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# The Influence of Local Anesthetics on Molecular Organization in Phosphatidylethanolamine Membranes

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SUMMARY

The influence of the local anesthetics tetracaine (TTC) and procaine (PRC) on bilayers of specifically deuterated phosphatidylethanolamines (PE) has been studied by $^{2}H$ and $^{31}P$ NMR. Dimyristoylphosphatidylethanolamines (DMPE), deuterated at positions 2, 4, and 14 of the *sn*-2 chain, position 2 of the *sn*-1 chain, and in the ethanolamine headgroup, were mixed 1:1 with a semisynthetic egg PE and the effect of measured quantities of TTC and PRC on the $^{2}H$ quadrupole splittings, spin-lattice relaxation times, and $^{31}P$ chemical shift anisotropy were observed. Experiments were performed at pH 5.5, when the anesthetics are primarily charged, and at pH 9.5, when they are uncharged. Tetracaine was observed to disorder the hydrocarbon region of the bilayer and to induce a conformational change in the PE headgroup. Conversely, procaine had little or no effect on the hydrocarbon region and induced only a small change in the headgroup. These conformational changes and disordering effects, when adjusted for anesthetic partitioning, are essentially independent of the charge on the anesthetic. However, at pH 5.5 and low TTC/PE molar ratios (<0.1), the $^{2}H$ NMR spectra showed two lipid environments—one corresponding to free PE and the other to PE in contact with TTC. Continued addition of TTC resulted in the eventual disappearance of the free PE signal and the corresponding growth of the signal from PE in contact with TTC. At pH 9.5, when TTC is uncharged, only one signal is observed. This indicates that at low pH, when TTC is primarily charged, it has a much slower rate of lateral diffusion in the PE bilayer. In mixtures of PE and phosphatidylserine, a conformational change in the headgroup was noted which was similar to that seen in the pure PE; however, there was no evidence for slow lateral diffusion of the anesthetics. The effects of TTC and PRC on the PE bilayer, when combined with our earlier study of the labeled anesthetics [Kelusky, E.C., and I.C.P. Smith, *Biochemistry*, **22**:6011–6017 (1983)], indicate that TTC penetrates into the hydrocarbon portion of the bilayer whereas PRC sits only in the headgroup region.

## INTRODUCTION

The interaction of local anesthetics with the lipid portion of the nerve membrane has been the subject of much study and speculation in recent years. Although the exact nature of the molecular mechanism of anesthesia is still unknown, many models have been proposed involving an interaction of the anesthetic with the lipid matrix (1). To complement our earlier studies on PC[2] (2, 3), we have chosen to investigate the interaction of two amino ester anesthetics, TTC and PRC, with model bilayers of PE, another common membrane lipid.

The $^{2}H$ NMR studies of specifically deuterated TTC and PRC in semisynthetic egg PE, reported recently (4), provided a clear example of an anesthetic in slow exchange between a lipid site and a free (solution) site. The experiments also indicated a depth of anesthetic penetration into the bilayer which was independent of the charge on the anesthetic. The only apparent dependence on anesthetic charge was in the observation of a strong angular-dependent linewidth, when TTC is charged, while uncharged TTC shows an angular-independent linewidth. The strong angular dependence can be correlated with a slow diffusion of TTC over a surface of finite curvature, whereas the angular-independent linewidth found at high pH suggests a faster rate of lateral diffusion.

The results in PE are in sharp contrast to the work on

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[2] The abbreviations used are: PC, phosphatidylcholine; TTC, tetracaine; PRC, procaine; PE, phosphatidylethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; PS, phosphatidylserine; CSA, chemical shift anisotropy.

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labeled TTC in egg PC (2, 5, 6), where TTC is observed to exist in three sites: strongly bound in the membrane, weakly bound in a site of low order, and free in solution. The location of the strong binding site for TTC in the PC bilayer has also been studied by observing the effect of TTC on the $^2H$ NMR spectra of specifically labeled DPPC (3). This work showed a strong dependence of the anesthetic location on pH. At low pH, charged TTC sits high in the bilayer interacting with the PC headgroup. However, at high pH, the uncharged TTC intercalates more deeply into the bilayer. A similar location for procaine, in egg PC/cholesterol bilayers, has been suggested from X-ray diffraction results (7). The local anesthetic benzyl alcohol has also been observed to disorder the acyl chains of specifically deuterated DMPC (8), indicating a hydrocarbon environment for the bound benzyl alcohol.

In order to explore more fully the interaction of TTC with PE bilayers, specifically labeled DMPE species were prepared. They were deuterated in the ethanolamine headgroup (3-[1′,2′-$^2H_4$]DMPE), on the *sn*-1 chain (1-[2′-$^2H_2$]DMPE), and on the *sn*-2 chain (2-[2′-$^2H_2$]DMPE, 2-[4′,14′-$^2H_5$]DMPE). The DMPE was mixed 1:1 with a semisynthetic egg PE or bovine PS, and the influence of TTC and PRC on the $^2H$ and $^{31}P$ NMR spectra was studied. The addition of a natural lipid ensured a fatty acid distribution more comparable with that in our work on labeled TTC and PRC in PE (4). As in our earlier work, experiments were performed at both pH 5.5, when TTC and PRC are primarily charged, and at pH 9.5, when they are uncharged (TTC $pK_a$ = 7.5, PRC $pK_a$ = 9.0) (2, 5).

## MATERIALS AND METHODS

*Syntheses.* [2-$^2H_2$]Myristic acid was prepared by $^2H_2O$ exchange, under pressure (9). [12-$^2H_3$]Lauric acid was purchased from Larodan Lipids, Sweden, and labeled at position 2 to give [2,12-$^2H_5$]lauric acid (9). The labeled lauric acid was reduced to the alcohol with $LiAlH_4$, and the mesylate was prepared with methanesulfonyl chloride (10). The mesylate was then used to alkylate sodium diethyl malonate in xylene, saponified, and decarboxylated (11) to give [4,14-$^2H_5$]myristic acid. [1,2-$^2H_4$]Ethanolamine was prepared by the action of Raney active nickel catalyst, in $^2H_2O$, (No. 28, Grave Davidson Chemicals) on ethanolamine (12). Semisynthetic egg PE (hereafter called egg PE) was prepared by phospholipase D-mediated headgroup exchange of ethanolamine with egg yolk PC (4). Specifically deuterated DMPC was prepared using the labeled myristic acid (13, 14); the corresponding DMPE was prepared by phospholipase D-mediated headgroup exchange (4, 14).

*Sample preparation.* Egg PE and a labeled DMPE (1:1), in chloroform/methanol, were added to a glass tube containing the anesthetic. A stream of nitrogen was used to blow off most of the solvent, and the tube was dried by pumping overnight on a vacuum line. A borate-phosphate-citrate (BPC) buffer, in $^1H_2O$ (4) with pH adjusted to 5.5 or 9.5, was added and the mixture was vortexed extensively. The buffer consisted of citric acid (3.8 mM), boric acid (2.9 mM), sodium hydroxide (17.1 mM), and 85% $H_3PO_4$ (2.4 mM) in $^1H_2O$. Samples were heated to approximately 60° to ensure hydration of the PE (15) and then subjected to at least five freeze-thaw-vortex cycles to ensure complete equilibration of anesthetic between water and lipid (16). The measured partition coefficients (4) for the anesthetics between water and PE are as follows: TTC, pH 5.5 ($K_p$ = 46), pH 9.5 ($K_p$ = 71); PRC, pH 5.5 ($K_p$ = 3.3), pH 9.5 ($K_p$ = 4.1).

*$^2H$ NMR.* The $^2H$ NMR spectra were obtained on a Bruker CXP-300 spectrometer operating at 46.063 MHz using 10-mm tubes in a home-built probe. Spectra were acquired using the quadrupole echo sequence (17) with full-phase cycling of the *rf* pulses. Pulse spacing was typically 50 μsec, the $\pi/2$ pulse length 5 μsec and the recycle time greater than 5 × $T_1$. Spectra were acquired on resonance, using quadrature detection, but were folded about the center frequency in order to increase the signal-to-noise ratio. In each case the spectra were checked prior to folding, in order to ensure that the folding did not introduce artifacts in the lineshape. $T_1$ values were determined using a modified inversion recovery sequence ($180°_x$-$\tau$-echo sequence).

Spectral de-Paking (18) was done on a Nicolet 1280 data station connected to the Bruker ASPECT-2000 computer. Three iterations on 750 data points gave good convergence.

All of the $^2H$ NMR spectra were acquired at 40°, which is approximately 5° above the gel-liquid crystal transition temperature of our egg PE/DMPE mixtures.

The $^{31}P$ NMR experiments were performed on a Bruker CXP-300 spectrometer operating at 121.47 MHz. Spectra were acquired using a Hahn echo and phase cycling so as to obtain undistorted lineshapes (19). Proton decoupling was used for all spectra, using the low-power decoupler on the Bruker CXP-300. Gated broadband decoupling was used with the decoupling field on from the start of the refocusing 90° pulse until the end of the acquisition. A $^1H$ field strength of 1.9 G gave complete decoupling. Spectra were acquired with a 125 kHz spectral window and a recycle time of 2 sec.

## RESULTS AND DISCUSSION

*Headgroup.* The $^2H$ NMR spectrum of a multilamellar dispersion of neat 3-[1′,2′-$^2H_4$]DMPE, above its phase transition temperature of 53°, shows two quadrupole splittings of 10.4 and 4.3 kHz. These quadrupole splittings are similar to those observed for specifically labeled DPPE and are assigned to the deuterons adjacent to the oxygen ($\alpha$) and the deuterons adjacent to the nitrogen ($\beta$), respectively (20). When mixed 1:1 with the semisynthetic egg PE, the temperature of the gel-to-liquid crystal phase transition is lowered to approximately 35°. The $^2H$ NMR spectrum of this mixture, at 40°, showed only liquid crystalline phase, with quadrupole splittings of 10.5 and 4.4 kHz at pH 5.5, and 10.6 and 4.3 kHz at pH 9.5. The consistent nature of these quadrupole splittings with variations in the pH and the type of fatty acid indicates that the orientation of the headgroup is essentially unchanged over the pH range and fatty acid mixtures used.

The addition of TTC and PRC to the PE dispersion causes a decrease in the splitting of the $\alpha$ deuterons and an increase in the quadrupole splittings of the $\beta$ deuterons. Figure 1 shows the variation in quadrupole splittings, at pH 9.5, when TTC and PRC are added. The ratio of $[\text{anesthetic}]_{\text{bound}}/[\text{lipid}]$ is calculated from the amount of anesthetic, water, and lipid and the known partition coefficients (4). Therefore, the effect of equal amounts of TTC and PRC bound in the lipid can be compared directly. Figure 1 also shows the effect of TTC and PRC on the headgroup labeled PE mixture at pH 5.5. At this low pH, TTC and PRC are primarily in their charged forms (2), but they show only a slightly greater effect on the PE bilayer than at pH 9.5, where they are primarily uncharged. At low TTC concentrations, the $^2H$ NMR spectrum shows three superimposed quadrupole patterns, one from the $\beta$ deuterons and two from the $\alpha$ deuterons (see Fig. 2). Of the two splittings arising from the $\alpha$ deuterons, one is essentially unchanged from the

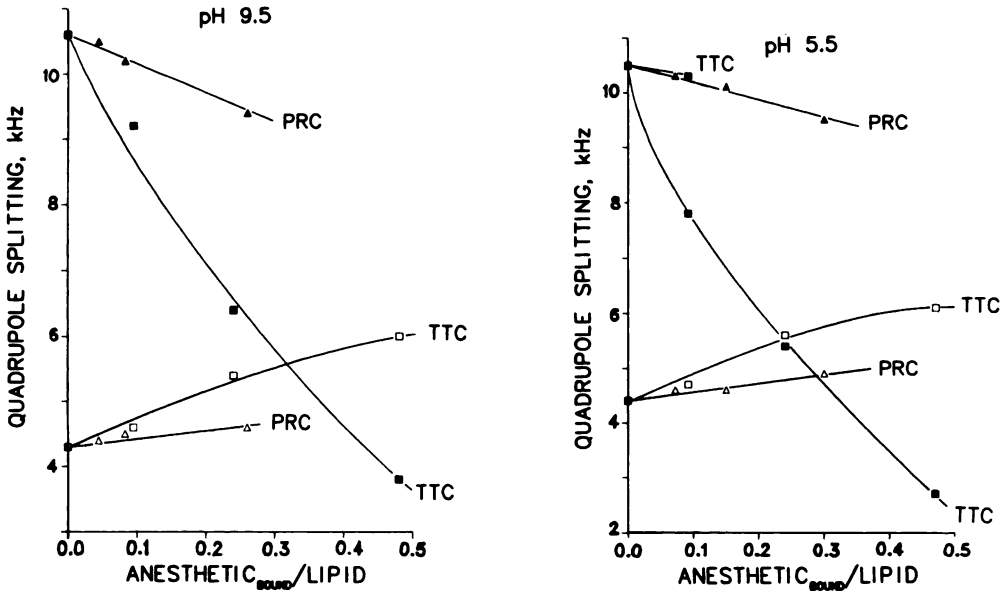


FIG. 1. *Variation of the quadrupole splittings of headgroup-labeled PE with the addition of TTC and PRC at pH 9.5 (left) and pH 5.5 (right)* ▲, $\alpha$ Deuterons with PRC; △, $\beta$ deuterons with PRC; ■, $\alpha$ deuterons with TTC; □, $\beta$ deuterons with TTC.

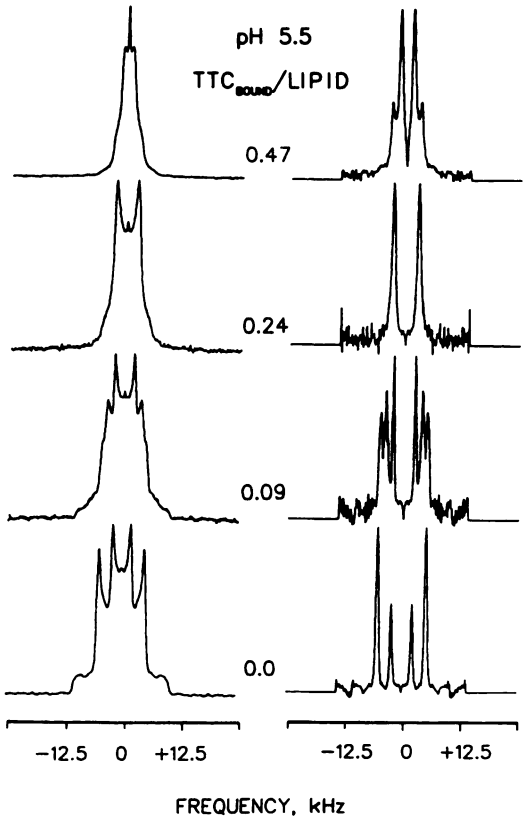


FIG. 2. *$^2$H NMR spectra (46 MHz) of a 1:1 mixture of 3-[1′,2′-$^2H_4$] DMPE and egg PE at pH 5.5 with varying amounts of TTC*

The TTC$_{bound}$/lipid ratio is indicated on the spectrum, and the de-Paked spectra are shown on the right.

neat PE sample, whereas the other has a substantially reduced quadrupole splitting. The assignment of the quadrupole splittings can be made from the integrated areas of the de-Paked spectra. With increased concentrations of TTC it is clear that the outer doublet for the $\alpha$-deuterons eventually disappears, whereas the inner doublet experiences an increase in intensity and a decrease in its quadrupole splitting. Concomitantly, the $\beta$ deuterons show a more gradual increase in their quadrupole splitting with added TTC. For the $\alpha$ deuterons, addition of very high concentrations of TTC (≈1/1) causes the quadrupole pattern to reduce to a narrow line and then increase again, indicating a change in the sign of the quadrupole splitting.

It is clear from the changes in the quadrupole splittings that the effect of TTC on the headgroup of PE is very pronounced, whereas PRC exerts only a very small effect. Since the size of the $^2$H quadrupole splitting is determined by both an average conformation and the amplitude of the fluctuations about the average conformation, the results with TTC must be explained by a change in the average conformation of the PE headgroup. A change in the amplitude of the fluctuations alone should change both of the quadrupole splittings in the same direction. The decrease in the $\alpha$ quadrupole splittings and the increase in the magnitude of the $\beta$ splittings when TTC is added are indicative of a conformational change which alters the torsional angles in the ethanolamine headgroup. Similar effects have been noted for the addition of the local anesthetic dibucaine and the ions Eu(III) and La(III) to headgroup-deuterated DPPE (21). The actual conformational change necessary to cause the observed changes in quadrupole splittings need not be large. Brown and Seelig (22) have shown that even subtle modifications of the ethanolamine torsional angles (<5°) can produce substantial changes in the observed quadrupole splittings.

The $^{31}$P NMR spectra of the neat semisynthetic egg PE, with and without anesthetics, were also recorded. The spectra at both pH values and at all concentrations studied were only of the type usually found for bilayer structures (20). Addition of anesthetic resulted in an

increase in the absolute value of the $^{31}P$ CSA (Table 1). This increase in the CSA is much more pronounced for TTC than PRC.

These data suggest that the anesthetic-induced conformational change also includes alterations in the phosphate orientation. Similar behavior has been observed in the interaction of several local anesthetics with PE and PC model membranes. Chlorpromazine, dibucaine, TTC, and PRC were observed to increase the CSA of natural egg PE (as opposed to the semisynthetic egg PE used in this work) at pH 7.0 (23). Diethazene and chlorpromazine, at neutral pH, cause a similar effect on DPPC (24). TTC (3) and dibucaine (21) increased the CSA of DPPC bilayers at pH 7.0, whereas at high pH TTC induced only a small change.

The effect of TTC on the PE headgroup is in sharp contrast to the effect of cholesterol. Cholesterol causes a reduction in the magnitude of the quadrupole splittings of the $\alpha$ and $\beta$ deuterons and induces only a small change in the $^{31}P$ CSA (22, 25). Although cholesterol increases the order of the hydrocarbon region (25, 26), it merely acts as a spacer between adjacent headgroups (22).

The spin-lattice relaxation times of the $\alpha$ and $\beta$ deuterons of the DMPE headgroup at pH 9.5 were also measured. Whereas the quadrupole splittings provide information on the amplitude of the anisotropic motion, the spin-lattice relaxation times ($T_1$) can give information on the rates of these motions (27). Addition of TTC (0.1/1) resulted in only a small reduction (≈10%) of the $T_1$ values (see Table 2), whereas PRC at the same concentrations showed no significant effect. At PRC/PE ratios of 0.5/1, the $T_1$ values were reduced, but by less than 10%. Clearly, the rates of motion of the headgroup are not significantly affected by the addition of anesthetics.

TABLE 1

*$^{31}P$ CSA for egg PE with added anesthetic*

| Anesthetic | Anesthetic/lipid ratio[a] | CSA |
|---|---|---|
| | | *ppm* |
| pH 5.5 | | −39.5 |
| TTC | 0.05 | −40.5 |
| | 0.24 | −42.4 |
| | 0.34 | −43.7 |
| PRC | 0.14 | −40.0 |
| pH 9.5 | | −37.2 |
| TTC | 0.05 | −39.9 |
| | 0.08 | −40.5 |
| | 0.23 | −43.0 |
| | 0.47 | −44.7 |
| PRC | 0.14 | −40.1 |

[a] Anesthetic/lipid ratios are based on bound anesthetic.

TABLE 2

*Spin lattice relaxation times ($T_1$) of a 1:1 mixture of 3-[1′,2′-$^2H_4$] DMPE and egg PE*

| | No anesthetic | TTC/PE[a] ≃ 0.1 | PRC/PE[a] ≃ 0.05 | PRC/PE[a] ≃ 0.25 |
|---|---|---|---|---|
| | | *msec* | | |
| $\alpha$ Deuterons | 23.5 | 19.7 | 22.8 | 21.4 |
| $\beta$ Deuterons | 22.5 | 20.8 | 22.7 | 20.9 |

[a] Anesthetic/PE ratios are based on bound anesthetic, calculated from the partition coefficients.

Experiments similar to those described above were also performed on equimolar mixtures of the 3-[1′,2′-$^2H_4$]DMPE and bovine PS. Figure 3 shows the effect of TTC and PRC on the quadrupole splittings of the labeled DMPE at pH 9.5 and 5.5. As in the PE experiments, TTC showed a much more pronounced effect than PRC, and this effect was, again, only slightly greater at pH 5.5 than at 9.5. However, there was no evidence for a third quadrupole splitting, as was observed in the neat PE experiments. The effect of the local anesthetics on DMPE/bovine PS mixtures is quantitatively similar to the reduction in quadrupole splittings observed in the DMPE/egg PE mixtures.

*Hydrocarbon region.* The effect of anesthetics on the hydrocarbon region of the PE bilayer has been studied using several specifically deuterated DMPE species. The DMPE was deuterated at position 2 of the *sn*-1 chain (1-[2′-$^2H_2$]DMPE), position 2 of the *sn*-2 chain (2-[2′-$^2H_2$] DMPE), or simultaneously at positions 4 and 14 of the *sn*-2 chain (2-[4′,14′-$^2H_5$]DMPE). These were mixed 1:1 with the semisynthetic egg PE, and the $^2H$ NMR spectra were recorded at both pH 5.5 and 9.5 with the addition of PRC and TTC.

The addition of PRC to the DMPE/egg PE mixtures at pH 9.5 caused no measurable spectral change for position 2 of either chain. However, the quadrupole splittings at positions 4 and 14 were reduced slightly (≈6%). At pH 5.5 a similar effect was noted, with the exception that position 2 on the *sn*-1 chain showed a very slight (≈3%) reduction in quadrupole splitting. The variation of quadrupole splittings with the addition of PRC is shown for both pH 9.5 and 5.5 data in Fig. 4.

The influence of TTC on the PE hydrocarbon region is much more pronounced than that of PRC at similar concentrations of bound anesthetic. At pH 9.5 the quadrupole splittings for all chain positions are reduced, with the most pronounced reductions occurring at positions 4 and 14 (Fig. 5). At pH 5.5, both positions 2 experience reductions in quadrupole splitting which are almost identical with those observed at pH 9.5. However, positions 4 and 14 each show two quadrupole patterns at low TTC concentrations. This is entirely analogous to the effect of TTC on the $\alpha$ deuterons of the headgroup at pH 5.5. For each of positions 4 and 14, one of the quadrupole patterns shows only a very small reduction in the splitting, but a large decrease in intensity as TTC is added. At concentrations greater than 0.1/1 ($TCC_{bound}$/lipid), this pattern disappears completely. The other pattern shows a substantial reduction in splitting with added TTC and an increase in intensity until it is the only signal observed. Figure 6 shows the $^2H$ NMR spectra of a 1:1 mixture of 2-[4′,14′-$^2H_5$]DMPE and egg PE at pH 5.5 with added TTC. The de-Paked spectra clearly show the two components for each position and the changes in intensity.

*Influence of pH.* All of the $^2H$ and $^{31}P$ NMR experiments showed a surprising similarity in results at high and low pH. With the exception of the observation of a

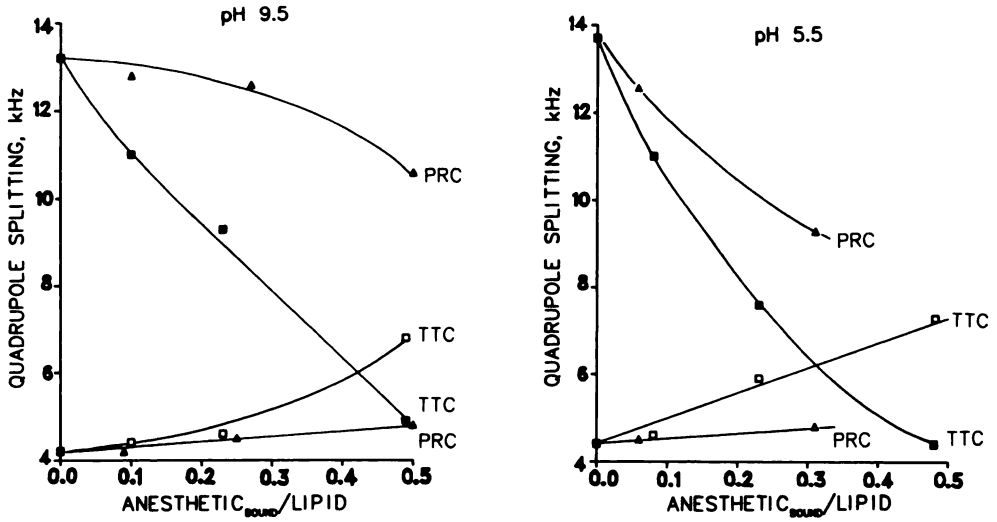


FIG. 3. *Dependence upon anesthetic bound of the quadrupole splittings of headgroup-labeled DMPE, in a 1:1 mixture with PS, at pH 9.5 (left) and pH 5.5 (right)*

▲, α Deuterons with PRC; △, β deuterons with PRC; ■, α deuterons with TTC; □, β deuterons with TTC.

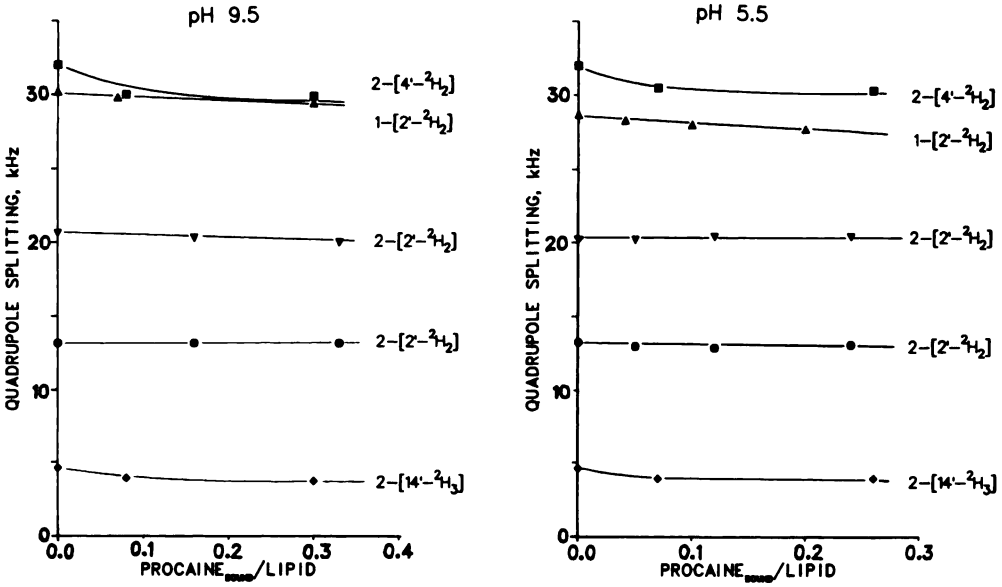


FIG. 4. *Influence of PRC on the quadrupole splittings of PE hydrocarbon region at pH 9.5 (left) and pH 5.5 (right)*

portion of the PE that is unaffected by low concentrations of TTC at pH 5.5 (*vide infra*), both the $^2H$ quadrupole splittings and the $^{31}P$ CSA at high and low pH show variations in magnitude which are quantitatively very similar. This similarity extends to the DMPE/PS mixtures, where only a slightly larger effect is noted at pH 5.5.

The results of these experiments are in sharp contrast to those for the interaction of TTC with labeled PC, where substantially larger effects were observed at pH 5.5 than at pH 9.5 (6). A similar, strong pH dependence has been noted in the $^2H$ NMR spectra of labeled TTC in egg PC (2, 5, 6). It has been postulated that in PC the charged TTC sits higher in the bilayer and interacts more strongly with the headgroup, whereas uncharged TTC penetrates more deeply into the hydrocarbon region. The deeper penetration of TTC results in closer contact of the bulky benzoid moiety with the PC acyl chains. The proximity of TTC thus opposes any disordering effort due to increased intermolecular separation. This results in quadrupole splittings which are much less affected at pH 9.5 than at pH 5.5 (6).

In the present study the similarity between the results at high and low pH indicates a similar depth of penetration for the charged and uncharged forms of the anesthetics. This is in agreement with the results of our studies with labeled anesthetics (4), which were also supportive of a charge-independent location for TTC and PRC in the bilayer.

*TTC lateral diffusion.* The presence of two signals for the 4, 14, and head group α positions at pH 5.5 and low TTC concentration can be interpreted as a manifestation of slow lateral diffusion of TTC through the bilayer. If the diffusion is slow on the $^2H$ NMR time scale, not all of the PE molecules will experience the influence of the anesthetic at low TTC/PE ratios. As the TTC concen-

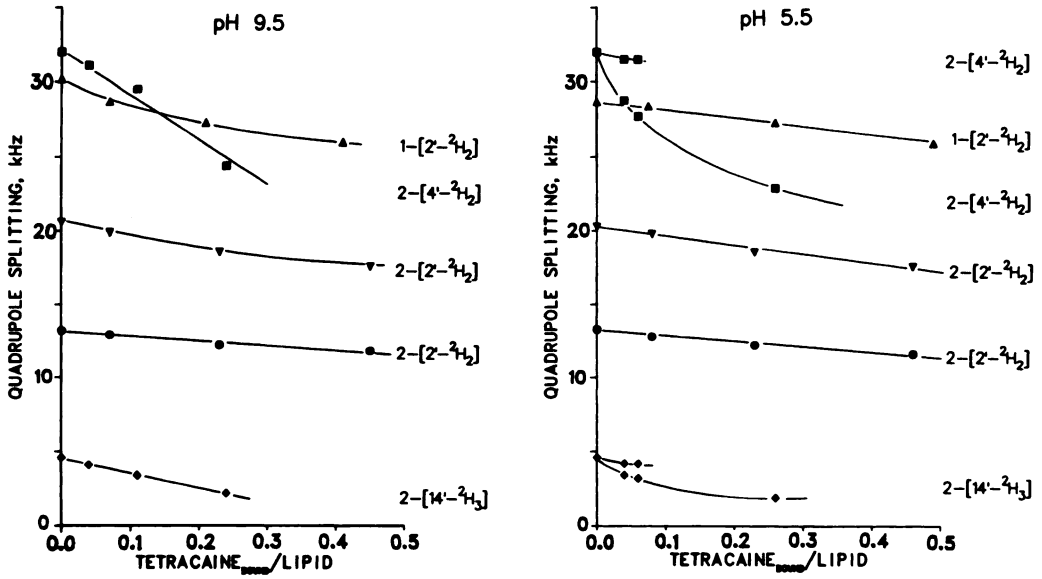


FIG. 5. *Influence of TTC on the quadrupole splittings of the PE hydrocarbon region at pH 9.5 (left) and pH 5.5 (right)*

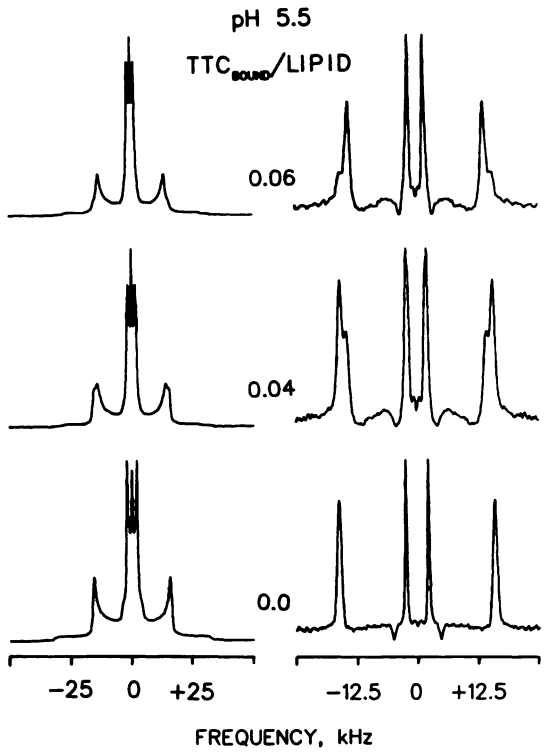


FIG. 6. *$^2H$ NMR spectra (46 MHz) of a 1:1 mixture of egg PE and 2-[4',14'-$^2H_5$]DMPE at pH 5.5 with varying amounts of TTC*

The TTC$_{bound}$/lipid ratio is indicated on the spectrum, and the de-Paked spectra are shown on the right.

tration is increased, the proportion of PE unaffected by TTC decreases and, finally, disappears (Figs. 2 and 6). For position 2 and the $\beta$ deuterons of the headgroup, TTC addition does not reduce the quadrupole splittings sufficiently to allow resolution of two signals. However, careful examination of the de-Paked spectra does show a slight asymmetry to the lineshape, suggesting the presence of two components.

This relatively slow diffusion could arise as a result of two factors. First, since TTC is charged at pH 5.5, there could be an electrostatic interaction between TTC and the zwitterionic PE. This would retard lateral diffusion of TTC at pH 5.5, but not at pH 9.5, when TTC is uncharged, as observed. A second cause for the slow diffusion could be increased interaction between neighboring PE headgroups at the low pH (the pK of PE is ≥ 9.8). This would result in a "tighter" lipid matrix which would act to prevent lateral diffusion. It has recently been suggested (28) that the strong hydrogen bonds occurring between the headgroups in the crystal structure of DL-PE (29) and proposed for the PE headgroups in the gel state (30, 31) are also present, albeit reduced in strength, in the liquid crystalline phase. These interactions, which are not present in comparable PC systems, could also account for the increased quadrupole splittings of the acyl chain region of PE bilayers as compared with the corresponding PC (28, 32). Further evidence for the increased headgroup interactions comes from the observation of the specific volumes of PE and PC (33). In the gel state, dilatometry shows very similar results for the specific volume of PE and PC, but in the liquid crystalline phase the PC volume is larger than that of PE, suggesting stronger PE interactions.

An increased PE-PE interaction, as described above, would also act to slow the diffusion of the PE molecule itself. Since TTC and PE are present in the samples at similar concentrations (i.e., neither is in a large excess), the actual requirement of a "slow diffusion of TTC" will be a complex interplay of the diffusion of both TTC and the lipid. A more exact description is that in the lipid:TTC matrix the *net* diffusion must be such that on the $^2H$ NMR time scale, discrete areas of lipid in contact with TTC, and areas of free lipid, must exist. This slow diffusion could also account for the observation of two quadrupole patterns for each of the $\alpha$ and $\beta$ deuterons of headgroup-labeled DPPE in the presence of charged dibucaine (21).

It may also be that TTC induces localized changes in

the PE bilayer structure and that a slow exchange of lipids occurs between these two different phases (one with TTC and one without). The new phase (containing TTC) would certainly not be hexagonal, since that would result in a reduction by 2 in the quadrupole splittings, but possibly something more subtle such as a "ripple phase." Regardless, this still requires a slow diffusion of TTC between the two phases since two signals are observed.

*Membrane location of TTC and PRC.* The influence of TTC and PRC on the $^{2}H$ NMR and $^{31}P$ NMR spectra of labeled PE, in combination with the results for the labeled anesthetics (4), can be used to ascertain the location of the anesthetic in the bilayer. The similarity of the $^{2}H$ NMR quadrupole splittings, regardless of whether TTC is primarily in its charged or uncharged forms (ignoring the diffusion effects), indicates that the depth of intercalation of TTC into the bilayer must be very similar at high and low pH. The very small effects noted for position 2 of the *sn*-2 chain suggests that the bulky aromatic ring is located at, or just above, that level (Fig. 7). Since this pushes neighboring lipids apart, the acyl chains below have more room and less motional constraint. The reduced quadrupole splittings at positions 4 and 14 are consistent with this hypothesis. Location of the aromatic ring at the level of the glycerol backbone permits the amine headgroup of TTC to interact with the PE headgroup. The similarity of the results at high and low pH supports the molecular shape model for the interaction of TTC with PE, as proposed in our earlier work (4). It is clear that shape and volume considerations, and not anesthetic charge, govern the location of TTC within the PE bilayer.

This model contrasts with that for the interaction of TTC with egg PC (2, 5, 6). In egg PC, TTC sits higher in the bilayer when it is charged and it penetrates more deeply when it is uncharged. For TTC interacting with PC it is clear that the anesthetic charge is a dominant factor in determining the anesthetic location, and the molecular shapes are less so.

Although PRC induces only minor changes in the $^{2}H$ and $^{31}P$ NMR spectra of the labeled PE, the anesthetic does interact with the bilayer. The observation of a quadrupole pattern for PRC, specifically deuterated in the aromatic ring (4), in the presence of egg PE indicates an ordered environment for bound PRC and not just a loose surface effect. However, this quadrupole splitting is almost 40% less than that observed for the corresponding label on TTC. This would be consistent with a PRC molecule whose aromatic ring sits higher in the PE

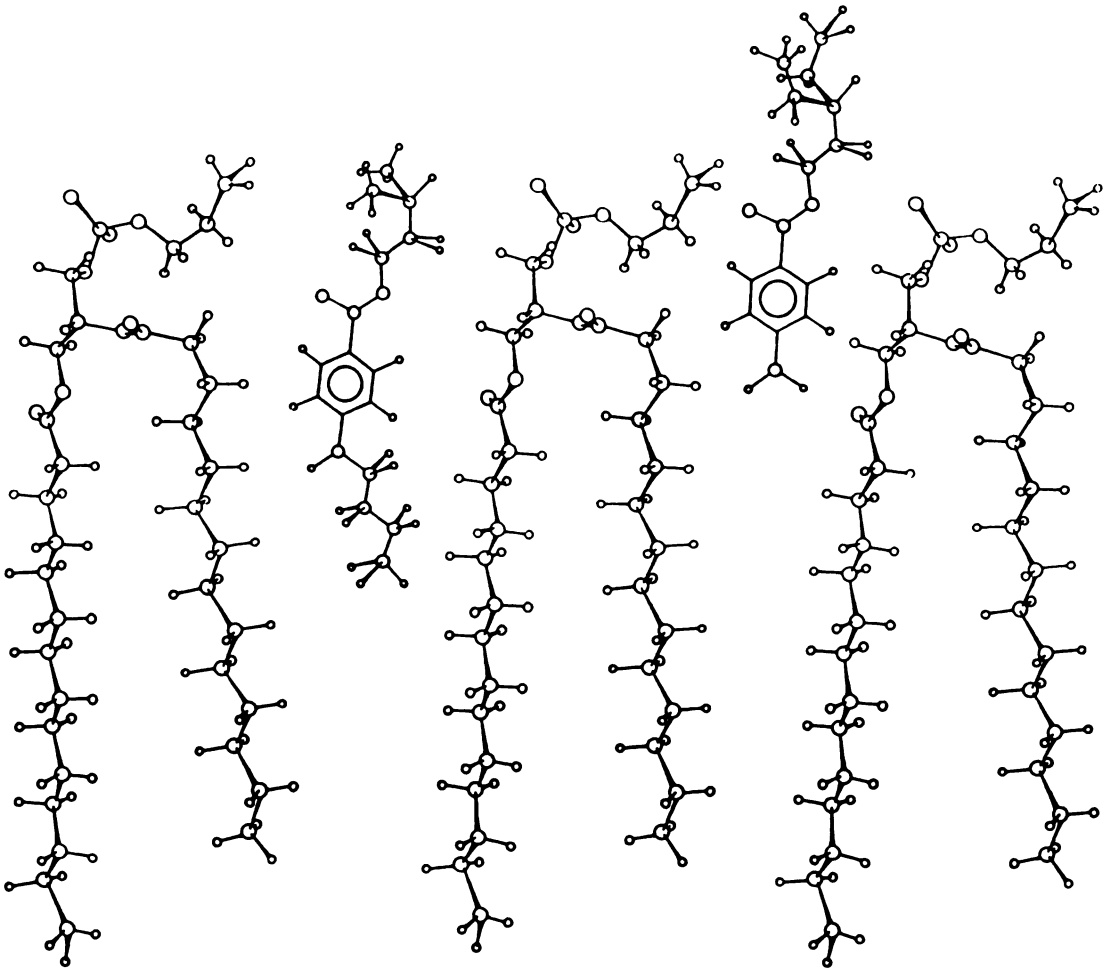

FIG. 7. *Proposed locations of TTC (left) and PRC (right) in the PE bilayer*

bilayer than does TTC (Fig. 7). This higher position for PRC also explains the lack of a quadrupole splitting for PRC-$d_4$. The *N*-ethyl groups of PRC probably reach out into the bulk water enveloping the membrane, and are therefore subject to substantial motional averaging. It would appear, therefore, that PRC does not penetrate into the hydrocarbon region, preferring to locate only in the hydrophilic headgroup region.

## CONCLUSIONS

The studies of the effect of TTC and PRC on PE bilayers have demonstrated the importance of the butyl group to TTC. Although the anesthetics are otherwise very similar, only TTC is able to penetrate significantly into the bilayer. Both induce changes in the ethanolamine headgroup. It would appear that both the charged and uncharged forms of TTC penetrate to the same depth in the PE bilayer and that this depth is governed mainly by the molecular shapes of TTC and PE, and not by the charge on TTC.

The one substantial difference induced by pH changes is in the lateral diffusion rates. At pH 5.5 the *net* diffusion rate (a combination of the TTC and PE rates) is slow on the $^2$H NMR time scale, and two environments for PE can be detected. The slower diffusion likely arises from increased headgroup interactions between neighboring PE molecules and between PE and TTC. Since examples of slow lateral diffusion are quite rare, we suggest that studies on other phenomena, such as lipid-protein interactions, might be more successful with PE than with the currently popular PC.

Finally, because of the known preference of PE to occupy the inside of many membranes (34) and the propensity for charged amine anesthetics to be more effective when applied to the intracellular side of the membrane (35), these results are quite interesting. It may well be that for a heterogeneous site of anesthesia, of the type described by Trudell (1), PE is localized near the sodium channels. In such a case, the interactions observed might modulate the ability of the sodium channel to function and thus prevent the propagation of a nerve signal.

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