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Molecular Details of Anesthetic–Lipid Interaction As Seen by Deuterium and Phosphorus-31 Nuclear Magnetic Resonance†

Yvan Boulanger,‡ Shirley Schreier,§ and Ian C. P. Smith*

ABSTRACT: Phosphatidylcholines (PC) deuterated in the fatty acyl chain (*sn*-2 chain, carbons 2, 6, 12, and 16) and in the head-group methyl (PC-d_9) and methylene (PC-d_4) moieties have been synthesized. Addition of the local anesthetic tetracaine hydrochloride (TTC) to the lipid dispersions produced different effects, depending on whether the anesthetic was positively charged (pH 5.5) or uncharged (pH 9.0), as monitored by the deuterium and phosphorus-31 nuclear magnetic resonance (^{2}H and ^{31}P NMR) of the phospholipid. A decrease in the ^{2}H NMR quadrupole splittings of the PC fatty acyl chains was observed at both pH values, the relative disordering effect becoming larger with the depth in the bilayer of the carbon segment. The decrease in order was more pronounced at low pH. At pH 5.5, the effect of TTC on the head group deuterated PC dispersions was to increase the quadrupole splittings for the two most peripheral segments (*N*-methyl and methylene groups) but to decrease that of the *O*-methylene segment. At pH 9.0, TTC decreased slightly the quadrupole splittings for all three head-group segments. The ^{31}P residual chemical shift anisotropy of the PC phosphate group increased upon addition of TTC at low pH and remained unchanged at high pH. The results are interpreted in terms of a model that depicts the charged dimethylammonium moiety of the anesthetic as being located in the head-group region, while the uncharged form penetrates more deeply into the membrane. The changes observed at low pH in the ^{2}H and ^{31}P NMR spectra of the phospholipid head group resemble those caused by ions and suggest that the head-group portion of the molecule undergoes a conformational change upon interaction with the anesthetic. Similar results were obtained for a 1:1 phosphatidylcholine:phosphatidylserine mixture. The anesthetic-induced disordering leads to an increase in phospholipid cross-sectional area and a decrease in membrane thickness, which could be related to the membrane expansion of one proposed mechanism of anesthesia. The results corroborate the conclusions obtained in previous work using deuterated local anesthetics [Boulanger, Y., Schreier, S., Leitch, L. C., & Smith, I. C. P. (1980) *Can. J. Biochem. 58*, 986–995].

The lipid structure of natural and model membranes has been investigated with techniques such as X-ray diffraction (Janiak et al., 1979), electron microscopy (Branton, 1969), neutron diffraction (Büldt et al., 1978), electron spin resonance (ESR)[1] (Schreier et al., 1978), and proton (Sheetz & Chan, 1972), carbon-13 (Lee et al., 1974), deuterium (Smith et al., 1978; Seelig, 1977), and phosphorus-31 (Cullis & de Kruijff, 1979) nuclear magnetic resonance (NMR). Broad-band ^{31}P NMR has been mostly used to distinguish between bilayer, hexagonal, and isotropic phases (Seelig, 1978; Cullis & de Kruijff, 1979), whereas ^{2}H NMR has been applied to several phospholipid dispersion systems and biological membranes and constitutes an excellent probe of the degree of molecular order (Seelig & Browning, 1978; Smith et al., 1979). Phosphatidylcholine (PC), in particular, has been deuterated in every position and the extent of order experienced by each deuterated group monitored by ^{2}H NMR under varying conditions such as temperature (Ulmius et al., 1977; Davis, 1979), ionic strength (Brown & Seelig, 1977), degree of unsaturation of the fatty acyl chains (Seelig & Waespe-Šarčevič, 1978), protein content (Rice et al., 1979), and cholesterol addition (Stockton & Smith, 1976). However, except in the case of the local anesthetic benzyl alcohol (Turner & Oldfield, 1979) and of the tertiary amine local anesthetics procaine and tetracaine (Boulanger et al., 1980), no ^{2}H NMR study has been reported on the influence of anesthetics.

Several theories have been proposed for the mechanism of action of local anesthetics. They have been suggested to act at the membrane level by interaction with either proteins or lipids. Theories involving proteins have focused on the interaction with a specific receptor (Hille, 1980) or on nonspecific binding to hydrophobic sites (Richards et al., 1980). As for interaction with lipids, changes in overall organization of the bilayer (Butler et al., 1973) as well as effects on lipid phase transition (Hill, 1974; Jain & Wu, 1977) and lateral phase separations (Trudell, 1977) have been postulated to play a role. The phenomenon of membrane expansion caused by anesthetics (whether related to the lipid and/or the protein membrane components) has also been proposed to be involved with the mechanism of anesthesia (Seeman, 1975). As pointed out by Ritchie (1975), many experiments do indicate that more than one of these theories could apply. Most likely, the

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[1] Abbreviations used: PC, phosphatidylcholine; PC-d_9, phosphatidylcholine deuterated in the head-group methyl groups; PC-d_4, phosphatidylcholine deuterated in the head-group methylene moieties; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; PS, phosphatidylserine; TTC, tetracaine hydrochloride; BPC buffer, borate–phosphate–citrate buffer; NMR, nuclear magnetic resonance; ESR, electron spin resonance; CSA, chemical shift anisotropy.

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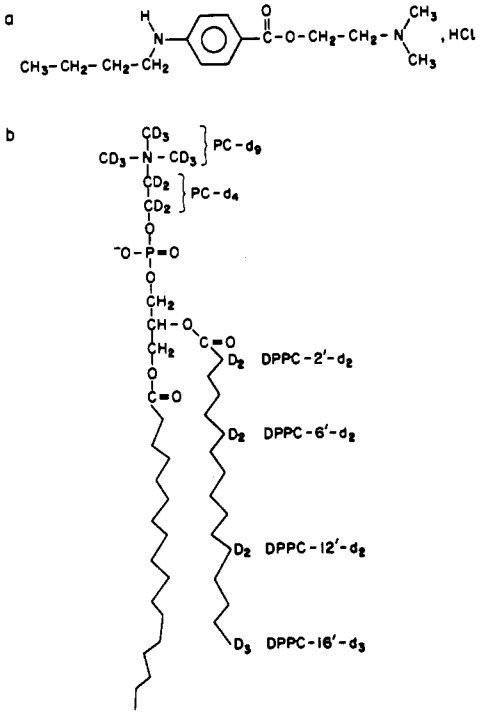

FIGURE 1: Structure of (a) the local anesthetic tetracaine hydrochloride (TTC) and (b) phosphatidylcholine (PC) deuterated in the *N*-methyl groups (PC-d_9, egg phosphatidylethanolamine fatty acyl chain composition) and in the head-group methylenes (PC-d_4, egg PC fatty acyl chain composition) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine deuterated at positions 2, 6, 12, and 16 of the *sn*-2 fatty acyl chain (DPPC-*2'*-d_2, DPPC-*6'*-d_2, DPPC-*12'*-d_2, and DPPC-*16'*-d_3).

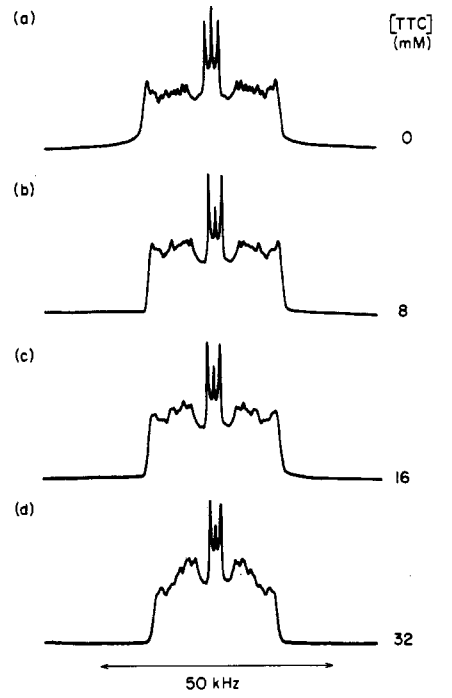

FIGURE 2: ^{2}H NMR spectra (46.06 MHz) of 65 mM DPPC-d_{62} multilamellar dispersions in BPC buffer at pH 5.5 with (a) no TTC, (b) 8 mM TTC, (c) 16 mM TTC, and (d) 32 mM TTC. The spectra were recorded at 47 °C by using the quadrupole echo technique (τ_1 = 60 μs, τ_2 = 50 μs), a 0.25-s recycle time, a 100-kHz spectral width, 4096 data points, a 5-μs 90° pulse, and 4000 scans.

mechanism of anesthesia consists of a series of steps, among which the above phenomena could be included. In addition, the possibility has to be taken into account that different anesthetics act by different mechanisms. Since very few studies have been done at the molecular level, we have chosen to study in detail the interaction between tertiary amine local anesthetics and model lipid membranes by use of magnetic resonance techniques. We have reported the results obtained with deuterated procaine and tetracaine (Boulanger et al., 1980). Here we present the effects of tetracaine on phospholipid membranes as monitored by ^{2}H NMR of perdeuterated and selectively deuterated phosphatidylcholines and by ^{31}P NMR.

The experiments were performed at pH 5.5 where the local anesthetic exists mostly in the positively charged form and at pH 9.0 where it exists in the uncharged form (Boulanger et al., 1980). The present data confirm the results obtained with deuterated anesthetics which indicate that the two forms bind to different membrane sites (Boulanger et al., 1980). The structures of tetracaine and specifically deuterated phosphatidylcholines are given in Figure 1.

Materials and Methods

Tetracaine hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO. 1,2-Dipalmitoyl-d_{62}-*sn*-glycero-3-phosphocholine was obtained from Lipid Specialties, Boston, MA. Phosphatidylethanolamine-*1,1,2,2*-d_4 (with egg PC fatty acid composition) was a generous gift of M. G. Taylor, National Research Council, Ottawa, Canada. Phosphatidylserine (PS, beef brain) was a product of Lipid Products, South Nutfield, United Kingdom. Deuterium-depleted water was purchased from Aldrich Chemical Co., Milwaukee, WI.

PC-d_9 was synthesized by deuteriomethylation of egg phosphatidylethanolamine as reported earlier (Boulanger et al., 1980). PC-d_4 was synthesized by exchange of the choline head group of egg PC with ethanolamine-d_4 by using phospholipase D (Taylor & Smith, 1981), followed by N-methylation according to the synthesis of PC-d_9 (Boulanger et al., 1980). The synthesis of specifically deuterated DPPC by established methods has been reported (Boulanger, 1980).

Lipid solutions in chloroform–methanol were evaporated under nitrogen and placed under vacuum overnight to remove the residual solvent. The dry phospholipid was then dispersed in a BPC buffer (0.02 N sodium citrate, 0.02 N sodium phosphate, 0.017 N sodium borate, and 0.1 N sodium chloride) at a temperature above that of the gel to liquid crystal phase transition. The sample pH values were measured and corrected if necessary. TTC was added in solid form, the sample vortexed, and the pH adjusted. All spectra were recorded above the gel to liquid crystal phase transition of the phospholipid: 30 °C for egg PC and 47 °C for DPPC.

^{2}H and ^{31}P NMR spectra were recorded on a Bruker CXP-300 spectrometer operating in the Fourier transform (FT) mode at 46.06 and 121.39 MHz, respectively. Some ^{2}H NMR spectra (spectral width less than 10 KHz) were obtained on a Varian XL-100 spectrometer modified for wide sweeps, radio frequency phase alternation, and accurate short-pulse intervals, operating in the FT mode at 15.36 MHz and 29 °C. The quadrupole echo technique (Davis et al., 1976) was used on both spectrometers.

Results

DPPC Perdeuterated in the Acyl Chains. Spectra of DPPC-d_{62} dispersions display a broad envelope arising from the superposition of the quadrupole splittings of all the deuterons of the palmitoyl chains (Figure 2a). It has been shown previously (Seelig & Seelig, 1974) that in DPPC the order parameter is high and constant for the first ten segments of the palmitoyl chains and decreases rapidly with increasing distance from the carboxyl group to low values near the terminal methyl group. The intense shoulders at the edges of

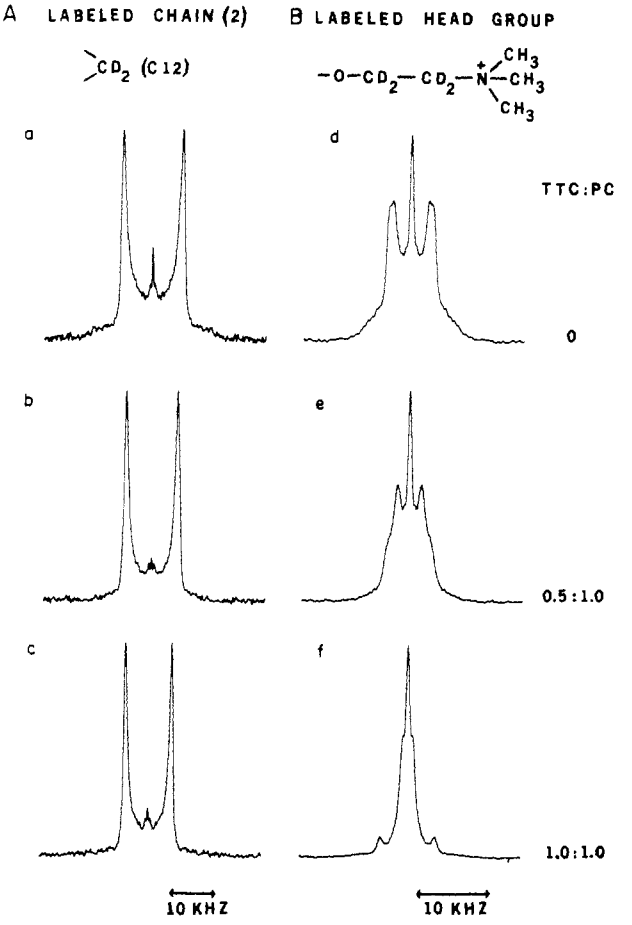

FIGURE 3: ^2H NMR spectra (46.06 MHz) of (a–c) PC-*12'*-d_2 at 47 °C and (d–f) PC-d_4 at 30 °C. The mole ratio of TTC:PC is indicated to the right of the spectra. All spectra were obtained at pH 5.5 in BPC buffer (32 mM lipid in 1.0 mL of buffer). The spectra were acquired with the quadrupole echo technique (τ_1 = 60 μs, τ_2 = 50 μs), 2048 data points, a 500-kHz spectral width, a 4.8- (PC-*12'*-d_2) or 8-μs (PC-d_4) 90° pulse, 50000 (PC-*12'*-d_2) or 15000 (PC-d_4) scans, and an 0.08- (PC-*12'*-d_2) or 0.2-s (PC-d_4) recycle time.

the ^2H spectrum are due to the deuterons in the region of high and constant order (Davis, 1979). Addition of increasing concentrations of TTC at pH 5.5 results in a decrease in intensity of these shoulders, with a buildup of intensity at frequencies corresponding to smaller order parameters (Figure 2b–d). Thus, it appears that insertion of TTC into the DPPC bilayer leads to a decrease in the extent of the region of high and constant order, with an overall disordering effect on the acyl chains. Due to the presence of two deuterated acyl chains and the overlap of quadrupole splittings from different positions, an accurate assessment of the disordering effect on individual positions is not possible, although an estimate could be made via the method of moments used by Davis et al. (1980). The one unequivocal quadrupole splitting, that due to the terminal methyl groups at the center of the spectrum, decreases steadily with increasing amounts of TTC bound to the DPPC.

At high pH a similar behavior is observed, except that lower TTC concentrations are required to produce the same effect due to the increased partition coefficient for the uncharged form (in egg PC, 30 °C, K_p = 660 at pH 9.5 and 22 at pH 5.5; Boulanger et al., 1980). In order to quantitate the disordering effects more precisely, we have studied specifically deuterated DPPC.

DPPC Specifically Deuterated in the sn-2 Acyl Chain. The structures of the compounds used are shown in Figure 1. Figure 3 shows the ^2H NMR spectra of DPPC-*12'*-d_2 in the absence and in the presence of TTC at pH 5.5, and Figure 4a shows the dependence of the quadrupole splittings on TTC concentration. At this position, as for the others studied along the chain, the quadrupole splitting was reduced upon addition of TTC. At pH 9.0, where the TTC is in its uncharged form, a decrease was observed for DPPC-*6'*-d_2, DPPC-*12'*-d_2, and DPPC-*16'*-d_3; however, for DPPC-*2'*-d_2, a very small increase was measurable (Figure 4b). The slopes of the curves of the quadrupole splitting values (Dq) as a function of concentration of TTC bound (calculated from the partition coefficient values of Boulanger et al., 1980) were greater for the charged (low pH, Figure 4a) than for the uncharged (high pH, Figure 4b) form.

The relative variation of the quadrupole splitting in the presence of TTC (Dq/Dq_0, where Dq_0 is the quadrupole splitting in the absence of TTC) is plotted in Figure 5 as a function of the amount of TTC bound. The relative effect of TTC on the different groups in the fatty acyl chain increases with the position of the carbon segment at both low and high pH.

Calculation of Phospholipid Dimensions. Deuterium order parameters can be used to calculate the effective length of a hydrocarbon chain with n segments (Schindler & Seelig, 1975). The length calculated for hydrocarbon chain segments 2–16 in the DPPC bilayer decreased from 12.7 to 12.2 Å upon

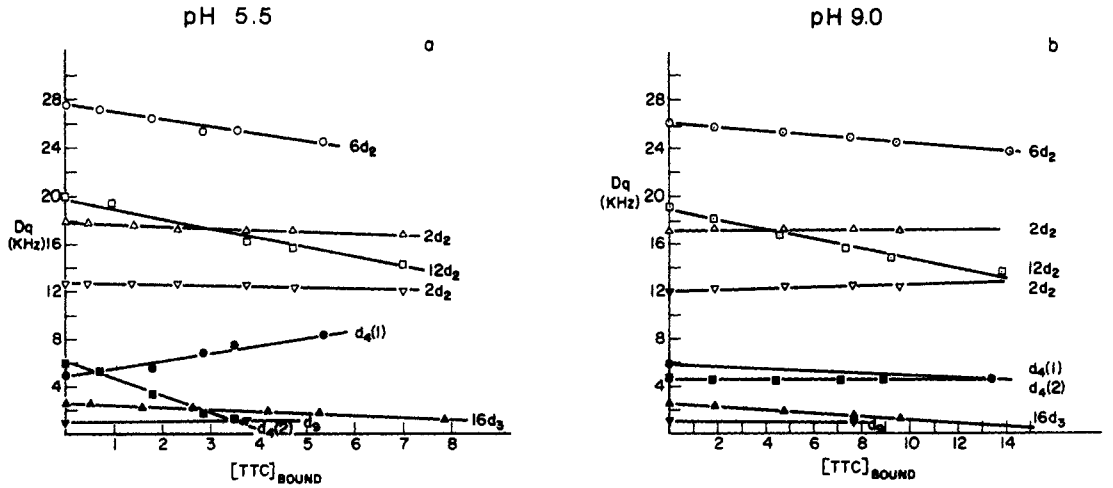

FIGURE 4: Graph of quadrupole splittings (Dq) of deuterated PC dispersions as a function of the amount of TTC bound at (a) pH 5.5 and (b) pH 9.0 (each unit corresponds to 0.05 mol of TTC/mol of PC). The spectra were acquired at 47 °C for DPPC samples and at 30 °C for egg PC samples (see Figure 1).

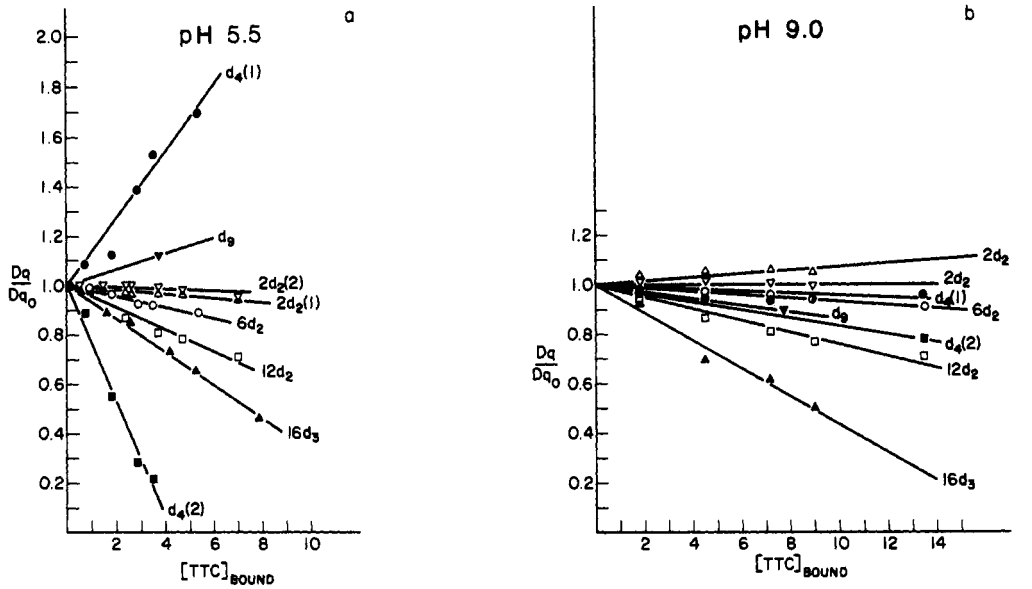

FIGURE 5: Graph of the ratios of quadrupole splittings (Dq/Dq_0) of deuterated PC dispersions as a function of the amount of TTC bound at (a) pH 5.5 and (b) pH 9.0 (each unit corresponds to 0.05 mol of TTC/mol of PC). Values calculated from data of Figure 4.

incorporation of 0.1 mol of TTC per mol of DPPC at pH 5.5. At pH 9.0, this length changed from 12.6 to 12.3 Å when the same amount of TTC was added. In terms of surface area per lipid molecule, this represents a variation from 63.4 to 66.0 Å^2 at pH 5.5 and from 63.7 to 65.4 Å^2 at pH 9.0. The charged species has a greater disordering effect on the phospholipid chain region and perturbs more the dimensions of this region than does the uncharged species.

Head Group Deuterated PC. Gally et al. (1975) have assigned the quadrupole splittings for the two choline methylene groups. Upon addition of TTC at pH 5.5, the quadrupole splitting of the *O*-deuteriomethylene (attached to the phosphate) decreases, whereas that of the *N*-methylene increases (Figure 3b). An increase in *Dq* was also observed for PC-d_9 (*N*-methyl groups) with TTC at low pH. At pH 9.0, however, a reduction in the quadrupole splittings of the three head group deuterated positions was recorded, the magnitude of the effect being much smaller for the two methylene groups than at low pH.

Phosphate Group of PC. ^{31}P NMR spectra of egg PC multilamellar dispersions display an increase in chemical shift anisotropy of approximately 8% at pH 5.5 upon addition of 0.5 mol of TTC/mol of PC (i.e., 0.34 mol of TTC bound/mol of PC) (Figure 6). No variation was observed at pH 9.0 in the presence of the same amount of TTC.

The single narrow resonance observed on the ^{31}P NMR powder pattern before addition of local anesthetic (Figure 6) arises from the buffer phosphate ions. The increase in intensity of that single resonance at low pH and the appearance of a second peak at high pH (Figure 6d) are indicative of the formation of an isotropic phase (possibly small vesicles) in minor amount. No fundamental structural change, such as from a bilayer to an hexagonal arrangement, is observed in the structure of the membrane.

Deuterated PC:PS (1:1). Addition of TTC to a DPPC-d_{62}:PS (1:1) dispersion produced disordering effects at both low and high pH, analogous to those observed in the absence of PS, as monitored by ^{2}H NMR. At pH 5.5 the head group deuterated PC-d_4–PS and PC-d_9–PS dispersions displayed a behavior identical with that of the PC system. At pH 9.0, the *Dq* values of both the PC-d_9–PS and the *N*-methylene group of the PC-d_4–PS systems increase in the presence of TTC, whereas the converse is true for the *O*-methylene in PC-d_4–PS.

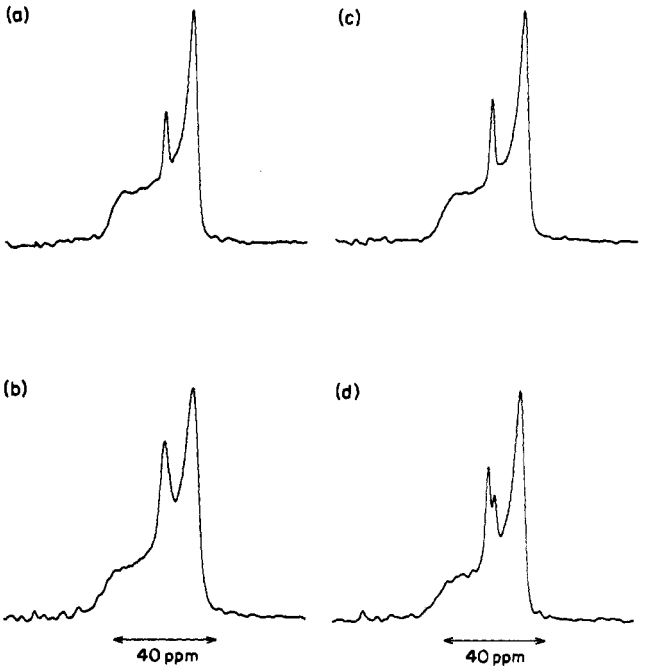

FIGURE 6: Proton-decoupled ^{31}P NMR spectra (121.39 MHz) of (a) 260 mM PC dispersions at pH 5.5, (b) 130 mM TTC in 260 mM PC dispersions at pH 5.5, (c) 173 mM PC dispersions at pH 9.0, and (d) 87 mM TTC in 173 mM PC dispersions at pH 9.0. Spectra were acquired with 4096 data points, a 125-kHz spectral width, a 2-s recycle time, 2500 scans, and a 4-μs 90° pulse length.

These changes are in the same direction as, but much smaller than, those observed at pH 5.5 (Figure 5b).

Discussion

Tetracaine perturbs membrane structure at both low and high pH. At low pH it affects both the head-group and the acyl chain region of PC membranes, whereas at high pH the changes are minor in the former region and, although considerable, are smaller than at low pH in the hydrocarbon region of the bilayer (Figures 3–5).

At pH 5.5, the quadrupole splittings of the *N*-methyl and *N*-methylene groups increase, but that of the *O*-methylene group decreases, upon addition of TTC (Figures 4 and 5). This is suggestive of a conformational change which modifies the torsional angles at the PC head group. It has been calculated

Table I: Percent Variation of ^2H Quadrupole Splittings for Methylene Groups, and of Chemical Shift Anisotropy for Phosphorus, at the Head Group of Phosphatidylcholines in the Presence of Anesthetic, Inorganic Ions, and Cholesterol

agent	phospholipid: agent mole ratio	-NCD_2-	-$POCD_2$-	^{31}P
TTC (pH 5.5)	10:1	25.6	−43.6	13.0[a]
	5:1	43.6	−93.6	
Eu(III)	(2.7-3):1	63.6	−83.1	*b*
La(III)	(2.7-3):1	56.8	−100	22.7
$[Fe(CN)_6]^{3-}$	(2.7-3):1	15.9	−33.9	15.9
TTC (pH 9.0)	10:1	−2.8	0	0
	5:1	−5.6	0	0
	2:1	−16.7	0	0
cholesterol	2:1	−13.6	−1.7, −8.5[c]	0
	1:1	−45.5	−1.7, −10[c]	0

[a] The molar ratio of phospholipid to bound anesthetic was 2.5:1. [b] Eu causes the CSA to change from −44 ppm to +44 ppm. [c] Cholesterol addition leads to two quadrupole splittings for these deuterons.

that a small modification of the head-group torsion angles can produce a substantial variation in the quadrupole splittings of deuterated groups in that region (Seelig et al., 1977). An effect analogous to that of TTC at low pH on the head group deuterated DPPC has been reported for ions such as $[Fe(CN)_6]^{3-}$, La(III), and Eu(III) (Brown & Seelig, 1977).

In Table I we compare the percent variation of *Dq* values for the choline methylene groups and of the ^{31}P chemical shift anisotropy (CSA), caused by TTC at pH 5.5 (this work), by the trivalent ions Eu(III), La(III), and $[Fe(CN)_6]^{3-}$ (Brown & Seelig, 1977), and by cholesterol (Brown & Seelig, 1978). The data in Table I are for 0.1 and 0.2 mol of *bound* anesthetic per mol of lipid, for a (2.7–3):1 mole ratio of DPPC:ion in the aqueous phase, and for 0.5:1 and 1:1 mole ratios of cholesterol:phospholipid. At pH 5.5 TTC causes changes in *Dq* values and CSA comparable in sign and magnitude to those caused by the ions [except for the CSA in the presence of Eu(III)]. Cholesterol, in contrast, changes *Dq* values for the *N*-methylene groups in an opposite direction and leaves that for the *O*-methylene group and the ^{31}P CSA almost unaffected.

The charged form of TTC thus behaves very much like the inorganic ions. It has been proposed by Brown & Seelig (1977) that the observed changes in spectral parameters induced by the ions could be related to conformational changes at the phospholipid head group, whereas the lack of a cholesterol effect on the *O*-CD_2 *Dq* value and on the ^{31}P CSA would indicate that the sterol does not alter the head-group conformation, acting only as a spacer.

It is conceivable that the charged form of TTC would affect the head-group conformation. The physical insertion of anesthetic between the lipid molecules could be partly responsible, and electrostatic interaction between the phospholipid phosphate and the anesthetic dimethylammonium moieties could alter the existing intra- and intermolecular electrostatic interactions.

The effect of TTC on the acyl chain ordering is to produce a pronounced decrease of the *Dq* values at low pH (Figure 4). The relative change of *Dq* (Dq/Dq_0, Figure 5) increases with the distance of the carbon atom from the head-group region. Taken in conjunction with the results obtained for the head group, these data suggest that the charged portion of the anesthetic molecule is located in the polar head-group region of the bilayer, with the alkyl tail descending into the acyl chain region. Since TTC is shorter than the phospholipid, the insertion of the anesthetic between phospholipid molecules would lead to an increase in the probability of gauche conformations at positions further along the acyl chain and, thus, to the observed lower values of *Dq*.

Uncharged TTC decreases *Dq* for the *N*-CD_2 moiety of PC, whereas the quadrupole splitting for the *O*-CD_2 and the ^{31}P CSA are unchanged (Table I). This behavior is very similar to that of cholesterol (0.5:1 mole ratio, Table I). However, unlike cholesterol, the anesthetic disorders the hydrocarbon chain at both low and high pH (Figure 4).

The small effect of TTC on the head-group spectral parameters at pH 9.0 suggests that the uncharged anesthetic penetrates more deeply in the membrane than does the charged form. Further support for this location is provided by the smaller decrease in *Dq* at pH 9.0 than at pH 5.5 for the deuterons at carbon atoms 6, 12, and 16 on the phospholipid acyl chain. A deeper penetration of the benzenoid ring into the bilayer would reduce the effective length of the region of the acyl chains with increased freedom for the formation of the bulkier gauche conformers. The small effect of TTC on the quadrupole splittings of the deuterons at position 2, at both pH 5.5 and 9.0, suggests that the contact between the anesthetic and the phospholipid is closest in this region of the acyl chain and that the phospholipid–anesthetic packing at this position does not differ much from phospholipid–phospholipid packing. This implies that the rigid benzenoid region of the anesthetic is located in this region.

Figure 7 is a schematic representation of the possible membrane location of the charged and uncharged forms of tetracaine. On the assumption that the cross-sectional molecular area of the phospholipid and the membrane thickness can be calculated from the molecular order parameters, the data indicate an increase of phospholipid cross-sectional area of 4.1% and 2.7% at pH 5.5 and 9.0, respectively, when 0.1 mol of anesthetic is bound per mol of phospholipid. Concomitant decreases in membrane thickness also result at both pH values; the charged form of tetracaine has the larger effect. Such phenomena have been invoked in the mechanism of anesthesia (Ritchie, 1975). The decrease in membrane thickness is in agreement with the conclusions of the ^2H NMR study using benzyl alcohol (Turner & Oldfield, 1979) but not with measurements of black lipid membrane capacitance and conductance which implied an increase in membrane thickness when using the same local anesthetic (Ashcroft et al., 1977).

We have previously examined the ^2H NMR spectra of deuterated TTC in PC membranes and found that multiple equilibria exist for the anesthetic between membrane and water (Boulanger et al., 1980). The data were indicative of membrane-bound anesthetic molecules in both fast (weakly bound) and slow (strongly bound) exchange with anesthetic in water. The two types of binding and exchange rate appeared to occur for both the charged and uncharged forms of the anesthetic but were more noticeable in the former case. We believe that the effects observed in the present study are due to the strongly bound form of TTC, at both low and high pH. It is possible that the weakly bound charged anesthetic resides in the electrical double layer at the membrane–water interface. If it is assumed that this weakly bound species behaves like other monovalent ions, it should not be expected to affect the head-group conformation (Brown & Seelig, 1977). Thus, the changes in lipid structure, both at the head group and at the acyl chain, can be ascribed to strongly bound TTC located between phospholipid molecules.

In the presence of PS at low pH, the ^2H NMR results indicate a location of TTC similar to that in PC alone. At pH 9.0, the changes in the ^2H NMR spectra of the head group deuterated PC–PS dispersions are in the same direction as,

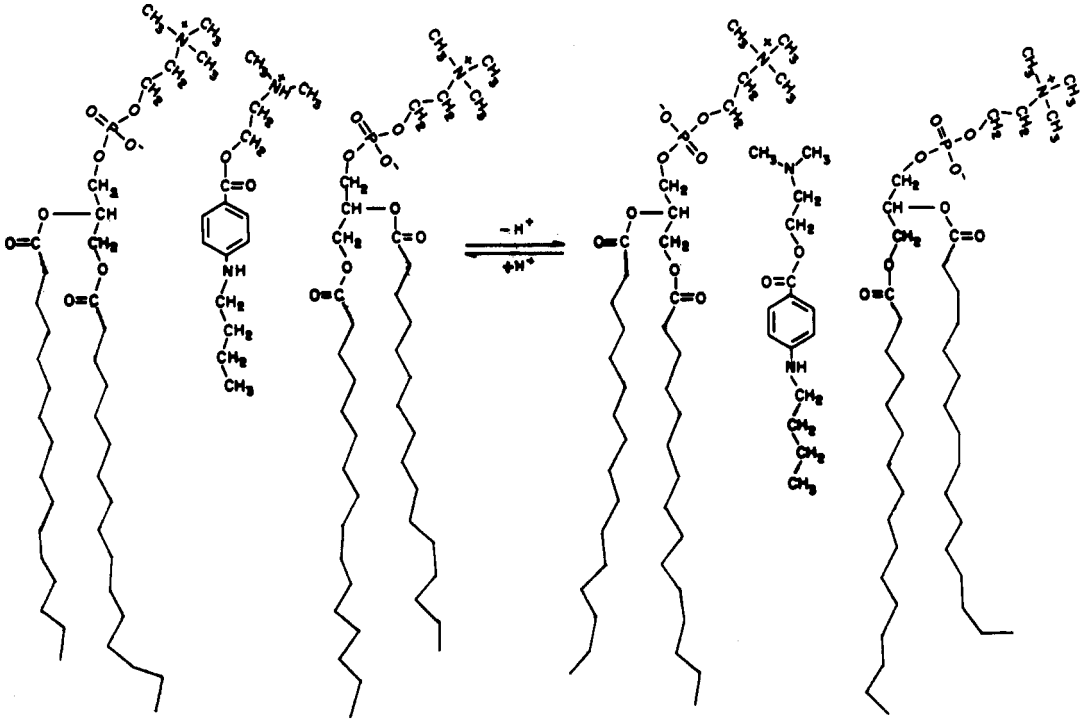

FIGURE 7: Model for the interaction of the local anesthetic TTC in PC lamellar dispersions. The positively charged TTC at low pH (left) remains mostly at the phospholipid head-group level, and the uncharged TTC at high pH is intercalated partly in the head-group region and partly in the fatty acyl chains of the phospholipid.

but much smaller than, those observed at pH 5.5. Although these data could be explained by a location comparable to that of TTC at low pH, it seems more likely, considering the difference in the quadrupole splittings of PC in the presence of PS (Smith et al., 1978) and the deuterated TTC data (Boulanger et al., unpublished experiments), that such is not the case. On the basis of the available data, we conclude that the positions of TTC in PC–PS dispersions at pH 5.5 and 9.0 are the same as those in PC dispersions at the corresponding pH.

The ^2H and ^{31}P NMR results for PC are in excellent agreement with those obtained previously with specifically deuterated TTC (Boulanger et al., 1980). For example, the quadrupole splitting of TTC deuterated on the benzenoid nucleus (TTC-d_2, ortho to the nitrogen substituent) decreases when the pH is increased. If the benzenoid moiety of the TTC molecule moves from the glycerol backbone region at low pH to the fatty acyl chain region at high pH (Figure 7), there should be such a decrease in the degree of order of the TTC aromatic nucleus following the change in order observed in the phospholipid itself (Stockton et al., 1976; Browning & Seelig, 1980). The quadrupole splitting of TTC deuterated in the two *N*-methyl groups (TTC-d_6) at low pH is very similar to that displayed by the choline *N*-methyl groups, which supports the location of both moieties in the same region and a similar electrostatic interaction with the phosphate group. We suggest that the lower value of Dq displayed by TTC-d_6 at high pH is due to the disappearance of the electrostatic interaction with the phosphate group leading to an increased freedom of motion concomitant with a change in location of the anesthetic molecule. In addition, a change in orientation of the anesthetic C–D vector with respect to the director of molecular ordering could play a role. The spectrum of TTC deuterated at the terminal methyl group of the butyl chain (TTC-d_3) does not yield a quadrupole splitting at low pH; the large degree of disorder of the phospholipid acyl chains in this region, combined with the intrinsic mobility of the anesthetic methyl group, would explain the reduction of the quadrupole splitting to a single resonance. At high pH, because of the lesser anesthetic-induced decrease in order in the fatty acyl chains, TTC-d_3 gives rise to a small quadrupole splitting (Boulanger et al., 1980).

Our results are in agreement with an ESR study of the effect of local anesthetics on planar multibilayers of ox brain white matter lipids (Butler et al., 1973). In the concentration range used in our studies, tetracaine disordered the lipid both above and below the pK_a of the anesthetic dimethylamino group.

It is necessary to demonstrate that the types of effect observed here are relevant at the concentrations of local anesthetic used clinically. Doses of up to 50 mL of 2% (9 mM) aqueous solutions have been used (de Jong, 1977). The concentration at the nerve site in vivo is difficult to ascertain. However, with isolated nerves, minimum blocking concentrations of 1–10 mM are reported (de Jong, 1977). The present effects are observed at similar concentrations.

The determination of the location of tetracaine in a phospholipid bilayer, and the details of its perturbation of the membrane structure, provides valuable information necessary for the understanding of anesthetic–membrane interaction. At present there is no consensus on the mechanism of anesthesia, but it seems clear that lipids are involved either directly or indirectly via lipid–protein interaction. Application of the techniques reported here to more complex membranes, including the protein components, should add significant, further understanding of the mechanism.

Added in Proof

The disordering effects noted here are consistent with the recent X-ray diffraction data (Coster et al., 1981), although the relative concentrations of lipid, water, and anesthetic were very different in the two studies.

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