Biochemical Pharmacology, Vol. 34, No. 11, pp. 1917–1924, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECT OF POSITIVELY CHARGED LOCAL ANESTHETICS ON A MEMBRANE-BOUND PHOSPHATASE IN *ACHOLEPLASMA LAIDLAWII**

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(*Received* 13 *May* 1983; *accepted* 26 *September* 1984)

Abstract—The plasma membrane *p*-nitrophenylphosphatase activity of *Acholeplasma laidlawii* was stimulated by the spin-labeled local anesthetic 2-[*N*-methyl-*N*- (2,2,6,6-tetramethylpiperidinooxyl)]ethyl *p*-hexyloxybenzoate, abbreviated as C6SL, and its methylated quaternary analog, C6SLMeI. The tertiary amine C6SL (at a concentration of 5×10^{-5} M) was more potent at pH 6.5 than at pH 7.7. In contrast, the permanently-charged C6SLMeI was equally potent, independently of pH. These results suggest that cationic forms of the anesthetics are responsible for stimulating the enzyme. Electron spin resonance studies of C6SL- and C6SLMeI-labeled membranes showed that these anesthetics in their cationic forms interacted electrostatically with components of the *Acholeplasma* membrane. For C6SL, this interaction was pH dependent and correlated with the pH dependency of the anesthetic-induced enzyme stimulation in the *Acholeplasma* membranes. Further, studies using 5-doxylstearic acid labels and non-spin-labeled anesthetics at various pH values showed that the membrane-fluidizing effect of anesthetics was not correlated with anesthetic-induced pNPPase stimulation. Our observations are consistent with the hypothesis that electrostatic interactions between cationic local anesthetics and anionic membrane components may lead to functional changes mediated by membrane proteins.

A variety of membrane functions mediated by membrane channels, receptors, and enzymes are sensitive to amine local anesthetics [1–4]. The wide range of functions affected by these amine compounds suggests that local anesthetics must act by affecting essential molecular properties common to many biological membranes. Furthermore, the reversible nature of anesthetic action suggests that the anesthetic-induced changes are not permanent alterations in membrane structure but are, instead, perturbations of dynamic processes in the membrane. The molecular mechanism by which membrane functions are altered by anesthetics could, therefore, provide important clues to these dynamic processes essential to membrane function. Thus, the action of local anesthetics on membrane enzymes is interesting in its own right and, in addition, may provide information on molecular mechanisms of membrane function.

Local anesthetics are small, amine amphiphiles with pK values close to the physiological pH. Therefore, both charged and uncharged forms may interact with membranes. Anesthetics intercalated in the membrane bilayer can diffuse laterally and interact with various membrane components including membrane phospholipids and proteins [5]. Physico-chemical studies have led to anesthetic mechanisms which are based on the ability of anesthetics to fluidize lipid bilayers [6, 7]. In contrast, physiological studies have suggested that anesthetics act by binding to membrane lipoproteins [8]. To test these hypotheses, we must seek structural and functional correlates of anesthetic action. If fluidization of lipid-bilayer indeed leads to changes in membrane function, we would expect a positive correlation between the changes in function and fluidization. On the other hand, if binding of anesthetic by certain membrane components is to be an important feature in the anesthetic mechanism, we would then expect to observe a correlation between such binding and the anesthetic effect.

Spin labels, which are also potent local anesthetics, as shown by voltage-clamped study in squid giant axons [9], have been synthesized [10] to study the binding of local anesthetics to membrane components. These local anesthetics, intercalated in the membrane bilayer, have been shown to be particularly useful for detecting interactions with membrane lipids and proteins at the polar–apolar interphase [5, 11].

We have searched for a membrane enzyme which shows well defined sensitivity towards local anesthetics. In this report, we describe the effects of local anesthetics on a membrane enzyme, *p*-nitrophenylphosphatase (pNPPase), in the *Acholeplasma ladilawii* membrane. We explore both the structural and biochemical correlates of local anesthetic–enzyme interaction. We show that tertiary amine local anesthetics stimulated the enzyme and that the cationic form was responsible for the stimulation.

* Supported in part by the Public Health Service (GM 28087), the National Science Foundation (PMC 75-23478), and the Senate Faculty Research Committee, UCSC.

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Electron spin resonance studies showed that this cationic form was partially immobilized and that the immobilization correlated with the stimulation of the pNPPase. In addition, studies using doxylstearate spin labels showed a fluidizing effect in the hydrocarbon region of the membrane in response to the presence of local anesthetics. However, the fluidization effect did not correlate with the stimulation of pNPPase.

MATERIALS AND METHODS

Acholeplasma laidlawii strain B cells (culture obtained from NIH, Research Resources) were grown on a modified Edwards medium [12] without lipid extraction. Membranes were isolated from the cells by osmotic lysis and differential centrifugation as described by Silvius *et al.* [13]. The membranes were washed twice with diluted buffer (2.5 mM Tris, 7.8 mM NaCl) and stored on ice until assayed. 2-Mercaptoethanol was omitted from the wash buffer because it may interfere with spin-label studies. All membrane preparations were used within 3–4 days.

Phosphatase activity was assayed at 37° in 100 mM Tris–MOPS* buffer, 5 mM $MgCl_2$, 100 mM NaCl, with 30–80 μg protein/ml using the discontinuous assay of Ne'eman *et al.* [14]. The reaction was stopped with 0.03% sodium laurel sulfate in 0.5 M Tris. In some experiments, activity was assayed by measuring A_{420} continuously with an Hitachi 110 spectrophotometer. NaCl was omitted from some assays; its presence or absence did not affect enzyme activity. The extinction coefficient of *p*-nitrophenol was measured as a function of pH and absorbance data reduced accordingly.

The local anesthetic 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl *p*-hexyloxybenzoate (C6SL), its quaternary analog (C6SLMeI), and a non-spin-labeled anesthetic (C6; Fig. 1) were synthesized in this laboratory [10]. All three compounds are potent local anesthetics at micromolar concentrations [9, 10]. The membrane/buffer partition coefficient at pH 7 was about 6000 for C6SLMeI and 6600 for C6SL in erythrocyte ghosts.† The pK values of both C2SL and C4SL (ethoxy and butoxy analogs of the local anesthetic used in these experiments) were approximately 7.2 to 7.4 as determined by the procedure of Tencheva *et al.* [15]. The C6SL itself was not sufficiently soluble for accurate determination, but the pK of its tertiary amine was expected to be the same as that of C2SL and C4SL.

For all ESR experiments, spin labels were added to membranes in assay buffer (at a lipid to spin-label ratio of 100:1). Anesthetic spin labels were usually added to the membrane suspension followed by mild agitation. Doxylstearate labels were dried from solvent on a rotary evaporator to form a thin film in a small round bottom flask; membranes in assay buffer were then placed in the flask and rotated on the evaporator for 20 min without vacuum. The membranes were centrifuged at 38,000 *g* for 40 min followed by two washes in buffer, and part of the pellet was sealed in a 100-μl capillary pipet. The ESR spectra were recorded (typically at about 3300 G, 9.5 GHz, and 10 mW power) with a Varian E-3 spectrometer interfaced to a PDP 11/10 computer with 12-bit resolution at the analog to digital conversion. Each scan was collected over 6 min through a scan range of 130 G. The noise filtration time constant was 0.1 sec. The modulation was 100 kHz at an amplitude of 1 G. Spectra were normalized to the same total number of spins (second integral of the ESR spectra).

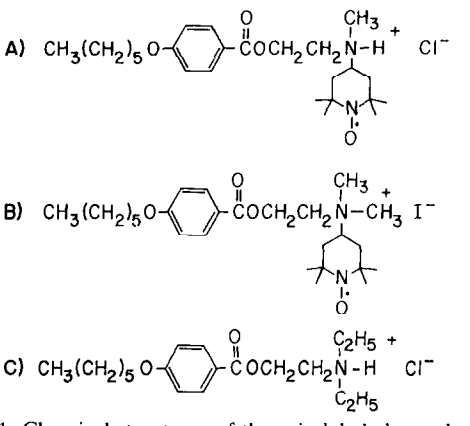

Fig. 1. Chemical structures of the spin-labeled anesthetics used in this study. (A) Tertiary amine (C6SL). (B) Quaternary amine (C6SLMeI). (C) Non-spin-labeled local anesthetic (C6).

Because of variation in the absolute activity of different enzyme preparations and of individual preparations with pH, velocities are frequently expressed as percent of control. Biochemicals were purchased from Calbiochem or Sigma. Protein was determined by the method of Bradford [16], using bovine serum albumin as a standard.

RESULTS

The local anesthetic C6SL and its permanently charged analog C6SLMeI both stimulated the pNPPase (Fig. 2). The pNPPase was stimulated at the same range of anesthetic concentration (25–100 μM) as that required for blocking sodium conductance in squid giant axons [9]. The stimulation by C6SLMeI was independent of pH in the range 6.4 to 7.7 (Fig. 2) while that by C6SL varied with pH (Fig. 2) in proportion to the charged form of the anesthetic weak base which has a pK_a of about 7.3 in aqueous solution. At pH 6.4, approximately 90% of the C6SL in the medium was charged and stimulation was slightly less than that by C6SLMeI. At pH 7.0, 70% of the C6SL in the medium was charged and stimulation was less than that of C6SLMeI. At pH 7.7, only 30% of the C6SL in the medium was

* Abbreviations: MOPS, 3-(*N*-morpholino)-propanesulfonic acid; HEPPS, *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; C6SL, 2-[*N*-methyl-*N*-(2,2,6,6-tetramethylpiperidinooxyl)]ethyl *p*-hexyloxybenzoate; C6SLMeI, methylated quaternary analog of C6SL (see Fig. 1); C2SL, ethoxy analog of C6SL; C4SL, butoxy analog of C6SL; and ESR, electron spin resonance.

† The values of the partition coefficients of C6SL and C6SLMeI were determined by Gary Blickenstaff in our laboratory; this result together with other material will be published separately.

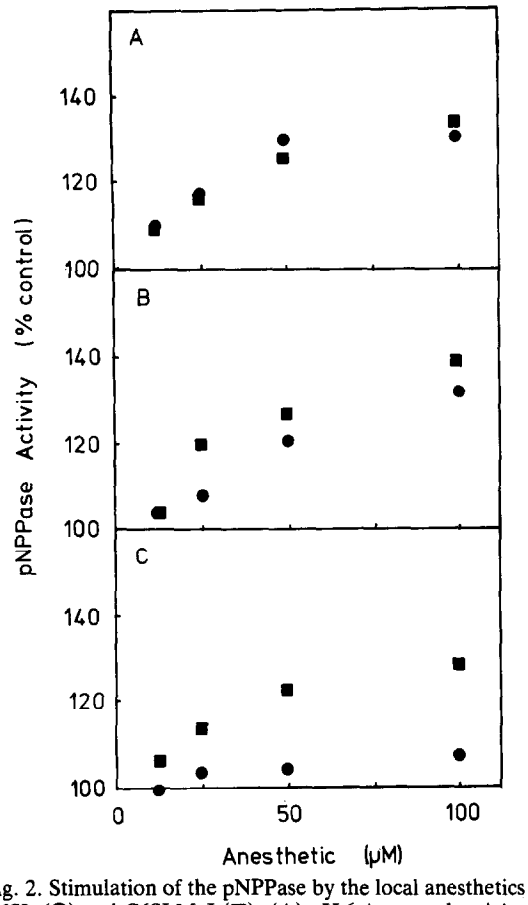

Fig. 2. Stimulation of the pNPPase by the local anesthetics C6SL (●) and C6SLMeI (■). (A) pH 6.4, control activity 0.160 A/min per mg protein, substrate concentration 0.2 mM (0.4 K_{app}). (B) pH 7.0, control activity 0.226 A/min per mg protein, substrate concentration 1.25 mM (0.5 K_{app}). (C) pH 7.7, control activity 0.278 A/min per mg protein, substrate concentration 5 mM (0.45 K_{app}).

charged and there was only weak stimulation. Lineweaver–Burk plots further confirmed this observation (Fig. 3). Relative stimulation by C6SL decreased as the pH rose (Fig. 3A–C), while stimulation by the permanently charged C6SLMeI was independent of pH (Fig. 3D–F). The observation that the permanently charged C6SLMeI was able to stimulate pNPPase over the entire pH range tested is confirmatory evidence that the stimulatory effect depends on the cationic form of the anesthetics. Possible stimulatory effects due to pH-induced changes in membrane or substrate structure are thus not a factor.

The pNPPase was similar to many alkaline phosphatases in that the K_{app} increased drastically with pH (Fig. 4B). V_{max} showed a weak optimum between pH 7 and 8 (Fig. 4A). At 10 mM substrate, the apparent pH optimum was below pH 7. This apparent optimum varies with substrate concentration because K_{app} varies with pH. Tris buffer did not stimulate the activity at higher pH values, as it does some alkaline phosphatases, because activity was the same for Tris–MOPS and HEPPS–MOPS buffer (data not shown).

This strong dependence of K_{app} on pH influenced the apparent stimulation by local anesthetics because the major effect of the anesthetics was to lower K_{app} (Fig. 3). For example, very little stimulation by C6SLMeI occurred at pH 6.4 when the substrate concentration was 10 mM, which was well above the initial, unstimulated K_{app} of 0.4 mM, whereas a larger stimulation occurred at pH 7.7 in which the drug lowered K_{app} from 12.6 to 9.4 mM at 50 μM anesthetic (Fig. 5 is included to illustrate this effect). Thus, the stimulation may appear to increase with pH when the substrate concentration is kept constant. Such apparent stimulation was even greater at high pH (8.6). This increase in apparent stimulation with pH also occurred with the local anesthetics dibucaine and tetracaine and with chlorpromazine (Fig. 6). As found with C6SL, all three of these drugs lowered K_{app} with small or negligible change in V_{max}; consequently, these drugs did stimulate pNPPase with a pH dependency consistent with that found for C6SL (data not shown).

To reveal the true effects of the drugs and their charged and uncharged forms at various pH levels, the measurement must distinguish between the effects of drugs and pH variations in the kinetic constants of the unstimulated enzyme activity (K_{app} and V_{max}). Because the major effect of the anesthetics is to lower K_{app} with little effect on V_{max}, we decided to measure anesthetic stimulation at the same relative position (substrate concentration) on initial velocity curves. Figure 5 shows the initial velocity as a function of substrate concentration at low and high pH. Stimulation by the permanently charged C6SLMeI was the same at both pH levels, although the substrate concentrations varied. If apparent stimulation were measured at 10 mM substrate concentration, very little effect would be seen at pH 6.4 where the velocity of the reaction has nearly saturated, while a larger effect would be seen at pH 7.7 where the unstimulated velocity is about $1/2V_{max}$. For this reason percent stimulation in Fig. 2 was measured with substrate concentrations of 0.4 to 0.5 K_{app} at each pH. When plotted this way, the effect on activity is obvious: C6SL stimulation decreased as the pH increased and the proportion of its charged form decreased. In contrast, C6SLMeI stimulation was constant, independently of pH.

This stimulation was also evident in the Lineweaver–Burk plots using a range of substrate concentrations. Stimulation by C6SL was marked at pH 6.4 where the charged form predominates. However, stimulation by C6SL decreased as the pH rose, consistent with the hypothesis that the charged form is responsible for the observed stimulation. Experiments with C6SLMeI confirmed this interpretation. The effects of both C6SL and C6SLMeI were comparable at pH 6.4 where C6SL in the medium was primarily charged and stimulation by the permanently charged form had no pH dependence. With the data normalized for pH-dependent changes in K_{app} and V_{max} of the unstimulated enzyme, Lineweaver–Burk plots for C6SLMeI were identical at all pH values (Fig. 3D–F).

Control experiments were also conducted to insure that we had not modified the enzyme by manipulating the pH values. Membranes were preincubated at

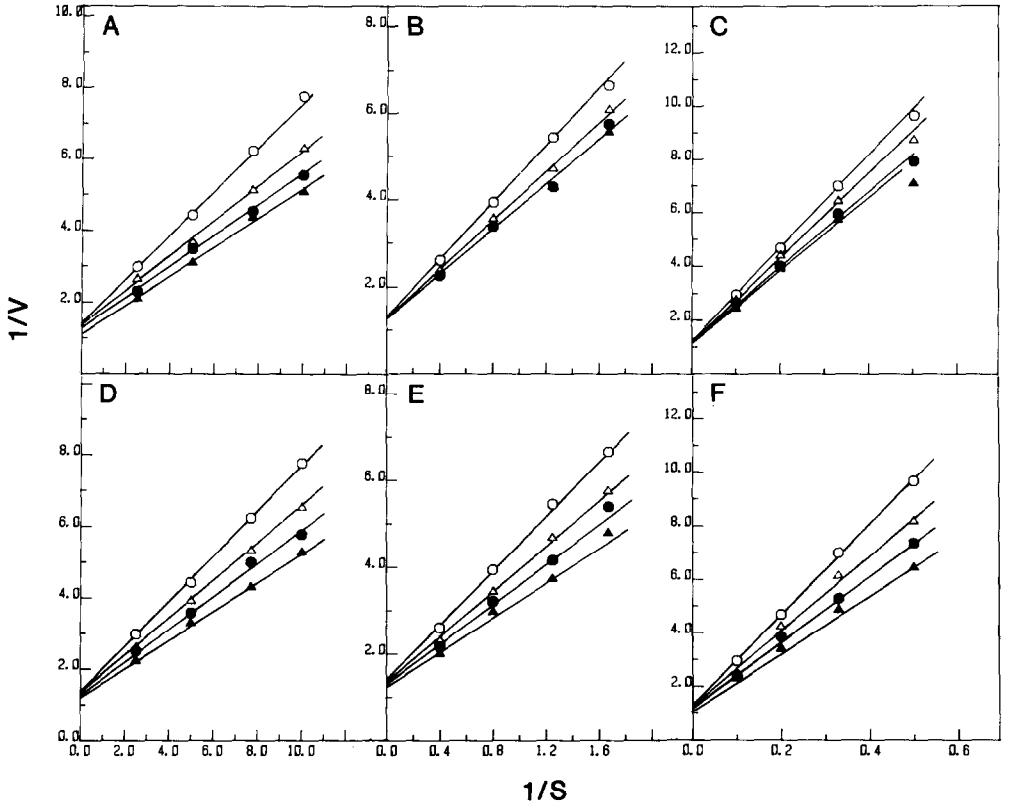

Fig. 3. Effects of C6SL and C6SLMeI on pNPPase activity at pH 6.4, 7.0, and 7.7 (Lineweaver–Burk plots). The ordinate ($1/V$) is (A/min per mg protein)$^{-1}$; the abscissa ($1/S$) is (mN pNPP)$^{-1}$. (A) Stimulation by C6SL at pH 6.4; (B) stimulation by C6SL at pH 7.0; (C) stimulation by C6SL at pH 7.7; (D) stimulation by C6SLMeI at pH 6.4; (E) stimulation by C6SLMeI at pH 7.0; (F) stimulation by C6SLMeI at pH 7.7. Key: (○) control activity without anesthetic; (△) 25 μM anesthetic; (●) 50 μM anesthetic; (▲) 100 μM anesthetic.

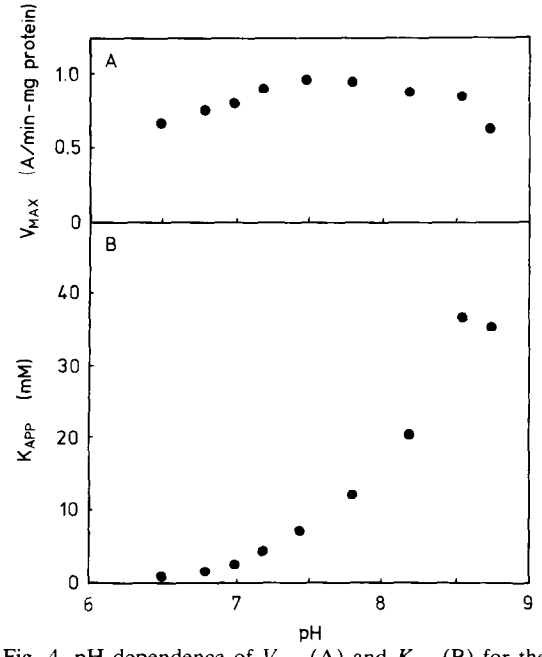

Fig. 4. pH dependence of V_{max} (A) and K_{app} (B) for the pNPPase in Tris–MOPS buffer. A Lineweaver–Burk plot was constructed at each pH and fitted using the method of least squares. V_{max} and K_{app} were calculated from this plot.

pH 6.4, 7.4 and 8.5, respectively, for 1 hr before washing and assaying at pH 7.4. No significant differences were observed between the various samples.

Nonionic detergents such as Lubrol WX also stimulated the membrane bound enzyme independently of the anesthetic effects. Lubrol WX stimulated the membrane bound enzyme (Fig. 7) at a concentration just above its critical micell concentration [17]. At higher concentrations of Lubrol, enzymatic activity was depressed, probably due to structural breakdown in the membrane. The result is typical for nonionic detergents and is in agreement with the report by Ne'eman *et al.* [18] of initial stimulation followed by inhibition. Similar stimulation by detergent of other membrane enzymes, such as the ($Na^+ + K^+$)-ATPase, has been reported previously [19]. Kinetic effects on the enzyme were investigated at several detergent concentrations. Double-reciprocal plots of the stimulation do not depend on pH and, in the range in which stimulation occurs, increase V_{max} 2- to 3-fold with a smaller effect on K_m. These kinetic effects differ from those observed for local anesthetics.

Although the effects on the kinetic parameters of the enzymes were different, we further examined the possibility that local anesthetics stimulate pNPPase by a non-specific detergent effect. We found that the permanently charged anesthetic C6SLMeI caused an

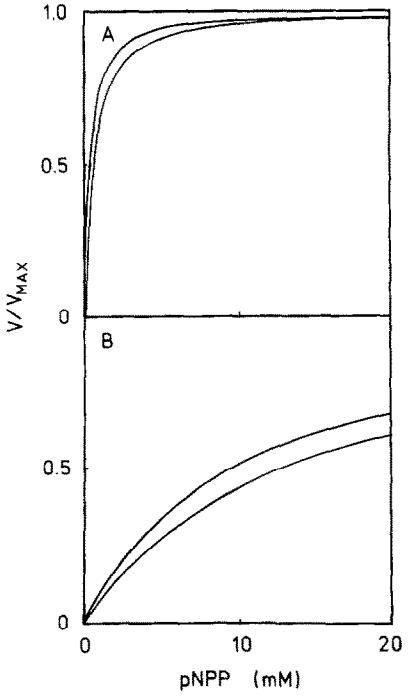

Fig. 5. Initial velocity curves for the pNPPase in the presence of 0 and 50 μM C6SLMeI. (A) pH 6.4, K_{app} = 0.48 and 0.32 mM for 0 and 50 μM C6SLMeI respectively; (B) pH 7.7, K_{app} = 12.6 and 9.4 mM for 0 and 50 μM C6SLMeI respectively.

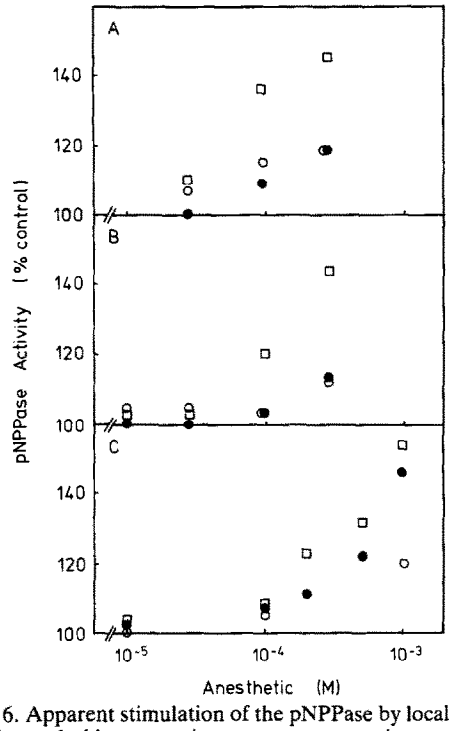

Fig. 6. Apparent stimulation of the pNPPase by local anesthetics and chlorpromazine at a constant substrate level of 10 mM pNPP. (A) Chlorpromazine: (○) pH 6.4, (●) pH 7.5, and (□) pH 8.2: control activities, 1.03, 0.75 and 0.38 A/min per mg protein respectively. (B) Dibucaine: as for chlorpromazine. (C) Tetracaine: (○) pH 7.2, (●) pH 7.8, and (□) pH 8.0: control activities, 0.56, 0.50, and 0.43 A/min per protein respectively (see text for interpretation of apparent pH dependence).

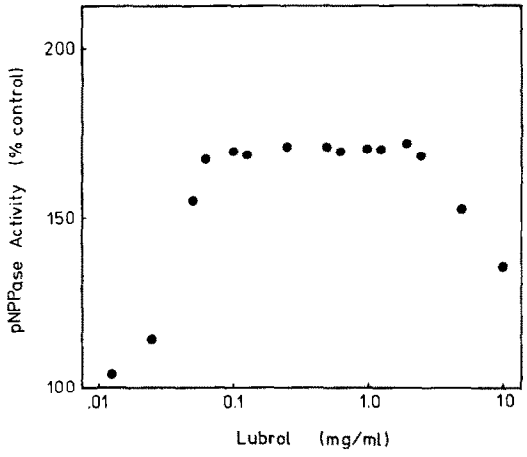

Fig. 7. Stimulation of the pNPPase by Lubrol WX with 10 mM p-nitrophenylphosphate as substrate, pH 8.0. Representative experiments, control activities range from 0.30 to 0.52 A/min per mg protein.

additional, independent stimulation. This result was explored over a range of Lubrol concentrations. As shown in Fig. 7, the detergent-induced stimulation of the pNPPase saturates at Lubrol concentrations just near 0.1 mg/ml. At Lubrol concentrations both above and below this saturation condition, the local anesthetic C6SLMeI further stimulated the pNPPase (Table 1). Further stimulation by C6SLMeI above the Lubrol-stimulated level was dose dependent; increasing the C6SLMeI concentration from 100 to 150 μM brought a substantial increase in pNPPase activity (Table 1). The C6SLMeI-induced changes remained stable despite a large fluctuation in Lubrol concentration (0–1 mg/ml; solubilization occurred near 1 mg/ml). C6SLMeI stimulation continued up to at least 200 μM. These results further support our interpretation that the local anesthetic-induced changes shown in Figs. 2, 3, and 6 were related to the cationic characteristics of local anesthetics, not to be mistaken as detergent effects.

In the presence of C6SL, *Acholeplasma* membranes gave rise to ESR spectra with line shapes that were pH dependent (Fig. 8A). At pH 6.4, the spectrum was a composite exhibiting two easily identifiable components of different widths plus a negligibly small free solution spectrum. As the pH increased, the broader (or more constrained) of the two components decreased relative to the more

Table 1. C6SLMeI-induced effects in the presence of Lubrol MX

Lubrol (mg/ml)	Change in pNPPase activity* (%)	
	100 μM C6SLMeI	150 μM C6SLMeI
0	38	57
0.1	29	50
1.0	25	39

* Percent control activity in the presence of detergent and anesthetic minus percent control activity in the presence of detergent alone.

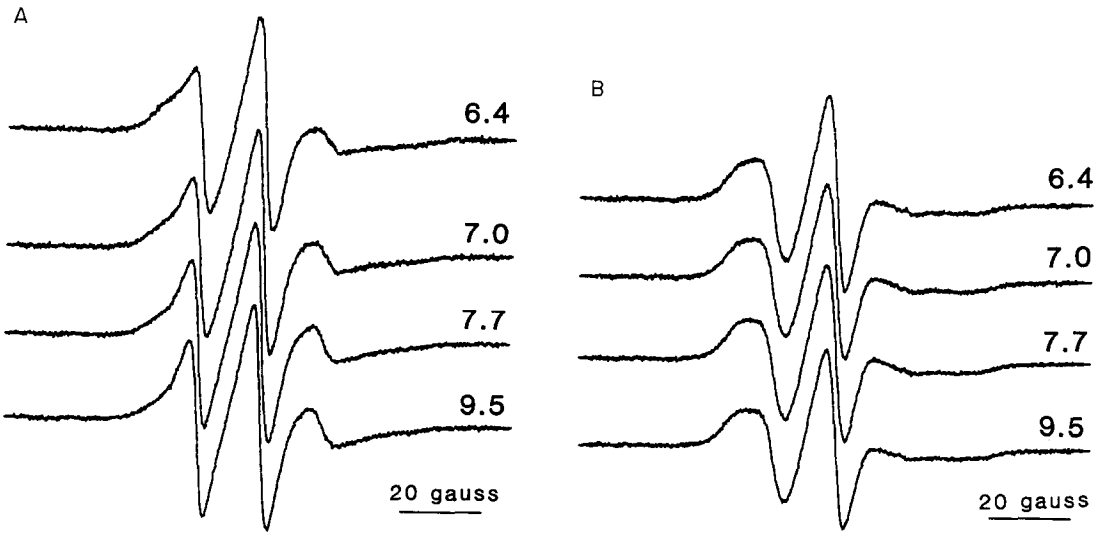

Fig. 8. (A) ESR spectra of C6SL-labeled membranes in assay buffer at pH 6.4, 7.0, 7.7, and 9.5. (B) ESR spectra of C6SLMeI-labeled membranes in assay buffer at pH 6.4, 7.0, 7.7, and 9.5.

mobile component. At pH 9.5, only the mobile component was observable. This result is consistent with the interpretation that the positively charged C6SL interacted ionically with membrane constituents to yield the constrained component. Spectral subtraction shows that at pH 6.4 the constrained component represented about 40% of the composite spectrum.

In contrast, membranes labeled with the permanently charged C6SLMeI gave spectra that were pH independent (Fig. 8B). This result is a strong indication that the pH effect on C6SL-labeled preparations is a reflection of the anesthetic charge, ruling out possible structural changes in the membrane as an explanation. Furthermore, the dominance of the constrained components in the ESR spectrum of C6SLMeI-labeled membranes suggests that most of the positively charged anesthetics interact with negatively charged membrane constituents.

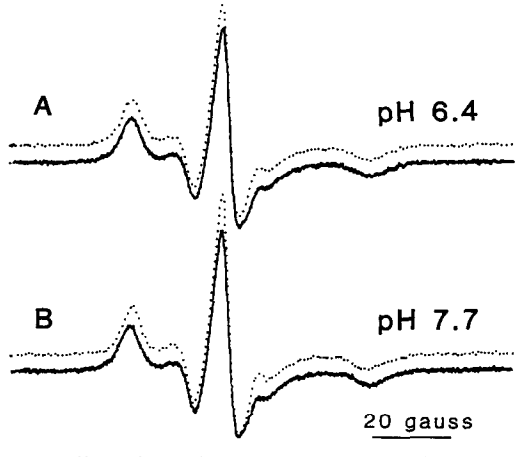

Fig. 9. Effect of anesthetics on membrane fluidity. Membrane fluidity of *Acholeplasma* membrane was monitored by 5-doxylstearic acid. Solid line: control experiment, with no anesthetic; dotted line: membranes in the presence of a 50 μM concentration of the anesthetic C6. (A) At pH 6.4, there was no significant difference (for both spectra $2A\| = 60.8$, and $2A\perp = 16.7$), but for (B) at 7.7, the anesthetic had an obvious fluidizing effect (solid line: $2A\| = 61.6$, $2A\perp = 16.7$; dotted line: $2A\| = 59.2$, $2A\perp = 17.5$).

At pH 6.4, C6SL ($pK_a = 7.3$) should be 90% charged. At the membrane, however, only 40% were observed to be constrained. This result may be interpreted in two way: either that not all charged anesthetics are constrained or that the anesthetic exhibits a lower apparent pK when associated with the membrane. Judging from the highly constrained spectrum obtained from C6SLMeI-labeled membranes, it would appear that most of the charged anesthetics are constrained. Thus, the latter interpretation is probably correct. A lowered apparent pK_a would incorporate corrections for any surface phenomenon which affects surface pH or pK_a [11, 20].

It has been suggested that anesthetics may affect enzyme activities indirectly by fluidizing hydrophobic regions of the bilayer. Experiments were carried out using 5-doxylstearic acid as the spin label to monitor membrane fluidity. A non-spin-labeled local anesthetic, C6 (Fig. 1C), was used to treat the membrane. At pH 6.4, there was little detectable difference between the spectrum of membrane treated with 5×10^{-5} M C6 and that of the control (Fig. 9A). In contrast, at pH 7.7 the spectrum of C6-treated membrane was significantly different from the control (Fig. 9B); the outer hyperfine splitting of the control was greater than that of the C6-treated membranes. This result suggests that, although C6 is capable of fluidizing the membrane, it is much more effective at higher pH. The pH dependency of the fluidizing effect is thus not correlated with pNPPase stimulation.

DISCUSSION

The local anesthetic C6SL and its permanently charged analog C6SLMeI both stimulated pNPPase

and did so at physiologically relevant concentrations. This stimulatory effect has not been reported previously and is of particular interest in that local anesthetics are usually known to inhibit enzymes [2, 21]. Our observation is not limited to spin-labeled local anesthetics since tetracaine, dibucaine, and chlorpromazine all produced similar stimulation of the pNPPase. This stimulation can be associated with the positively charged form of the anesthetic. Permanently charged C6SLMeI stimulated the enzyme independently of pH, whereas the weak-base C6SL stimulated in proportion to its charged form. The observed effect cannot be attributed to solubilization by detergent-like amphiphiles. Although the anesthetics are capable of fluidizing the membrane bilayer, this effect does not correlate with the pH dependency of pNPPase stimulation. A fluidizing effect is, therefore, not a satisfactory mechanism for explaining the observed pNPPase stimulation.

The ESR experiments distinguished two components (constrained and mobile) in the spectrum of C6SL which may be associated, respectively, with the charged and uncharged forms of the anesthetic. Stimulation of the enzyme by C6SL and C6SLMeI correlated with the amount of the charged, constrained, component present in the membrane. This result is consistent with the hypothesis that an electrostatic interaction between positively charged anesthetics and membrane constituents is responsible for the observed pNPPase stimulation.

Modulation of membrane enzyme activity by amphiphiles may be an important feature in cellular regulation. Several enzymes are known which depend on acidic phospholipids for activity or have their activity modulated by acidic phospholipids and which are affected by local anesthetics. The ATPase of *A. laidlawii* requires a small amount of PG for activity, as removal of the last 10% of the PG from the membrane by phospholipase digestion abolishes enzyme activity [22]. C6SL and C6SLMeI inhibited this enzyme (unpublished work). In contrast, removal of PG stimulates the pNPPase [22]. Another example of lipid–protein interactions is the calcium-activated, phospholipid-dependent protein kinase recently found in mammalian tissues [35]. This soluble protein is activated by association with acidic phospholipids in the cell membrane. In particular, PS activates the protein kinase at micromolar concentrations of calcium. Local anesthetics competitively inhibit this activation. Neither anesthetic nor phospholipid interacts with the active site of the enzyme. Finally, local anesthetics inhibited mitochondrial cytochrome oxidase, affecting both V_{max} and K_m. These effects varied with pH and have been interpreted in terms of anesthetic interactions with the protein and with its associated phospholipids [21].

Local anesthetics are amphiphilic molecules with pK values close to the physiological pH. Therefore, both charged and uncharged forms may interact with the membranes, although Schreier *et al.* [20] found that the apparent pK for the anesthetic at the membrane surface is lower. Considerable evidence also supports the idea that local anesthetics partition into the membrane by intercalating into lipid bilayers with the polar amine-head-group located at the polar–apolar interface of the membrane [23–25]. The hydrophobic tail group of the local anesthetic appears to be situated in the hydrocarbon region of the lipid bilayer [25–29]. Our own studies on the quenching of various fluorescent probes by local anesthetics also support this general, molecular orientation of amphiphilic local anesthetics in the membrane [30–32]. Indeed, this view agrees with the original findings of Skou [33] based on monolayer studies.

Rapid translational motion of membrane components in the plane of the bilayer [34] permits interaction between these components and intercalated local anesthetics. One possible consequence of such interactions is ionic binding between positively charged anesthetics and anionic membrane components such as phospholipids or proteins. Electrostatic binding of cationic local anesthetics to anionic sites on membrane components has been observed, using the local anesthetic spin labels C6SL and C6SLMeI [5, 11]. The anesthetics intercalated in the biological membrane can thus bind to phospholipids and membrane proteins under equilibrium conditions. In such a model, the concentration of anesthetics in the buffer medium is related to that in the membrane by the partition coefficient. Interactions between the anesthetic and various membrane components, in addition to membrane anesthetic concentration, further depend on the equilibrium binding constant for the respective interaction. If a charge interaction is relevant, as it is in the case of pNPPase, then pH and apparent pK_a are also determining factors. We are thus modeling the membrane as a two-dimensional reaction surface in which lateral diffusion permits equilibrium interactions between local anesthetics and membrane constituents.

We have found that the experimental data are consistent with the hypothesis that binding of cationic anesthetics to membrane constituents leads to pNPPase stimulation. Two general models for stimulation of the pNPPase by charged local anesthetics are possible: either electrostatic binding of cationic local anesthetics to phospholipids, or electrostatic binding of cationic local anesthetics directly to proteins in the membrane. In the first case, the interaction may perturb the normal lipid–protein interactions which modulate protein function. Interaction of local anesthetics with phospholipids has been demonstrated previously [5], thus charge interaction between anesthetics and phospholipids (such as phosphatidylglycerol) no doubt takes place. However, whether such interaction actually is responsible for the stimulatory effect is not yet established. Alternatively, direct anesthetic binding to the protein may change protein conformation. However, it is also possible that binding of cationic anesthetics alters the charge environment of the protein with consequent changes in protein function.

Acknowledgements—We thank Gary Blickenstaff for reading the manuscript and for providing the data on the partition coefficients of C6SL and C6SLMeI.

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