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THE EFFECT OF ANESTHETIC CHARGE ON ANESTHETIC-PHOSPHOLIPID INTERACTIONS

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Cationic and uncharged forms of a tertiary amine local anesthetic are reported to have different properties and potencies as nerve blocking agents. However, the relative capacities of each form of the local anesthetic to perturb the properties of different model membrane systems is unknown. For this reason we have studied the effects of uncharged lidocaine (high pH) and its quaternary amine analogue (W49091) on the phase transition properties of DMPS, DPPE and DPPC liposomes using high-sensitivity differential scanning calorimetry. We report that neutral lidocaine interacts similarly with all three phospholipids. This interaction results in a decrease in the temperature of the gel → liquid crystalline phase transition (T_m), an increase in the enthalpy of the transition (ΔH), and a slight decrease in the cooperativity of melting. Quaternary lidocaine (W49091), on the other hand, interacts significantly with only DMPS; the result being again a decrease in the temperature of DMPS melting, an increase in ΔH, and a slight decrease in the cooperativity of the phase transition. These results are interpreted to indicate that uncharged lidocaine enters the membrane during the DPPE and DPPC phase transitions. In the case of DMPS, an influx of both charged forms of lidocaine must occur at T_m. These anesthetic fluxes at the lipid's phase transition are suggested to be responsible for the observed elevated enthalpies of the respective transitions. The observation that the cationic form of lidocaine does not significantly modify the behavior of DPPC and DPPE liposomes suggests that these lipids are not important components of the anesthetic's site in nerve membranes. However, the dramatic perturbation of the properties of DMPS by W49091 suggests that phosphatidylserine may comprise part of this inhibitory site.

Introduction

Based on the premise that local anesthetics function in the lipid regions of the nerve membrane, a large number of studies have focused on the interactions of local anesthetics with model phospholipid systems. The results of these studies have revealed several anesthetic-induced perturbations of model membrane systems which appear to correlate with an anesthetic's ability to block neurotransmission. From these model studies several explanations of anesthesia have arisen which attribute a compound's nerve blocking potency to its ability to: (i) melt the sodium channel's boundary lipid [1–4], (ii) reduce the cooperativity of the channel's associated lipid phase [5], (iii) disrupt an essential gel ⇌ liquid-crystalline mixed phase domain surrounding the sodium channel [6], (iv) fluidize the nerve membrane [7,8], (v) thicken the nerve membrane [9–11], or (vi) expand the nerve membrane [12].

One aspect of these model studies which has received little attention has been the effect of anesthetic charge on the perturbation of the lipid bilayer by the local anesthetic. While a detailed description

Abbreviations: DMPS, dimyristoyl phosphatidylserine; DPPC, dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl phosphatidylethanolamine; PS, phosphatidylserine; Tricine, *N*-tris-(hydroxymethyl)methylglycine.

of anesthetic binding to lipid membranes does exist [13,14], little is known concerning the relative disruptive influences of the charged and uncharged forms of the anesthetic on the behavior of these model membrane systems. Since the cationic and uncharged forms of the tertiary amine local anesthetics differ considerably in their properties and potencies as nerve blocking agents [15–22], a thorough understanding of their relative potencies as perturbers of model membrane behavior would seem to be necessary before a critical evaluation of the various explanations of local anesthesia (vide supra) can be achieved.

The purpose of this investigation has been to obtain accurate information on the effects of uncharged lidocaine and a quaternary amine analogue of lidocaine, W49091, on the calorimetric properties of several phospholipid dispersions. We report that significant differences exist between the effects of the two charged forms of lidocaine on the temperatures, cooperativities and enthalpies of the DPPC, DPPE and DMPS phase transitions. These data allow a more critical examination of the various proposed mechanisms of local anesthesia which are based on model membrane studies.

Materials

Lidocaine hydrochloride and W49091 were the generous gifts of Dr. Bertil Takman of Astra Pharmaceutical Products, Inc., Worcester, MA. Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Calbiochem-Behring Corp. and dimyristoyl phosphatidylserine (DMPS) was purchased from Avanti Chemical Co. Dipalmitoyl phosphatidylethanolamine (DPPE) and *N*-tris(hydroxymethyl)methylglycine (Tricine) were both purchased from Sigma Chemical Co.

Methods

Preparation of phospholipid suspensions. DPPC and DPPE liposomes were prepared by weighing the desired amount of dry phospholipid (usually 3–4 mg) into a 25-ml conical flask. The phospholipids were then dissolved in either warm chloroform (for DPPC) or chloroform/methanol (3 : 2, v/v) (for DPPE). DMPS was purchased in $CHCl_3$ solution so that the required volume of solution was added directly to the 25-ml conical flask. (The amount of added DMPS was more accurately quantitated using the phosphorus assay of Bartlett [23]). In each case the solvent was removed on a rotary evaporator at 40°C, and the lipid-coated flask was placed in a vacuum chamber for two additional hours to remove any residual solvent. The lipid-coated flask was then heated to a temperature 10 degrees (10 K) higher than the melting temperature of the lipid and prewarmed Tricine buffer (20 mM Tricine plus 120 mM NaCl adjusted to the desired pH) was introduced into the flask to achieve a final lipid concentration of 0.5–1.0 mg/ml. The suspension was vortexed for 30 s at the elevated temperature (10°C above the phase transition temperature) and used the same day as a 'stock suspension' for calorimetric experiments. Directly preceding each scan, 0.8 ml of the stock suspension were mixed with 0.4 ml of the same Tricine buffer solution containing the desired concentration of anesthetic. After mixing, the pH of the suspension was readjusted to the correct value and the calorimetry was carried out. In the case of DPPC and the quaternary lidocaine analogue W49091, complete equilibration of the anesthetic with the liposomes was not achieved within the period (generally 1 or 2 h) between addition of the anesthetic and the calorimetry. Thus, in this case, the W49091 solution was introduced directly into the flask containing the dried lipid film and the liposomes were prepared in the presence of the anesthetic. Also, since the DPPE liposomes were found to rapidly precipitate out of solution at neutral pH, the DPPE suspensions were invariably prepared and stored at pH 9.5 until directly before the calorimetry. At this time the suspensions were adjusted to the desired pH. Unless this precaution was followed, highly variable enthalpies of melting were observed.

Calorimetry. The calorimetry was performed on a Microcal-1 differential scanning calorimeter (Amherst, MA), at a heating rate of 1.0–1.08 K/min. Baseline noise with this instrument was always less than 0.5% of the transition peak height. Reproducibility of the gel to liquid-crystalline phase transition temperature was typically better than ±0.1 K. Enthalpies of each transition were determined by comparison of the area under the calorimetric endotherm with the corresponding area of a DPPC transition which was assumed to have an enthalpy of 8.7

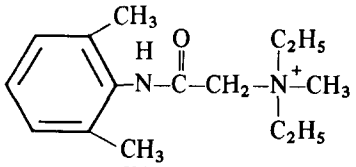
kcal/mol [5,24]. ΔH measurements on control samples drawn from different stock solutions were always reproducible to ±4% and were often reproducible to ±1% (for DPPC).

Buffers. Since the pK_a of lidocaine (7.87, Ref. 25) has a temperature dependence of –0.022/K (personal observations), a buffer had to be chosen which had a similar temperature dependence so that the relative concentrations of the charged and uncharged forms of lidocaine would not change during the calorimetric scan. Tricine, which has a temperature dependence of –0.021 K [26] was chosen for this purpose. Thus, although the pH conditions reported for each experiment describe only the pH of the suspension at room temperature, the concentration of neutral lidocaine which was present at this pH will remain constant throughout the scan.

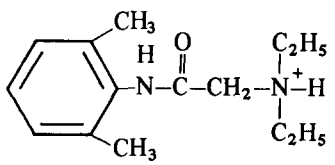
Results

The effect of lidocaine's charge on the DPPC phase transition

A comparison of the calorimetric properties of model membranes in the presence of cationic and uncharged lidocaine can be conducted in two ways. First, the influence of lidocaine (pK_a 7.87) on lipid behavior can be compared at pH 6.5 and pH 9.2 where the anesthetic is protonated and unprotonated, respectively. This procedure, however, suffers from the disadvantage that the head groups of phosphatidylethanolamine and phosphatidylserine may partially titrate over this pH range, thus introducing an extra variable into the interpretation of the experimental results. Alternatively, the lipid perturbations of lidocaine can be monitored at a single high pH, substituting the quaternary amine analogue of lidocaine (W49091) for cationic lidocaine:



W49091



lidocaine at pH 6.5

This procedure assumes that W49091 accurately mimics the effects of cationic lidocaine on liposomal systems. We have found that if sufficient time is allowed for anesthetic equilibration, the effects of W49091 and lidocaine at low pH are essentially indistinguishable. For this reason all further comparisons of anesthetic effects on liposomal properties were made between lidocaine and W49091 at high pH, i.e., where lidocaine is predominantly uncharged.

A comparison of the effects of 15 mM lidocaine and 15 mM W49091 on the calorimetric transition of DPPC liposomes buffered at pH 8.9 is shown in Fig. 1. The control scan has a temperature of maximum heat capacity (T_m) of 41.35°C, an enthalpy of 8.7 kcal/mol (see Methods), and a width at half-height of 0.2°C. The number of phospholipid molecules per cooperative unit (n) can be determined from the following relationship [5]:

$$n = 4RT_m^2\, Cp_{max}/\Delta H^2 \qquad (1)$$

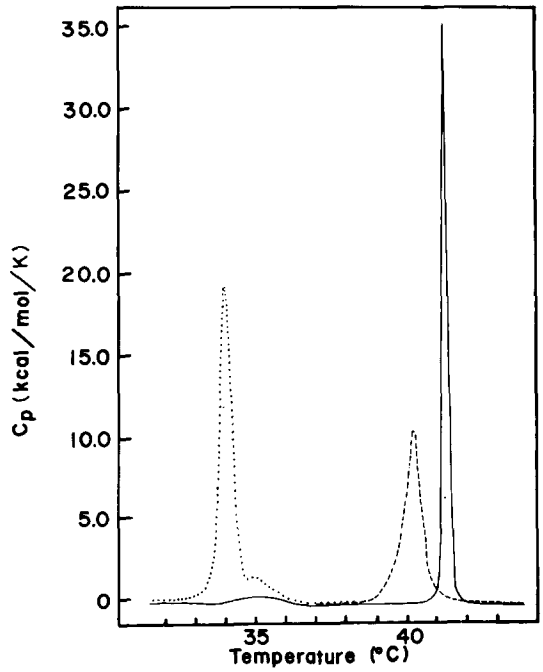


Fig. 1. Heat capacity profiles of multilamellar dispersions of DPPC liposomes suspended in 20 mM Tricine and 120 mM NaCl buffer pH 8.9, containing no anesthetic (———), 15 mM lidocaine (· · · · ·), or 15 mM W49091 (- - - - -). Heating rate 1.08 K/min.

where Cp_{max} is the excess heat capacity at T_m, and ΔH is the enthalpy of the transition. Using this relationship the value of n is calculated to be approx. 360 phospholipids for the control scan at the scanning rate used in these experiments (1 K/min). At slower scanning rates, e.g., 0.25 K/min, the apparent cooperativity of the control scan increases and the width at half-height decreases to 514 molecules/unit per 0.14 K, respectively. Because of the approximate inverse relationship between the cooperativity of a transition (n) and its width at half-height ($W_{1/2}$), we have used $W_{1/2}$ as a rough measure of $1/n$ throughout this paper.

As shown in Fig. 1 both W49091 and lidocaine at pH 8.9 exert measurable effects on the properties of the DPPC phase transition. However, the cationic and uncharged forms of lidocaine differ significantly in their perturbations of two of the three major properties of the DPPC transition. Firstly, lidocaine, which is approx. 90% uncharged under the conditions of these experiments, is much more effective at lowering the melting temperature of DPPC than is the cationic analogue, W49091. Thus 15 mM lidocaine depresses T_m by approx. 7.5 K, while W49091 lowers T_m by only approx. 1 K. This marked influence of anesthetic charge is also clearly illustrated in the concentration study outlined in Fig. 2a. W49091 is seen to be relatively ineffective at lowering the T_m of DPPC liposomes at all concentrations studied, while the depression of T_m by uncharged lidocaine increases with concentration. These data agree with the light scattering experiments of Ueda et al. [25] which suggest that the T_m of DPPC is perturbed primarily by the unprotonated form of lidocaine.

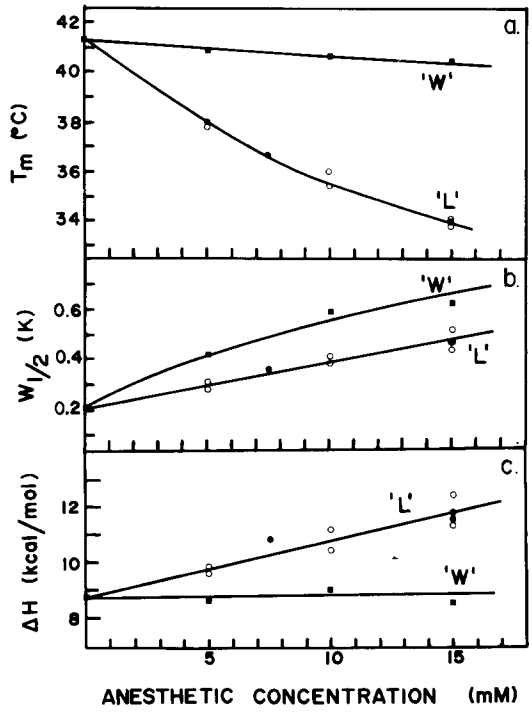


Fig. 2. Temperature of maximum heat capacity (T_m), width at half-maximal height ($W_{1/2}$) and enthalpy (ΔH) of the phase transition of DPPC liposomes: (i) prepared in the presence of W49091 (■), (ii) prepared in the presence of lidocaine (●), or (iii) prepared before addition of the lidocaine solution (○) (see Methods). The suspending buffer contained 20 mM Tricine and 120 mM NaCl, pH 8.9.

Lidocaine and W49091 also appear to perturb the enthalpy of the DPPC phase transition differently. Thus, the area under the excess heat capacity curve of DPPC is unaltered by the presence of 15 mM W49091, despite the transition's shorter, more broadened appearance (Fig. 1). On the other hand, the presence of 15 mM lidocaine increases the apparent enthalpy of the DPPC transition by approx. 31%, i.e., from 8.7 kcal/mol to 11.4 kcal/mol. This striking increase in enthalpy was found to be independent of the method of introducing the anesthetic into the liposomes and was roughly linearly correlated with the concentration of lidocaine in the suspension (Fig. 2c). Thus again, the uncharged form of lidocaine is found to be the more potent membrane perturbing agent.

In contrast to their disparate effects on the above two properties, lidocaine and W49091 provoke relatively similar changes in the cooperativity of the DPPC phase transition. For example, 15 mM concentrations of lidocaine and W49091 decrease the size of the cooperative unit (n) from 360 to 128 and 115 phospholipids, respectively. These decreases in cooperativity are illustrated graphically in Fig. 2b where the width at half-height of each transition is plotted as a function of the concentration of anesthetic in the liposomal suspension. Both charged forms of lidocaine promote relatively minor, yet measurable losses of cooperativity.

Finally, we have noticed the initial stages of a lidocaine-induced phase separation in the DPPC liposomal system at high concentrations of uncharged lidocaine.

The small shoulder on the high temperature side of the main transition shown in Fig. 1 was present in the calorimetric scan at high pH regardless of whether lidocaine was introduced during or subsequent to liposome formation. Thus, while the shoulder is clearly not an equilibration artifact, its origin is presently unknown.

The effect of lidocaine's charge on the DPPE phase transition

Because of the partial deprotonation of DPPE at high pH, it was decided to compare the effects of lidocaine and its cationic analogue (W49091) at pH 7.9, where DPPE is completely zwitterionic [27,28].

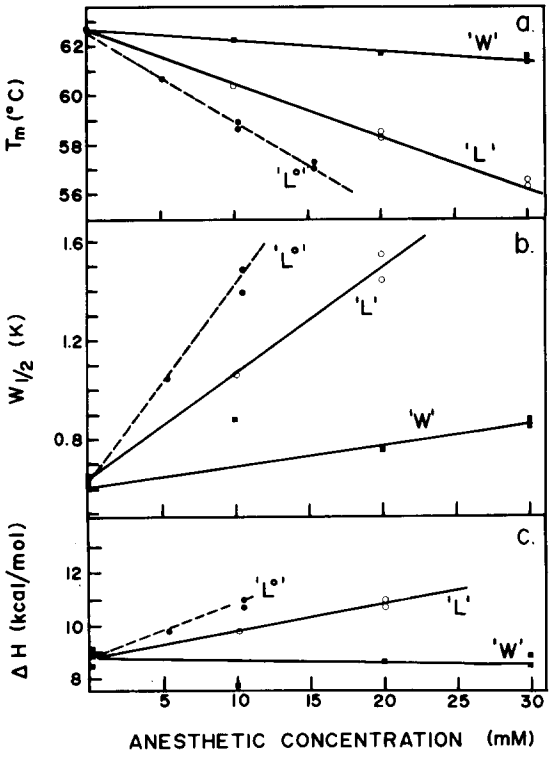


Fig. 3. T_m, $W_{1/2}$ (width at half-height) and ΔH of the gel → liquid crystalline phase transition of a vortexed dispersion of DPPE after addition of lidocaine (○) or W49091 (■). The data were obtained calorimetrically at a heating rate of 1.0 K/min. The suspension contained 20 mM Tricine and 120 mM NaCl, and was adjusted to pH 7.9 after addition of the anesthetic. The dotted lines, labeled 'L^0', represent the influence of solely the neutral form of lidocaine on the calorimetric parameters T_m, $W_{1/2}$ and ΔH. These lines were calculated by a procedure described in the Results.

Unfortunately, lidocaine (pK_a 7.87) is approx. 50% protonated at pH 7.9, thus rendering the evaluation of the perturbing potency of solely the uncharged form of the anesthetic very difficult. For this reason, the data concerning the effect of lidocaine ('L') on the properties of the DPPE phase transition have also been plotted as a function of the concentration of uncharged lidocaine ('L^0') (Fig. 3). These replots were obtained by (i) calculating the concentration of the protonated (cationic) form of lidocaine at each lidocaine concentration using the Henderson-Hasselbalch equation, (ii) subtracting the magnitude of the perturbation which is caused by a concentration of W49091 equal to this calculated concentration of cationic lidocaine (this residual perturbation should represent the contribution of uncharged lidocaine to the total observed perturbation) and, (iii) plotting this residual perturbation as a function of the calculated concentration of uncharged lidocaine in the suspension. By this procedure, the plots labelled 'L^0' should reflect the perturbations due solely to the uncharged form of the anesthetic.

The data in Fig. 3 indicate that the relative perturbations of DPPE by charged and uncharged lidocaine are qualitatively similar to the perturbation patterns observed for DPPC. Thus, the uncharged form of lidocaine is the only form which can significantly depress the melting temperature of the DPPE transition (Fig. 3a). Similarly, the unprotonated form of the anesthetic is solely responsible for lidocaine's modification of the enthalpy of the transition. Furthermore, as with DPPC, the apparent enthalpy of the transition rises as the concentration of uncharged lidocaine is increased. Thirdly, both lidocaine and W49091 reduce the cooperativity of the DPPE transition, although in this case the uncharged form is somewhat more potent. Thus, the cooperativity of the DPPE transition is reduced from a control value of 105 phospholipids per cooperative unit to 50 and 95 phospholipids per cooperative unit in the presence of 20 mM lidocaine or W49091, respectively. Finally, treatment of DPPE liposomes with lidocaine concentrations greater than 30 mM induces a phase separation in the lipid which is observed as a broad, new transition on the low temperature side of the main transition. While the T_m of the gel to liquid-crystalline phase transition is still clear in this scan, the ΔH and cooperativity measurements are somewhat

ambiguous due to the partial overlap from the new transition. For this reason no data points for ΔH or $W_{1/2}$ are reported in Fig. 3b and c at 30 mM lidocaine.

The effect of lidocaine's charge on the DMPS phase transition

Unlike the situation with DPPC and DPPE, dimyristoyl phosphatidylserine (DMPS) is similarly perturbed by both W49091 and lidocaine at high pH (Fig. 4). In the presence of 15 mM lidocaine (pH 8.9) or W49091 the temperature of the DMPS phase transition is reduced from 35.7°C to 26.35°C and 23.45°C, respectively. These changes in T_m are the largest we have observed at this lidocaine concentration for any

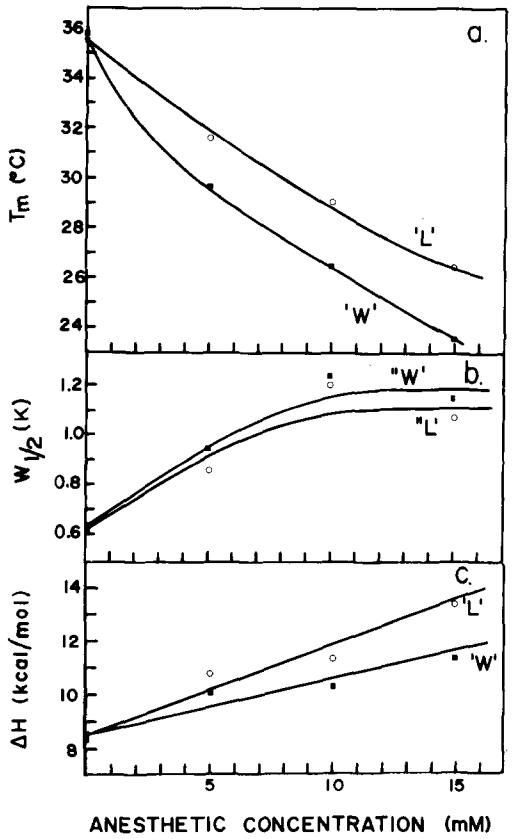


Fig. 4. T_m, $W_{1/2}$ (width at half-height) and ΔH of the phase transition of DMPS liposomes after addition of lidocaine (○) or W49091 (■). The heating rate of the calorimeter was 1.08 K/min. The DMPS was suspended in a solution of 20 mM Tricine, 120 mM NaCl and 2 mM EDTA. After addition of the anesthetic the suspension pH was adjusted to pH 8.9.

phospholipid. Likewise, the effect of lidocaine and its quaternary amine analogue on the enthalpy of the DMPS transition are larger than those previously encountered. For example, at 15 mM concentrations, W49091 and lidocaine elevate the enthalpy above the control value (8.5 kcal/mol) by 34% and 58%, respectively. For comparison, these same concentrations of lidocaine and W49091 were observed to raise the enthalpy of the DPPC phase transition by only 0% and 31%, respectively. Thirdly, the cooperativity of the DMPS transition is altered to a similar extent by both forms of lidocaine. The control value of n (approx. 100 lipids per cooperative unit) decreased to 40 lipids per cooperative unit in the presence of 15 mM lidocaine (pH 8.9) and 50 lipids per cooperative unit in 15 mM W49091. However, unlike the situation with the neutral phospholipids, the loss of cooperativity for DMPS appeared to saturate at anesthetic concentrations greater than 10 mM. The reason for this behavior is not clear since the effects of the anesthetics on T_m and ΔH continue to increase at concentrations greater than this value.

Finally, before DMPS became commercially available, a similar set of experiments to those shown in Fig. 4 was conducted on hydrogenated bovine brain phosphatidylserine. The results of these studies were qualitatively very similar to those reported in Fig. 4. Since saturated bovine brain phosphatidylserine is composed predominantly of stearoyl and longer fatty acid chains [29] and since the T_m of this hydrogenated phospholipid is 65.8°C, the differences in behavior between DMPS and DPPC or DPPE cannot be ascribed to differences in either the chain lengths or melting temperatures of these phospholipids.

Discussion

Explanation of lidocaine's effects on phase transition properties

The effect of neutral lidocaine on the behavior of the liposomal suspensions of DPPC, DPPE and DMPS can be explained by the following argument. We have shown that uncharged lidocaine depresses the temperature of the DPPC, DPPE and DMPS phase transitions. These observations indicate that the anesthetic must interact preferentially with the melted state of the respective phospholipid dispersions rather than with the gel (solid) state. It follows, therefore, that

the partition coefficient of uncharged lidocaine will increase significantly at temperatures where fluid patches of phospholipid first appear in the bilayer, leading to an influx of lidocaine into the membrane at T_m. The entry of lidocaine into the membrane will necessarily be accompanied by at least a partial dehydration of the anesthetic and some sort of accomodating rearrangement of the surrounding membrane lipids. In this view, the measured values of ΔH at each lidocaine concentration would ultimately derive from two sources: (i) the melting of the phospholipid, and (ii) the enthalpy changes accompanying the entry and positioning of lidocaine in the membrane. If the former contribution were similar to the ΔH of melting of the control liposomes (i.e., approx. 8.7 kcal/mol phospholipid in the case of DPPC), then the latter contribution (approx. 2.7 kcal/mol phospholipid at 15 mM lidocaine, pH 8.9) would amount to approx. 3.8 kcal/mol of uncharged lidocaine entering the membrane *. This latter value appears to be too large to be entirely accounted for on the basis of the heat of transfer of lidocaine from water to the membrane [31,32], and therefore, some additional endothermic event must occur during insertion of the anesthetic into the membrane. It may be that the separation of adjacent phospholipids with the concomitant loss of headgroup-headgroup or acyl chain-acyl chain interactions could account for the unidentified excess enthalpy.

Using the above hypothesis, the effect of W49091 on the temperatures and enthalpies of the DPPC, DPPE and DMPS phase transitions can also be explained. In the cases of neutral phospholipids, i.e., DPPC and DPPE, the negligible perturbation of T_m suggests that the cationic analogue of lidocaine either interacts weakly with the lipid or does not discriminate between the solid and fluid states. In either situation, there should be no major redistribution of W49091 during the phase transition, and therefore, the anesthetic's effect on the enthalpy of the transitions should be minor. In the case of DMPS, however, W49091 shows strong preference for the fluid state of the lipid. Thus, the influx of W49091 during the phase transition should readily contribute to the observed ΔH of the transition.

Surprisingly, both charged forms of lidocaine exert only a minor influence on the cooperativities of the three phase transitions; i.e., the $W_{1/2}$ of DPPC at 15 mM lidocaine (approx. 0.5 K) is not significantly broader than most published control values for DPPC [33]. Thus, at anesthetic concentrations sufficient to reduce T_m by approx. 6–7 K, the sizes of the cooperative units of the three lipid dispersions are reduced by less than 70%. For comparison, Mountcastle et al. [5] report that halothane, an inhalation anesthetic, reduces the size of the cooperative unit of DPPC 10-fold at the concentration required to depress T_m by approx. 6–7 K. This difference in effect on cooperativity may suggest that lidocaine interacts much less with the gel (solid) state of DPPC than does halothane.

Implications concerning the proposed mechanisms of local anesthesia

While it is generally recognized that the uncharged form of a tertiary amine local anesthetic is the permeant species in the membrane [15–17,19,22] there remains some controversy concerning whether this form also contributes significantly to nerve blockade [17,18,20,21]. However, there is good agreement that the cation must be responsible for part of a tertiary amine's anesthetic potency since their quaternary amine analogues have been shown to be highly effective nerve blocking agents when perfused inside the axon [16–19]. Thus, the inability of the quaternary amine analogue of lidocaine to significantly perturb the behavior of DPPC and DPPE liposomes implies that these two lipids do not constitute an important functional component of the anesthetics' inhibitory site.

The observation that W49091 interacts strongly with DMPS naturally selects phosphatidylserine (PS) as a possible component of the inhibitory site. This idea has been inferred from the model studies of others [2,34]. Furthermore, there is evidence to suggest that PS surrounds the sodium channel [35,36]. However, if a disruption of a PS domain is responsible for the cation's anesthetic potency, then clearly the high pH form of the anesthetic should be similarly

* This estimate assumes that the partition coefficient of uncharged lidocaine for DPPC vesicles above T_m is 76 [25], that the partial specific volume of the lipid above T_m is 0.972 ml/g [30], and that the amount of uncharged lidocaine dissolved in the lipid below T_m is small compared to the amount which enters during the phase transition.

active, since our studies demonstrate that this form perturbs PS as effectively as W49091. The diminished potency of uncharged lidocaine in perfused axons [15,22] thus seems to be inconsistent with our model membrane studies. However, this apparent inconsistency may be reconciled in two ways. Firstly, the lower anesthetic potency of uncharged lidocaine might simply reflect its lack of selectivity in nerve membrane binding. Our studies suggest that uncharged lidocaine should not discriminate among different lipids, but should dilute itself into all hydrophobic regions of the nerve membrane. Cationic lidocaine, on the other hand, should concentrate into patches of anionic phospholipid, i.e., possibly at the inhibitory site.

Alternatively, the inability to correlate anesthetic potency in nerves with anesthetic modification of lipid behavior could indicate that a lipid perturbation is not the primary event responsible for local anesthesia. Thus, the anesthetic may distort an essential protein's structure [37,38], obstruct a cation channel [17,18], or disrupt the critical Ca^{2+} balance in the nerve cell [39–41]. This latter property seems to correlate nicely with anesthetic potency, regardless of the charge or structure of the local anesthetic [41].

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References

1 Lee, A.G. (1976) Nature 262, 545–548
2 Lee, A.G. (1976) Biochim. Biophys. Acta 448, 34–44
3 Lee, A.G. (1979) Anesthesiology 51, 64–71
4 Lee, A.G. (1977) Mol. Pharmacol. 13, 474–487
5 Mountcastle, D.B., Biltonen, R.L. and Halsey, M.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4906–4910
6 Trudell, J.R. (1977) Anesthesiology 46, 5–10
7 Hubbell, W.L., Metcalfe, J.C., Metcalfe, S.M. and McConnell, H.H. (1970) Biochim. Biophys. Acta 219, 415–427
8 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 321–327
9 Ashcroft, R.G., Coster, H.G.L. and Smith, J.R. (1977) Nature 269, 819–820
10 Ashcroft, R.G., Coster, H.G.L. and Smith, J.R. (1977) Biochim. Biophys. Acta 469, 13–22
11 Haydon, D.A., Hendry, B.M., Levinson, S.R. and Requena, J. (1977) Nature 268, 356–358
12 Seeman, P. (1972) Pharmac. Rev. 24, 583–655
13 McLaughlin, S. and Harary, H. (1976) Biochemistry 15, 1941–1948
14 Lee, A.G. (1978) Biochim. Biophys. Acta 514, 95–104
15 Narahashi, T., Frazier, D.T. and Yamada, M. (1970) J. Pharmacol. Exp. Ther. 171, 32–44
16 Frazier, D.T., Narahashi, T. and Yamada, M. (1970) J. Pharmacol. Exp. Ther. 171, 45–51
17 Schwarz, W., Palade, P.T. and Hille, B. (1977) Biophys. J. 20, 343–368
18 Hille, B. (1977) J. Gen. Physiol. 69, 497–515
19 Hille, B. (1977) J. Gen. Physiol. 69, 475–496
20 Bianchi, C.P. and Strobel, G.E. (1968) Trans. N.Y. Acad. Sci. 30, 1082–1092
21 Ritchie, J.M. and Greengard, P. (1966) Annu. Rev. Pharmacol. 6, 405–430
22 Ritchie, J.M., Ritchie, B. and Greengard, P. (1965) J. Pharmacol. Exp. Ther. 150, 160–164
23 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
24 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3862–3866
25 Ueda, I., Tashiro, C. and Arakawa, K. (1977) Anesthesiology 46, 327–332
26 Good, N.E., Winget, G.D., Winter, W., Connolly, T.N., Izawa, S. and Singh, R.M.M. (1966) Biochemistry 5, 467–477
27 Michaelson, D.M., Horwitz, A.F. and Klein, M.P. (1974) Biochemistry 13, 2605–2612
28 Träuble, H. and Eibl, H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 214–219
29 Yabuuchi, H. and O'Brien, J.S. (1968) J. Lipid Res. 9, 65–67
30 Watts, A., Marsh, D. and Knowles, P.F. (1978) Biochemistry 17, 1792–1801
31 Krishnan, C.V. and Friedman, H.L. (1969) J. Phys. Chem. 73, 1572–1574
32 Tanford, C. (1980) in The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd edn., pp. 21–28, Wiley-Interscience, New York
33 Lentz, B.R., Freire, E. and Biltonen, R.L. (1978) Biochemistry 17, 4476–4480
34 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504–519
35 Cook, A.M., Low, E. and Ishijimi, M. (1972) Nature New Biol. 239, 150–151
36 Hille, B., Woodhull, M. and Shapiro, B.I. (1975) Phil. Trans. R. Soc. Lond. 270, 301–318
37 Ueda, I., Kamaya, H. and Eyring, H. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 481–485
38 Woodbury, J.W., D'Arrigo, J.S. and Eyring, H. (1975) in Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), Vol. 1, pp. 253–275, Raven Press, New York
39 Blaustein, M.P. and Goldman, D.E. (1966) Science 153, 429–432
40 Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 265, 169–186
41 Low, P.S., Lloyd, D.H., Stein, T.M. and Rogers, J.A. (1979) J. Biol. Chem. 254, 4119–4125