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Effects of Anesthetics on Lipid Polymorphism

P. R. Cullis, A. P. Hornby, and M. J. Hope

Department of Biochemistry, University of British Columbia, Vancouver V6T IW5, British Columbia, Canada

The molecular mechanism(s) whereby anesthesia is induced represents a fascinating problem to students of membrane structure and function. This is because anesthetics display an ability. to not only inhibit the Na+ conductance necessary for nerve transmission, but also inhibit many facilitated transport processes (18). As the possibility exists that the Na+ channel may be considered to be a facilitated transport complex itself, an understanding of structure-function relationships in this system could lead to a clearer understanding of facilitated transport processes in general.

Further, from the point of view of those interested in the functional roles of lipids in biological membranes, the actions of anesthetics are particularly intriguing. This is because the available literature suggests a direct relationship between solubility in a hydrocarbon (lipid) environment and anesthetic potency (13). This information, when correlated with the vast variety of chemically disparate drugs able to induce nerve block (18), has important implications for the composition and function of the Na+ channel. In particular, these observations suggest that the Na+ channel exists as a lipid-protein complex, where the action of anesthetics corresponds to a (nonspecific) disruption of the lipid participating in this complex.

It is logical that these and associated observations and conjectures have led to many investigations of the influence of anesthetics on the physical properties of lipids in model membrane (protein-free) systems. Particular attention has been paid to the influence of anesthetics on membrane fluidity, as the possibility exists that (integral) protein function may be modulated by the viscosity of the surrounding lipid matrix. In the case of the Ca^{2+}-dependent ATPase of sarcoplasmic reticulum, for example, enzyme activity is inhibited in the presence of gel-state phospholipids (22). Thus, the demonstrations that local (15) and neutral (14) anesthetics can "fluidize" phospholipid bilayers (as indicated by an ability to depress the gel-liquid crystalline hydrocarbon transition temperature) have led to models such as that due to Lee (9), which suggests that the functional Na+ channel is "stabilized" by annular gel-state lipids. It is then hypothesized that the fiuidization effects resulting from the presence of anesthetic molecules results in "destabilization" of the Na^+ channel, corresponding to inhibition of Na^+ conductance.

These hypotheses suffer from two basic difficulties. First, given the unsaturated nature of nerve membrane lipids (23), there is no evidence for the existence of gel-state phospholipids at physiological temperatures. Indeed, there is little or no evidence for the presence of gel-state lipids in eukaryotic cell membranes in general. Secondly, the anesthetic concentrations required to induce significant "fluidization" are usually an order of magnitude larger than required to inhibit the action potential *in vitro* (1). In fact, such concentrations often have lytic effects.

Given this background, the need for an examination of the influence of anesthetics on alternative physical properties of lipids that may be relevant to transport processes is apparent. One such property, which has not received the serious attention it deserves in recent years, concerns the ability of lipids to adopt a variety of polymorphic phases (in addition to the bilayer phase) on hydration. As we point out here and elsewhere (4), the availability of these structural alternatives allows the direct participation of lipids in a variety of membrane mediated processes, including facilitated transport. In this chapter we show that representative anesthetics can strongly influence this polymorphism and, further, that such effects are observable at pharmacological aqueous concen-

trations of these drugs. On the basis of this information, certain speculations are made regarding potential roles of "nonbilayer" lipids in facilitated transport processes, which may include the Na+ channel.

LIPID POLYMORPHISM

The bilayer arrangement of hydrated lipids is only one of a great variety of polymorphic phases available. Alternative configurations include the hexagonal H, and H,, phases, the micellar and inverted micellar phases, as well as those phases exhibiting cubic or rhombic structure. For a detailed description of the characteristic dimensions and symmetries of these latter configurations the reader is referred to references 10 to 12. The bilayer and hexagonal H,, configurations are depicted in Fig. 1.

^{31}P nuclear magnetic resonance (NMR) has been found to be a convenient technique for the identification of the phase assumed by phospholipids in pure or mixed lipid systems (2,7), as is indicated in Fig. 1. Briefly, phospholipids in the (liquid crystalline) bilayer configuration exhibit broad, asymmetric ^{31}P NMR spectra with a low field shoulder, whereas hexagonal (H,,) phase phospholipids exhibit characteristic lineshapes that have reversed asymmetry compared with the bilayer spectra, and are narrower by a factor of 2. Finally, phospholipids in micellar, inverted micellar or cubic and rhombic phases show narrow, symmetric ^{31}P NMR signals.

It is remarkable that many lipid species found in significant concentrations in biological membranes (including the nerve cell membrane) preferentially adopt nonbilayer configurations-particularly the hexagonal (H_{II}) phase-on hydration. Notable examples include unsaturated phosphatidylethanolamines (3) as well as cardiolipin and phosphatidic acid in the presence of Ca^{2+} (16,17). Further, other lipids such as cholesterol (2) and unsaturated fatty acids (5) can also induce H,, phase structure in certain mixed lipid systems.

In order to illustrate both the polymorphic behavior of an unsaturated phosphatidylethanolamine, as well as the sensitivity of the ^{31}P NMR technique to this polymorphism, the temperature-dependent behavior of erythrocyte phosphatidylethanolamine is illustrated in Fig. 2. As the temperature is increased through 10°C a bilayer to hexagonal H,, transition is clearly observed. This is a remarkable observation, as it indicates that at 37°C, the bilayer configuration is not preferred by 30% of the endogeneous phospholipid of the erythrocyte membrane. This represents a direct

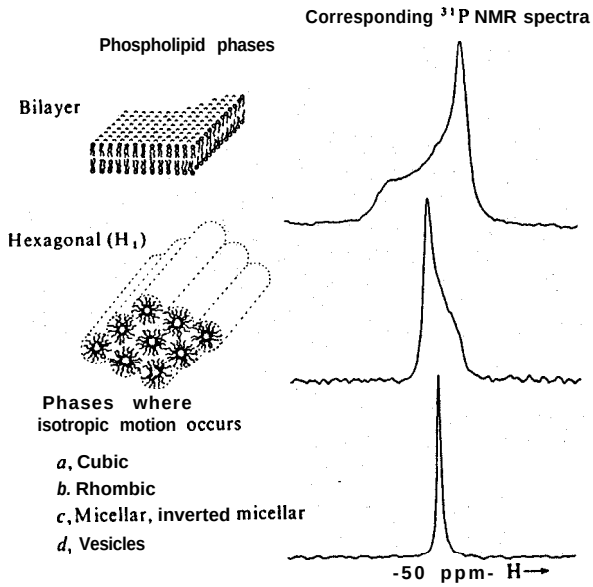


FIG. 1. Phospholipid phases and corresponding (36.4 MHz) ^{31}P NMR spectra. The bilayer spectrum was obtained from aqueous dispersions of egg yolk phosphatidylcholine, whereas. the hexagonal (H,,) phase spectrum was obtained from soya phosphatidylethanolamine. The "isotropic motion" spectrum was obtained from a mixture of 85 mole % soya phosphatidylethanolamine and 15 mole % egg yolk phosphatidylcholine. All spectra were taken at 30°C.

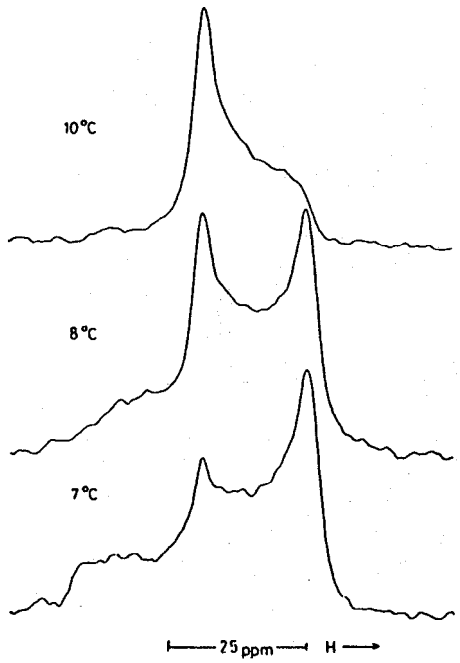


FIG. 2. Temperature-dependent behavior of the (36.4 MHz) ^{31}P NMR spectra arising from aqueous dispersions of human erythrocyte phosphatidylethanolamine. For details of lipid isolation and sample preparation see ref. 3.

challenge to the view that the major, if not the only, functional role of lipids is to provide a bilayer matrix, as the phosphatidylethanolamine component may be considered to actively destabilize such an arrangement. It is logical to suppose that phosphatidylethanolamine, together with other "nonbilayer" lipids, satisfy other functional roles in biomembranes.

The existence of "nonbilayer" lipid in biological membranes clearly establishes that the lipid component cannot, a priori, be assumed to be a bilayer configuration. Further, suggestive evidence that a portion of the lipids assume nonbilayer structures in membrane systems such as the (rat liver) endoplasmic reticulum (8,19) has recently become available. Thus the question is rapidly becoming "what functional roles do nonbilayer lipids and their associated structures play in biological membranes?" As we have indicated elsewhere, a variety of processes ranging from "flip-flop" (2) to fusion (5) to facilitated transport (4) could depend on nonbilayer lipid intermediates. Viewing the Na+ channel as a facilitated transport complex, we have naturally been led to investigate the effects of anesthetics on lipid polymorphism, and the results of these investigations are summarized in the next section.

EFFECTS OF ANESTHETICS ON LIPID POLYMORPHISM

The fact that local anesthetics such as dibucaine and chlorpromazine can have strong effects on the polymorphic phase adopted by phospholipids was first illustrated for model membrane systems comprised of cardiolipin, a major component of the inner mitochondrial membrane. These results are summarized in Fig. 3, where it is noted that both dibucaine and chlorpromazine trigger formation of the hexagonal H_{II} phase at anesthetic/cardiolipin ratios of 2:1. The fact that Ca^{2+} produces similar effects at equimolar concentrations (Fig. 3a) was then used to suggest that the local anesthetics interact with the (negatively charged) lipid in their (positively) charged form, inducing their effects by charge neutralization (7).

Dibucaine also exhibited strong effects on the polymorphism exhibited by mixtures of (egg yolk) phosphatidylethanolamine and (beef brain) phosphatidylserine (6), but in this case the effects observed are antagonistic to the influence of Ca^{2+}. As illustrated in Fig. 4, Ca^{2+} induces formation of the H_{II} phase, whereas the subsequent introduction of dibucaine (equimolar with the phosphatidylserine) produces a complete reversion to the bilayer phase (Fig. 4c).

These results are certainly suggestive. However, as indicated in the introductory section, it is important to establish that such anesthetic induced modulation of lipid structure can occur at pharmacologically relevant concentrations, and the results of preliminary investigations in this regard are illustrated in Fig. 5. The influence of three representative anesthetics (gaseous, neutral, and charged) on the polymorphism of appropriate model membrane systems is denoted in this figure. In the case of chloroform it is clear that 5 mM concentrations, which corresponds to the minimum blocking concentration (MBC) (18) produce strong effects on the polymorphism of soya phosphatidylethanolamine, favoring formation of the hexagonal (H_{II}) phase at -5°C. Alternatively, it is equally clear from Fig. 5b that 500 mM concentrations of ethanol, again corresponding to the MBC, also produce strong effects at 5°C. However, in contrast to the situation for chloroform, ethanol stabilizes the bilayer arrangement over the hexagonal H_{II} phase. Finally, 0.5 mM concentrations of dibucaine, which produce anesthesia *in vivo* are observed to produce strong bilayer stabilization effects for phosphatidylethanolamine-phos-

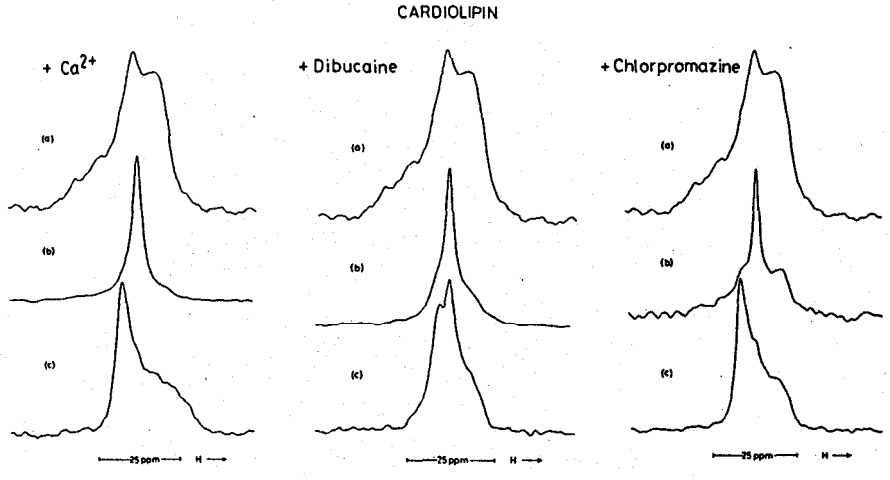


FIG. 3. Influence of Ca^{2+}, dibucaine, and chlorpromazine on the (36.4 MHz) ^{31}P NMR spectra arising from aqueous dispersions of beef heart cardiolipin. The (a), (b), and (c) figures for the Ca^{2+} also correspond to indicate Ca^{2+}/cardiolipin ratios of 0, 0.6, and 1.0, respectively. In the case of the dispersions to which anesthetics were added. the molar ratios of anesthetic to cardiolipin were: (a) 0; (b) 0.9; and (c) 2.0. Spectra were taken at 30°C.

phatidylserine systems, where hexagonal (H_{II}) phase structure has been induced by the presence of Ca^{2+}. This is equivalent behavior to that shown in Fig. 4b and 4c.

Two aspects of these results deserve further discussion. First, a ubiquitous narrow ^{31}P NMR component at the position corresponding to isotropic motional averaging, is visible in many of the spectra. As indicated earlier, such a component can arise from many structures available to hydrated phospholipids, including inverted micellar, cubic, rhombic, and so forth. In addition, such a spectral feature is commonly observed for phospholipid systems undergoing a transition from the bilayer to the H_{II} arrangements, or vice versa (see Fig. 3). The structure of this intermediate is not well characterized, although in the case of cardiolipin, freeze-fracture studies suggest an intrabilayer inverted micellar lipid structure (7,21).

Secondly, it is clear from the results in Figs. 3 to 5 that the same drug (e.g., dibucaine) can exhibit markedly different effects depending on the model system employed. In the case of cardiolipin, dibucaine induces the H_{II} phase, whereas in the phosphatidylethanolamine - phosphatidylserine - Ca^{2+} systems dibucaine inhibits the H_{II} phase and promotes formation of the bilayer. Alternatively, different anesthetics can induce opposite effects in the same model system, as indicated by the ability of chloroform to promote H_{II} phase formation in soya phosphatidylethanolamine dispersions, whereas ethanol stabilizes the bilayer. Although it is too early to try to correlate such differences with the differing pharmacological effects of these agents, it is clear that a potential for a variety of effects exists depending on drug type and distribution.

The physical basis for this ability of the anesthetics investigated to modulate lipid polymorphism is not well understood as yet. However, in

FIG. 5. 81.0 MHz ^{31}P NMR spectra arising from model systems incubated in the presence of various aqueous concentrations of anesthetic molecules. a: Influence of chloroform on the polymorphism of soya phosphatidylethanolamine at − 5°C. b: Influence of methanol on the polymorphic phase adopted by soya phosphatidylethanolamine at 5°C. c: Influence of dibucaine on the phase adopted by aqueous dispersions of 20 mole % soya phosphatidylserine-80 mole % soya phosphatidylethanolamine in the presence of 5 mM Ca^{2+} at 20°C. Methanol and chloroform were introduced into the lipid by dialysis of the lipid dispersion (40 mg phospholipid in 1 ml) against 500 ml 10 mM Tris-HCl (pH 7.2) containing an appropriate amount of dissolved ethanol or chloroform. The dibucaine was introduced by dissolving the appropriate amount in 200 ml 10 mM Tris-HCl, 5 mM Ca^{2+} to which the (hydrated) lipid dispersion was added and subsequently concentrated for ^{31}P NMR studies by centrifugatton.

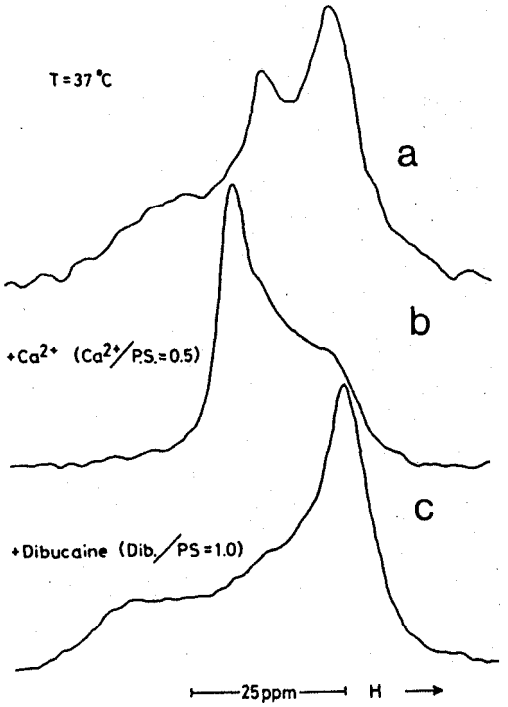
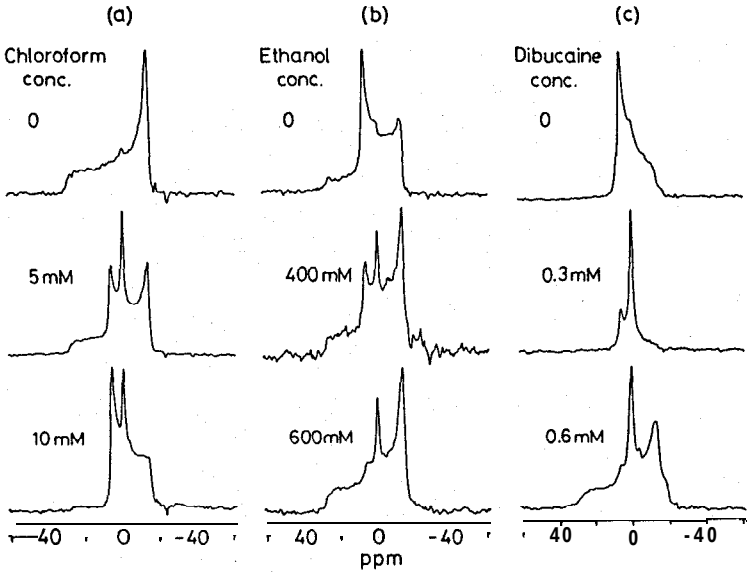


FIG. 4. 36.4 MHz ^{31}P NMR spectra at 37°C arising from an aqueous dispersion of 20 mole % (beef brain) phosphatidylserine and 80 mole % (egg yolk) phosphatidylethanolamine (a) in the absence of Ca^{2+} or dibucaine; (b) in the presence of Ca^{2+} (Ca^{2+} to phosphatidylserine ratio = 0.5); (c) in the presence of Ca^{2+} and dibucaine (dibucaine to phosphatidylserine ratio = 1.0). For additional detail see ref. 6.

another work (4) it has been suggested that to a first approximation the (dynamic) molecular shape of lipids dictates the polymorphic phase assumed. Within this framework, lipids that exhibit a larger cross-sectional area in the headgroup as opposed to the acyl chain region ("inverted cone"-shaped) adopt phases such as the micellar, whereas lipids with opposite geometry ("cone" shape) such as unsaturated phosphatidylethanolamine adopt the H_{II} phase. Finally, lipids with a cylindrical shape would be compatible with the familiar bilayer phase. On this basis the action of anesthetics may be suggested to arise from factors such as charge neutralization (which would result in an effective decrease in the area per molecule in the headgroup region) or would be a consequence of where the anesthetic molecules reside (i.e., at the lipid-water interface or in the hydrocarbon region). In the case of ethanol, for example, the relatively polar nature of this molecule would suggest a location at the lipid-water interface, which would correspond to a net increase in the area at this interface. This would be consistent with the stabilization of the bilayer over the H_{II} arrangement. Alternatively, chloroform would be expected to reside in the hydrocarbon region, which would be consistent with a tendency to favor the H_{II} arrangement.

SPECULATIONS

In this chapter we have shown that anesthetics can strongly affect the polymorphic phases adopted by lipids, and further that such effects are



observable at pharmacological concentrations of these drugs. Given such a situation, it is obviously tempting to speculate on the possible roles of nonbilayer lipids in facilitated transport processes (which may include the Na+ channel). Here we give way to this temptation, but wish to emphasize, however, that the models suggested below are very speculative and should be viewed with suspicion.

A role of H_{II} phase lipids as channel formers is directly suggested by the characteristic aqueous channel inherent to this arrangement (see Fig. 1) and such possibilities have been recognized for some time (10). On the basis of least energy considerations, however, it is unlikely that a short region of H,, phase lipid could assume an orientation perpendicular to the plane of the surrounding bilayer, and it would appear necessary to postulate a role of protein to stabilize this arrangement, as indicated in Fig. 6a. From the point of view of the Na+ channel a difficulty with this mode1 is that the diameter of the H_{II} phase aqueous pore is too large (-20 A), suggesting that any "selectivity filter" would have to be incorporated into the protein caps.

An alternative transport mechanism where the lipid acts as a carrier is indicated in Fig. 6b. This model makes use of the fact that a general characteristic of any carrier system must be an ability to form a lipid soluble intermediary complex with the agent to be transported-a demand that would be satisfied for lipids that adopt short intrabilayer cylinders (H_{II} arrangement) or inverted micelles in response to the presence of the transported agent. Alternatively, membrane protein may modulate such an arrangement. Such speculations are consistent with the observation that lipids such as cardiolipin and phosphatidic acid, which form the hexagonal (H_{II}) phase in the presence of Ca^{2+} (I 6,17), act as Ca^{2+} ionophores in model systems (20).

In summary, we wish to reemphasize the more important aspect of our results, which indicates that pharmacological concentrations of anesthetic strongly affect lipid polymorphism. Within the terms of the above models, the effects of anesthetics would be to disrupt or inhibit a nonbilayer lipid component vita1 to Na+ channel function. Although we expect that these models will be shown to be at least partially incorrect (particularly as they apply to Na+ conductance), it is clear that these observations offer an exciting new direction for anesthetic research.

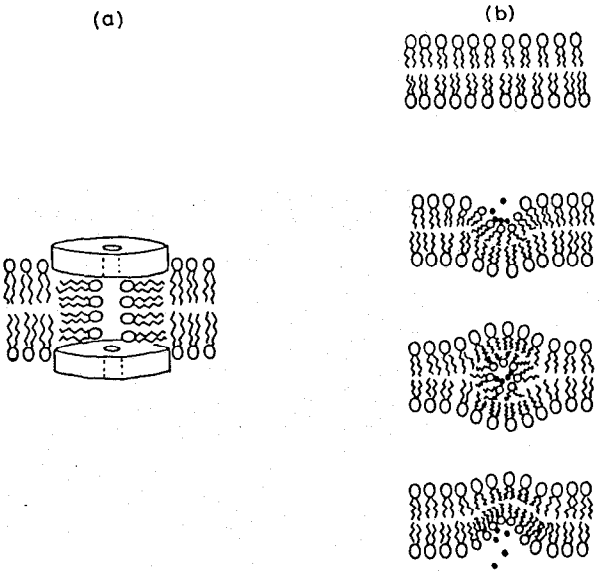


FIG. 6. Two speculative models whereby transport of polar molecules may be facilitated by the availability of nonbilayer lipid structures. a: Transport is facilitated by a lipid-protein complex employing a cylindrical section of H_{II} phase lipid forming the central channel. This is represented by the lipid oriented perpendicular to the plane of the surrounding bilayer lipid, and is hypothetically stabilized in this configuration by doughnut-shaped proteins with hydrophobic and hydrophilic sides. Transport is envisaged to occur through the central pore. b: Transport is facilitated for an agent which induces formation of (transitory) intrabilayer H_{II} or inverted micelles (see text).

ACKNOWLEDGMENTS

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