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Local Anesthesia: The Interaction between Phospholipids and Chlorpromazine, Propranolol, and Practolol

A. G. LEE

Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU, England

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SUMMARY

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Chlorpromazine and propranolol have been shown to reduce the temperature of the gel to liquid-crystalline phase transition in dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine, at concentrations comparable to those that block sodium conductance in nerve. Effects on the transition temperatures with increasing concentration are nonlinear because of the buildup of positive charge on the liposomes. Addition of myristic acid to the liposomes reduces this charge effect and so increases the binding of chlorpromazine and propranolol; the effect of myristic acid is masked by addition of Ca^{2+}. In contrast, practolol at concentrations up to 20 mM has no effect on lipid phase transition temperatures, and has little local anesthetic activity. These anesthetic effects are discussed in terms of the annular transition model; the sodium channel is postulated to be surrounded by an annulus of lipid in the gel state, the rigid lipid microenvironment ensuring maintenance of the optimum configuration for the channel. Addition of anesthetics triggers a change in the lipid annulus from the gel to the liquid-crystalline state, with concomitant relaxation of the protein and reduction in the size of the channel.

INTRODUCTION

A large variety of organic molecules show local anesthetic activity. This suggests that local anesthesia must result from a relatively nonspecific interaction, rather than from specific binding of the drug to a receptor in the membrane. A possible mechanism has been suggested elsewhere (1): the sodium channel in nerve is embedded in a rigid lipid environment, and addition of local anesthetics causes a transition of the lipid into the liquid-crystalline state, allowing relaxation of the sodium channel with concomitant reduction in the sodium conductance. This paper reports on the correlation between the local anesthetic effects of two antihypertensive agents and chlorpromazine, and their effects on the phase transition temperature of lipids.

Chlorpromazine is a major tranquilizer, and acts to suppress hallucinations in conditions such as schizophrenia, probably by interfering with dopaminergic transmission in the limbic system of the brain. The effects of chlorpromazine could be due either to nonspecific blockade of the dopamine-releasing neurons or to the specific inactivation of dopamine receptors. There is evidence in favor of both modes of action. Thus the dopamine receptor is thought to activate an adenylate cyclase, and chlorpromazine does indeed block the dopamine-sensitive adenylate cyclase (2-4). However, there is a poor correlation between the concentrations of the major

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tranquilizers required for blocking the adenylate cyclase and the clinical dosages used in the control of acute schizophrenia. Seeman *et al.* (5) found a much better correlation between clinical dosages and the concentrations required for nerve blockade. Chlorpromazine and related compounds have been shown to be very potent local anesthetics, blocking sodium conductance in nerve at a concentration of approximately 10–100 μM (5–10), which makes chlorpromazine a more potent local anesthetic than, for example, dibucaine or tetracaine.

In a number of papers Seeman and coworkers have demonstrated the strong, nonspecific hydrophobic interaction between chlorpromazine and biological membranes (see ref. 11). Since local anesthesia results from such low concentrations of chlorpromazine, it seems unlikely that blockade of sodium conductance could follow simply from a general increase in "fluidity" for the lipid component of the membrane resulting from the binding of chlorpromazine. This is particularly so since lipid in the liquid-crystalline state is already highly fluid: the "microviscosity" at the center of a bilayer of dimyristoylphosphatidylcholine in the liquid-crystalline state is about equal to that in *n*-decane at 31°, and the viscosity near the glycerol head group of the lipid is only approximately 15 times greater than for *n*-decane (12). A study has therefore been carried out on the effects of chlorpromazine on the temperatures of the phase transitions in bilayers of phosphatidylcholines and phosphatidylethanolamines, and on mixtures of these lipids.

Antihypertensive agents have also been shown to possess local anesthetic activity. Although it is thought that *beta* adrenergic blockers such as propranolol block the catecholamine stimulation of adenylate cyclase by binding to the catecholamine receptor (13), nonreceptor site binding of labeled propranolol in most tissues is approximately 60–80% of the total (14, 15). It has been suggested (13) that the nonreceptor binding could be connected with the local anesthetic effects of propanolol (16, 17). Interestingly, however, the *beta* blocker practolol has practically no local anesthetic activity (17). These two drugs therefore constitute an interesting pair of compounds with which to test any theory of local anesthetic action. Their structures are shown in Fig. 1.

MATERIALS AND METHODS

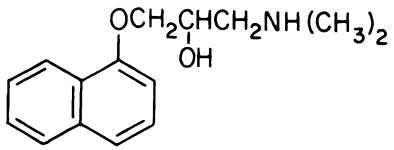
Dipalmitoylphosphatidylcholine was obtained from Koch-Light; dipalmitoylphosphatidylethanolamine, from Fluka; and myristic acid, from Sigma. Chlorophyll *a* was prepared as reported previously (18). Samples were prepared by dissolving lipids (0.6 μmole) plus chlorophyll *a* (1.6 nmoles) in chloroform in 10-ml stoppered flasks and evaporating them to dryness under a stream of nitrogen. Buffer (0.01 M Tris-HCl) was added together with the appropriate volume of a stock solution of drug in buffer to give a final volume of 4 ml at the required pH (usually 7.2). The mixture was shaken on a Vortex mixer.

Fluorescence measurements were made on an Aminco-Bowman SPF fluorometer, exciting chlorophyll *a* fluorescence at 420 nm and recording at 670 nm. The temperature of the sample was continuously monitored with a thermocouple inserted into the fluorescence cell.

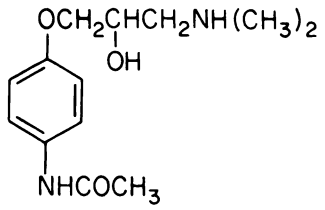
Propranolol and practolol were gifts from ICI Pharmaceuticals.

RESULTS

Effects on uncharged lipid bilayers. In previous studies (18, 19) it was shown that chlorophyll *a* incorporated into liposomes at a chlorophyll to lipid molar ratio of 1:400 did not affect the observed temperature of the gel to liquid-crystalline phase transition. As shown in Fig. 2, plots of the fluorescence intensity of chlorophyll *a* in liposomes as a function of temperature showed abrupt decreases in magnitude at temperatures corresponding to the calorimetrically determined phase transition. This has been attributed to the formation of nonfluorescent, aggregated species of chlorophyll *a* (18). If the transition temperature is defined as the midpoint of the fluorescence transition curve, the transition temperature for dipalmitoylphosphatidylcholine was observed to be 40.5°, agreeing



Propranolol



Practolol

FIG. 1. *Structures of propranolol and practolol*

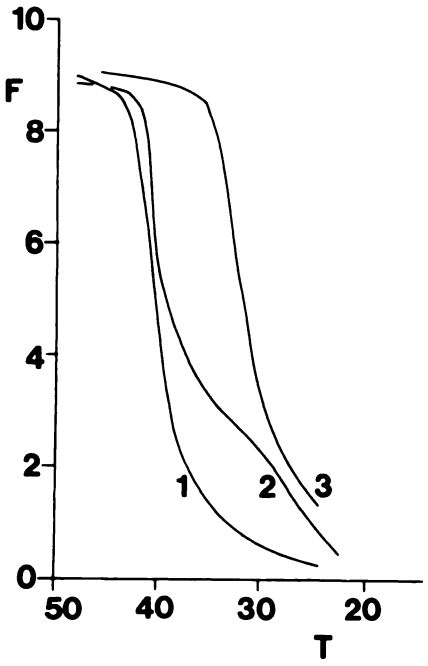


FIG. 2. *Fluorescence intensity at 670 nm for chlorophyll a in liposomes of dipalmitoylphosphatidylcholine plus 11 mole % myristic acid (curve 1), dipalmitoylphosphatidylcholine (curve 2), and dipalmitoylphosphatidylcholine plus 11 mole % myristic acid in the presence of 0.22 mM chlorpormazine (curve 3)*

Fluorescence intensity (*F*) is shown in arbitrary units.

exactly with that obtained by an electron spin resonance technique measuring the partition of the spin label tempo (20) and being close to the value of 41.75° obtained calorimetrically (21). As well as the midpoint temperature, the actual range of the transition is important, and this was readily determined from the intersections of the straight lines drawn through the three distinct portions of each curve. Immediately below the main transition, there was a second, more gradual transition, centered at approximately 29° and usually referred to as the pretransition.

Addition of chlorpromazine at pH 7.2 to dipalmitoylphosphatidylcholine shifted the temperature of the main lipid phase transition to lower values, in a nonlinear fashion (Fig. 3A). The width of the transition remained fairly constant, and there was relatively little effect on the pretransition. The effects of chlorpromazine on dipalmitoylphosphatidylethanolamine were significantly smaller than on dipalmitoylphosphatidylcholine (Fig. 3B).

Addition of propranolol at pH 8.5 to liposomes of dipalmitoylphosphatidylcholine also caused a marked decrease in the temperature of the phase transition, defined as the midpoint of the transition (Fig. 4A). As with chlorpromazine, the effects of increasing concentrations of propranolol were nonlinear, presumably because of the buildup of positive charge on the liposomes. Addition of propranolol at pH 7.0 had a slightly smaller effect than at pH 8.5: thus, at pH 7.0, 2 mM propranolol lowered the lipid transition temperature to 35°, whereas at pH 8.5 it lowered it to 34°.

Effects of pH were more marked for the interaction of propanolol with dipalmitoylphosphatidylethanolamine (Fig. 4B). At pH 7.2 propanolol had relatively little effect on the temperature of the phase transition, even in the presence of 11 mole % myristic acid. At pH 8.5 the effects of propanolol on the transition temperature of dipalmitoylphosphatidylethanolamine were considerably greater than for dipalmitoylphosphatidylcholine.

In contrast to the effects of propranolol, addition of practolol to liposomes of dipalmitoylphosphatidylcholine produced virtually no change in the temperature of the phase transition. In the presence of 20 mM practolol at pH 7.4, the temperature of the phase transition was 39.5°, a decrease of only 1°, and in the presence of 11 mole %

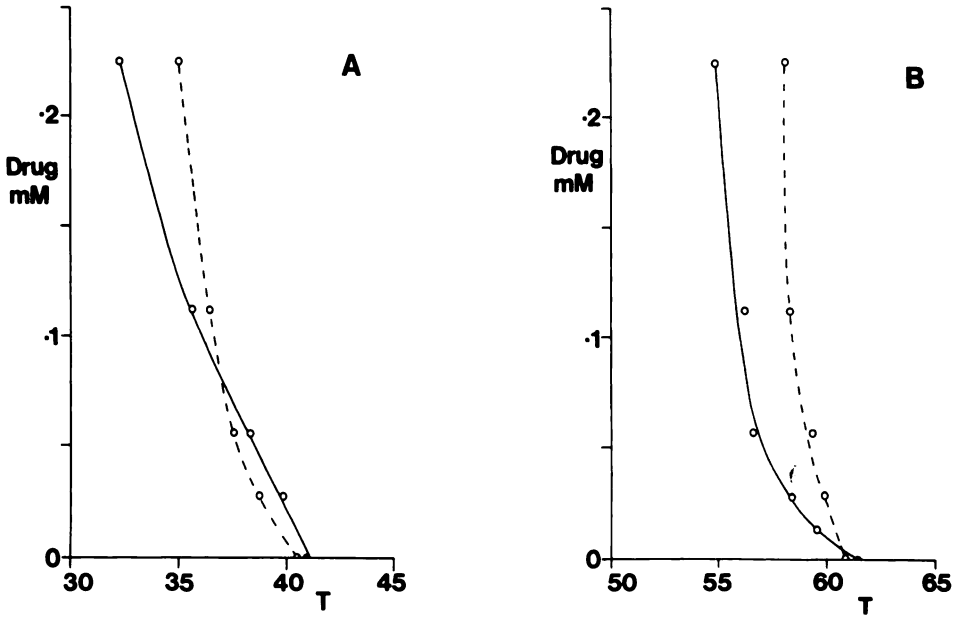


FIG. 3. *Effect of chlorpromazine on phase transition temperatures*

A. - - -, dipalmitoylphosphatidylcholine; ——, dipalmitoylphosphatidylcholine plus 11 mole % myristic acid. B. - - -, dipalmitoylphosphatidylethanolamine; ——, dipalmitoylphosphatidylethanolamine plus 11 mole % myristic acid.

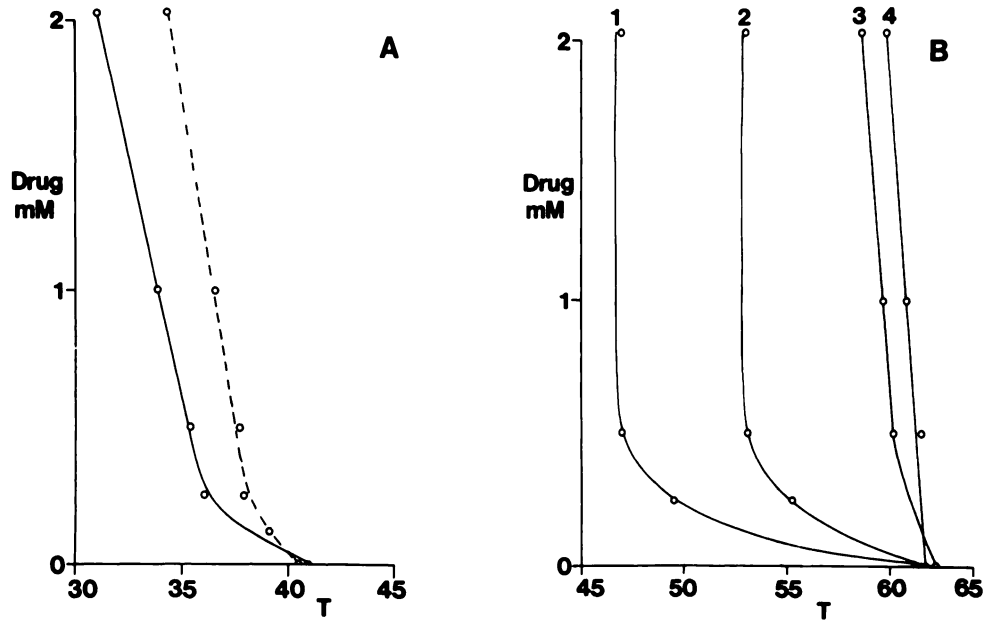


FIG. 4. *Effect of propranolol on phase transition temperatures*

A. - - -, dipalmitoylphosphatidylcholine; ——, dipalmitoylphosphatidylcholine plus 11 mole % myristic acid, both at pH 8.5. B. Dipalmitoylphosphatidylethanolamine at pH 8.5 (line *2*) and pH 7.2 (line *4*) and dipalmitoylphosphatidylethanolamine plus 11 mole % myristic acid at pH 8.5 (line *1*) and pH 7.2 (line *3*).

myristic acid (see below) the transition temperature was 40°, again a decrease of 1°. Practolol at this concentration had no effect on the temperature of the phase transition in dipalmitoylphosphatidylethanolamine.

As described elsewhere (19), plots of the fluorescence of chlorophyll *a* as a function of temperature when incorporated into lipid mixtures provide a series of temperatures corresponding to the onset and completion of gel phase formation. These can then be plotted to give what can be loosely termed "phase diagrams" (22). The effects of chlorpromazine on the transition temperatures in mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine are presented in Fig. 5.

Effects on charged lipid bilayers. The nonlinear effects of increasing chlorpromazine concentrations on lipid transition temperatures can be attributed to the buildup of charge on the liposomes, caused by binding of the positively charged chlorpromazine. Incorporation of negatively charged lipid into the bilayers should neutralize some of this charge, and thus increase the effect of chlorpromazine. Figure 2 shows that incorporation of 11 mole % myristic acid into liposomes of dipalmitoylphosphatidylcholine caused a slight increase in the temperature of the main phase transition to 41°, and also removed the pretransition. The pretransition has been attributed to a change in the orientation of the lipid fatty acid chains from being tilted with respect to the plane of the bilayer to being oriented perpendicular to the bilayer plane (23), thus allowing rotation about their long axes (24), and has previously been shown to be removed by long-chain alcohols (25). Addition of myristic acid to dipalmitoylphosphatidylcholine or dipalmitoylphosphatidylethanolamine caused a considerable increase in the effect of chlorpromazine on the phase transition temperatures (Fig. 3). The effect of the negatively charged fatty acid could be reversed by the addition of divalent metal ions. Addition of more than about 0.5 mM Ca^{2+} to dipalmitoylphosphatidylcholine containing 11 mole % myristic acid in the absence of monovalent metal ions caused an increase in temperature of the phase transition in the presence of 0.22 mM chlorpromazine from 32.2° to 34.5°.

Similar results were obtained with propranolol, the effect of propranolol being much increased by addition of myristic acid (Fig. 4), with masking by approximately 0.2 mM Ca^{2+}.

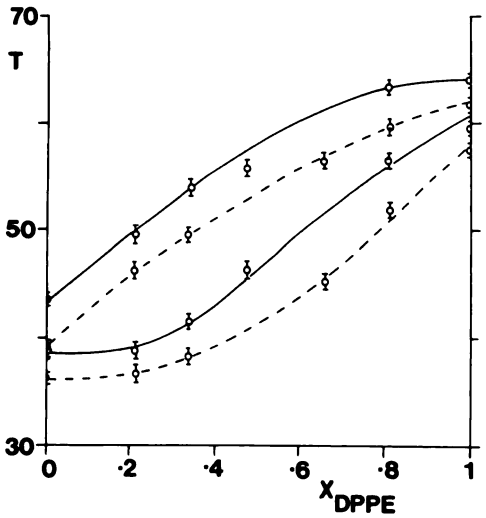


FIG. 5. *Temperatures of onset and completion of solid-lipid separation in mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine as a function of the mole fraction (X_{DPPE}) of dipalmitoylphosphatidylethanolamine*

——, no chlorpromazine; - - -, in the presence of 0.06 mM chlorpromazine.

DISCUSSION

Theories of local anesthetic action. A relationship between local anesthetic potency and lipid solubility has been observed so often that it is generally believed that the primary effect of local anesthetics is on the lipid component of the membrane, and that direct effects on membrane proteins are irreversible and occur only at high, lytic concentrations (26). It has been observed that for most local anesthetics, anesthesia results when either a certain critical concentration or molar volume of anesthetic is reached within the membrane (27). However, although this might represent a useful correlation, it does not constitute a theory of anesthetic action.

One possibility that has been suggested is that local anesthetics increase the "fluidity" of the membrane lipids, thereby somehow interfering with passage of sodium ions through the sodium channels of the nerve. However, although membrane proteins involved in ion transport are known to be sensitive to the nature of their

lipid environment, in all cases so far reported it seems that activity increases with increasing lipid fluidity (28). Furthermore, although there is no doubt that high enough concentrations of anesthetics can disturb membrane structure, it is, of course, essential to demonstrate that effects occur at concentrations comparable to those used clinically. This generally appears not to be so. Thus, for example, using spin label techniques, Boggs *et al.* (29) have shown that alcohols and other anesthetics have no detectable effect on lipid fluidity at concentrations which block nerve conduction. Similarly, Miller and Pang (30) have recently reported that 46 mM 1-octanol causes a decrease in order parameter of 0.02 for 5-doxylstearic acid incorporated into bilayers of lecithin. Since the effects were reported to be linear with temperature, this means that at its anesthetic concentration of approximately 1 mM, 1-octanol would cause a change in order parameter of about 0.0004. To put this in context, the same change in order parameter is caused by an increase in temperature of about 0.06° (31). As pointed out by Boggs *et al.* (29), the concentration of anesthetic at which significant increases in fluidity occur corresponds more closely to those causing lysis of erythrocyte ghosts than to anesthesia.

One way of magnifying the effect of the anesthetic has been suggested by Trudell *et al.* (32). If the nerve membrane contains lipids in both the gel and liquid-crystalline phases, the addition of anesthetic could, by lowering the lipid phase transition temperature, trigger a change from the gel phase to the liquid-crystalline state. This would result in an increase in fluidity of the membrane. If, however, anesthesia followed simply from a general increase in the proportion of liquid-crystalline phase lipid in the membrane, it would be difficult to explain the selective effects of anesthetics. Thus the majority of anesthetics affect the sodium channel at concentrations lower than those required to affect potassium currents, and the $(Na^+ + K^+)$-ATPase of red blood cells (33) and synaptosomes (34) is unaffected at high, almost lytic, concentrations of anesthetics. Furthermore, the lipids of nerve plasma membranes contain an unusually high percentage of unsaturated lipid (35, 36), so that the proportion of gel phase lipid in the membrane at ambient temperatures will be low. Addition of anesthetic can therefore produce only a relatively small increase in the general fluidity of the membrane, even if it does trigger lipid phase transitions.

Many of these problems can be overcome if it is assumed that, rather than interacting with membrane lipids, anesthetics interact directly with the sodium channel. However, this creates new problems, largely because of the surprising unselectivity that has to be attributed to the interaction between the protein and anesthetics. Thus neutral, positively charged, and negatively charged molecules (37), together with inert gases (38), can all act as local anesthetics. Also surprising are the observations that long-chain alkyl compounds such as 1-octanol (26) and neutral detergents (39) are very potent local anesthetics at concentrations below those that lyse the membrane. Since the sodium channel presumably spans a lipid bilayer section of the nerve membrane, it might be expected to be relatively insensitive to lipid-like molecules; it has already been mentioned that very high anesthetic concentrations have no effect on the $(Na^+ + K^+)$-ATPase (33, 34). In contrast to the lack of selectivity that would have to be postulated for interactions with anesthetics, very minor changes in the structures of tetrodotoxin, which is known to bind specifically to the outside surface of the sodium channel, cause complete loss of activity (40).

To overcome these difficulties, a new model for the action of local anesthetics was recently proposed (1). It was suggested that the lipid surrounding the sodium channel in nerve was in the gel state, and that it was the rigidity of its environment which maintained the sodium channel in an open condition. Addition of local anesthetics might then trigger a change in the surrounding lipids to the liquid-crystalline phase, the new fluid environment then allowing the sodium channel to relax into a more stable state, in which the sodium channel is effectively closed (1). This

model was designed to incorporate the best features of the previous theories of local anesthesia. First, the relatively unselective nature of local anesthetics is explained, since the anesthetic is postulated to interact with the lipid component of the nerve membrane. Second, by postulating that the anesthetic interacts with gel phase lipid, the effect of the anesthetic will be magnified, as suggested by Trudell *et al.* (32). Third, the anesthetic will have a large effect only on proteins surrounded by gel phase lipid: for metal ion pumps to work effectively, the surrounding lipid has to be in the liquid-crystalline state (28), and so anesthetics would be expected to have relatively little effect. Fourth, if the sodium channel has the structure postulated by Hille (41), the model provides, for the first time, a simple picture of how a local anesthetic might block the sodium current through nerve.

Effects of anesthetics on lipid phase transitions. One obvious requirement of the above model is that local anesthetics should be able to produce a significant decrease in lipid transition temperatures, at concentrations that produce local anesthesia. In a previous paper it has been shown that alcohols up to 1-octanol produce a decrease in phase transition temperature of approximately 3° at the concentration required for local anesthesia (25).

The amine anesthetics have also been shown to reduce the temperatures of lipid phase transitions (42). Again, for the neutral benzocaine, the concentration required for a 3° drop in phase transition temperature is comparable to that required for local anesthesia. For the charged amines, the relationship is more complex because of the buildup of positive charge on the liposomes. However, incorporation of negatively charged lipid markedly increases the binding of the amines in the absence of Ca^{2+}. The anesthetic effect of the amines can then be explained if the annular ring of lipid around the sodium channel includes negatively charged lipid. The presence of negatively charged lipid close to the sodium channel has been concluded from electrophysiological studies (43), and approximately 10% of the lipid in the nerve membrane is phosphatidylserine (35, 44). It has also been shown that the charged form of the amine anesthetics is most active when applied to the inside surface of the nerve (37). This can be explained since, although it seems that negatively charged lipid is present on both sides of the membrane close to the channel (43), the concentration of Ca^{2+} inside the nerve has been estimated (45) to be only a few micromolar, whereas it is present at typically 2 mM on the outside. This simple picture is complicated somewhat by the presence of Mg^{2+}, which has been estimated to be present at about 3 mM inside the nerve (46). However, there is much evidence for differences between the effects of Mg^{2+} and Ca^{2+} on negatively charged lipids, significant effects commonly appearing at about 0.5 mM Ca^{2+} and 5 mM Mg^{2+} (47, 48). It has also been shown that barbiturates lower the phase transition temperatures of lipids at concentrations comparable to those causing local anesthesia, and that the barbiturates are more effective at lower pH values, where more of the barbiturate is present in uncharged form (49).

Last, it has been shown that *n*-alkanes such as decane and dodecane have no effect on the temperature of the gel to liquid-crystalline phase transition,[1] and these compounds appear to have no local anesthetic effects (50). These studies have now been extended to include chlorpromazine and the *beta* adrenergic blockers propranolol and practolol.

Effects of chlorpromazine, propranolol, and practolol. Chlorophyll *a* has been shown to be a convenient probe for detecting phase transitions in lipid bilayers. Both a monomeric, fluorescent form and an oligomeric, nonfluorescent form of chlorophyll *a* are present in the bilayers, the proportion of oligomeric chlorophyll *a* increasing when the lipid is transformed from the fluid, liquid-crystalline phase to the crystalline, gel phase (18). Addition of chlorpromazine to lipid bilayers in the liquid-crystalline state produces no significant change in fluorescence intensity (Fig. 2). This suggests that the ratio of monomeric and oligomeric forms of chlorophyll

[1] A. G. Lee, unpublished observations.

a is little affected, so that the fluidity of the lipid bilayer in the liquid-crystalline state is not changed by the addition of chlorpromazine.

Addition of chlorpromazine does, however, have a very marked effect on the phase transition temperatures of phosphatidylcholines and phosphatidylethanolamines. For uncharged molecules such as the alcohols (25) and benzocaine (42), plots of anesthetic concentration against transition temperature are linear, as expected for ideal or close-to-ideal behavior. For chlorpromazine, effects are clearly nonlinear (Fig. 3) because of the buildup of positive charge on the liposomes: the pK_a of chlorpromazine is approximately 8.2 (6), so that at the pH of 7.2 used in these experiments, chlorpromazine will be present predominantly in a charged, protonated form. The binding constant for chlorpromazine can be written as

$$K = K_0 \exp(F\psi_0/kT) \quad (1)$$

where K_0 is the binding constant at high ionic strength, F is the Faraday constant, and ψ_0 is the surface potential. Buildup of positive charge on the liposomes will thus cause a reduction in the binding constant K. It follows from the above equation that incorporation of negatively charged lipid into the bilayer should increase the binding of chlorpromazine. The data presented in Fig. 3 show that this does indeed occur. Furthermore, addition of 0.5 mM Ca^{2+} has been shown to "mask" effectively the effect of the negatively charged lipid. The mechanism of the masking effect has not been definitely established, but the low concentration of Ca^{2+} required suggests direct binding of Ca^{2+} to the negative charges in the bilayer, rather than a simple screening effect.

Similar effects have been observed with propranolol (Fig. 4). The pK_a of propranolol is 9.45 (28), so that 70% and 100% are present in the protonated, charged form at pH 8.5 and 7, respectively. The smaller effect of propranolol on the temperature of the phase transition for dipalmitoylphosphatidylcholine at pH 7 than at pH 8.5 is also most probably due to a more rapid buildup of charge on the liposomes at pH 7. An alternative explanation, suggested by a reviewer, is that only the uncharged form of propranolol binds to the membrane, and that at pH 7 no uncharged base is available to produce an effect. However, for the following reason this seems very unlikely for a neutral bilayer. For an uncharged bilayer, the pH at the membrane surface should be very close to that in the bulk solution. The proportion of ionized $[AH^+]$ and un-ionized $[A]$ base is given as a function of pH by the Henderson-Hasselbalch equation,

$$\log \frac{[A]}{[AH^+]} = pK_a - pH$$

This equation must hold both for the bulk phase and for the drug bound to the membrane, with possible differences between the pK_a and pH values between the two environments.

To say that only the uncharged form of the base binds to the membrane must then imply that the pH at the membrane surface is at least 2 pH units higher than the pK_a value of the drug. This means that if the pK_a of propranolol is unchanged at 9.45, the pH at the surface of the neutral phosphatidylcholine bilayer must be about 11.5, which is clearly unreasonable. Also, of course, this surface pH would have to be independent of the bulk pH, so that there would no longer be any obvious explanation for the larger effect of propranolol at a bulk pH of 8.5 than at one of 7. Alternatively, if the pH at the surface were equal to the bulk pH, the pK_a value of propranolol bound to the membrane would have to decrease to be approximately 2 units lower than the bulk pH. The implication of this would be a continual variation of the pK_a value of the bound drug in order for it to remain at least 2 units lower than the bulk pH, at whatever value chosen for the latter. There is no obvious mechanism for such a process. Furthermore, it again would mean that there is no obvious explanation for the observed increased effect of propranolol at pH 8.5. In conclusion, and as is always assumed, it seems that both charged and uncharged forms of the drug must bind to the membrane, the relative proportions being dependent both on the pK_a of the drug when bound to the membrane and on the pH at the membrane surface. If both charged and uncharged

forms of the drug bind, a positive charge will build up on the surface, resisting further binding, as expressed in Eq. 1.

Interestingly, the effect of pH was much more marked for bilayers of dipalmitoylphosphatidylethanolamine than for dipalmitoylphosphatidylcholine. At pH 7.2 propranolol had less effect on the transition temperature than for dipalmitoylphosphatidylcholine, whereas at pH 8.5 it had a greater effect. The relatively small effects at pH 7.2 can be attributed to tighter packing of the phosphatidylethanolamine molecules in the bilayer as compared with phosphatidylcholine molecules, caused by strong interaction between the phosphatidylethanolamine head groups, with the positively charged amine group of one molecule interacting electrostatically with the negatively charged phosphate group of an adjacent molecule. The greater effect of propranolol at pH 8.5 can be attributed to a partial negative charge on the phosphatidylethanolamine at this pH: although the pK_a of phosphatidylethanolamine does not seem to have been measured, Michaelson *et al.* (51) observed effects in nuclear magnetic resonance spectra that could be attributed to ionization at approximately pH 9. Effects attributed to ionization were also observed in a study of the interaction between barbiturates and dipalmitoylphosphatidylethanolamine at pH 8.5 (49).

The effect of chlorpromazine on mixtures of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine is presented in the form of a "phase diagram" in Fig. 5. Although, as discussed elsewhere (22), there are problems in equating these diagrams of the temperatures of onset and completion of gel phase formation with phase diagrams as defined for macroscopic systems, the analogy can be helpful. Thus the primary effect of chlorpromazine is to shift the upper, fluidus curve and the lower, solidus curve to lower temperatures. The over-all effect is to increase the amount of fluid lipid present. Thus, for example, in an equimolar mixture of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine at 45°, all of the lipid is in the gel state in the absence of chlorpromazine, but in the presence of 0.06 mM chlorpromazine 30% of the lipid is in the fluid state.

It has therefore been shown that the anesthetic effect of chlorpromazine can be explained by the same model used for the other anesthetics. Addition of chlorpromazine lowers the temperature of the lipid phase transition, and so triggers a change in the annulus of lipid around the sodium channel from the gel state to the liquid-crystalline state. The sodium channel can then relax to a lower-energy state, with closing of the oxygen-lined slit through which the sodium ions pass, and a reduction of the sodium current. The concentration of chlorpromazine required for a drop of approximately 3° in the phase transition temperature of dipalmitoylphosphatidylcholine is 0.05 mM, the concentration required for nerve blockade (5–10).

For propranolol, the concentrations required to produce a 3° drop in transition temperature at pH 8.5 are approximately 0.7 and 0.2 mM in the absence and presence, respectively, of 11 mole % myristic acid. Sasa *et al.* (17) found that 0.2 mM propranolol was required to block conduction in lobster giant axon at pH 9. This correlates very well with the lipid transition data, particularly if positively charged propranolol is postulated to interact with negatively charged lipid close to the sodium channel on the inside surface of the membrane.

The effect of propranolol on both phosphatidylcholine and phosphatidylethanolamine is greater at pH 8.5 than at pH 7. It is therefore of interest that Sasa *et al.* (17) found that 0.6 mM propranolol is required to block nerve conduction at pH 7.2, compared with 0.2 mM required at pH 8.5. This correlation may, however, prove to be artifactual, since it has been suggested that the primary effect of decreasing external pH is to decrease the amount of uncharged drug, which alone is able to permeate the membrane to the site of action on the inside surface (52).

Sasa *et al.* (17) have also shown that increasing the external Ca^{2+} concentration antagonizes the local anesthetic effect of propranolol in lobster giant axon. Although the effects of varying Ca^{2+} concentration around excitable membranes are complex (53), an increase in external Ca^{2+}

concentration is expected to lead to an increase in internal Ca^{2+} (45), which would serve to mask negative charges around the sodium channel on the inside surface of the membrane.

In contrast to these effects of propranolol, 20 mM practolol at pH 7.4 produces only a 1° drop in lipid transition temperature, in both the presence and absence of myristic acid. This is most likely due to a smaller partitioning of practolol into the lipid bilayer, caused by the presence of a hydrophilic amide group. However, differences in partitioning into the bilayer are not the only sources of differences in effect. Specific charge-charge interactions between the drug and the lipid molecules might be important. Differential solubility in gel phase lipid could also be important, since Hill (54) has shown that the greatest decrease in transition temperature will be caused when the drug is completely insoluble in gel phase lipid. Interactions with gel phase lipid have been postulated to involve insertion of the foreign molecules into vacancies present in gel phase lipid (55). Jain *et al.* (56) have shown that there is no simple correlation between the effects of adamantane derivatives on phase transition temperatures of lipids and calculated hydrophobicities.

The important observation for our purposes, however, is the finding by Sasa *et al.* (17) that practolol at concentrations up to 15 mM at pH 7.2 did not block conduction in lobster giant axon.

Although racemic propranolol was used in these and the electrophysiological experiments (16, 17), both optical isomers would be expected to have similar potencies as local anesthetics, since the nonreceptor binding of propranolol to various tissues has been shown not to be stereospecific (13, 15). In general, optical isomers of local anesthetics do not seem to differ in potency (57), although small differences, of a factor of about 2, have been found for the local anesthetic potencies *in vitro* of the enantiomers of *N*-aminoalkyl derivatives of Tetralin 1-spirosuccinimide (58) and of aminoacylephedrines (59). This is consistent with a lipid binding site for the anesthetics, since protein receptor binding often shows absolute stereospecificity whereas binding to the simpler lipids would be expected to show no stereospecificity and binding to more complex lipids, such as the cerebroside sulfates, has been shown to exhibit small stereospecific effects (60).

The greater effect of chlorpromazine than of the amine anesthetics on phase transition temperatures presumably is the result of a greater hydrophobicity for chlorpromazine. Molecular models of chlorpromazine and dibucaine are shown in Fig. 6. Cerbon (61) has shown that tetracaine interacts with phospholipids, with the positively charged amine group lying close to the lipid head groups and the remainder of the molecule buried within the membrane, parallel to the fatty acid chains. Interactions of both chlorpromazine and dibucaine with phospholipids should be similar: the distances between the charged amine and the aromatic rings in the two compounds are equal. The greater effect of chlorpromazine is probably related to its lower water solubility. Hill (59) has shown that for ideal behavior the product of the anesthetic concentration at aqueous saturation S and the partition coefficient P should be equal to 2. Thus, for ideal behavior, the lower the water solubility of an anesthetic, the greater its partition coefficient into the membrane and thus the greater its effect on lipid phase transition temperatures.

Although the presence of negative charge in the bilayer increases the effect of chlorpromazine on transition temperatures, the effect is not seen in bilayers of dipalmitoylphosphatidylcholine until a concentration of about 0.1 mM is reached. The effect of negatively charged lipids, however, is seen more clearly in bilayers of dipalmitoylphosphatidylethanolamine, presumably because of the tighter packing that has been suggested in bilayers of this lipid (22). The effects of negatively charged lipid are reversed by a Ca^{2+} concentration of approximately 0.5 mM. In general, the effects of negatively charged lipid on the binding of chlorpromazine are less marked than for dibucaine, for example. Thus, although chlorpromazine might be expected to be more active as a local anesthetic when applied to the inside surface of a

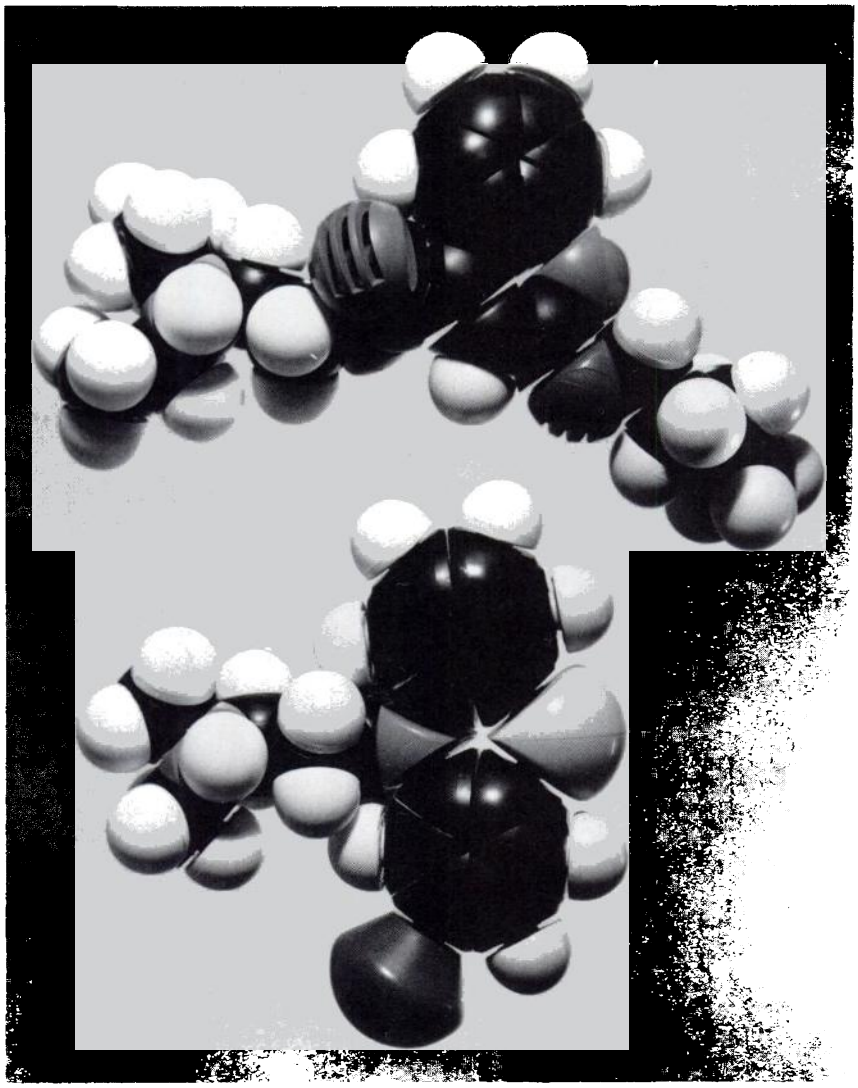


FIG. 6. *Molecular models of chlorpromazine (lower) and dibucaine (upper)*

nerve, where the Ca^{2+} concentration is lowest, the difference between internal and external application should be less than for dibucaine. This was indeed found to be the case. Gruener and Narahashi (9) showed that the quaternary methyl derivative of chlorpromazine (which presumably cannot pass from one side of the membrane to the other) is a better local anesthetic when applied to the inside of the nerve, but is also active from the outside.

The annular transition model can therefore account successfully for the anesthetic action of chlorpromazine. The alternative model that has been proposed for the action of the charged amine anesthetics, such as dibucaine, involves specific interaction between the anesthetic and a receptor in the membrane, probably the sodium channel itself. If the same receptor site were postulated to be involved in the action of dibucaine and chlorpromazine, it would have to be surprisingly unselective, since the only obvious common feature between these two drugs is the sequence hydrophilic region–spacer region–hydrophobic region. If, on the other hand, separate binding sites for dibucaine and chlorpromazine were postulated, it would be surprising that the effects of the two drugs

are so similar: this second binding site for chlorpromazine could not be postulated to be the lipid phase of the membrane, since if it were allowed that chlorpromazine could affect the sodium channel from the lipid phase, so could dibucaine, and there would then be no need for the postulated specific receptor site for dibucaine.

Effects of temperature. It has now been shown that the anesthetic action of a wide variety of molecules can be explained in terms of the model outlined above. One consequence should perhaps be discussed further, and that is the effect of temperature. Since the sodium channel is postulated to be surrounded by lipid in the gel phase, the actual channel through the membrane should be affected relatively little by a decrease in temperature, but should be inactivated by an increase in temperature. However, the effect of decreasing temperature must necessarily be complicated, because there is more to the channel than simply the "slit" through the membrane. In particular, if the channel gates fail to open, no current will flow, whatever the state of the slit through the membrane. Since even rates of water-soluble enzymes have large temperature coefficients (62), it is likely that there will be a relatively small temperature "window" within which to work. Nevertheless, there is much evidence that decreasing temperature has less effect on sodium conductivity than on many membrane proteins. Thus it has been shown in squid axon that decreasing temperature has very little effect on the Na^+ influx during an action potential, whereas there is a marked effect on the sodium pump (63-65). Again, in frog nerve the spike amplitude increases slightly on cooling from 25° to 0°, even though spin label studies have shown a considerable decrease in fluidity for the bulk lipids within the same temperature range (66, 67). In contrast, increasing temperature produces a reversible conduction block, whose temperature depends on the animal (68, 69). If a suitably small decrease in temperature is sufficient, it might be possible to reverse the effects of anesthesia by decreasing temperature: by lowering the temperature of the nerve below that of the phase transition in the presence of anesthetic, the lipid will be transformed to the gel phase with complete or partial exclusion of the anesthetic. Whether or not this exclusion of anesthetic by the annular lipid is detected as a decrease in the bulk partition coefficient into the membrane depends, of course, on the proportion of total lipid that is in the annulus surrounding the sodium channel (likely to be small) and on the effect of temperature on the rest of the membrane. Thus, although reversal of anesthesia by decreasing temperature is consistent with the model of anesthesia presented here, it can also easily be made to be consistent with the other theories as well. In fact, the partitioning of alcohols into red blood cells has been shown to decrease with decreasing temperature (70, 71), and Spyropoulos (72) has reported that the anesthetic effect of ethanol on squid axon at 22° was reversed completely by lowering the temperature to 4°. However, effects of temperature should, in general, be expected to be very complex. Thus Wang *et al.* (73) observed that decreasing temperature enhances the nerve-blocking action of allethrin, through effects on, for example, potassium conductance and the rate of sodium inactivation.

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