

n.e., no effect; n.d., not determined.

Anesthetic	$ED_{0.01}$ (mM)	
	30 °C	18 °C
Ethanol	770 ± 130	2200 ± 140
Propanol	150 ± 16	590 ± 48
Butanol	54 ± 13	44 ± 5.0
Pentanol	11 ± 0.6	17 ± 1.1
Hexanol	4.4 ± 0.3	2.4 ± 0.7
2-Chloroethanol	150 ± 5.8	n.d.
2,2-Dichloroethanol	36 ± 6.2	n.d.
2,2,2-Trichloroethanol	5.5 ± 0.4	n.d.
Chloroform	3.8 ± 0.7	n.d.
Diethyl ether	130 ± 5.0	n.d.
Urethane	210 ± 15	270 ± 15
Acetone	520 ± 20	n.e. (2 M)
Ethyl acetate	72 ± 4.6	n.d.

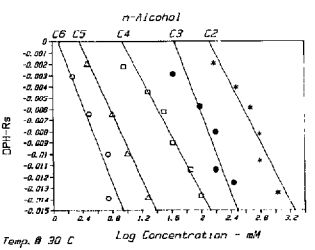

Fig. 1. Changes induced by *n*-alcohols (C_2 to C_6) on DPH r_s in DMPC multilamellar liposomes. All experiments were performed at 30 °C or 6 C° above the main phase transition temperature. The baseline r_s at this temperature was 0.085 ± 0.002. Increasing concentrations of the alcohols were added until a decrease in r_s of at least 0.02 was produced. Data are presented as the mean of four to eight experiments.

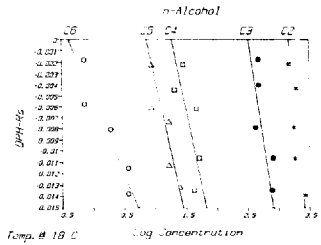

Fig. 2. Changes induced by *n*-alcohols (C_2 to C_6) on DPH r_s in DMPC multilamellar liposomes. Experiments were performed at 18 °C or 6 C° below the main phase transition temperature.

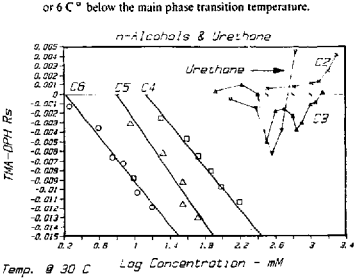

Fig. 3. Changes induced by *n*-alcohols (C_2 to C_6) and urethane on TMA-DPH r_s in DMPC multilamellar liposomes. Details as in legend to Fig. 1 except that for propanol, ethanol and urethane, a decrease in TMA-DPH r_s of more than 0.01 was not obtained.

TABLE II

Summary of anesthetic effects on TMA-DPH fluorescence anisotropy in DMPC liposomes

For abbreviations, see legend to Table I.

Anesthetic	$ED_{0.01}$ (mM)	
	30 °C	18 °C
Ethanol	n.e. (1.5 M)	
Propanol	n.e. (1.5 M)	390 ± 10
Butanol	110 ± 8.5	74 ± 12
Pentanol	33 ± 2.1	14 ± 1.2
Hexanol	11 ± 0.9	4.2 ± 1.0
2-Chloroethanol	n.e. (1.5 M)	n.d.
2,2-Dichloroethanol	n.e. (1.5 M)	n.d.
2,2,2-Trichloroethanol	55 ± 13	n.d.
Chloroform	26 ± 7.1	n.d.
Diethyl ether	200 ± 26	n.d.
Urethane	n.e. (1 M)	n.d.
Acetone	n.e. (2 M)	n.d.
Ethyl acetate	170 ± 2.4	n.d.

significant effect (increase or decrease) on TMA-DPH r_s over a wide range of concentration (100–1500 mM) (data not shown). However, unlike the 30 °C effects, at 18 °C, propanol significantly decreased TMA-DPH r_s and the potency was significantly greater than that required to decrease DPH r_s (Table II). The $ED_{0.01}$ data for TMA-DPH show that pentanol and hexanol were significantly more potent at 18 °C as compared to 30 °C.

Effects of chlorinated ethanols on DPH and TMA-DPH r_s

The data in Table I show that, as expected, the potency of the chlorinated ethanols to decrease DPH r_s, increased with each Cl addition. 2-Chloroethanol was significantly more potent than ethanol as evidenced by the 80% decrease in the $ED_{0.01}$. 2,2-Dichloroethanol was 4.2-times more potent than 2-chloroethanol and 2,2,2-trichloroethanol was 6.5-times more potent than 2,2-dichloroethanol (Table I). Over a wide range of concentrations, 2-chloroethanol (100–500 mM) increased TMA-DPH r_s modestly ($\Delta = 0.005$ at 0.5 M) and 2,2-dichloroethanol (10–100 mM) had no significant effect on TMA-DPH r_s (data not shown). In contrast, 2,2,2-trichloroethanol significantly decreased r_s with a potency similar to that seen for pentanol (Table II).

Effects of other anesthetics on DPH and TMA-DPH r_s

Five additional non-electrolyte anesthetics were examined. They were chloroform, diethyl ether, urethane, acetone and ethyl acetate. The order of potency for the DPH $ED_{0.01}$ at 30 °C was chloroform (3.8 mM) > ethyl acetate (72 mM) > diethyl ether (128 mM) > urethane (209 mM) > acetone (523 mM) (Table I). Urethane had a biphasic effect on TMA-DPH r_s. Up to concentrations of 470 mM, r_s decreased ($\Delta = -0.006$) (Fig. 3). With further increases in concentration, r_s increased such that at 670 mM, r_s was increased by 0.007. Acetone was found to induce a small increase in r_s, such that at 1.3 M, r_s was increased 0.007 (data not shown). Chloroform, diethyl ether and ethyl acetate all significantly decreased TMA-DPH r_s. As with the changes in DPH r_s, ethyl acetate and diethyl ether were of approximately the same potency and both were somewhat less potent than butanol. Chloroform was quite potent to decrease TMA-DPH r_s ($ED_{0.01} = 26$ mM), with a potency somewhat intermediate between that of pentanol and hexanol.

Selectivity vs. sensitivity

Collander [21–23] has made the empirical observation that there are systematic relationships among the partition coefficients for non-electrolytes measured between different non-polar solvents and water. The relationship could be approximately defined by

$$\log K_{i,y} = S_{x,y} \log K_{i,x} + r_{x,y} \quad (1)$$

where $K_{i,y}$ or $K_{i,x}$ is the partition coeffient of compound 'i' between solvent 'x' and water or solvent 'y' and water. $S_{x,y}$ is the selectivity constant; values of $S_{x,y} > 1$ suggest greater selectivity of solvent 'y' as opposed to solvent 'x'. Values of $S_{x,y} > 1$ can also be interpreted to indicate that the partitioning of solutes into solvent 'y' occurs into a more hydrophobic domain than in solvent 'x'. Diamond and Katz [14] have utilized this concept of selectivity to compare the partitioning of non-electrolytes into DMPC liposomes and into a variety of non-polar solvents, e.g., octanol and olive oil. To the extent that there is a strong correlation between anesthetic potency and membrane partitioning for non-electrolyte solutes [1], the concept of selectivity as developed for partitioning theory should also apply to the mechanisms of anesthetic action. Assuming that r_s is a reliable reporter of anesthetic (solute) effects that occur after partitioning, it is proposed that Eqn. 1 can be rewritten as

$$\log P_{i,y} = S_{x,y} \log P_{i,x} + R_{x,y} \quad (2)$$

where $P_{i,y}$ or $P_{i,x}$ is a relational measure of the perturbation of the membrane (or domain) 'y' or 'x' caused by solute 'i' e.g., the $ED_{0.01}$. $S_{x,y}$ retains the concept of selectivity or, more properly, the spread of selectivity. Practically, $R_{x,y}$ ($= \log C_{x,y}$) expresses a solute-independent difference in sensitivity between two membranes or membrane domains for perturbation (assuming Eqn. 2 is a valid approximation). Thus, $R_{x,y}$ assumes a different meaning for perturbation as opposed to partitioning theory. The factors underlying the differences in membrane sensitivity to perturbation are likely to be quite complex. $R_{x,y}$ may reflect solute-independent dif-

TABLE III

Summary of selectivity and sensitivity data

Comparison	$S_{x,y}$ (mean ± S.E.)	$C_{x,y}$	R^2
$ED_{0.01}$-30 °C vs. 18 °C for *n*-alcohols (DPH)	1.31 ± 0.14	0.46 ± 0.18	0.99
$ED_{0.01}$ (DPH) vs. $ED_{0.01}$ (TMA-DPH)- at 30 °C for seven anesthetics [a]	0.65 ± 0.14	8.71 ± 1.62	0.82

[a] Butanol, hexanol, 2,2,2-trichloroethanol, chloroform, diethyl ether and ethyl acetate.

ferences in such factors as the zone of maximum partition, the resistance to permeation and diffusion within the membrane bilayer to the domains occupied by the fluorescence dyes.

For the *n*-alcohols, the $ED_{0.01}$ data at 18 and 30 °C were compared from the perspective of selectivity and sensitivity (Table III). Both selectivity and sensitivity were increased in the gel as compared to the liquid crystalline phase ($S_{x,y} = 1.31$; $C_{x,y} = 0.46$). Data on both DPH and TMA-DPH r_s at 30 °C were obtained for seven compounds (butanol, pentanol, hexanol, 2,2,2-trichloroethanol, diethyl ether and ethyl acetate). Selectivity for the $ED_{0.01}$ was significantly decreased for TMA-DPH as opposed to DPH ($S_{x,y} = 0.65$). On average, the DMPC liposomes were more than 8-times less sensitive to change in TMA-DPH r_s as opposed to DPH r_s ($C_{x,y} = 8.7$).

Discussion

Previous NMR data have suggested that ethanol has differential effects on the interior and surface of rat synaptic plasma membranes; the membrane interior was disordered (as expected) but the membrane surface particularly in the region of the PC choline moiety was ordered [4]. The fluorescence data reported here modestly support this suggestion but are not as strong as the NMR data. This difference may reflect the intrinsic differences in the NMR and fluorescence experiment e.g., the time-scale of the motions monitored [24]. In addition, the ordering phenomenon detected in the NMR experiment was focused on the choline moiety of PC which is at or near the membrane surface. In contrast, TMA-DPH reports from more interior domains [18]. Ethanol produced a small but significant ordering effect at 30 °C (Fig. 2) but the concentration required (> 1 M) was considerably higher than the concentration (200 mM) required to demonstrate ordering in the NMR experiment. Such ethanol data are, however, suspect, since at concentrations above 1 M, ethanol may be causing the formation of new membrane structures, e.g., the interdigitated state [25]. At lower concentrations, which are more relevant to our understanding of anesthetic action, ethanol has no significant effect on the TMA-DPH monitored domains.

The effect of temperature on the membrane perturbations induced by the *n*-alcohols and other nonelectrolyte anesthetics is of interest for several reasons. (i) Bradley and Richards [26] have examined the effect of decreasing temperature (from 20 to 4 °C) on the nerve blocking action of ten different anesthetics. These authors noted that cooling had little effect on the anesthetic action of the short-chain alcohols but markedly increased the potency of the long-chain alcohols, benzyl alcohol, benzocaine and pentobarbitone. Since membrane partitioning decreases with decreasing temperature, these data were taken to be incompatible with the lipid perturbation hypothesis of anesthetic action. (ii) In contrast to the results of Bradley and Richards [26]. Alkana and colleagues have observed that decreasing the ambient temperature markedly attenuates the effects of ethanol in a variety of behavioral paradigms [27–29]; these authors have argued that such data support the membrane perturbation hypothesis. (iii) The partitioning of ethanol to the surface and interior of both DMPC liposomes and synaptic plasma membranes is markedly temperature-dependent. Although increasing temperature increases partitioning to both sites, partitioning to the membrane surface increases disproportionately in comparison to the membrane interior, due to the greater enthalpy of the surface binding [4].

In the present study the effect of temperature on membrane perturbations induced by the *n*-alcohols was examined from the extreme positions of 6 C° above and below the main phase transition temperature. In the gel phase (18 °C) as compared to the liquid crystalline phase (30 °C), the DPH $ED_{0.01}$ for both ethanol and propanol markedly increased. Differently, there were only modest changes for butanol and pentanol and the $ED_{0.01}$ for hexanol significantly decreased. Analyzing the $ED_{0.01}$ data for 18 and 30 °C from the perspective of selectivity, suggests that selectivity markedly increases in the gel phase i.e., partitioning of the *n*-alcohols in the gel phase is occurring into a more hydrophobic domain. This conclusion is consistent with the results of Diamond and Katz [14] who have shown for DMPC liposomes that partitioning in the gel phase occurs almost entirely into the hydrophobic core, the most fluid domain under these conditions. This contrasts with the situation in the liquid crystalline phase, where partitioning occurs primarily into a more superficial and hydrophilic domain [14]. It is of further interest to note that membrane anesthetic sensitivity, as approximately defined by $R_{x,y}$, also increases in the gel phase. The partitioning of the *n*-alcohols directly into the 'DPH-like' domains may account for this phenomenon.

In view of the above discussion, it is possible to examine the data of Bradley and Richards [26] from a somewhat different perspective. A review of Fig. 2 in their paper reveals that a major change upon cooling was to change the selectivity of the rat sciatic nerve membrane to anesthetics and by inference to change the site(s) of anesthetic action. The observation that the potencies of neither ethanol nor propanol were affected by cooling could be explained if there was a increase in membrane sensitivity which offset the selectivity effect. Given that such an increase in sensitivity was found with cooling in the DMPC membranes, it is not unreasonable to assume that a similar increase of sensitivity occurs in the intact nerve membrane. Thus, one could conclude that the increase of anesthetic potency with increasing temperature as observed by Bradley and Richards [26] is not inconsistent with the lipid perturbation hypothesis of anesthetic action.

The question arises as to whether the differential effects of the short- and long-chain alcohols on membrane structure (Fig. 2), are unique to the *n*-alcohols or may be extended to a comparison of all hydrophilic and hydrophobic non-electrolyte anesthetics. Superficially, the chlorinated ethanols should be an interesting series of substituted *n*-alcohols that could be used to address this question. With each Cl addition there was increasing potency of the compounds to disorder the domains monitored by DPH; however, these compounds had only modest effects on TMA-DPH r_s. Thus, 2-chloroethanol was of approximately equal potency to propanol from the perspective of DPH but unlike propanol. 2-chloroethanol had no significant effect on TMA-DPH r_s. Similarly, 2,2-dichloroethanol was approximately equipotent to butanol from the perspective of DPH but, unlike butanol, did not decrease TMA-DPH r_s. 2,2,2-trichloroethanol did significantly decrease TMA-DPH r_s, but the magnitude of the effect was much less than would have been expected based on the DPH data. (Similar to 2,2,2-trichloroethanol, chloroform had a high TMA-DPH-to-DPH potency ratio i.e., 7.) Thus, although the addition of each Cl substituent increases the membrane partition coefficient by approximately the same factor as by extending the chain-length by a single carbon, one cannot generalize from the *n*-alcohol data regarding the effect of the chlorinated ethanols on TMA-DPH r_s. The data with the chlorinated ethanols suggest that the differential effect on non-electrolyte anesthetics on DPH and TMA-DPH r_s is not solely related to the membrane partition coefficient but may also have structural considerations. It also should be noted that increasing the chainlength of the *n*-alcohols [30], but not the addition of chlorine atoms to ethanol, will increase the extent of membrane orientation. Evidence that the more hydrophilic compounds are remarkably less potent to disorder the membrane domains monitored by TMA-DPH as compared to the DPH domains is supported by the data obtained with the 'miscellaneous' group of non-electrolyte anesthetics examined. Urethane had a DPH potency similar to propanol and like ethanol increased the TMA-DPH r_s at high concentrations. Acetone, which has a DMPC partition coefficient intermediate between that of propanol and ethanol, had a DPH potency similar to ethanol but unlike ethanol and propanol did not increase TMA-DPH r_s even at concentrations up to 2 M. The more hydrophobic compounds, diethyl ether and ethyl acetate, decreased both DPH and TMA-DPH r_s. The DPH/TMA-DPH potency ratios for these compounds were approx. 2 and in this regard were similar to those seen for butanol, pentanol and hexanol. It is of some interest to compare the potencies of ethyl acetate and butanol. In their study of partition coefficient into DMPC liposomes, Diamond and Katz [14] have concluded that ethyl acetate should have a slightly lower partition coefficient than butanol because the -OH group will form stronger hydrogen bonds than the ester group. Paralleling this consideration, ethyl acetate was somewhat less potent than butanol in decreasing DPH and TMA-DPH r_s.

The present data clearly demonstrate that hydrophilic and hydrophobic non-electrolyte anesthetics have differential effects on the perturbation of membrane structure. Are such differential effects of biological significance or merely an epiphenomenon of the technology employed? Data to support the proposition that the differences are significantly comes from studies of the long-sleep (LS) and short-sleep (SS) mice. The LS and SS mice differ significant in terms of their response to a challenge dose (4 g/kg) of ethanol; the difference in sleeping time may be more than 10-fold [16]. The differential anesthetic response between the LS and SS mice also extends to other hydrophilic anesthetics such as methanol, propanol, 2-chloroethanol and urethane. However, the differential response is lost with more hydrophobic molecules, such as chloroform, butanol and halothane [31]. This pattern of differential response between the LS and SS mice clearly suggests that the major difference between the two strains is one of anesthetic selectivity. The LS mice are less selective than the SS mice. In this regard it is of interest to note that ethanol is markedly more potent in perturbing the TMA-DPH monitored domain in the LS mice as compared to the SS mice [32].

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