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INTERACTION OF ANTIHISTAMINES WITH LIPID BILAYERS

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Abstract—Interaction between a variety of antihistamines and lipid bilayers has been studied. The H_1-antagonists all reduce the phase transition temperature of dipalmitoyl phosphatidylcholine, and these data have been used to calculate binding constants for the drug to lipid. The H_2-antagonists cimetidine and metiamide have no effect on the lipid phase transition temperatures.

One class of antihistaminic drug is thought to exert its action by successful competition with histamine for the allergic (H_1) receptor site on the walls of smooth muscle tissue. Many of these compounds are also known to be potent local anaesthetics as well as leading to central nervous system depression or stimulation, myocardial depression and direct smooth muscle spasmolytic actions [1, 2]. In contrast, the newer histamine H_2-receptor antagonists show no local anaesthetic activity [3, 4]. The local anaesthetic activity of many drugs has been attributed to effects following from non-specific binding to biological membranes [5] particularly the lipid component of the membrane [6, 7]. It seemed worthwhile therefore to study the binding of the antihistamines to lipid bilayers, to see whether there were significant differences between the two classes of antihistamine. Structures of the antihistamines used in this study are given on the following page.

MATERIALS AND METHODS

Dipalmitoyl phosphatidylcholine was obtained from Koch-Light, egg yolk phosphatidylcholine from Lipid Products, *N*-phenylnaphthylamine from Kodak and 12-anthroyl stearic acid from Sigma. The anti-histamines were obtained as follows: antazoline hydrochloride (CIBA), cyproheptadine hydrochloride (Merck, Sharp and Dohme), pheniramine maleate (Hoechst), triprolidine hydrochloride (Burroughs Wellcome), methdilazine hydrochloride (Duncan, Flockhart) dimethothiazine mesylate, mepyramine maleate and trimeprazine tartrate (May & Baker), diphenylpyraline hydrochloride, cimetidine and metiamide (Smith, Kline & French) and diphenhydramine hydrochloride (Parke Davis).

Chlorophyll *a* was prepared as previously described [8]. Samples were prepared by dissolving lipid plus chlorophyll *a* or *N*-phenylnaphthylamine in chloroform in 10 ml stoppered flasks and evaporating to dryness under a stream of nitrogen. Buffer (0·01 M Tris–HCl, 0.1 M NaCl) containing anaesthetic at the desired pH was added to the mixture and then shaken on a vortex mixer. Fluorescence measurements were made on an Aminco Bowman SPF Fluorimeter, with continuous monitoring of temperature via a thermocouple inserted directly into the fluorescence cell.

Measurements of fluorescence quenching of 12-anthroyl stearic acid were made by adding drug to a liposome suspension containing 12-anthroyl stearic acid at a lipid/stearic acid molar ratio of 7.5: 1. Fluorescence quenching was also studied in 1:1 ethanol–dimethyl sulphoxide mixtures, at an anthroyl stearic acid concentration of 19 μM. Fluorescence was excited at 370 nm and the fluorescence intensity was measured at 450 nm.

Fluorescence spectra of diphenyhydramine and mepyramine were recorded as exciting at 258 and 307 nm respectively and fluorescence maxima were 285 and 367 nm respectively (uncorrected). The observed decrease in fluorescence intensity with increasing pH was used to determine the drug pK. Values of 9.0 and 9.2 were observed for diphenhydramine and mepyramine respectively, in good agreement with literature values [9].

RESULTS

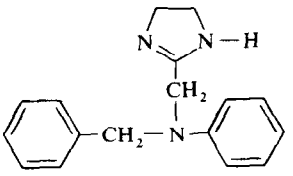
It has been shown in previous publications that changes in the fluorescence of chlorophyll *a* or *N*-phenylnaphthylamine incorporated into liposomes can be used to measure the temperature of lipid phase transitions [8]. Typical fluorescence plots are illustrated in Fig. 1. Where both probes were used, identical results were obtained. However, *N*-phenylnaphthylamine forms a highly fluorescent adduct with some of the drugs; in these cases it can not be used.

From the effect of drugs on the temperatures of the phase transitions, it is possible to calculate the amount of drug bound to the membrane [8].

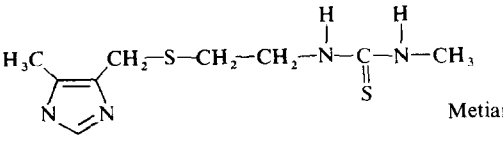
Many drugs have pK values close to physiological pH, and so will exist as a mixture of charged and uncharged forms, both of which will be able to bind to the membrane. In the following example the drug is assumed to exist as a mixture of neutral and cationic forms, true for all the antihistamines studied here. The binding can be described by Langmuir adsorption isotherms,

$$\sigma^{unch} = \frac{1}{K^{unch}}(\sigma^{max} - \sigma^{unch} - \sigma^{ch})[A]^{unch}_{x=0} \quad (1)$$

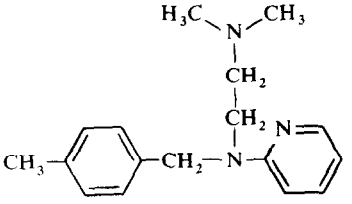
$$\sigma^{ch} = \frac{1}{K^{ch}}(\sigma^{max} - \sigma^{unch} - \sigma^{ch})[A]^{ch}_{x=0} \quad (2)$$



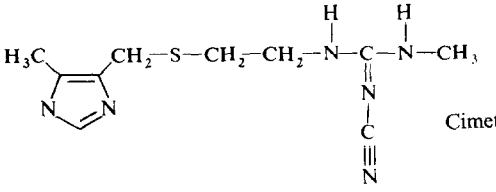
Antazoline



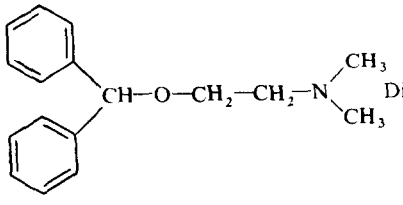
Metiamide



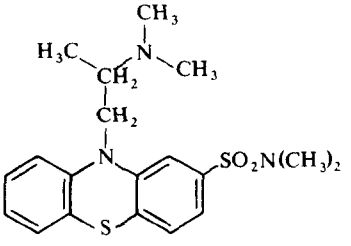
Mepyramine



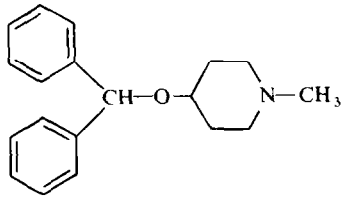
Cimetidine



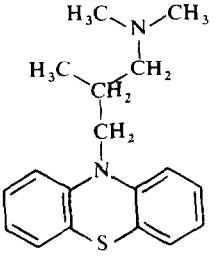
Diphenhydramine



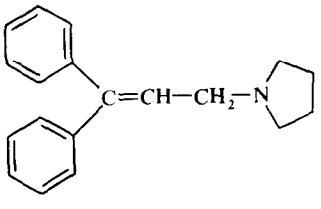
Dimethothiazine



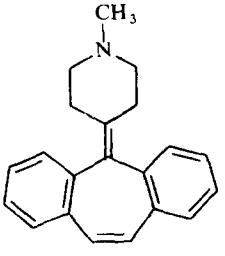
Diphenylpyraline



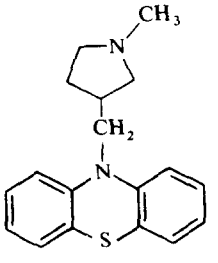
Trumeprazine



Triprolidine



Cyproheptadine



Methdilazine

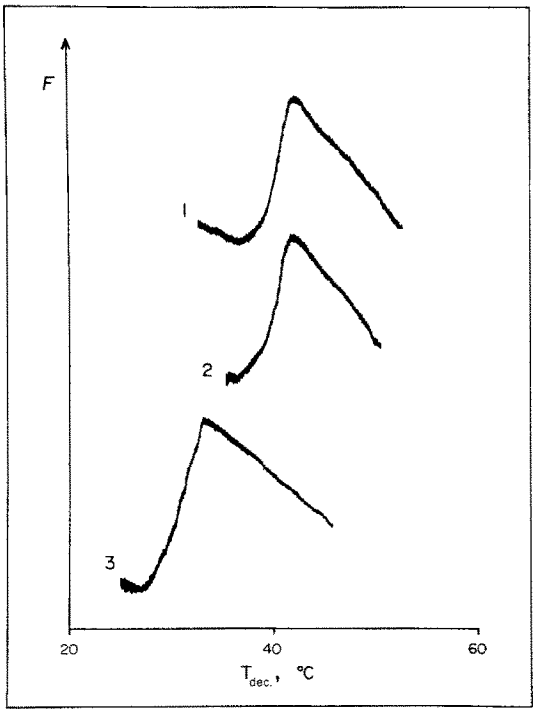


Fig. 1. The fluorescence intensity of *N*-phenylnaphthlamine incorporated into liposomes of dipalmitoyl phosphatidylcholine vs temperature: (1) in the absence of any drug; (2) in the presence of 10 mM cimetidine; (3) in the presence of 10 mM diphenylpyraline.

Here, σ^{unch} and σ^{ch} are, respectively, the number of uncharged and charged drug molecules adsorbed to the membrane per unit area, and σ^{max} is the maximum number of drug molecules that can be adsorbed per unit area. K^{unch} and K^{ch} are dissociation constants to describe the binding of the uncharged and charged forms of the drug respectively. Lastly, $[A]^{unch}_{x=0}$ and $[A]^{ch}_{x=0}$ are, respectively, the concentrations of uncharged and charged species at the membrane–solution interface. The binding constants for the uncharged and charged forms of the drug can be related by [8],

$$K^{unch}/K^{ch} = \exp(2.303/\Delta pK) \tag{3}$$

where ΔpK is the change in drug pK on binding to the membrane. The concentration of the uncharged form of the drug at the membrane–solution interface $[A]^{unch}_{x=0}$ will be equal to its bulk concentration, but the concentration of the charged form will be less because of charge effects, as described by the Gouy–Chapman theory:

$$[A]^{ch}_{x=0} = [A]^{ch} \exp(-F\psi_0/RT) \tag{4}$$

$$\sinh(F\psi_0/2RT) = 136.6\sigma^{ch}/\sqrt{c} \tag{5}$$

Here F is the Faraday, c is the electrolyte concentration, assuming that only monovalent electrolyte is present and $[A]^{ch}$ is the bulk concentration of the charged form of the drug. If the membrane contains negatively charged lipid, then equation (5) needs to be modified to:

$$\sigma^{ch} - \sigma^{neg} = \frac{\sqrt{c}\sinh}{136.6}(F\psi_0/2RT) \tag{6}$$

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

The depression ΔT of the lipid transition temperature can be calculated assuming that none of the drug is soluble in lipid in the gel phase:

$$\Delta T = \frac{RT^2}{\Delta H} x_{drug} \tag{7}$$

Here T is the transition temperature and ΔH is the enthalpy of the transition. The number of drug molecules bound, σ, is related to the mole fraction x_{drug} by:

$$x_{drug} = \frac{1}{1 + 60\sigma} \tag{8}$$

where the molecular area of the lipid in the liquid crystalline phase has been taken to be 60 Å^2.

The above equations are transcendental, but can be readily solved by digital computer, using the Bolzano method [10]. To illustrate the fitting procedure, we will consider the data for the effect of methdilazine on the phase transition temperature of dipalmitoyl phosphatidylcholine (Figs. 2–3). The value of σ^{max} has been fixed at 1/60 Å^2, corresponding to one drug molecule bound per lipid molecule [8]. The transition temperature and enthalpy of transition for dipalmitoyl phosphatidylcholine are 41.7° and 9.69 kcal mole^{-1}, respectively [11]. The variables in the fitting procedure are the drug pK, ΔpK (the shift in pK on binding) and one of the dissociation constants for binding, K^{unch}. Often the drug pK value is known from the literature. Although the effects of the three variables are interrelated, each does have a predominant effect on some particular aspect of the data. In plots of ΔT against drug concentration (Fig. 2) at a fixed pH the steepness of the plot depends on K^{unch}. In plots of ΔT against pH at a fixed drug concentration, the pH at which the plots show a large change in slope depends on the drug pK (Fig. 3); this is because the charged form of the drug binds less

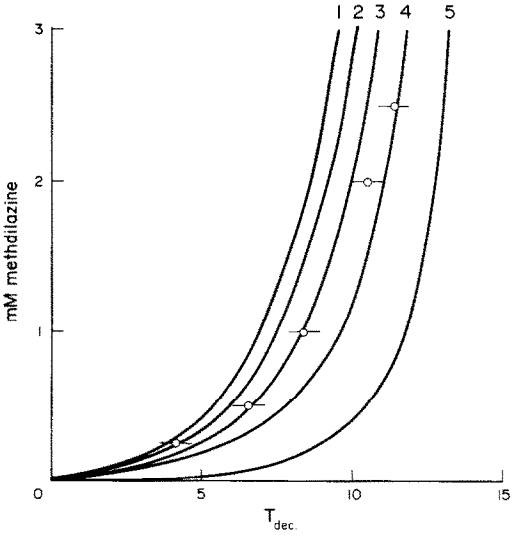


Fig. 2. Theoretical fits to the data for the effect of methdilazine on the transition temperature of dipalmitoyl phosphatidylcholine at pH 7.2. Curves calculated for pK 9.0, ΔpK = 0 and K^{unch} = : (1) 9 × 10^{-5} M; (2) 7 × 10^{-5} M; (3) 5 × 10^{-5} M; (4) 3 × 10^{-5} M; (5) 1 × 10^{-5} M.

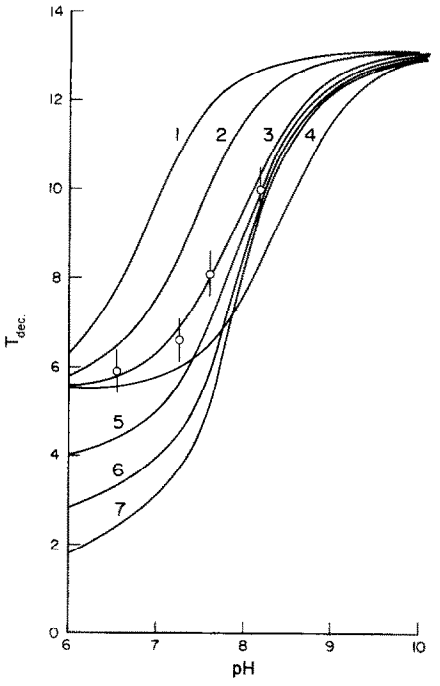


Fig. 3. Theoretical fits to the data for the effect of 0.5 mM methdilazine on the transition temperature of dipalmitoyl phosphatidylcholine as a function of pH. Curves calculated for $K^{unch} = 5 \times 10^{-5}$ M, $\Delta pK = 0$, pK = : (1) 8.0; (2) 8.5; (3) 9.0; (4) 9.5 and for $K^{unch} = 5 \times 10^{-5}$ M, pK = 9.0 and ΔpK = : (5) 0.5; (6) 1.0; (7) 1.5.

well than the uncharged form as a result of charge effects. Further, in the same plots of ΔT against pH, the difference in effects at low and high pH depends on ΔpK (Fig. 3); this is because a positive value of ΔpK corresponds to decreased binding of the charged form of the drug which will predominate at low pH. In this way, values of the parameters are chosen to give the best fit to the experimental data. The calculated values of drug pK agree within 0.5 units with literature values where available [8]. The fit is quite sensitive to the chosen values of K^{unch} and certainly a change by a factor of two or greater leads to a significantly worse fit. For most of the antihistamines, best fit to the data is obtained with $\Delta pK = 0$ but for diphenhydramine the best fit is obtained with $\Delta pK = 0.5$. A list of values obtained is given in Table 1.

In striking contrast to the effects observed with the H_1-antagonists, the H_2-antagonists cimetidine and metiamide have no effect on the transition temperature of dipalmitoyl phosphatidylcholine (Fig. 1).

The binding of the H_1-antagonists to the lipid bilayer could also be demonstrated by their quenching of the fluorescence of 12-anthroyl stearic acid incorporated into the lipid bilayer. The relative ability of these drugs to quench the fluorescence of 12-anthroyl stearic acid in solution in dimethyl sulphoxide–ethanol mixtures is illustrated in Fig. 4. It is clear that in this solvent quenching by mepyramine, trimeprazine and dimethothiazine all give reasonable straight line Stern–Volmer plots, whereas pheniramine and diphenhydramine produce no effect. The first three of these drugs also cause fluorescence quenching of 12-anthroyl stearic acid incorporated into lipid bilayers. Fig. 5 shows data for fluorescence quenching against drug concentration *within* the membrane, calculated using the dissociation constants listed in Table 1, and assuming that the negative charge introduced into the bilayer by the 12-anthroyl stearic acid can be treated by equation 6. Clearly, these plots are non-linear, suggesting a large component of static quenching; this would be expected if positively charged drugs were to bind preferentially in the region close to the negatively charged 12-anthroyl stearic acid. Such localised binding would also help to explain the relatively small differences in quenching ability of mepyramine and trimeprazine in these plots compared to those in organic solvent.

DISCUSSION

There are several categories of H_1-antagonist [12] examples each of which have been studied: derivatives of ethylenediamine (mepyramine and antazoline), aminoalkyl ethers (diphenyhydramine), cyclic basic chains (diphenylpyraline), monoaminopropyl groups (pheniramine and triprolidine) and tricyclic systems (cypro-

Table 1. Binding of anti-histamines to bilayers of dipalmitoyl phosphatidylcholine

Compound	Dissociation constant for binding, K^{unch}	pK*	ΔpK	Concentration (mM) for a 3° drop in transition temperature at pH 7.2
Mepyramine	2.5×10^{-3}	8.9	0.55	6
Antazoline	2×10^{-3}	10	0	2.8
Diphenyhydramine	6×10^{-4}	9.0	0.5	1.5
Diphenylpyraline	5×10^{-4}	(9)	0	0.6
Pheniramine	7×10^{-3}	(9.8)	0	8.5
Triprolidine	1.3×10^{-2}	(8.5)	0	10.25
Cyproheptadine	3×10^{-4}	(9.5)	0.4	0.85
Dimethothiazine	9×10^{-5}	(8.5)	0	0.08
Trimeprazine	3×10^{-5}	(9.5)	0	0.045
Methdilazine	5×10^{-5}	(9)	0	0.09
Cimetidine	—	—	—	>20
Metiamide	—	—	—	>20

* Values in brackets are pK values determined indirectly by best fits. The others are from ref. [9].

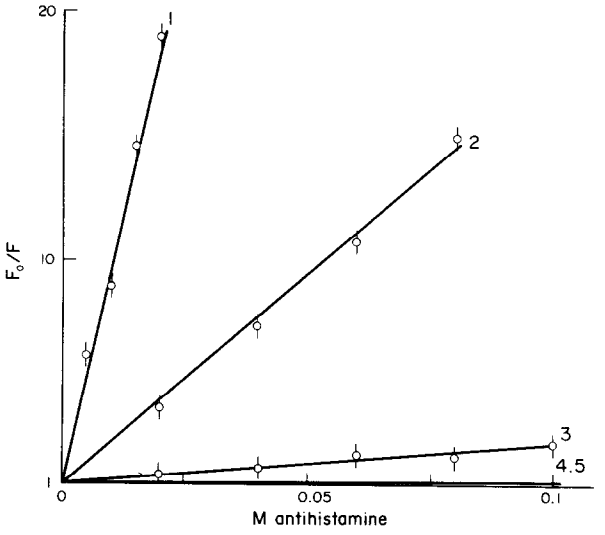


Fig. 4. Stern–Volmer plot for the fluorescence quenching of 12-anthroyl stearic acid by antihistamines in solution in dimethyl sulphoxide–ethanol 1:1 mixture. (1) Dimethothiazine; (2) trimeprazine; (3) mepyramine; (4) diphenhydramine; (5) pheniramine.

heptadine, dimethothiazine, trimeprazine and methdilazine). Local anaesthetic activity has so far been demonstrated, to our knowledge, for the following antihistamines: antazoline, chloropyramine, dimenhydrinate, dimethindene, diphenhydramine, mepyramine, methapyrilene, pheniramine, promethazine, pyrilamine and tripelennamine [1, 13–17]. Unfortunately, tests for local anaesthetic activity have generally been carried out using guinea-pig or human skin wheal, so that relative local anaesthetic potencies are not firmly established; but nevertheless all the above compounds are said to be more active than procaine. The H_2-antagonists, by contrast, show no local anaesthetic activity [3, 4].

All of the H_1-antagonists tested have distinct hydrophilic and hydrophobic portions and so would be ex-

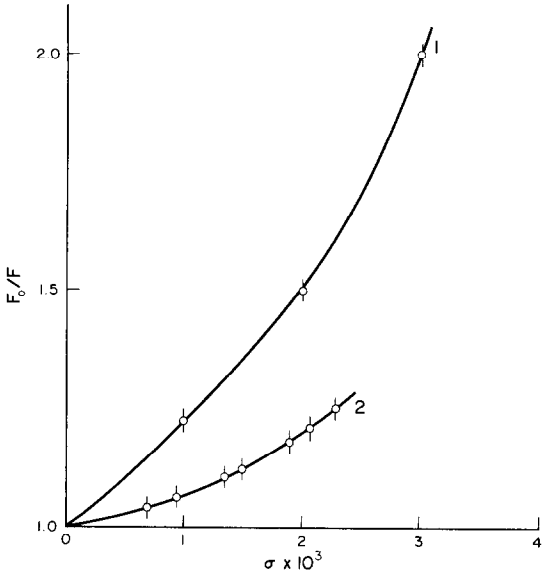


Fig. 5. Stern–Volmer plot for the fluorescence quenching of 12-anthroyl stearic acid incorporated into liposomes of egg phosphatidylcholine as a function of the calculated concentration of antihistamine in the membrane. (1) Trimeprazine; (2) mepyramine.

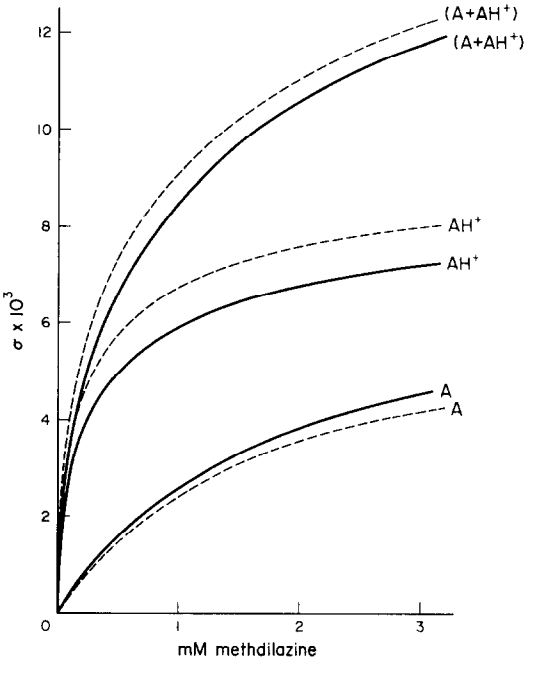


Fig. 6. Calculated surface concentration (No. of drug molecules/Å^2) of methdilazine in a lipid bilayer as a function of the aqueous concentration of methdilazine. Solid line, in the absence of negatively charged lipid. Broken line, in the presence of negatively lipid at 1.4×10^{-3} negative charges/Å^2. A represents the uncharged form of methdilazine, AH^+ the positively charged form and $(A + AH^+)$ the total concentration.

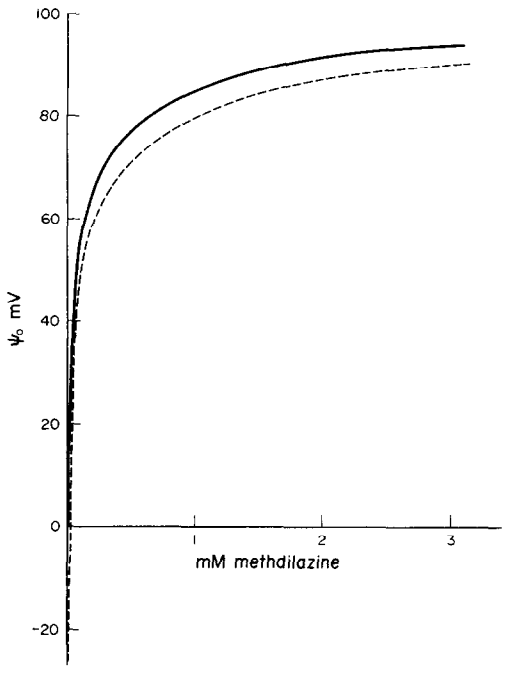


Fig. 7. The calculated surface charge (mV) on a lipid bilayer as a result of the binding of methdilazine as a function of the aqueous concentration of methdilazine. Solid line, in the absence of negatively charged lipid. Broken line, in the presence of negatively charged lipid at 1.4×10^{-3} negative charges/Å^2.

pected to bind well to lipid bilayers. This is confirmed by the marked decrease in the temperature of the lipid phase transition in the presence of these drugs (Table 1). In marked contrast the H_2-antagonists cimetidine and metiamide have no effect on transition temperatures (Table 1). In previous publications it has been shown that the concentrations of many drugs that lower the phase transition temperature of dipalmitoyl phosphatidylcholine by 3° are about equal to the concentration causing 50% block of sodium current in nerve (see ref. 7). The data presented in Table 1 would then suggest that all the H_1-antagonists would show local anaesthetic activity, whereas neither of the H_2-antagonists would. This is in agreement with the available data.

As shown elsewhere [8] it is possible to use the observed decrease in transition temperature to calculate binding constants for the drugs to lipid bilayers. Dissociation constants for all of the H_1-antagonists are smaller than that of procaine (7×10^{-3}), indicating stronger binding, except for triprolidine.

These dissociation constants for binding can be used to calculate some of the effects that will follow from non-specific binding to biological membranes. As an example, Fig. 6 shows how the amounts of the charged and uncharged forms of methdilazine bound to dipalmitoyl phosphatidylcholine vary with the concentration of drug in the aqueous phase at pH 7.2, and Fig. 7 shows the build-up of positive charge on the bilayer as a result of this binding. It is clear that a very large positive charge can build up on the bilayer. Most biological membranes of course normally carry a net negative charge due to the presence of negatively charged phospholipids and to the net preponderence of negative charges on proteins. If the common assumption is made that this negative charge can be treated as being 'smeared-out' over the membrane, then the effect of the negative charge can be treated by the Gouy–Chapman theory [9]. Chandler *et al.* [18] have estimated that the negative charge density on the inside face of the squid axon is 1.4×10^{-3} charges/Å^2. Figure 7 illustrates that this negative charge density results in increased binding of the positively charged form of methdilazine, and a reversal of the sign of the membrane surface potential at approx. 2 μM methdilazine. This could have a number of important consequences, since a number of membrane processes are likely to be sensitive to surface charge. In particular, Ca^{2+} concentration close to the membrane will be very sensitive to the charge on the membrane, and a large number of cellular processes are now thought to be triggered by the passage of Ca^{2+} through the cell membrane. For example, it has been shown that histamine increases the Ca^{2+} efflux from a microsomal fraction derived from cat small intestine and believed to be rich in histamine receptor [19]. Further, since histamine is present almost exclusively as the univalent cation at physiological pH, charge effects will also be important in determining its concentration close to the membrane surface. According to the Boltzmann relationship (equation 4) its concentration at the surface will decrease by a factor of 90 when the membrane potential is changed from −25 mV to +90 mV, the potential reached in the presence of 3 mM methdilazine. The importance of such charge effects is unclear. Presumably charge effects would be manifested by any positively charged drug binding to the membrane and thus it is of interest that many compounds of diverse structure are known to block or interfere with histamine responses [9, 20]. H_1-antagonists reduce both the slope and the maximum amplitude of histamine dose–response curves at concentrations in the micromolar range [21, 22], the effect on maximum amplitude being perhaps a reflection of the build-up of positive charge on the membranes as a result of non-specific binding.

Charge effects might also possibly explain the strange observations of Rocha e Silva which led to his 'charniere (hinge)' theory [23]. The experiments involved the effects of histamine and diphenhydramine on guinea pig ileum muscle. After exposure to the antihistamine, the bound antihistamine should slowly dissociate from the receptor with a characteristic sigmoid recovery curve. If during this recovery time a very large amount of agonist is applied (100 times normal), a supranormal response can be obtained even though the antagonist is still bound; once the excess agonist is washed out the preparation returns to the same level of blockade, as if no agonist had been added. Rocha e Silva attributed this to a simultaneous but partial occupation of the receptor by both agonist and antagonist. However, this is exactly the effect that would be expected if the antihistamine were having its effect largely through charge. From the Boltzmann relationship, a very large increase in the bulk concentration of histamine would be expected to increase the concentration of histamine close to the receptor despite the positive charge due to the bound antihistamine.

Although the above treatment of negative charge on the membrane is very approximate, it is likely to be an

under-estimate of the real situation as far as binding is concerned. Because the local charge density close to a negatively charged lipid is high, the positively charged form of the drug is likely to preferentially adsorb adjacent to the negatively charged lipid and give a relatively high local concentration of drug [24]. Some slight evidence for this is shown here by the relative abilities of mepyramine and trimeprazine to quench the fluorescence of 12-anthroyl stearic acid in liposomes and in organic solvent, and by the upward curve of the Stern–Volmer plots for lipid bilayers (Fig. 5) indicative of static quenching [25].

Of course, the primary effect of the antihistamines is likely to follow from specific binding to membrane receptors, and a high-affinity binding of mepyramine to a component of the membranes of intestinal smooth muscle has been demonstrated by Hill *et al.* [26]. These workers also demonstrated a large non-specific binding component for mepyramine consistent with the demonstration here of good binding to lipid bilayers. Such binding would be expected to produce a large change in fluidity for lipids in a gel-like state, with possible consequences for the activity of membrane proteins [6, 24], and would also produce large changes in surface charge, with possible consequences for a wide range of membrane-related events.

These observations may go some way to explain the wide range of side-effects observed with the H_1-antagonists.

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