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EFFECTS OF CHARGED DRUGS ON THE PHASE TRANSITION TEMPERATURES OF PHOSPHOLIPID BILAYERS

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Summary

An approach is presented which allows the description of drug binding to lipid bilayers, when the drug is present in both charged and uncharged forms. Binding is described by Langmuir adsorption isotherms, with the maximum number of binding sites being 1/60 $Å^2$. An estimate of the change in drug pK on binding is necessary, and is close to zero for most drugs binding to dipalmitoyl phosphatidylcholine, although $\Delta pK = 1.0$ for procaine. From the binding curves it is possible to calculate the drug-induced decreases in lipid phase transition temperature, assuming ideal behaviour. Good fits between experiment and theory are possible, giving values for the dissociation constant describing drug binding to the membrane.

Introduction

Many drugs exist as mixtures of charged and uncharged species at physiological pH values. Since many also possess hydrophobic groups they will adsorb to biological membranes, with the acidic or basic group of the drug probably located in the polar head group region. This non-specific binding can be expected to have two effects. Firstly, it will increase the fluidity of the lipid phase of the membrane, particularly if it can trigger a phase transition for lipid originally in the gel or solid-like phase [1]. Secondly, it will introduce charge into the membrane, with important consequences for a wide variety of functions of the membrane. It is important, therefore, to develop a theory to describe such binding.

In a recent paper, McLaughlin and Harary [2] showed how the Gouy-Chapman theory could be used to describe the binding of charged molecules to lipid bilayers. They showed that experimental data on the extent of binding of 2,6-toluidinylnaphthalene-sulphonate ions, and on the surface potential created by such binding, were in good agreement with theoretical expectations.

Here I extend the approach of McLaughlin and Harary [2] to the study of the binding of mixtures of charged and uncharged species, and show how the theory can be fitted to data on drug-induced decreases in transition temperatures of lipids.

If, as an example, we consider a drug containing an amine group, both neutral and positively charged forms of the drug will be present in aqueous solution,

$$A + H^+ \rightarrow HA^+$$

The relative proportions of the two forms of the drug are given by the Henderson-Hasselbalch equation,

$$pH = pK + \log[A]/[HA^+] \tag{1}$$

Both forms of the drug will bind to the membrane. McLaughlin and Harary [2] show that this binding can be described by the Langmuir adsorption isotherm. In our case, adsorption isotherms are necessary for both the charged and uncharged forms and these take the form

$$\sigma^A = \frac{1}{K^A}(\sigma^{max} - \sigma^A - \sigma^{HA})[A]_{x=0} \tag{2}$$

$$\sigma^{HA} = \frac{1}{K^{HA}}(\sigma^{max} - \sigma^A - \sigma^{HA})[HA^+]_{x=0} \tag{3}$$

where σ^A and σ^{HA} are, respectively, the number of molecules of A and HA adsorbed to the membrane per unit area, σ^{max} is the maximum number of molecules adsorbed per unit area, K^A and K^{HA} are dissociation constants for A and HA, respectively, and $[A]_{x=0}$ and $[HA^+]_{x=0}$ are the aqueous concentrations of A and HA^+, respectively, at the membrane solution interface, $x = 0$.

The ratio of K^A and K^{HA} can be related to changes in drug pK on binding. Aveyard and Haydon [3] have shown that K can be related to $\Delta\mu^\circ$, the change in standard chemical potential on adsorption

$$K^A = \exp\left(\frac{\Delta\mu^\circ_A}{RT}\right) \tag{4}$$

$$K^{HA} = \exp\left(\frac{\Delta\mu^\circ_{HA}}{RT}\right) \tag{5}$$

A shift in pK on binding can occur as the result of the preferential binding of one form of the drug, either the charged or the uncharged form. The drug pK in the aqueous phase can be written as

$$pK^w = \frac{1}{2.303\,RT}(\mu^{ow}_{H^+} + \mu^{ow}_A - \mu^{ow}_{HA}) \tag{6}$$

where μ°'s are standard chemical potentials in the aqueous phase. Similarly, the pK for drug bound to the membrane is given by

$$pK^m = \frac{1}{2.303\,RT}(\mu^{ow}_{H^+} + \mu^{om}_A - \mu^{om}_{HA}) \tag{7}$$

where the standard chemical potentials are now referred to water for the proton, but to the membrane phase for A and HA^+. The shift in pK due to binding is given by

$$\Delta pK = pK^m - pK^w \tag{8}$$

$$= \frac{1}{2.303\,RT}(\mu_A^{om} - \mu_A^{ow}) - \frac{1}{2.303\,RT}(\mu_{HA}^{om} - \mu_{HA}^{ow})$$

$$= \frac{1}{2.303\,RT}(\Delta\mu_A^o - \Delta\mu_{HA}^o)$$

We thus see that,

$$K^A/K^{HA} = \exp(2.303\ \Delta pK) \tag{9}$$

For drugs with spectroscopic properties that change on ionisation, it is possible to measure the drug pK when bound to a membrane. For a number of such drugs it has been found that ΔpK is close to zero when the drug adsorbs onto liposomes of dipalmitoyl phophatidylcholine (Lee, unpublished observations). Further, as will be shown later, the binding data allows an estimate to be made of ΔpK.

The concentration of A at the membrane solution interface will be equal to that in bulk solution. The concentration of HA^+ at the membrane solution interface can differ from the bulk concentration, however, if the surface is charged. The concentration is then assumed to be given by the Boltzmann relationships

$$[HA^+]_{x=0} = [HA^+] \exp(-F\psi_o/RT) \tag{10}$$

where $[HA^+]$ is the bulk concentration and ψ_o is the electrostatic potential in the aqueous phase adjacent to the membrane. Now, if it is assumed that the membrane was initially neutral, then the charge density on the surface of the membrane is equal to the surface concentration of adsorbed HA^+, so that the membrane potential is given by

$$\sinh(F\psi_o/2\,RT) = A\sigma^{HA}/\sqrt{c} \tag{11}$$

This equation is true in the presence of monovalent electrolytes of concentration c (mol/l). The value of A is 136.6 when σ^{HA} is expressed in ions per Å^2, and $F/2RT$ is 25.7 mV at 25° C. If the membrane contained negatively charged lipids, then Eqn. 11 can be modified to,

$$\sigma^{HA} - \sigma^{neg} = \frac{\sqrt{c}}{136.6}\sinh(F\psi_o/2\,RT) \tag{12}$$

where σ^{neg} is the surface charge density of negatively charged lipid.

Eqns. 2 and 3 can be combined to eliminate σ^A. Combination with Eqns. 1 and 10 then eliminates $[HA^+]$, and substitution into Eqn. 11 gives a transcendental equation relating σ^{HA} and the total drug concentration at any given pH. This is readily solved on a digital computer, using the Bolzano method [4].

The resulting number of adsorbed molecules can readily be used to calculate the expected decrease in lipid transition temperature. Defining

$$\sigma^{total} = \sigma^A + \sigma^{HA}, \tag{13}$$

the mol fraction of drug in the membrane can be written as

$$x_{\text{membrane}}^{\text{drug}} = \frac{1}{1 + 60\,\sigma^{\text{total}}} \tag{14}$$

where the molecular area of dipalmitoyl phosphatidylcholine in the liquid crystalline phase has been taken to be 60 Å^2 [5]. If it is assumed that the drug is totally immiscible with lipid in the gel state, then the depression ΔT of lipid transition temperature can be written as [6,7].

$$\Delta T = \frac{RT^2}{\Delta H}\, x_{\text{membrane}}^{\text{drug}} \tag{15}$$

where T is the transition temperature and ΔH is the enthalpy of the transition.

Materials and Methods

Dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine were obtained from Koch-Light and myristic acid from Sigma. Chlorophyll *a* was prepared as reported previously [8], and *N*-phenylnaphthylamine was from Kodak. Transition temperatures were determined from fluorescence-temperature plots as described elsewhere [9]. Samples were prepared by dissolving lipid ($3 \cdot 10^{-7}$ mol) plus fluorescence probe in chloroform in 10-ml stoppered flasks and evaporating to dryness under a stream of N_2. Buffer (4 ml; 0.01 M Tris-HCl/0.1 M NaCl) of the appropriate pH was added together with the anaesthetic and the mixture shaken on a Vortex mixer. Fluorescence measurements were made on an Aminco Bowman SPF fluorimeter, the temperature being monitored with a thermocouple inserted into the fluorescence cell. The drug-induced decrease in transition temperature for the gel to liquid crystalline phase transition measured from chlorophyll *a* or *N*-phenylnaphthylamine fluorescence were identical, where both probes were used. For some drugs, however, *N*-phenylnaphthylamine was found to form a highly fluorescent adduct and so could not be used.

Results

The first step in applying the theory outlined above is to obtain an estimate for $\sigma^{\max}$. As described elsewhere [8] the *n*-alcohols cause a decrease in lipid transition temperature which is linear with concentration up to a decrease of approx. 6°C in transition temperature. At higher concentrations there is some non-linearity in the plots, particularly marked for 1-butanol (Fig. 1). Such non-linearity is suggestive of saturation and can be readily fitted to a Langmuir adsorption isotherm. Fig. 1 shows the best fits to the experimental data that can be obtained from Eqns. 15 and 2 (putting $\sigma^{HA} = 0$), for various assumed values of $\sigma^{\max}$. The best fit is obtained for a value of $\sigma^{\max}$ about equal to 1/60 Å^2, and since this corresponds to one binding site per lipid molecule, it will be the value used here: in their analysis McLaughlin and Harary [2] used a value of $\sigma^{\max}$ of 1/70 Å^2.

Figs. 2 and 3 compare theoretical and experimental data [7] for the effect of tetracaine on the phase transition temperature of dipalmitoyl phosphatidyl-

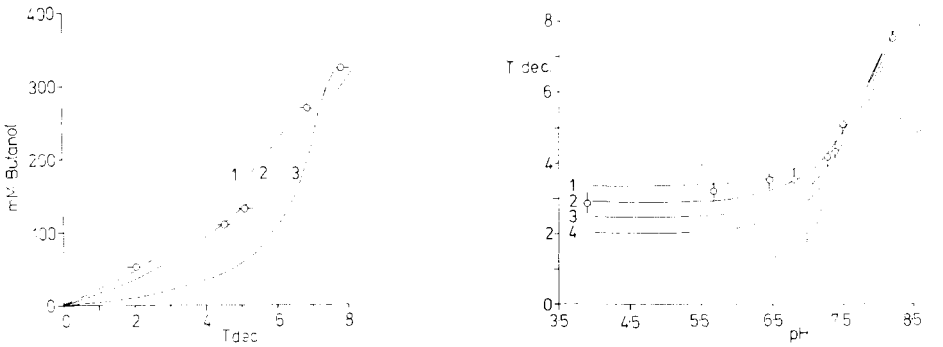


Fig. 1. The decrease in phase transition temperature (Tdec) for dipalmitoyl phosphatidylcholine caused by butanol, as a function of butanol concentration (mM). Circles, experimental; solid lines, theoretical calculations with 1, $\sigma^{max} = 1/30$, $K = 1.14$; 2, $\sigma^{max} = 1/60$, $K = 0.34$; 3, $\sigma^{max} = 1/120$, $K = 0.05$.

Fig. 2. The decrease in phase transition temperature (Tdec) for dipalmitoyl phosphatidylcholine caused by 2 mM tetracaine as a function of pH, in 0.1 M NaCl. Circles, experimental; solid lines, theoretical calculations with $K = 1 \cdot 10^{-3}$ and 1, $\Delta pK = 0$; 2, $\Delta pK = 0.2$; 3, $\Delta pK = 0.4$; 4, $\Delta pK = 0.6$.

choline. The transition temperature for dipalmitoyl phosphatidylcholine was taken to be 41.7°C and the transition enthalpy ΔH 9.69 kcal · mol^{-1} [10]. Studies of the pH dependence of the effect of tetracaine give some idea of the relative magnitudes of K^A and K^{HA}: at very low pH values all of the drug is present in the charged form, whereas at pH values closer to the drug pK value, both charged and uncharged forms are present (it is not possible to approach too closely to the pK value because of precipitation of the drug). As shown in Fig. 2, the data for tetracaine is best fitted assuming that K^A and K^{HA} are very

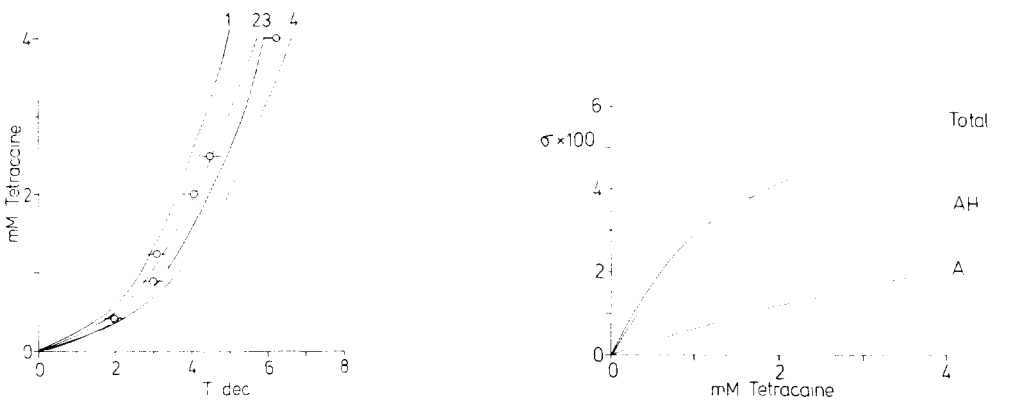


Fig. 3. The decrease in phase transition temperature (Tdec) for dipalmitoyl phosphatidylcholine caused by tetracaine at pH 7.2 in 0.1 M NaCl, as a function of tetracaine concentration (mM). Circles, experimental; solid lines, theoretical calculations with $\Delta pK = 0$ and, 1, $K = 1.6 \cdot 10^{-3}$; 2, $K = 1.2 \cdot 10^{-3}$; 3, $K = 1 \cdot 10^{-3}$; 4, $K = 0.8 \cdot 10^{-3}$.

Fig. 4. The binding curves for the uncharged (A), charged (HA) and total forms of tetracaine as a function of concentration (mM), calculated with $K = 1 \cdot 10^{-3}$ and $\Delta pK = 0$.

similar, that is, that ΔpK is close to zero, with $K^A = 1 \cdot 10^{-3}$. Fig. 3 shows the concentration dependence of the effect at fixed pH. With $\Delta pK = 0$, the best fit is again obtained with $K = 1 \cdot 10^{-3}$. Agreement is quite sensitive to variation in K, and certainly varying K by a factor of two leads to a very much poorer fit: agreement is generally better than to within $\pm\frac{1}{2}^{o}$, a reasonable estimate of the experimental error. Fig. 4 shows how the binding of the charged and uncharged forms of the drug vary with concentration.

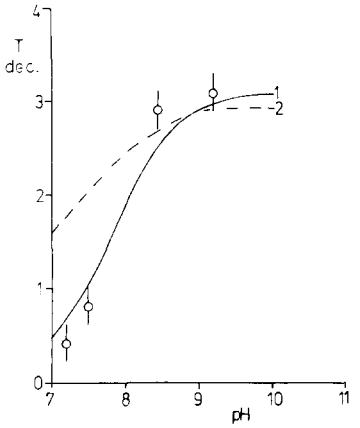
For the majority of drugs tested it appears that ΔpK is zero for binding to dipalmitoyl phosphatidylcholine. Dissociation constants giving best fits to the experimental data are listed in Table I. For diphenylhydantoin, the best fit is obtained with $\Delta pK = 0.2$ (Fig. 5), although the improvement over that with $\Delta pK = 0$ is relatively small. For procaine, however, effects are greater and, as shown in Fig. 6, data obtained at high and low pH values cannot be fitted to the same value of the dissociation constant K, if it is assumed that ΔpK is zero. However, a good fit is obtained with $\Delta pK = 1.0$ and $K^A = 0.007$. The same parameter values give a good fit to the concentration dependence of the effect

TABLE I

CALCULATED DISSOCIATION CONSTANTS, ASSUMING $\sigma^{max} = 1/60$ Å^2 AND $\Delta pK = 0$

Drug	pK	Dissociation constants for (mol/l)	
		Dipalmitoyl phosphatidyl-choline	Dipalmitoyl phosphatidyl-ethanolamine
Butanol	—	0.34	
Pentanol	—	$7.2 \cdot 10^{-2}$	
Benzocaine	—	$5.4 \cdot 10^{-3}$	
Procaine [a]	9 [b]	$7 \cdot 10^{-3}$	
Lidocaine [a]	7.9 [b]	$1.3 \cdot 10^{-2}$	
Dibucaine	8.5 [b]	$2.4 \cdot 10^{-4}$	$6 \cdot 10^{-3}$
Tetracaine	8.5 [b]	$1 \cdot 10^{-3}$	
Org. 6001	—	$5 \cdot 10^{-4}$	
Chlorpromazine	9.3 [c,d]	$3.5 \cdot 10^{-5}$	$2.8 \cdot 10^{-4}$
Diphenylhydantoin [e]	8.3 [f]	$3 \cdot 10^{-4}$	
Propranolol	9.45 [g]	$8 \cdot 10^{-4}$	$1.2 \cdot 10.2$
Benztropine	10.0 [h]	$5 \cdot 10^{-4}$	
Pentobarbitone	8.0 [i]	$1.1 \cdot 10^{-2}$	
Phenobarbitone	7.3 [i]	$7 \cdot 10^{-2}$	
Benzimidazole	—	$5.3 \cdot 10^{-2}$	
2-Aminobenzimidazole	7.5 [j]	$1.5 \cdot 10^{-2}$	
Decyltrimethylammonium bromide	—	$2.6 \cdot 10^{-4}$	
Dodecyltrimethylammonium bromide	—	$2.1 \cdot 10^{-5}$	

[a] With $\Delta pK = 1.0$.
[b] ref. 11.
[c] ref. 12.
[d] ref. 13.
[e] With $\Delta pK = 0.2$.
[f] ref. 14.
[g] ref. 15.
[h] ref. 16.
[i] ref. 17.
[j] ref. 33.



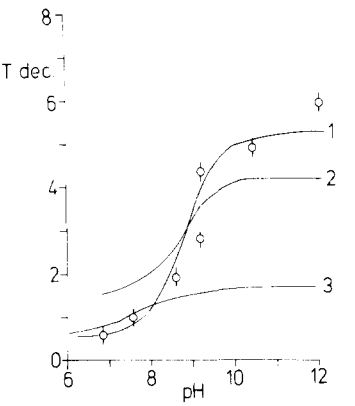
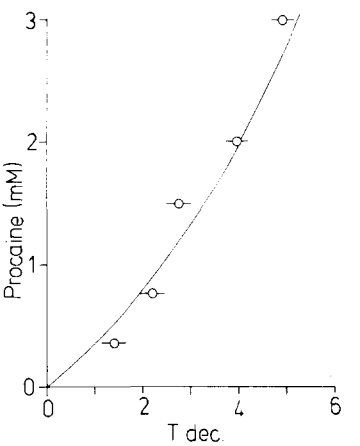


Fig. 5. The decrease in phase transition temperature for dipalmitoyl phosphatidylcholine caused by 0.1 mM diphenylhydantoin in 0.1 M NaCl. Circles, experimental; solid lines, theoretical calculations with 1, $K = 3 \cdot 10^{-4}$, $\Delta pK = 0.2$; 2, $K = 4 \cdot 10^{-4}$, $\Delta pK = 0$.

Fig. 6. The decrease in phase transition temperature for dipalmitoyl phosphatidylcholine caused by procaine as a function of pH, in 0.1 M NaCl. Circles, experimental; solid lines, theoretical calculations with 1, $K = 7 \cdot 10^{-3}$; $\Delta pK = 1$; 2, $K = 1 \cdot 10^{-2}$, $\Delta pK = 0$; 3, $K = 3 \cdot 10^{-1}$, $\Delta pK = 0$.

of procaine (Fig. 7). Similarly, a good fit to the data for lidocaine requires a value of ΔpK of 1 (Fig. 8).

Effects of drugs on the transition temperature of dipalmitoyl phosphatidylethanolamine are less marked than on dipalmitoyl phosphatidylcholine. However, similarly good fits can be obtained, using a transition temperature of 63° C and a transition enthalpy of 8 kcal · mol^{-1} [18]. Results are listed in Table I.



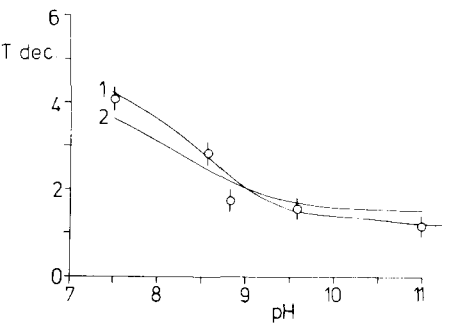


Fig. 7. The decrease in phase transition temperature for dipalmitoyl phosphatidylcholine caused by procaine at pH 10.4 in 0.1 M NaCl, as a function of procaine concentration (mM). Circles, experimental; solid lines theoretical calculations with $K = 7 \cdot 10^{-3}$ and $\Delta pK = 1$.

Fig. 8. The decrease in phase transition temperature for dipalmitoyl phosphatidylcholine caused by 2.6 mM lidocaine in 0.1 M NaCl. Circles, experimental; solid lines, theoretical calculations with 1, $K = 1.3 \cdot 10^{-2}$, $\Delta pK = 1.0$; 2, $K = 1.4 \cdot 10^{-2}$, $\Delta pK = 0$.

Introduction of negative charge into the bilayer will increase the proportion of the charged form of the drug that binds, as a result of reduction of the positive charge built up on the membrane. Attempts to fit data for bilayers containing myristic acid [23], however, gave poor agreement if it was assumed that the dissociation constant K and the lipid transition enthalpy were unchanged by addition of myristic acid. This could be a reflection of localised charge effects, with the positively charged drug preferentially accumulating in the vicinity of the negatively charged myristic acid.

Discussion

The goodness of fit between theory and experiment obtained with a wide variety of drugs is very satisfying, particularly since the fit was normally obtained with a single free variable K^A. Unfortunately, there are little other data in the literature to compare with the values for the dissociation constants obtained here. From their analysis of the binding of 2-6-toluidinylnapthalene sulphonate ions to dioleoyl phosphatidylcholine, McLaughlin and Harary [2] obtained a value of $K = 2 \cdot 10^{-4}$, comparable to those obtained for the more hydrophobic of the drugs listed in Table I.

The analysis of the data presented here makes the assumption that drugs are totally immiscible with lipid in the gel state. Although this is probably true for the short chain *n*-alcohols it is not true for the longer chain alcohols [6,8,20]. The partition coefficients calculated for the long chain alcohols are about half those expected if behaviour had been ideal [6,20], attributable to partial miscibility in the gel state. Similarly, using the reported aqueous solubility of benzocaine [21] the product of the determined partition coefficient for benzocaine and its aqueous solubility is $P \cdot S = 0.8$, compared to the ideal value of 2. At the same time, partial miscibility of the drug and lipid in the gel state has been suggested to be the basis for the increase in width of the transition observed with some drugs [20]. The concentrations of long chain alcohols and benzocaine causing a doubling in width for the transition are about equal to those causing a 5°C fall in transition temperature. For the charged drugs, however, the concentrations causing a doubling in transition width are very much greater than those causing a 5°C fall in transition temperature, the width of the transition being determined both from fluorescence-temperature plots and by using differential scanning calorimetry [22]. For example, the concentration of chlorpromazine causing a doubling of transition width is 4.5 mM [22] and that causing a 5°C fall in transition temperature is 0.2 mM [23]. The charged drugs are not totally immiscible with lipid in the gel phase, however, as Singer [24] has shown that dibucaine can slightly reduce the negative potential on liposomes of dipalmitoyl phosphatidylcholine containing dicetylphosphate in the gel state. Errors due to binding in the gel state are, therefore, likely to be small, at the very worst the reported dissociation constants being a factor of 2 too high because of non-ideality, but even then non-ideality would be expected to have smaller effects on the ratios of dissociation constants for different drugs.

An independent test of the dissociation constants is possible in terms of the charge on the liposomes. Singer [24] has measured the electrophoretic mobility

of liposomes of dipalmitoyl phophatidylcholine containing 5 mol% dicetylphosphate in the presence and absence of 1 mM dibucaine. When the lipid is in the liquid crystalline state, addition of dibucaine changes the electrophoretic mobility from −1 to +2.5 arbitrary units. Under his conditions the calculated surface potential is −24 mV in the absence of dibucaine, so that addition of 1 mM dibucaine would, from the electrophoresis data, change the surface potential to approx. +60 mV. That, calculated theoretically using the dissociation constant in Table I, is +61 mV. The agreement is pleasing.

It has been suggested that local anaesthetics could block Na currents in nerve by dissolving in the lipid component of the nerve membrane and triggering a phase transition for the annular lipid from a solid-like to a fluid-like state [1]. In contrast, others have suggested that positively charged amines act as local anaesthetics by binding within the sodium channel itself, possibly interacting with a particularly narrow part of the channel, referred to as the 'selectivity filter' [25,26]. Arguments against direct binding to the channel have been rehearsed elsewhere [27] and here we simply note the wide variety of chemically distinct species that can act as local anaesthetics. We also note the observation of Harvey and Rang [28] that skeletal muscle fibre membranes undergo extensive changes after chronic denervation, and that a partial resistance develops to tetrodotoxin, but affects of local anaesthetics remain the same as in normal muscle.

Because benzimidazole contains an amidine group and 2-aminobenzimidazole a guanidine group, it has been suggested that they could bind to sodium channels at the site of interaction of the guanidinium group of tetrodotoxin. These compounds have been shown to cause 50% block of the action potentials in frog sciatic nerve at concentrations of approx. 10—20 mM [29]. Elsewhere [27] it has been shown that the concentrations of many drugs causing 50% nerve block is equal to that causing a 3°C drop in transition temperature of dipalmitoyl phosphatidylcholine. The concentrations of benzimidazole and 2-aminobenzimidazole causing a 3°C drop in transition temperature at pH 7.1 are 10 mM and 5 mM, respectively. There seems, therefore, to be no reason for suggesting any special mode of action for these compounds. It is also of interest that 4-aminopyridine has been reported to block potassium channels in nerve, but leave the sodium channels unaffected [30]: this compound has no effect on the phase transition temperature of dipalmitoyl phosphatidylcholine at concentrations up to 10 mM, the limit of aqueous solubility. Decyl trimethylammonium bromide causes a 3°C drop in transition temperature at a concentration of 0.5 mM, and in a preliminary report, Yeh [31] has reported that it is a local anaesthetic effective in the millimolar range.

Diphenylhydantoin has also been shown to block sodium currents in squid axon [32].

The concentration reported to cause 50% nerve block is 0.16 mM, and, under the same conditions of pH and ionic strength, the concentration that causes a 3°C drop in transition temperature for dipalmitoyl phosphatidylcholine is also 0.16 mM.

This good correlation between local anaesthetic activity and effect on lipid phase transition appears to break down for procaine and lidocaine. Here, the

concentrations required to produce a 3°C drop in transition temperatures at pH values close to neutral are very large. This is at least in part a consequence of the shift in the pK value on binding to the membrane (Table I). Correspondingly 6 mM QX314 (a quaternary derivative of lidocaine) causes only a 0.5°C drop in transition temperature. For these relatively non-hydrophobic molecules it is likely that charge-charge interactions will be important, and it is possible that interaction with negative charges near the sodium channel could significantly increase the amount of drug bound near the channel.

Finally, the data reported in Table I suggest that drug binding to phosphatidylethanolamines will generally be weaker than drug binding to phosphatidylcholines. If these lipids are asymmetrically distributed across the membrane, then drug binding on the two halves of the membrane will also be asymmetric.

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