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INTERACTION OF DIBUCAINE AND PROPRANOLOL WITH PHOSPHOLIPID BILAYER MEMBRANES—EFFECT OF ALTERATIONS IN FATTY ACYL COMPOSITION

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Abstract—Sodium 22 efflux was measured, at various temperatures both in the presence and absence of dibucaine or propranolol, in multilamellar liposomes composed of different phosphatidylcholines and dicetylphosphate. Such vesicles display a marked increase in sodium permeability in the temperature region of the phase transition of the component phospholipid. Both local anesthetics lower the temperature of onset of this permeability increase, the extent of the reduction being dependent upon the anesthetic concentration. The liposomes used in these experiments possess a negative charge due to the presence of dicetylphosphate. When exposed to dibucaine, these vesicles develop a significant positive surface potential at the same temperature at which this anesthetic increases ^{22}Na efflux. In a parallel series of experiments, a spin-labeled fatty acid ester was incorporated into similar liposomes and the mobility of the label used as a measure of lipid hydrocarbon chain motion. The phase transition of a phospholipid is associated with a marked increase in fatty acyl chain motion. Both anesthetics lower the temperature at which the lipid chains display significant motional freedom. These observations indicate that dibucaine and propranolol interact with liposomes so as to bring about the phase transition (i.e. melt the hydrocarbon chains) of the membrane lipids at a temperature below that at which this event normally occurs.

Local anesthetics block nerve conduction by interfering with the increased sodium conductance associated with the rising phase of the action potential [1]. Even though their mechanism of action is not completely understood, a number of studies have drawn attention to a correlation between anesthetic potency and the ability to interact with phospholipids [2]. Although biological membranes, such as that of the nerve cell, are complex structures, current evidence suggests that a phospholipid bilayer forms the backbone of the membrane with proteins embedded to a variable depth in this lipid matrix [3]. The correlation noted above, together with the important role of phospholipids in cell membrane structure, suggests that the main site of action of these agents is the lipid matrix of the nerve cell membrane. Cationic local anesthetics display a rather complex interaction with phospholipid membranes. These agents can adsorb onto both neutral and negatively charged membranes, resulting in marked changes in both surface charge and ion permeability [4]. However, other observations indicate that these same agents can also significantly perturb the hydrocarbon core of such membranes so as to "fluidize" this region of the bilayer [5, 6]. It is this perturbation that is explored more fully in this paper.

MATERIALS AND METHODS

Materials. Egg phosphatidylcholine (PC) was extracted from fresh eggs and purified by alumina and silicic acid chromatography [7]. Dimyristoyl and dipalmitoyl PC were purchased from CalBiochem, Calif. Each of these phospholipids showed a single spot on thin-layer chromatography. All three phospholipids were stored as stock chloroform solutions under N_2 at $-20°$. Dicetylphosphate (DCP) was obtained from Sigma Chemical Co., St. Louis, MO, and ^{22}Na as the chloride salt from Amersham/Searle. Dibucaine was purchased from K & K Laboratories while propranolol was generously supplied by Ayerst Laboratories, Montreal, Canada. All other chemicals were of reagent grade wherever possible. Twice distilled water was used for all experiments.

Preparation of lipid vesicles (liposomes). Egg PC–DCP liposomes were prepared by previously described methods [7]. Briefly, appropriate aliquots of stock chloroform solutions of egg PC and DCP to give a mole ratio of 95% PC, 5% DCP, were dried under vacuum in a glass tube. The required salt solution in a volume of 1 ml/15 μmoles lipid and containing tracer amounts of $^{22}NaCl$ if efflux measurements were to be made, was then pipetted into the tube and the mixture mechanically shaken on a rotamixer. This step was carried out at room temperature and continued until all of the lipid was suspended. The resulting milky dispersion was left overnight at room temperature under N_2.

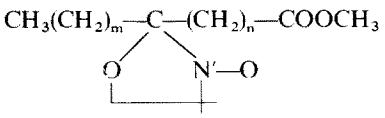
The preparation of dimyristoyl or dipalmitoyl PC (95%)–DCP (5%) liposomes required a modification of the above described method. These saturated phospholipids form characteristic vesicles only when the temperature exceeds their phase transition temperature (23° for dimyristoyl PC, 41° for dipalmitoyl PC). Hence, the salt solution, in which the lipid was dispersed, was pre-heated to 50° and the tube, when not applied to the rotamixer, was immersed in a water bath also at 50°. The final suspensions were left overnight under N_2 at either room temperature for dipalmitoyl PC–DCP vesicles or at 5° for dimyristoyl PC–DCP vesicles.

Measurement of ^{22}Na efflux. After overnight equlibration, a given lipid dispersion was passed down a

column of Sephadex G-50 coarse (Pharmacia, Montreal) to remove excess tracer not trapped within the liposomes. Dimyristoyl PC–DCP vesicles are very leaky in the temperature region of their phase transition, which is close to room temperature. Therefore, these liposomes were eluted from "chilled" Sephadex using a salt solution previously cooled to 5°. This was done to reduce the loss of trapped isotope while this particular lipid was still on the column. One-ml portions (about 1 μmole) of eluted lipid were pipetted into dialysis bags, which were then sealed and dropped into stoppered-glass tubes containing 10 ml of aqueous solution. All tubes were placed in a shaking water bath maintained at the desired temperature. The efflux rate was measured over a 3-hr period. At the termination of each experiment, the ^{22}Na content of the various tubes and bags was counted on a Nuclear–Chicago gamma scintillation counter. Effluxes are expressed as the percentage of initial trapped radioactivity lost over the 180 min. This 3-hr flux period was chosen primarily to allow sufficient counts to collect in the bulk aqueous phase in those experiments performed at low temperatures and hence involving small leakage rates.

Electrophoretic measurements. The electrophoretic mobility of different liposome populations was measured at various temperatures in a cylindrical microelectrophoresis chamber [8]. Under the influence of an electric field, the vesicles will migrate toward the electrode of opposite polarity. For a given lipid particle, the mobility is expressed as the velocity ($\mu m \cdot sec^{-1}$)/unit potential gradient ($V \cdot cm^{-1}$). For each sample, the mobility of at least ten to fifteen liposomes was measured and the results were averaged. The range of values was never greater than ± 10 per cent of the mean.

Electron spin resonance (ESR) spectroscopy. Two spin-labeled fatty acid esters, with the following general formula, were obtained from Syva Corp., Palo Alto, Calif.



in which I (m,n) = I (12,3) and II (m,n) = II (5,10).

The label in a stock chloroform solution was added to the lipid prior to drying at a concentration of 1 mole/100 moles total lipid. Hence, the composition of the liposomes by mole ratio was PC 94%, DCP 5% and probe 1%. Electron spin resonance spectra were recorded on a Varian E3 X-band spectrometer with 100 kHz modulation using a special quartz flat cell placed in a custom-designed quartz temperature control probe [9]. The temperature of the sample was maintained at better than ±1° during the measurement.

RESULTS

Permeability experiments. Dimyristoyl, dipalmitoyl, and dimyristoyl–dipalmitoyl PC–DCP membranes show a sharp increase in permeability in the region of their phase transition temperatures (Figs. 1 and 2). When dibucaine or propranolol is present, the increase in ^{22}Na efflux appears at a temperature about 5° lower than that which occurs in untreated liposomes.

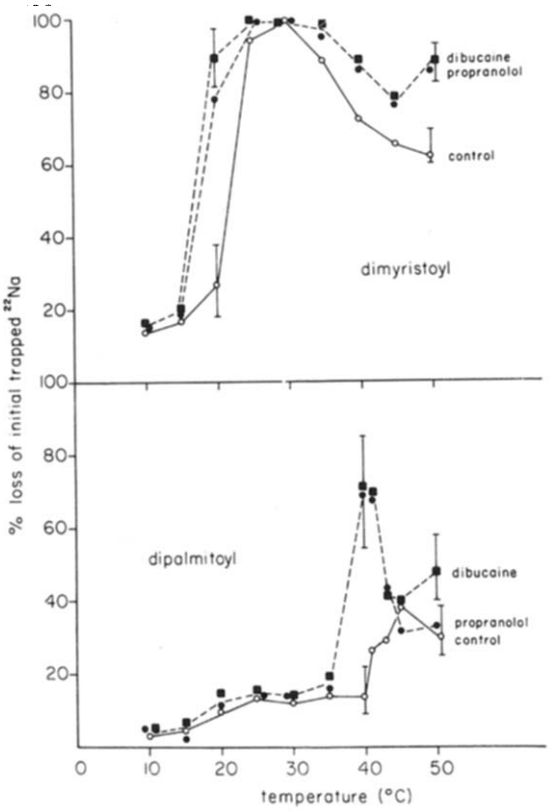


Fig. 1. Effect of dibucaine and propranolol on ^{22}Na efflux. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5% (upper half) or dipalmitoyl PC 95%, DCP 5% (lower half) were dispersed in 50 mM NaCl, $^{22}NaCl$ and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into three portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and in the presence of either dibucaine (1 mM) or propranolol (1 mM). The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points for each temperature represent a separate experiment performed in quadruplicate. The results illustrated are the means with error bars indicating the range of values obtained. To simplify the figure the error bars have been given for only some of the points.

Vesicles containing egg PC, which is above its transition temperature over the whole temperature range examined, show no permeability maximum either in the presence or absence of the two local anesthetics (Fig. 2).

The experiments described above were all performed at a fixed anesthetic concentration of 1 mM. This concentration was chosen on the basis of previous work carried out in this laboratory [4]. Figure 3 summarizes a series of experiments illustrating the effects of higher concentrations of dibucaine on the ^{22}Na permeability of dimyristoyl PC–DCP liposomes. Dibucaine (1 mM), as already noted, causes a significant increase in ^{22}Na efflux at 20° but not at 15°. A 2 mM concentration significantly increases sodium transport at 20°, but also produces a modest increase at 15°. Dibucaine (4 mM) markedly enhances sodium permeability even at 15°.

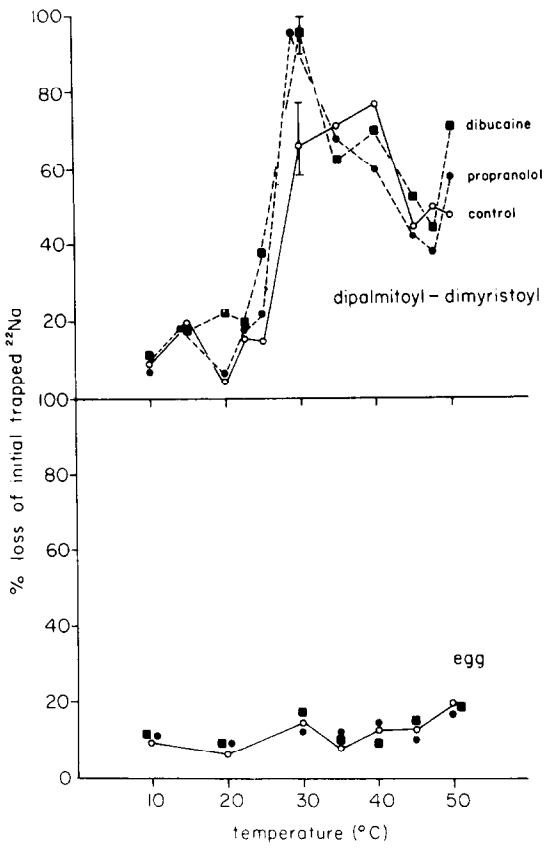


Fig. 2. Effect of dibucaine and propranolol on ^{22}Na efflux. Liposomes composed (by mole ratio) of dimyristoyl PC 47.5%, dipalmitoyl PC 47.5%, DCP 5% (upper half) or egg PC 95%, DCP 5% (lower half) were dispersed in 50 mM NaCl, $^{22}NaCl$, and 6 mM Tris-HCl, pH 7.5. Each liposome preparation was divided into three portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and in the presence of either dibucaine (1 mM) or propranolol (1 mM). The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points for each temperature represent a separate experiment performed in quadruplicate. The results illustrated are the means with error bars indicating the ranges of values obtained. To simplify the figure the error bars have been given for only several points.

Electrophoretic mobility measurements. Negatively charged liposomes of identical composition to those used in the permeability experiments were electrophoresed at different temperatures both in the presence and absence of dibucaine (Fig. 4). Both dimyristoyl and dipalmitoyl PC–DCP "control" liposomes become slightly more negatively charged as the temperature is increased. However, no significant discontinuities occur over the temperature ranges examined. In the presence of dibucaine, an abrupt change in electrophoretic mobility occurs at about 18° for dimyristoyl PC–DCP vesicles and at about 37° for dipalmitoyl PC–DCP vesicles. In both cases, the liposomes develop a significant positive charge over a narrow temperature interval. It is also evident from Fig. 4 that at temperatures below the phase transition temperature some anesthetic molecules still adsorb onto the membrane surface since the electrophoretic mobility of the lipid particles is less negative in the presence of dibucaine than in its absence.

Spin probe experiments. Two different spin probes were used to label the liposomes. Assuming that the probe molecules align themselves parallel to the phospholipid hydrocarbon chains with their polar groups at the membrane–water interface, then the two spin labels will monitor motion at different planes within the bilayer. The nitroxide group of probe I (12,3) will lie close to the lipid–water interface, while the nitroxide group of II (5,10) will be buried deeper within the hydrocarbon core. The main information sought from the ESR spectra was the effect of the two local anesthetics on probe, and hence fatty acyl chain, motion. Since only relative changes in mobility were examined, two empirical parameters were used to semi-quantitate spin label motion. For ESR spectra performed at low temperatures the separation between the low and high field extrema ($2T_{\|}$) was measured. Increasing molecular motion is characterized by a narrowing of this separation [10]. With sufficient probe motion, however, the high field deflection disappears making it impossible to measure $2T_{\|}$. For these spectra the rotational correlation time τ_c was calculated according to the following formula [11]:

$$\tau_c = 6.5 \times 10^{-10}\, Wo[(ho/h - 1)^{1/2} - 1]$$

where *Wo*, *ho*, $h - 1$ are all defined in Fig. 5.

This equation is only valid for times less than about 2 nsec [12]. Increasing probe mobility is associated with a smaller value for τ_c.*

Figure 5 illustrates some representative spectra obtained with dipalmitoyl PC–DCP vesicles labeled with II (5,10) and measured in the presence and absence of dibucaine. The "motion parameters" $2T_{\|}$ and τ_c for all of the spectra are summarized in Fig. 6.

In the case of dipalmitoyl PC–DCP liposomes, both probes display a moderate increase in mobility (as indicated by a decrease in $2T_{\|}$) over the temperature range of 10–30°. Above this temperature, probe mobility significantly increases as manifested by a disappearance of the upper field deflection and by a progressive decrease in τ_c. Label II (5,10) shows a rather marked increase in motion between 35 and 40°. The deeper probe, II (5,10), displays more molecular motion than the superficial one. In the presence of the two anesthetics, the above described changes in probe motion occur at a lower temperature. In the case of I (12,3), the upper field deflection disappears after 25° and in the case of II (5,10) it disappears

*Unfortunately, neither of these two parameters, $2T_{\|}$ or τ_c, could be used throughout the whole temperature range. Actually the spectra obtained from labeled dipalmitoyl or dimyristoyl PC–DCP liposomes at temperatures below the phase transition temperature appear to be a composite of two populations of spin probes. For example in the spectra of Fig. 5, measured at 20°, the low and high field deflections measured by the separation $2T_{\|}$ are characteristic of a highly immobilized nitroxide group. The sharper lines encompassed by the low and high field extrema, however, are more characteristic of a mobile nitroxide group. Most likely, when the lipid is in the gel state, some of the probe molecules partition into the water phase, giving rise to this mobile population.

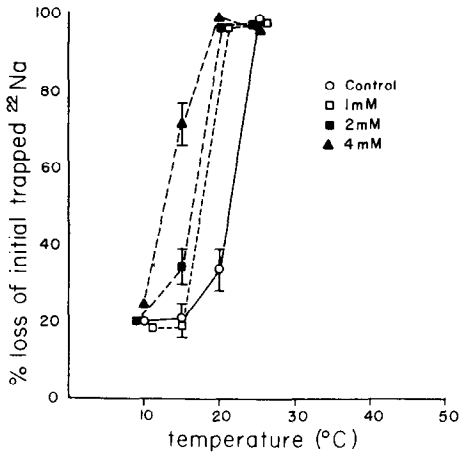


Fig. 3. Effect of different concentrations of dibucaine on ^{22}Na efflux. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5%, were dispersed in 50 mM NaCl, ^{22}NaCl, and 6 mM Tris–HCl, pH 7.1. Each liposome preparation was divided into four portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and in the presence of three different concentrations of dibucaine. These experiments were performed at pH 7.1, since a 4 mM solution of dibucaine could not be prepared at a higher pH. The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points for each temperature represent a separate experiment performed in quadruplicate. The results illustrated are means with error bars indicating the ranges of values obtained.

after 20° with propranolol and 25° with dibucaine. The rotational correlation times are lowered by the anesthetics but become equal to those of control liposomes by 45°.

Dimyristoyl PC–DCP vesicles display qualitatively similar results. Probe motion increases modestly over the range 10–20°. Above this temperature, the upper field deflection disappears and τ_c decreases with rising temperatures. Again the deeper probe possesses more motion than the superficial one at all temperatures. The parameter $2T_{\parallel}$ decreases significantly for I (12,3) between 15 and 20° in the presence of both anesthetics. In the case of II (5,10), the upper field deflection disappears after 15° in the presence of propranolol and after 10° in the presence of dibucaine. Above 20° both anesthetics have no consistent significant effect on probe motion.

DISCUSSION

The purpose of this study was to examine the interaction of cationic local anesthetics with phospholipid bilayer membranes and in particular with the hydrocarbon interior of such membranes. The two anesthetics used in these experiments were chosen for several reasons. First, the pK values of these two agents are different [4]. In the case of dibucaine the pK is 8.5, while for propranolol it is 9.45. Hence at pH 7.5, which is the pH at which most of the permeability and all the spin label experiments were performed, the concentration of uncharged dibucaine molecules will be about ten times greater than the concentration of uncharged propranolol molecules. The observation that dibucaine and propranolol were equally effective in both the permeability and ESR spectroscopic experiments would exclude the possibility that only the uncharged molecule is the active species. However, it is possible that either the charged species alone or that some combination of charged and uncharged forms is responsible for the observed effects. Second, space filling models of these two molecules [4] are remarkably similar except for the presence of a 4-carbon group projecting from the ring structure of dibu-

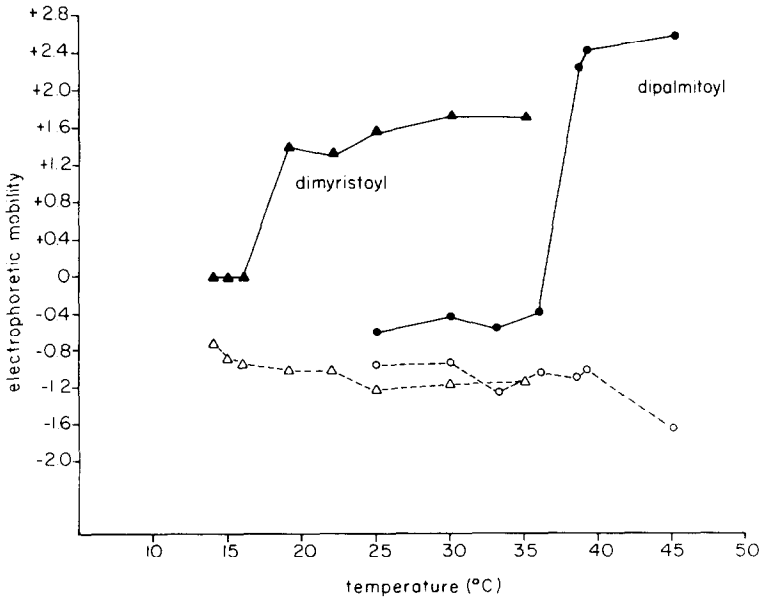


Fig. 4. Effect of dibucaine on electrophoretic mobility. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5% or dipalmitoyl PC 95%, DCP 5% were dispersed in 50 mM NaCl and 6 mM Tris–HCl, pH 7.5. The electrophoretic mobility of the lipid vesicles was measured at different temperatures both in the absence (open symbols) or presence (closed symbols) of 1 mM dibucaine. Each point represents the mean of at least ten to fifteen measurements with the range of values being within ± 10 per cent of the mean. The ordinate has the units $\mu m\ sec^{-1} \cdot V^{-1} \cdot cm$.

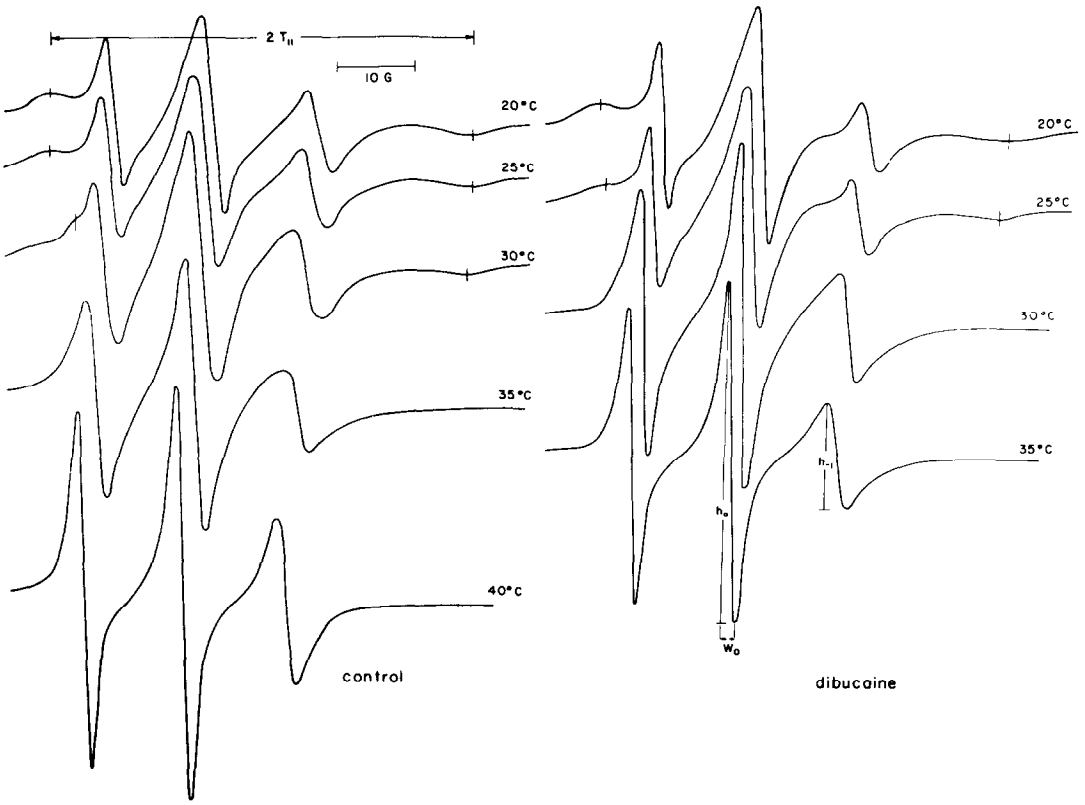


Fig. 5. Effect of dibucaine on spin-labeled dipalmitoyl PC–DCP vesicles. Liposomes composed (by mole ratio) of dipalmitoyl PC 94%, DCP 5%, spin label II (5,10) 1%, were dispersed in either 50 mM NaCl and 6 mM Tris–HCl, pH 7.5 (control) or 50 mM NaCl, 6 mM Tris–HCl, pH 7.5, and 1 mM dibucaine. Electron spin resonance spectra were recorded at the indicated temperatures.

caine. Most likely these molecules are oriented with their polar ends at the membrane–water interface and their "lipophilic" portion extending into the bilayer. By virtue of this 4-carbon tail, dibucaine should penetrate more deeply into the membrane than propranolol. Again the observation that these two anesthetics were equally effective in the permeability and spin label experiments implies that the deeper penetration of dibucaine is not essential to bring about the effects described in this study.

The model system used in these experiments consists of an aqueous suspension of lipid vesicles or liposomes. The physical characteristics and properties of liposomes have recently been reviewed by Bangham *et al.* [13]. Each of these vesicles consists of a series of concentric unbroken bimolecular phospholipid membranes, separated by discrete and isolated internal aqueous compartments. One particularly attractive feature of this model membrane is that it lends itself to a number of different physical measurements. In this study for instance, identical liposome populations were used to measure ^{22}Na efflux, to assess changes in surface charge, and to monitor hydro-carbon chain motion via the spin label technique. Since the same membrane system was used for all of these different measurements, the results become directly comparable.

Liposomes were formed from three phosphatidylcholines differing in their fatty acyl composition. Dimyristoyl PC has a phase transition temperature (T_c) of 23° while the T_c values for dipalmitoyl and egg PC are 41 and about −10° respectively [13, 14]. An equimolar mixture of dimyristoyl and dipalmitoyl PC possesses a single transition temperature intermediate between that of the two individual phospholipids [14]. The T_c of a phospholipid represents the temperature at which the gel to liquid crystal phase transition occurs. In the gel condition, the lipid chains are in a quasi-crystalline state while these same chains become much more mobile and "fluid" above the transition temperature. In essence, the fatty acyl chains "melt" as the lipid is heated above its T_c. Recent studies [15, 16] have demonstrated the existence of a maximum in the permeability of both electrolytes and non-electrolytes in the vicinity of the transition temperature of the membrane lipids. This permeability increase is a function of such variables as the length of the hydrocarbon chains being less marked for dipalmitoyl than dimyristoyl PC membranes. In the region of the T_c some of the lipid will be present in the gel state, while some will already be melted and present in the liquid crystal form. It is postulated that the increased permeability is due to enhanced diffusion across discontinuities present at the boundary regions between liquid and solid lipid domains. Dimyristoyl, dipalmitoyl and dimyristoyl–dipalmitoyl PC–DCP liposomes all display a local maximum in ^{22}Na efflux in the region of their phase transition temperatures. In the presence of the two local anesthetics, the onset of the increase in ^{22}Na transport is shifted to a lower temperature, the shift being greater, the higher the anesthetic concentration.

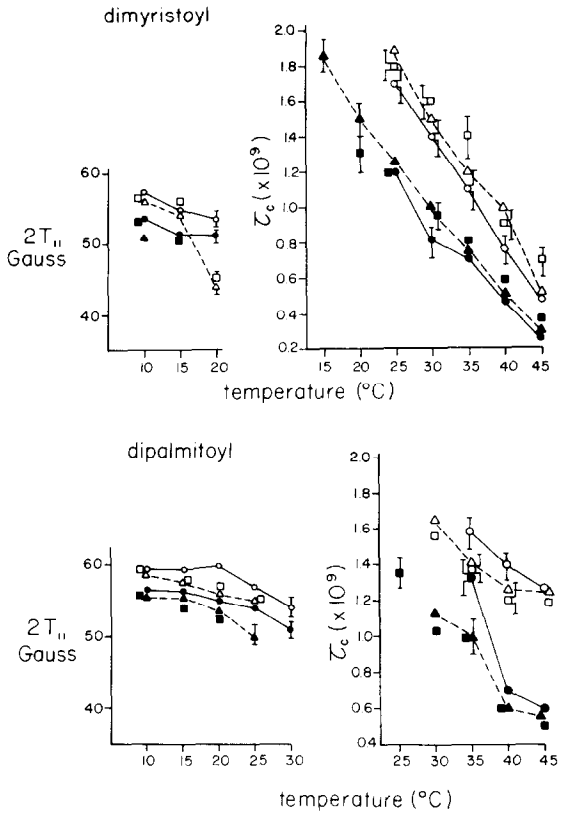


Fig. 6. Effect of dibucaine and propranolol on the "motion parameters" $2T_{\parallel}$ and τ_c. Upper half: Liposomes composed (by mole ratio) of dimyristoyl PC 94%, DCP 5% and either spin label I (12,3) or II (5,10) 1%, were dispersed in 50 mM, NaCl and 6 mM Tris–HCl, pH 7.5. Electron spin resonance spectra were recorded at the temperatures indicated on the abscissa both in the absence (control) and presence of dibucaine (1 mM) or propranolol (1 mM). The parameters $2T_{\parallel}$ and τ_c are described in the text. Symbols used are as follows: open symbols: liposomes containing probe I (12,3); closed symbols: liposomes containing probe II (5,10); circles: no anesthetic (control); triangles: dibucaine 1 mM; and squares: propranolol 1 mM; Each point is the mean value obtained from five separate spectra. Error bars denote the ranges of values obtained and to simplify the figure have been given for only some of the points. However, in the case of the parameter τ_c the data were reproducible to within 8 per cent. Lower half: These spectra were recorded from spin-labeled liposomes containing dipalmitoyl PC. Otherwise, experimental details and symbols used are the same as those described above.

These observations indicate that the anesthetics have caused the lipid fatty acyl chains to melt at a lower temperature than that which normally occurs. This conclusion is supported by the results of the spin label experiments. Two fatty acid ester spin probes were incorporated into liposomes to monitor lipid chain motion. The underlying assumption of this technique is that the mobility of the spin-labeled probe is a valid measure of the molecular motion of the surrounding phospholipid fatty acyl chains. Spin-labeled dimyristoyl PC–DCP vesicles show a large increase in probe mobility as the temperature exceeds 20°. This change corresponds closely with the T_c of dimyristoyl PC of 23°. At all temperatures, molecular motion increases toward the centre of the membrane consistent with the fluidity gradient hypothesis [17]. Dipalmitoyl PC–DCP liposomes display an increase in probe mobility above 30° with the deeper probe showing a rather marked increase in mobility between 35 and 40°. These changes occur at a slightly lower temperature than the T_c of dipalmitoyl PC, which is 41°. As with dimyristoyl PC–DCP liposomes, the deeper probe possesses more molecular motion than the superficial one. For both types of liposomes, probe motion increases at a lower temperature in the presence of the anesthetics than in their absence.

In the case of dimyristoyl PC DCP liposomes, there is good agreement between the spin label studies and the permeability experiments. Anesthetic-treated vesicles demonstrate an increase in probe motion above 15° and an increase in ^{22}Na efflux between 15 and 20°. For dipalmitoyl PC–DCP liposomes the correlation is not as good. The anesthetics induce an increase in probe motion above 25°, yet ^{22}Na permeability does not change until a temperature between 35 and 40° is reached. These results indicate that, at least in this lipid, the anesthetics begin to "melt" the hydrocarbon chains at a temperature about 10° below that at which these agents increase ^{22}Na efflux.

The surface adsorption of anesthetic molecules was monitored by measuring changes in electrophoretic mobility. The term "surface adsorption" refers chiefly to the position of the charged group. However, as already discussed, these molecules are most likely oriented with their polar ends at the membrane–water interface and their non-polar part extending into the bilayer. In essence, the charged end is used in these experiments as a "marker" for the whole molecule. No attempt has been made to quantitate the change in electrophoretic mobility in terms of the number of dibucaine molecules adsorbed. It should be recalled that the electrophoretic mobility measures the electrical potential not at the actual membrane–water interface, but rather a finite distance away at the hydrodynamic slip plane of the lipid particle. In addition, the surface area of multilamellar liposomes is difficult to define precisely.

An abrupt increase in the surface concentration of anesthetic molecules occurs at about the same temperature at which these agents increase ^{22}Na efflux. The mechanism underlying this temperature-dependent process is probably as follows. At low temperatures a small number of anesthetic molecules adsorb onto the surface. At a certain temperature, dependent upon the lipid, the anesthetic begins to induce a "melting" of the bilayer. This change would result in an enhancement of the adsorption process perhaps by permitting more hydrophobic interactions between the non-polar part of the molecule and the membrane interior. The enhanced adsorption would result in more melting, which in turn would lead to more adsorption, etc. The melting–adsorption sequence is similar to an "autocatalytic" process. In the case of dipalmitoyl PC–DCP vesicles, the spin label studies support this mechanism. The anesthetics begin to increase lipid chain motion at 25°, about 10–12° below the temperature at which the electrophoretic mobility abruptly changes. Associated with the change in electrophoretic mobility, a further "melting" of the bilayer occurs as manifested by a marked increase in motion of probe II (5,10) between the temperatures 35 and

40°. For dimyristoyl PC–DCP liposomes the same mechanism probably applies, although the anesthetic-induced changes in spin label motion and electrophoretic mobility appear to occur, within experimental error, at the same temperature.

The adsorption of anesthetic molecules results in the development of a significant positive membrane surface charge. The charge reversal observed in the presence of dibucaine indicates that this agent binds to the membrane by forces other than those of purely electrostatic origin. As already alluded to, these non-electrostatic forces probably represent hydrophobic interactions between the non-polar part of the dibucaine molecule and the hydrocarbon interior of the bilayer. In addition, the observation that dibucaine renders the liposomes positively charged yet increases ^{22}Na permeability implies that the increased ^{22}Na movement occurs across regions of the membrane relatively unaffected by the surface charge. On the basis of charge considerations alone, sodium efflux should have been reduced.

The relationship between the capacity of these anesthetics to lower the phase transition temperature of saturated phospholipids and their ability to block nerve conduction must remain speculative at the moment. However, it is interesting that inhalation anesthetics also depress the phase transition temperature of bilayer membranes composed of dipalmitoyl PC [18]. As noted in the beginning, of this paper, the proteins of biological membranes are embedded to variable depths in a phospholipid bilayer matrix which can be viewed as a solvent system for these proteins. Furthermore, the state of fluidity of this solvent appears to be important for the proper functioning of these proteins, many of which are involved in transport processes [19]. Anesthetics, by changing the transition temperature of the phospholipids and hence the fluidity of the lipid solvent, might secondarily impair the function of those proteins which constitute the so-called sodium channel.*

*It should be recalled that the conductance of a phospholipid bilayer is extremely small when compared to the conductance of the usual biological membrane. Hence, a reduction in ion flow through the "sodium channel" will overshadow any small increase in permeability that may occur in the surrounding lipid bilayer.

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REFERENCES

1. P. Seeman, *Pharmac. Rev.* **24,** 583 (1972).
2. D. Papahadjopoulos, *Biochim. biophys. Acta* **265,** 169 (1972).
3. S. J. Singer, *Ann. N.Y. Acad. Sci.* **195,** 16 (1972).
4. M. A. Singer, in *Progress in Anesthesiology* (Ed. B. R. Fink), Vol. 1, p. 223. Raven Press, New York (1975).
5. D. Papahadjopoulos, K. Jacobson, G. Poste and G. Shepherd, *Biochim. biophys. Acta* **394,** 504 (1975).
6. K. W. Butler, *J. pharm. Sci.* **64,** 497 (1975).
7. M. A. Singer, *Can. J. Physiol. Pharmac.* **52,** 930 (1974).
8. A. D. Bangham, R. Flemans, D. H. Heard and G. V. F. Seaman, *Nature, Lond.* **182,** 642 (1958).
9. M. A. Singer, and J. K. S. Wan, *Can. J. Physiol. Pharmac.* **53,** 1065 (1975).
10. J. L. Rigaud, C. M. Gary-Bobo and C. Taupin, *Biochim. biophys. Acta* **373,** 211 (1974).
11. A. Keith, G. Bulfield and W. Snipes, *Biophys. J.* **10,** 618 (1970).
12. S. Eletr, M. A. Williams, T. Watkins and A. D. Keith, *Biochim. biophys. Acta* **339,** 190 (1974).
13. A. D. Bangham, M. W. Hill and N. G. A. Miller, in *Methods in Membrane Biology* (Ed. E. D. Korn), Vol. 1, p. 1. Plenum Press, New York (1974).
14. D. Chapman, J. Urbina and K. M. Keough, *J. biol. Chem.* **249,** 2512 (1974).
15. M. C. Blok, E. C. M. Van Der Neut-Kok, L. L. M. Van Deenen and J. De Gier, *Biochim. biophys. Acta* **406,** 187 (1975).
16. D. Papahadjopoulos, K. Jacobson, S. Nir and T. Isac, *Biochim. biophys. Acta* **311,** 330 (1973).
17. P. Post, L. J. Libertini, V. C. Hebert and O. H. Griffith, *J. molec. Biol.* **59,** 77 (1971).
18. J. R. Trudell and E. N. Cohen, in *Progress in Anesthesiology* (Ed. B. R. Fink), Vol. 1, p. 315. Raven Press, New York (1975).
19. R. E. Barnett and J. Palazzotto, *Ann. N.Y. Acad. Sci.* **242,** 69 (1974).