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Liquid membrane phenomenon in antihistamines

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Summary

The liquid membrane phenomenon in antihistamines has been studied. The drugs have been shown to generate liquid membranes in series with a supporting membrane and modify the permeability of histamine. The data on the modification in the permeability of histamine indicate that the liquid membranes generated by antihistamines may play a significant role in the mechanism of their action.

Three structurally dissimilar antihistamines, namely chlorpheniramine, diphenhydramine and tripelennamine, have been chosen for the present study.

Introduction

Formation of cell membranes and location of receptor proteins in lipid bilayer is a consequence of surface activity. Hence it is logical to expect that the drugs acting by altering permeability of cell membranes are likely to be surface-active. A wide variety of drugs are, in fact, known to be surface-active in nature (Florence, 1968; Felmeister, 1972). This does not appear to be a fortuitous coincidence. In a number of cases correlations between surface activity and biological effects have been demonstrated (Seeman, 1972; Ritchie and Greengard, 1966; Vilallonga and Phillips, 1980). While investigating the actions of drugs like reserpine, prenylamine, chlorpromazine, propranolol, etc., which inhibit catecholamine transport, it has been concluded (Palm et al., 1970) that, irrespective of chemical structure, the surface-activity of psychotropic drugs mainly determines their potency to affect all kinds of membranes, especially that of catecholamine-storing particles. Since the structural

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requirements for surface activity are often similar to those for interaction of drugs with receptor sites (Attwood et al., 1974), the correlations between surface-activity and biological effects appear to indicate that a common mode of action might exist for all surface-active drugs.

According to the liquid membrane hypothesis (Kesting et al., 1968) which was originally propounded in the context of water desalination by reverse-osmosis, a surface-active substance when added to aqueous phase generates a surfactant layer liquid membrane at the interface. As concentration of the surfactant is increased, the interface gets progressively covered with the liquid membrane and at the critical micelle concentration (CMC), it is completely covered. In view of the liquid membrane hypothesis, it was suspected that the liquid membranes generated at the site of action of the respective drugs, acting as a barrier to the transport of relevant permeants, might be an important step, common to the mechanism of action of all surface-active drugs. Recent studies from this laboratory on several drugs belonging to different pharmacological categories, e.g. haloperidol (Bhise et al., 1982), imipramine (Srivastava et al., 1982), reserpine (Bhise et al., 1983a), chlorpromazine (Bhise et al., 1983b), diazepam (Srivastava et al., 1983a) and local anesthetics (Srivastava et al., 1983b) have strongly substantiated this suspicion.

In the present communication are reported investigations on antihistamines—H_1-antagonists. Surface-activity of antihistamines is documented in the literature (Attwood, 1972; Attwood and Udeala, 1975b). Antihistamines have been shown in the present study to generate liquid membrane at the interface. Because antihistamines are known to be competitive antagonists of histamine (Douglos, 1980), data have been obtained on the transport of histamine through liquid membranes which were generated by the antihistamines, in series with a supporting membrane and discussed in the light of the mechanism of their action. A Sartorius cellulose acetate microfiltration membrane/aqueous interface has been deliberately chosen as site for the formation of liquid membranes so that active interaction of the drugs with constituents of biological membrane as a cause for modification in the permeabilities is ruled out and the role of passive transport through the liquid membranes is highlighted.

Three structurally dissimilar antihistamines (H_1-antagonists) namely chlorpheniramine maleate, diphenhydramine hydrochloride and tripelennamine hydrochloride, have been chosen for the present study. The choice of structurally dissimilar drugs within one pharmacological category makes the role of the liquid membranes in the mechanism of their action conspicuous.

Materials and Methods

Chlorpheniramine maleate (Sigma, U.S.A.), diphenhydramine hydrochloride (Parke-Davis, Bombay, India), tripelennamine hydrochloride (Ciba-Geigy, Bombay, India), histamine acid phosphate (BDH), *o*-phthalaldehyde (Sigma, U.S.A.) and distilled water, distilled once over potassium permangnate in an all-pyrex glass still were used for the present study.

TABLE 1

CRITICAL MICELLE CONCENTRATION DATA OF ANTIHISTAMINES IN AQUEOUS SOLUTIONS

	CMC		
	$mol \cdot l^{-1}$	$mol \cdot kg^{-1}$	$mol \cdot l^{-1}$
Chlorpheniramine maleate	(1×10^{-4}) *	–	–
Diphenhydramine hydrochloride	(1×10^{-3}) *	0.122 **	~ 0.05 ***
Tripelennamine hydrochloride	(1×10^{-3}) *	~ 0.20 **	–

* Present investigation, values are at 37°C.
** Attwood and Udeala (1975a), values are at 30°C.
*** Johnson et al. (1965), values are at 25°C.

The CMCs of aqueous solutions of the drugs were determined from the surface tension–concentration plots. The surface tensions were measured using a tensiomat (Fisher Tensiomat model 21). The CMCs of aqueous solutions of the drugs thus estimated are given in Table 1.

The all-glass cell described earlier (Bhise et al., 1982) was used for the transport studies. It is reproduced in Fig. 1 for ready reference and has been well labelled to make it self-explanatory. A Sartorius cellulose acetate microfiltration membrane (cat. no. 11107), of thickness 1×10^{-4} m and area 5.373×10^{-5} m^2 which acted as a

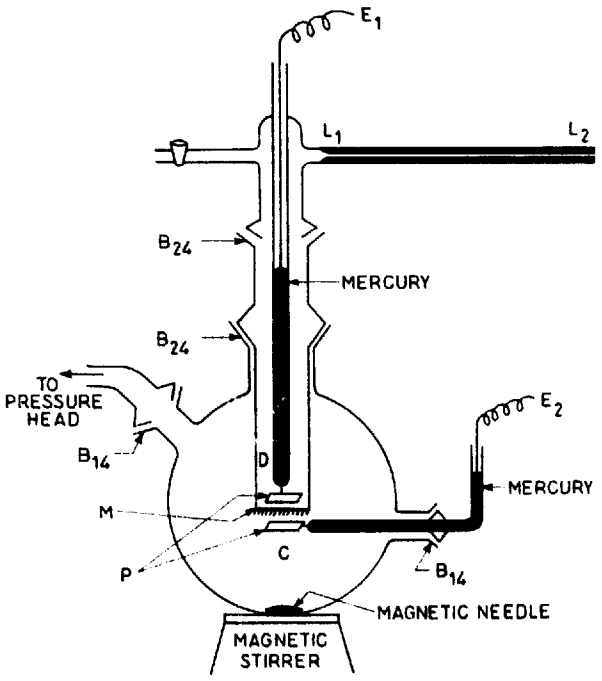

Fig. 1. The all-glass transport cell. M = supporting membrane (cellulose acetate microfiltration membrane cat. no. 11107 of thickness 1×10^{-4} m and area 5.373×10^{-5} m^2); P = bright platinum electrodes; E_1, E_2 electrode terminals; L_1L_2-capillary of length 17 cm and diameter 1.18×10^{-1} cm.

support for the liquid membrane separated the transport cell into two compartments C and D (Fig. 1).

The hydraulic permeability data at various concentrations of the drug, utilized to demonstrate the existence of the liquid membrane were obtained using the method described earlier (Bhise et al., 1982). Aqueous solutions of the drugs of varying concentrations were filled in the compartment C of the transport cell and compartment D was filled with distilled water. Known pressures were applied on compartment C by adjusting the pressure head and the resulting volume flux was measured by noting the rate of advancement of the liquid meniscus in capillary L_1L_2 using a cathetometer reading up to 0.001 cm and a stopwatch reading up to 0.1 s. The magnitude of the applied pressure difference was also measured by noting the position of pressure head, with a cathetometer reading up to 0.001 cm. During the volume flux measurements the solution in compartment C was well stirred and electrodes E_1 and E_2 were short-circuited so that the electro-osmotic back-flow that developed due to streaming potentials did not interfere with the observations. The volume flux, J_v, at various values of ΔP, the applied pressure difference, were calculated using the relation,

$$J_v = \frac{\pi r^2 \ell}{\pi R^2 t} = \left(\frac{r}{R}\right)^2 \frac{\ell}{t} \tag{1}$$

where r and R are radii of the capillary L_1L_2 and the membrane M (Fig. 1), respectively, and ℓ is the distance travelled by the liquid meniscus in the capillary L_1L_2 in time t. The concentration ranges selected were such that hydraulic permeability data were obtained both below and above the CMCs of the drugs.

For the measurement of solute permeability (ω) of histamine, two sets of experiments were performed. In the first set of experiments, a mixture of the aqueous solutions of histamine and one of the antihistamines under investigation was filled in compartment C and compartment D (Fig. 1) was filled with distilled water. In the second set of experiments, an aqueous solution of histamine was kept in the compartment C and compartment D was filled with antihistaminic drug solution. However, in control experiments no antihistamine was used.

The values of solute permeability (ω) were measured using the definition (Katchalsky and Kedem, 1962; Katchalsky and Curran, 1967),

$$\left(\frac{J_v}{\Delta\pi}\right)_{J_v=0} = \omega \tag{2}$$

where J_v and J_s are the volume flux and the solute flux per unit area of the membrane, respectively, and $\Delta\pi$ is the osmotic pressure difference. The condition of no net volume flux ($J_v = 0$) during the solute permeability (ω) measurements was attained by adjusting the pressure head attached to the compartment C of the transport cell so that the liquid meniscus in the capillary L_1L_2 remained stationary. After a known period of time, which was of the order of several hours, the concentration of the permeant in the other compartment was measured. The amount

of permeant gained by compartment D divided by the time and the area of the membrane gave the value of the solute flux, J_s, for use in the calculation of ω using Eqn. 2. The value of $\Delta\pi$ used in the calculation of ω was the average of the $\Delta\pi$-values at the beginning ($t = 0$) and at the end of the experiment.

For solute permeability (ω) measurements, the concentrations of the drugs taken were always higher than their CMCs. This was done to ensure that the supporting membrane was completely covered with liquid membranes generated by the drugs. Fifteen repeats were taken for each value of ω.

The orientation of the liquid membrane generated by the antihistamines, with respect to approaching permeant—histamine, will be different in the two sets. Since the hydrophobic ends of antihistamines, which are surface-active in nature, would be preferentially oriented towards the hydrophobic supporting membrane, in the first set of experiments the drug liquid membrane would present a hydrophilic surface to the permeant—histamine. Similarly, in the second set of experiments, histamine would face the hydrophobic surface of the liquid membrane generated by the drugs.

All measurements including CMC determinations were carried out at constant temperature using a thermostat set at 37 ± 0.1°C.

Estimations

The amount of histamine transported to compartment D was estimated by measuring fluorimetrically the fluorophor derivative from its reaction with *o*-phthalaldehyde (Redlich and Glick, 1965; Udenfriend, 1969). A Photovolt Fluorescence Meter model 540 was used for the estimations.

Results and Discussion

Literature values of CMC of antihistamines are also recorded in Table 1 along with values determined in the present investigation. In the case of diphenhydramine hydrochloride it has been concluded (Johnson et al., 1965) that the drug shows aggregation beyond 0.05 M concentration. Hence, the CMC should be above this concentration. The CMC of chlorpheniramine maleate is not documented in the literature. The CMC values determined in the present investigation, though lower than the literature values, were found to be consistent with the hydraulic permeability data. The resistance to volume flux in presence of the drugs increased with increasing concentration of the drugs up to the CMC values—presently determined —beyond which it became more or less constant. This implies that these are the concentrations at which a complete liquid membrane is generated in series with the supporting membrane, in accordance with the liquid membrane hypothesis (Kesting et al., 1968).

The liquid membrane formation

The hydraulic permeability data at various concentrations of the drug in the case of all 3 antihistamines were found to obey the linear relationship

$$J_v = L_p \cdot \Delta P \qquad (3)$$

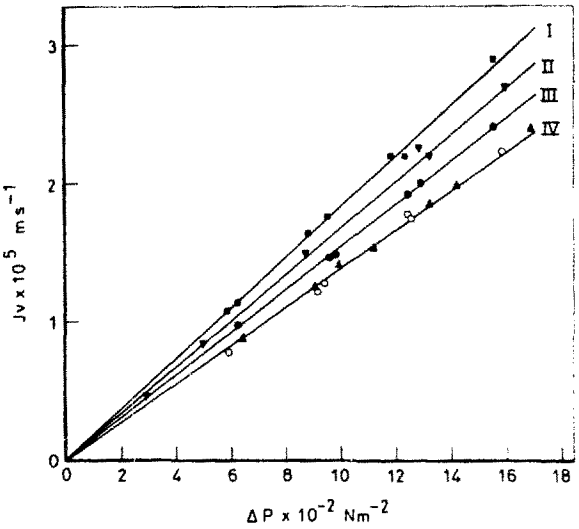

Fig. 2. Hydraulic permeability data at various concentrations of chlorpheniramine maleate J_v and ΔP have the same meaning as in Eqn. 3. Curves I, II and III are for 0, 0.25×10^{-4} M and 0.5×10^{-4} M concentrations of drug, respectively. Curve IV represents data for concentration equal to and higher than the CMC of the drug, viz. 1.0×10^{-4} M and 2.0×10^{-4} M.

where J_v represents the volume flux per unit area of the membrane, ΔP the applied pressure difference, and L_p the hydraulic conductivity coefficient. The data for chlorpheniramine maleate are plotted in Fig. 2. The values of L_p at various concentrations of chlorpheniramine maleate estimated from the slopes of the plots in Fig. 2, are given in Table 2. The values of L_p are expressed as arithmetic mean ± standard deviation. The value of L_p in Table 2 shows a progressive decrease with increase in concentration of the drug up to the CMC beyond which it becomes more or less constant. This trend is indicative of the progressive coverage of the supporting membrane with the liquid membrane generated by the drug in accordance with the liquid membrane hypothesis (Kesting et al., 1968). At the CMC, coverage of the supporting membrane with the drug liquid membrane is complete.

TABLE 2

VALUES OF L_p AT VARIOUS CONCENTRATIONS OF CHLORPHENIRAMINE MALEATE

	Chlorpheniramine maleate concentration $\times 10^4$ M				
	0	0.25 (0.25 CMC)	0.50 (0.5 CMC)	1.00	2.00
$L_p^a \times 10^8 (m^3 \cdot s^{-1} \cdot N^{-1})$	1.8560 ±0.0283	1.7215 ±0.0306	1.6470 ±0.0183	1.4307 ±0.0148	1.3860 ±0.0360
$L_p^a \times 10^8 (m^3 \cdot s^{-1} \cdot N^{-1})$	–	1.7500 ±0.0181	1.6436 ±0.0116	–	–

[a] Experimental values.

[b] Calculated values on the basis of mosaic model.

Analysis of the flow data in the light of the mosaic membrane model (Spiegler and Kedem, 1966; Sherwood et al., 1967; Harris et al., 1976) further supports the existence of the liquid membrane in series with the supporting membrane. Since, according to the liquid membrane hypothesis (Kesting et al., 1968) at CMC, the supporting membrane is fully covered with the surfactant layer liquid membrane, at concentrations lower than the CMC it will be only partially covered. The situation is pictorially depicted in Fig. 3. The equation for volume flux for such a situation can be written as:

$$J_v(A^s + A^c) = J_v^s A^s + J_v^c A^c \tag{4}$$

where A represents the area of the membrane denoted by the superscripts, and the superscripts s and c represent the bare supporting membrane and the supporting membrane covered with the liquid membrane, respectively. In view of the linear relationship between J_v and ΔP, i.e. Eqn. 3, Eqn. 4 can be transformed into

$$J_v = \left[L_P^s\left(\frac{A^s}{A^s + A^c}\right) + L_P^c\left(\frac{A^c}{A^c + A^s}\right)\right] \cdot \Delta P \tag{5}$$

Functionally L_P^s and L_P^c represent the value of L_P at 0 and CMC, respectively. The concept of progressive coverage in the liquid membrane hypothesis implies that at half the CMC, the fraction of the total area of the supporting membrane covered with the liquid membrane will be half, and hence the slope of J_v vs ΔP plot, in view of the Eqn. 5 should be equal to $(L_P^s + L_P^c)/2$. Similarly, when concentration of the surface-active agent is one-fourth its CMC, the value of the slope, should be equal to $[(3/4)L_P^s + (1/4)L_P^c]$ and so on. Thus in general terms if concentration of the surfactant is n times its CMC, n being less than or equal to 1, the value of the slope of J_v vs ΔP plots should be equal to $[(1 - n)L_P^s + nL_P^c]$. The values of L_P thus computed for 0.25 CMC and 0.5 CMC of chlorpheniramine maleate match favourably with the experimentally determined values (Table 2) furnishing additional evidence in favour of liquid membrane formation. Analysis of flow data in the case of other two antihistamines also gave similar results furnishing evidence for the formation of liquid membrane by them in series with the supporting membrane.

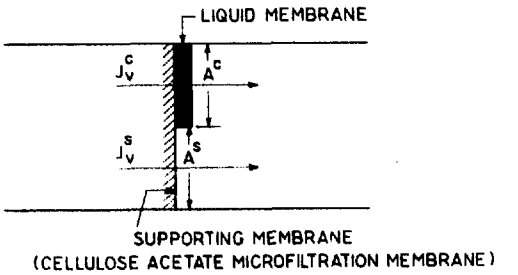

Fig. 3. The schematic representation of mosaic membrane formed when the concentration of the surfactant is lower than its critical micelle concentration. J_v^s, J_v^c, A^s and A^c have the same meaning as in Eqn. 4.

Role of liquid membrane in antihistamine action

Antihistamines are known (Douglos, 1980) to occupy histamine receptors causing exclusion of histamine from its site. The action is known to be competitive and reversible (Witiak and Cavestri, 1981). The antagonism is considered entirely on account of the specific interaction of antihistamine with the receptor. The present data, however, indicate that the liquid membranes generated by the drugs also contribute to the antihistaminic action.

The data on the solute permeability (ω) of histamine in the presence of the antihistamine drugs are recorded in Table 3. The values are expressed as arithmetic mean ± standard deviation—based on the 15 repeats for each value of ω. The differences between the various ω-values in Table 3 were found to be statistically significant. The data in Table 3 clearly indicate that the liquid membranes generated by antihistamines themselves impede the transport of histamine to a notable extent. Chlorpheniramine maleate is known to be most potent (Witiak and Cavestri, 1981) amongst all 3 antihistamines studied. This is consistent with the observation that the CMC of chlorpheniramine maleate is the lowest (Table 1) implying that it forms a complete liquid membrane at a much lesser concentration than the other two drugs. This prima facie indicates that the liquid membrane generated by the antihistamines at the site of action may play a role in the mechanism of their action.

Chlorpheniramine maleate which is known to be most potent of all the 3 drugs (Witiak and Cavestri, 1981) presently studied, impedes the transport of histamine more or less to the same extent in both the orientations—when the permeant faces the hydrophilic or the hydrophobic surface of the drug liquid membrane. The rest of the drugs, however, impede the transport of histamine more when the drug liquid membranes present their hydrophobic surface to the permeant than when the permeant faces the hydrophilic surface of the drug liquid membranes. Since, in the histamine receptor, existence of both hydrophilic and hydrophobic sites has been indicated (Korolkovas, 1970), it appears that chlorpheniramine maleate gets at-

TABLE 3

PERMEABILITY OF HISTAMINE[b] (ω) IN PRESENCE OF ANTIHISTAMINES[a]

Drug	$\omega_1 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$)	$\omega_2 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$)	$\omega_3 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$)
Chlorpheniramine maleate	5.1855 ±0.6379	3.0620 ±0.4604	3.3460 ±0.3398
Diphenhydramine hydrochloride	5.1855 ±0.6379	3.1063 ±0.3251	1.5250 ±0.1420
Tripelennamine hydrochloride	5.1855 ±0.6379	3.1579 ±0.2691	2.6607 ±0.3145

ω_1 = control value—when no drug was used; ω_2 = drug and histamine in the compartment C and water in the compartment D; ω_3 = drug in the compartment D and histamine in the compartment C.

[a] The concentrations of chlorpheniramine maleate, diphenhydramine hydrochloride, tripelennamine hydrochloride were: 2×10^{-4} M, 2×10^{-3} M and 2×10^{-3} M, respectively.

[b] Initial concentration of histamine 10 μg/ml.

tached to both hydrophilic and hydrophobic sites in the formation of liquid membrane, while the other two drugs get attached only to the hydrophilic sites. According to Waud (1968) 'potency may imply selectivity'. In other words, the more potent the drug is, the more selective it may be to the receptor. Thus the tendency of chlorpheniramine maleate to attach with both hydrophilic and hydrophobic sites implies its selectivity to histamine receptors, which is in keeping with Waud's statement.

It is interesting to note that structure–activity studies of H_1-antagonists have exhibited a relationship with partition characteristics (Brink and Lien, 1977; Testa and Murset-Rossetti, 1978) and association phenomena (Attwood and Udeala, 1976; Gettins et al., 1976) both of which are related to surface-activity.

Although the reduction in the permeability of histamine on account of liquid membranes generated by the antihistamines is passive in nature, it is likely to be accompanied by a consequent reduction in the active transport as well. This is because the presence of the liquid membrane generated by antihistamines is likely to reduce the access of histamine to its receptors.

The multiple effects (Douglos, 1980) associated with antihistamines, viz. anticholinergic effects, local anesthetic effects or sedation, may also be explained by modification in the transport of relevant permeants. The liquid membrane generated by antihistamines may offer a varying degree of resistance to the transport of relevant permeants. Detailed investigations, however, are called for to assess validity of the proposition.

Thus liquid membranes generated by the antihistamines at the site of action seem to contribute to the mechanism of their action.

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