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Effects of Anesthetics on Divalent Cation Binding and Fluidity of Phosphatidylserine Vesicles

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SUMMARY

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Divalent cation binding to sonicated phosphatidylserine (PS), as affected by added anesthetics, was examined by electron paramagnetic resonance, used in conjunction with the paramagnetic Ca^{2+} analogue Mn^{2+}. Long- and short-chain normal alkanols (up through pentadecanol), benzyl alcohol, chloroform, procaine, and tetracaine all inhibited Mn^{2+} binding to PS. With increasing chain length, the normal alcohols became more effective in displacing the divalent cation from the vesicles. This appeared to be related to the dependence of alcohol lipid/water partition coefficients on the number of carbons in the methylene chain. Chloroform, on the other hand, although reported to be comparable to hexanol in its partitioning characteristics and anesthetic potency, was a much weaker inhibitor of manganese binding than the latter. The perturbation of PS fluidity by the anesthetics was monitored through EPR spectral observations on a cholestane spin probe. When anesthetic concentrations were adjusted approximately to equalize effects on manganese binding, the lower normal alkanols, benzyl alcohol, and chloroform all fluidized the vesicles substantially, as evidenced by enhanced mobility of the probe. The higher alcohols and the amine anesthetics, in contrast, produced little or no fluidization of the vesicles. The results are discussed in terms of possible molecular explanations for the phenomena and suggested mechanisms for anesthesia.

INTRODUCTION

Evidence exists that the cationic amine anesthetics—e.g., procaine, tetracaine, and chlorpromazine—displace divalent cations from binding sites on biological membranes (1, 2) or from negatively charged phospholipids (3–7). On the basis of these results and the observed competitive effects of procaine and Ca^{2+} on the action potentials of lobster axons (8), it has been suggested that the pharmacological action of the anesthetics involves displacement of Ca^{2+} from sites on the axolemma, possibly acidic phospholipid head groups (3, 7, 8). This idea has recently been challenged (9, 10), but important questions remain concerning possible antagonistic or synergistic influences of Ca^{2+} and anesthetics on membranes of excitable tissue (11).

In contrast to the amines, many neutral anesthetics, including the normal alcohols,

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have been reported to enhance Ca^{2+} binding to membranes (12, 13), but it is unknown whether this represents binding to anionic or neutral phospholipids or to proteins.

In this study we have examined the effects of cationic and neutral anesthetics, especially the normal alcohols, on divalent cation binding to phosphatidylserine, the major anionic phospholipid of animal plasma membranes. In this study Mn^{2+} was employed as a paramagnetic Ca^{2+} analogue. This substitution appears justified in view of the similarity found between PS[1] affinities for the two ions (14).

Manganese-PS binding, as influenced by the anesthetics, was determined by EPR. The method, which has been widely used to monitor divalent cation association with biologically related ligands in aqueous media, is based on quantitating the narrow sextet signal due to free $Mn(H_2O)_6^{2+}$ (15). Manganese binding to phospholipid vesicles, as a function of lipid composition and monovalent salts, has been examined in this way (16).

In parallel with manganese binding measurements, the fluidity of the PS vesicles was followed with a nitroxide spin probe. Both sets of results are discussed in terms of possible mechanisms for anesthesia and in terms of fundamental molecular interactions of divalent cations and lipophilic perturbants with phospholipid bilayers.

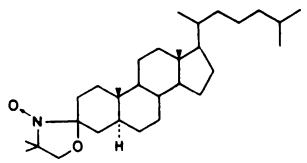
MATERIALS AND METHODS

Bovine brain PS and beef heart cardiolipin were obtained from Grand Island and Sigma, respectively. Each yielded a single spot on a thin-layer plate when run in two separate solvent systems. The cholestane spin label (I) was prepared by the method of Keana *et al.* (17) by Dr. F. H. Kirkpatrick. It should be noted that the N—O bond lies perpendicular to both the plane of the molecule and its long axis.

A23187 was furnished by Dr. R. Hamill of Eli Lilly. All other chemicals were reagent grade.

For measurements of manganese binding

(I)



in the presence of procaine or tetracine, individual samples were prepared, as described previously (16), by sonicating PS in Pyrex tubes containing 500 μl of the desired medium, including manganese and anesthetic. For reasons cited in the earlier study, the ratio of manganese to lipid phosphate was kept below 1:20. Free and bound manganese fractions were quantitated by EPR (15, 16).

Where neutral anesthetics were to be added, a slightly modified procedure was usually adopted in preparing samples. The latter yielded essentially equivalent results with respect to manganese binding but was more convenient and minimized variations in pipetting and sonication among samples. First, a stock suspension of vesicles was prepared by (a) drying down the phospholipid in a tube along with a small amount of the ionophore A23187, (b) removing trace solvent under vacuum, (c) adding several milliliters of HEPES-buffered NaCl medium containing manganese but no anesthetic, and (d) irradiating the tube in a sonicating bath for approximately 30 min under N_2.

Aliquots (480 μl) of the suspension were then transferred to separate vials, each containing a 20-μl mixture of the anesthetic and 95% ethanol. Ethanol at these concentrations produced no appreciable shifts in manganese binding to PS in either the presence or absence of other anesthetics. Samples were incubated for at least 1.5 hr to allow manganese re-equilibration by the ionophore. (The incubation period was chosen in light of results from controls, in which manganese was added to previously formed vesicles, in the presence of A23187, and the binding was monitored with time.) Manganese binding at room temperature in each sample was determined by EPR, as previously described (16).

EPR was also employed to assess the influence of the anesthetics on lipid fluidity. For this purpose, parallel samples were pre-

[1] The abbreviations used are: PS, phosphatidylserine; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

pared similar to those used in the manganese binding studies. Each sample contained a small amount of cholestane spin label (see above), which was dried down with the PS prior to vesicle formation. Also, because the small amounts of manganese and A23187 present in the binding studies had no effect on the cholestane signal except to reduce its amplitude through manganese-nitroxide spin-spin interactions, they were routinely omitted. The cholestane concentration, microwave power, and fluid modulation were adjusted downward until further reductions in any of these parameters left the observed line shapes of the spectra invariant.

RESULTS

Manganese binding to PS. In the experiments reported here, the PS binding sites were unsaturated with respect to divalent cations ([Mn]/[PS] < 0.05); hence the number of available sites and the surface potential of the vesicles were only slightly perturbed by the presence of manganese (16). Under these conditions the apparent manganese affinity is proportional to B/F, the ratio of bound to free manganese in the sample:

$$K = (B/F)[\mathrm{PS}]^{-1} \qquad (1)$$

where the concentration of sites has been arbitrarily set equal to the total concentration of PS. This normalization poses no difficulties as long as the sites are far from saturation (16).

Inhibition of binding by n-alkanols. Manganese binding to PS was decreased by added normal alcohols (Fig. 1). Regardless of the number of carbon atoms (n) per alcohol molecule, the measured affinity fell off monotonically from the control value K_0 with increasing alcohol concentration [A], but the longer-chain species were clearly more effective in reducing the binding.

It should be noted that [A] refers to the total alcohol concentration in a sample. In some samples containing long-chain alcohols, [A] exceeded the nominal alcohol solubility in water. In those instances, however, a high proportion of the alcohol appeared to be partitioned into the "lipid phase" (see below), so that little or no alcohol was present as insoluble aggregates.

The dependence on aliphatic chain length is summarized in Fig. 2, in which $[A]_{50}$, the respective alkanol concentration estimated to produce a 50% reduction in apparent manganese affinity (i.e., $[A]_{50}$ =

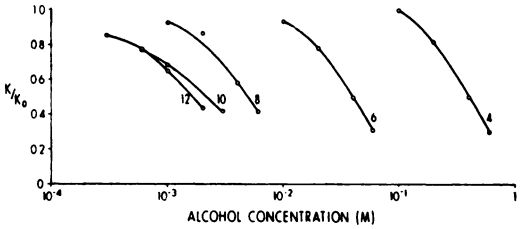


FIG. 1. *Affinity of manganese for PS vs. added n-alkanols*

The apparent manganese affinity, K, is plotted relative to K_0, its value in the absence of alcohol, as a function of [A] for several normal alcohols. The curves are labeled as to the number of carbons in each alcohol. Besides the indicated alcohols and up to 3.8% ethanol (see MATERIALS AND METHODS), the samples contained 1.52 mg/ml of PS, 110 mM NaCl, 14.4 mM sodium HEPES (pH 7.5), 62 μM $MnCl_2$, and 0.91 μM A23187. K_0 was determined to be approximately 5.9×10^3 M^{-1} from Eq. 1, assuming a molecular weight of 800 for PS.

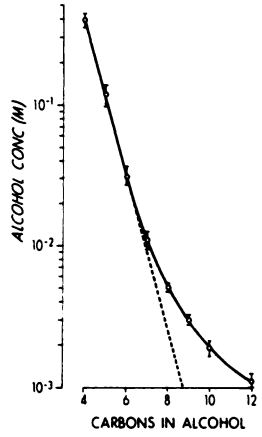


FIG. 2. *Manganese displacement vs. n-alkanol chain length*

Values of $[A]_{50}$ for each alcohol were estimated using data obtained under conditions like those in Fig. 1. In each case the affinity was plotted relative to the measured K_0 for the same vesicle suspension. $[A]_{50}$ was estimated (by interpolation) to be the alcohol concentration where $K/K_0 = ½$. The data points and error bars show the respective means and standard errors obtained from three to five determinations of $[A]_{50}$ for each alcohol.

[A] at the point where $K/K_0 = 1/2$), is plotted against n. As indicated by the dashed line, there was, for the lower alcohols, an approximate 3-fold decrease in $[A]_{50}$ with each additional carbon.

This behavior closely parallels the roughly 3-fold enhancement of nerve-blocking potency observed with each additional n-alkanol carbon (11, 18). It should be noted, however, that the estimated value of $[A]_{50}$ was, for a given alcohol, more than 5 times the corresponding nerve blocking threshold and about two orders of magnitude higher than the respective threshold for general anesthesia (11). [Decreases in manganese binding measured at nerve-blocking thresholds, e.g., 68 mM butanol (11), never exceeded 15% and were generally too small to be observed.]

A 3-fold rise in potency with each additional carbon is also reflected in numerous other alcohol-induced changes in membrane properties, including the increased conductance of black lipid membranes (19), the enhanced resistance of erythrocytes to osmotic shock (20), and the disordering of phospholipid hydrocarbon chains (21). All the variations with alcohol chain length referred to above can be accounted for in terms of an approximate 3-fold enhancement of the membrane/water partition coefficient of an alcohol with each additional carbon. The results in Fig. 2 thus suggest that, at least for the lower alcohols, manganese displacement from PS is principally a function of alcohol concentration in the membrane and is strongly dependent on the particular alcohol species only through its partition coefficient.

The curve in Fig. 2 begins to level off for $n \gtrsim 7$. This does not imply any fundamental difference between lower and higher alcohols, either with respect to their partitioning properties, discussed above, or with respect to their effects on divalent cation binding. Instead it may mostly reflect an approach toward 100% alcohol partitioning into the membrane phase. Supporting that interpretation are the results presented in Table 1, indicating that, as the PS concentration was raised, proportionally more long-chain alcohol was required to reduce K by 50%. On the other hand, it was found that the $[A]_{50}$ for butanol did not vary significantly with the concentration of PS.

TABLE 1

Decanol concentration required to produce 2-fold inhibition of manganese-PS binding, as a function of PS concentration

NaCl	[PS]	$[A]_{50}$	$[A]_{50}/[PS]^a$
mM	*mg/ml*	*mM*	
180	3.04	4.1	0.98
180	1.52	1.9	0.88
180	0.77	0.86	0.82
120	0.63	0.71	0.82
120	0.38	0.56	1.04

[a] Based on a molecular weight of 800 for PS. The molar ratio PS:Mn^{2+}:A23187 was 22.4:1:0.012 in all cases. Samples also contained 14.4 mM HEPES (pH 7.5) and NaCl as shown. The value of $[A]_{50}$ at each PS concentration was estimated (see Figs. 1 and 2) from a plot of K/K_0, measured by EPR, against added decanol.

Like the other alcohols, pentadecanol also inhibited manganese binding when added to sonicated aqueous suspensions of PS, but the results were highly variable. The variability may have been related to the low solubility of pentadecanol, since small particles adhered to the sides of the tubes even after resonication. This problem was alleviated somewhat by preparing sets of samples in which the alcohol was dried down together with the phospholipid. Manganese-buffer solution was added to each tube, and the samples were shaken on a Vortex mixer and sonicated. EPR determinations on these samples yielded results similar to those obtained with decanol and dodecanol ($[A]_{50} \sim 1.4$ mM).

Taken together, the results above suggest that, to a first approximation, $[A]_{50}$ varies inversely with f_n, the fraction of added n-carbon alcohol partitioned into the PS. (The partitioning, to some degree, may be cooperative; therefore f_n is more precisely defined as the fraction of alcohol in the lipid phase at the point where $K/K_0 = 1/2$.) Under the further assumption that $f_n \approx 1$ for the long-chain alcohols like dodecanol, the partitioning for other members of the series can be estimated. As an example, from Fig. 2 we infer that for pentanol, $f_5 \cong 10^{-2}$. P_5, the lipid/buffer partition coefficient for pentanol, is then calculated to be

$$P_5 \approx \frac{1 \text{ mole}/1.52 \text{ mg lipid}}{99 \text{ moles/ml buffer}}$$

$$\approx \frac{6.6 \text{ moles/g lipid}}{\text{moles/ml buffer}}$$

For comparison, the pentanol partitioning into erythrocyte membranes has been estimated to be ≈ 3.6 (moles/g of membrane)/(moles/ml of buffer) (11, 22). Assuming the alcohol to be partitioned mostly between the water and the lipid portion of the membrane, and noting further that the ghost is ≈43.6% lipid (23), we arrive at a pentanol partition coefficient of $P_5 \approx 8.3$ (moles/g of lipid)/(moles/ml of buffer). The qualitative agreement between this value of P_5 and that estimated above for the PS system, based on manganese binding data, further supports our earlier conclusions regarding *n*-alkanol inhibition of manganese binding to PS.

Alcohol inhibition of manganese binding to cardiolipin. As shown by the data in Table 2, the normal alcohols reduced manganese binding to cardiolipin vesicles in a manner similar to that seen with PS. The inhibition is therefore not peculiar to PS, but may reflect a general ability of the *n*-alkanols to displace divalent cations from acidic phospholipid binding sites.

Effect of alcohols on PS fluidity. PS vesicles treated with the cholestane spin label produced the spectrum shown in Fig. 3. The splitting parameter $2A_\perp'$ defined in Fig. 3 was used as a relative measure of fluidity (24). This approach seems justified in view of findings that, in cholestane-labeled PS vesicles, $2A_\perp'$ decreases monitonically with rising temperature but increases with PS fatty acid chain saturation or added cholesterol.[2] Although the spectrum in Fig. 3 appears highly immobilized, the measured $2A_\perp'$ at room temperature fell in the range 38.4 ± 0.4 G, compared with a total splitting of approximately 68 G when the vesicles were examined at 77°K. This is consistent with rapid motion about the long axis of the probe and some restricted motion about the other axes (25).

As shown for hexanol in Fig. 4, the addition of short-chain alcohols increased cho-

[2] Unpublished observations.

TABLE 2

Inhibition of manganese binding to cardiolipin by added normal alcohols

Values of $[A]_{50}$ were determined, as with PS, from plots of K/K_0 vs. $[A]$. Conditions were: cardiolipin, 1.54 mg/ml; manganese, 48 μM; NaCl, 86 mM; HEPES, 14 mM, pH 7.4; A23187, 1.3 μM.

Alcohol	$[A]_{50}$
	mM
Butanol	330
Hexanol	22
Decanol	1.8

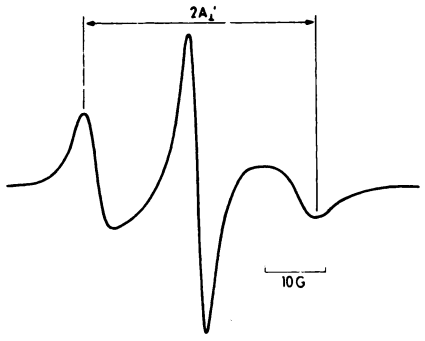


FIG. 3. *EPR spectrum of cholestane spin label in sonicated PS vesicles*

The cholestane probe (0.031 mg/ml) was sonicated together with PS (1.52 mg/ml) in a buffer containing 110 mM NaCl and 14.4 mM HEPES (pH 7.5). The spectrum was recorded at approximately 21° and 9.5 GHz. Modulation was 1.25 G, and microwave power to the dual cavity was 30 mW.

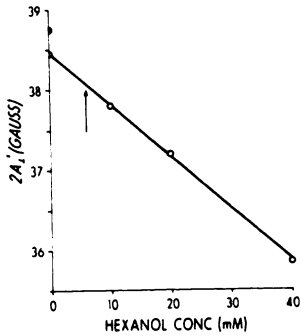


FIG. 4. *PS fluidity as a function of added hexanol*

The motion parameter $2A_\perp'$, defined in Fig. 3, was measured on a series of samples containing PS sonicated together with cholestane spin label. ○, hexanol was added in 20 μl of 95% ethanol to a 480-μl aliquot of lipid suspension, as in Fig. 1; ●, control, in which 20 μl of water were substituted for the alcohol. Conditions were otherwise the same as in Fig. 3. The arrow indicates the nerve-blocking threshold for hexanol.

lestane mobility, indicating presumably a more fluid bilayer. The long-chain alcohols, however, produced little or no fluidization of the vesicles. This point is illustrated in Table 3, which summarizes the changes observed in $2A_{\perp}'$ upon addition of a number of *n*-alkanols. To make the comparison meaningful, the measurements were carried out at the estimated value of $[A]_{50}$ for each compound, where the respective alcohol concentrations in the lipid are believed to be roughly equivalent (see above). [The presence of up to 3.8% ethanol in each sample (see MATERIALS AND METHODS) caused a slight spectral narrowing (Fig. 4). Controls showed that this change in $2A_{\perp}'$ was approximately linear with ethanol concentration and additive with the changes caused by the other alcohols. An appropriate subtraction was therefore made to arrive at the estimated shift in $2A_{\perp}'$ produced by the alcohol of interest alone.]

It appears then from Table 3 that the lower alcohols disrupt the ordered bilayer structure much more than do the longer-chain species. This conclusion is supported by other physical studies of *n*-alkanol interactions with phospholipids (26, 27) and has been advanced as an explanation why members of the series beyond dodecanol do not induce anesthesia (11, 26–28). The differential effects on lipid fluidity appear reasonable, since the higher alcohols can pack more tightly with phospholipid hydrocarbon chains, thus maximizing van der Waals attractions, while insertion of lower alcohols into the bilayer must necessarily involve dislocations from an ordered packing arrangement, which would permit enhanced mobility of membrane constituents.

Other anesthetics. Table 4 indicates how a number of other anesthetics affected manganese binding and cholestane mobility in PS vesicle suspensions.

As expected from previous work (3–7), the positively charged amine anesthetics procaine and tetracaine inhibited manganese-PS binding. The respective concentrations of these agents reducing manganese affinity by 50% were, moreover, comparable to those reported to cause 2-fold inhibition of Ca^{2+}-induced Na^{+} permeability in PS vesicles (6). Like the higher alcohols (see Table 3), procaine and tetracaine had little effect on fluidity. This conclusion appears consistent with a fluorescent probe study which indicated that the amine anesthetic dibucaine had only a very slight fluidizing effect on PS dispersions (29).

If the pharmacological action of neutral anesthetics is a consequence of divalent cation displacement from anionic phospholipids or of altered phospholipid fluidity, such compounds with similar nerve-blocking thresholds might be expected to show similar dose-response behavior with regard to their effects on manganese-PS binding

TABLE 3

Increased mobility of cholestane spin label in PS vesicles in the presence of normal alcohols

Except for the added *n*-alkanol, conditions were similar to those in Fig. 4. The tabulated decreases in the splitting parameter $2A_{\perp}'$ (indicating enhanced probe mobility) were determined relative to a standard without added alcohol (a correction was made in each case for the small amount of narrowing produced by the 0–3.8% ethanol in the samples).

Alcohol	$[A] = [A]_{50}$	$\Delta(2A_{\perp}')$
	mM	*G*
Butanol	400	−3.15
Pentanol	120	−3.55
Hexanol	32	−2.25
Octanol	5.0	−1.65
Decanol	1.9	−0.70
Dodecanol	1.1	0
Pentadecanol	1.4	0

TABLE 4

Effects of several anesthetics on manganese binding and fluidity of PS vesicles

Anesthetic	Effect on binding: $[A]_{50}$	Effect on fluidity	
		$[A]$	$\Delta(2A_{\perp}')$
	mM	*mM*	*G*
Procaine	11.5	11.5	−0.6
Tetracaine	0.29	0.29	−0.2
Benzyl alcohol	160	160	−2.9
		120	−2.3
Chloroform	—[a]	60	−2.1
		32	−1.1

[a] Chloroform, at its saturation point in water (approximately 60 mM), reduced *K* by only about 17%. Except for minor modifications in the case of the amines (see MATERIALS AND METHODS), essentially the same procedures and conditions were employed here as in Figs. 1 and 2 and Table 3 for determining $[A]_{50}$ and $\Delta(2A_{\perp}')$, respectively.

or on cholestane mobility, respectively. For example, pentanol and benzyl alcohol are reported to have nearly equal anesthetic potencies and membrane/buffer partition coefficients (11). Qualitatively, the two alcohols had a comparable influence on manganese binding as well, although slightly more benzyl alcohol was required to lower K by 50% (see Tables 3 and 4). Benzyl alcohol also caused a somewhat smaller fluidity change in the vesicles, whether the alcohols were compared at equal concentrations or at their respective $[A]_{50}$ values.

More definitive is a comparison between chloroform and hexanol, two other compounds having almost the same anesthetic potencies and membrane partitioning coefficients (11). Despite those similarities, hexanol was a much stronger inhibitor of manganese binding. Specifically, the estimated $[A]_{50}$ for hexanol was 32 mM, while 60 mM chloroform reduced manganese binding by only about 17%. Chloroform nevertheless had a considerable effect on cholestane mobility, although again less than that seen in the presence of an equal concentration of hexanol.

Chloroform is the smallest of the anesthetic molecules employed here and the only one that is not amphipathic. Hence chloroform may locally perturb the hydrocarbon core of a phospholipid bilayer but not interact directly with the head groups. This may help to explain how chloroform can affect PS fluidity without interfering appreciably with divalent cation binding. Such an interpretation is consistent with differential scanning calorimetry data (30).

In summary, all the compounds tested inhibited manganese binding to PS. With the important exception of chloroform, the degree of inhibition seemed to correlate well with the respective amounts of perturbant associated with the lipid. The data indicate, however, that when perturbant concentrations are normalized according to their effect on divalent cation binding, the higher alcohols and the amine anesthetics perturb fluidity much less than the lower n-alkanols, benzyl alcohol, or chloroform. From these differences it can be concluded that the displacement of divalent cations from PS by the anesthetics is probably not strongly dependent on the fluidity changes induced by these compounds. Fluidity changes may, however, still modulate cation binding to some degree.

DISCUSSION

Because of their similar charge distributions and ionic radii, the electrostatic binding characteristics of Ca^{2+} and Mn^{2+} to negative ligands are often similar. Specifically, measured affinities of PS for Ca^{2+} and Mn^{2+} are in close agreement (5). The conclusions reached here concerning anesthetic-induced shifts in Mn^{2+} binding should therefore be qualitatively correct for Ca^{2+} also.

All the anesthetics tested, neutral as well as cationic, inhibited manganese binding to PS (or cardiolipin) vesicles. Therefore the enhanced calcium binding to biological membranes observed in the presence of various alcohols (12, 13) probably does not result simply from an increased affinity for anionic phospholipids. This does not exclude the possibility that disruption of the membrane by an anesthetic might expose additional anionic phospholipid head groups to which divalent cations could bind.

The mechanism whereby neutral anesthetics such as the n-alkanols displace divalent cations from PS is unclear. The insertion of neutral molecules into the bilayer would, however, (locally) reduce the negative surface charge density and, consequently, the magnitude of the surface potential. This would lead to a lowered cation concentration in the aqueous interface region and a reduced apparent affinity of PS for cations (16, 31). In addition, intercalation of amphiphilic molecules into the bilayer might, as discussed previously, interfere with a 2-point attachment of a cation to phospholipid head groups (16).

In comparing the normal alcohols, there was, as previously found for the amine anesthetics (6, 8, 29), a good correlation between anesthetic potency and the ability to inhibit divalent cation binding to PS. This correlation may be fortuitous, however, reflecting the dependence of both phenomena on the membrane/aqueous phase partitioning coefficient.

In fact, some of the results presented

here militate against a direct causal relationship between divalent cation displacement and anesthesia. First, pentadecanol inhibited manganese binding but seems to be ineffective as an anesthetic (11). Second, thresholds for chloroform- and hexanol-induced nerve blockade are nearly equal (11), but hexanol was a much stronger inhibitor of manganese binding. Third, when alcohols were tested at their nerve-blocking thresholds (e.g., 68 mM butanol), the observed shift in manganese affinity was relatively small (about 15% or less). It should be noted in this connection that, for most neutral anesthetics, the threshold for general anesthesia is roughly an order of magnitude lower than for nerve blockade (11).

Nor do the findings here support the hypothesis that anesthesia results mainly from an over-all increase in lipid fluidity (32, 33). First, certain of the anesthetics (e.g., decanol and tetracaine) had very little fluidizing effect on the vesicles, even at concentrations where manganese binding data indicated extensive interaction between the drug and the phospholipid. Moreover, even those anesthetics producing substantial fluidization at concentrations near $[A]_{50}$ caused relatively small decreases in $2A_{\perp}'$ when tested near the threshold for local anesthesia (see arrow in Fig. 4). For comparison, larger reductions in $2A_{\perp}'$ are found in cholestane-labeled PS suspensions when the temperature is raised by only 5°.[2] These results then support the conclusion reached by Boggs *et al.* (34), in a previous spin label study, that phospholipid fluidity changes associated with anesthetic doses of neutral anesthetics are, by themselves, too small to be important pharmacologically. Anesthetic-induced shifts in fluidity or divalent cation binding of membrane phospholipids may nevertheless play a role in the toxicity of these compounds at high concentrations.

In conclusion, the results here demonstrate that anesthetics can affect both divalent cation binding to phospholipids and lipid fluidity, but there was no clear connection between these phenomena and anesthesia. It should be noted, however, that these experiments were carried out at low ratios of cation to phospholipid. Under physiological conditions, there is a large excess of divalent cations. By binding to acidic phospholipids, these cations can induce lateral phase separation of lipids in the bilayer (35) and rigidify certain of the lipids (36, 37). Conceivably, under these circumstances, a low concentration of lipophilic perturbant might displace enough divalent cations from their phospholipid binding sites to trigger a sequence of membrane structural changes that are involved in anesthesia.

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