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# LIQUID MEMBRANE PHENOMENA IN CHLORPROMAZINE ACTION

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Chlorpromazine has been shown, in the present study, to generate a liquid membrane at an interface in accordance with Kesting's hypothesis [5]. The specific orientation of chlorpromazine molecules in a liquid membrane with hydrophobic ends facing the permeable substances has been found to reduce the permeability of catecholamines and neurotransmitter amino acids. This observation is discussed in the light of the orientation of receptor proteins in general. The data on transport of catecholamines and neurotransmitter amino acids are discussed in the context of the mechanism of action of chlorpromazine.

## 1. Introduction

There is extensive evidence [1] to indicate that the biochemical and pharmacological actions of phenothiazines are related to the ability of these drugs to accumulate at biological membranes and modify their permeability characteristics. This certainly is a consequence of the surface-active nature of these drugs. The surface activity of phenothiazines, which can be altered by modifying their structure [2], has been shown to correlate with their clinical potencies [3]. All this indicates that the surface activity might play an important role in the mechanism of action of such drugs. Palm et al. [4] have concluded that in the case of psychotropic drugs, irrespective of chemical structure, it is the surface activity that mainly determines their potency.

According to Kesting's hypothesis [5], a surface-active substance when added to water or aqueous solutions generates a surfactant layer liquid membrane which completely covers the interface at the critical micelle concentration (CMC) of the surfactant. The liquid membranes thus generated modify material transport across the interface. Thus, it appears likely that the liquid membranes, which surface-active drugs are capable of generating at the interface, may be of consequence in the mechanism of action of such drugs. Prompted by this, studies on haloperidol [6], imipramine [7] and reserpine [8] were undertaken in this laboratory recently, and it was shown that the contribution of the liquid membranes generated by these drugs is significant to the mechanism of their action. In the present communication, studies on chlorpromazine, which is a surface-active neuroleptic drug, are reported. The existence of a liquid membrane generated by chlorpromazine at the interface has been demonstrated. The transport of biogenic amines and relevant neurotransmitter amino acids through the liquid membrane generated by chlorpromazine has been studied and the data have been discussed in the light of the mechanism of action of the drug. Data have also been obtained on the transport of small ions such as $Na^+$ and $K^+$ and neutral molecules like glucose through the liquid membrane to gain information on the specificity of the chlorpromazine liquid membrane to the permeability of neurotransmitters.

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In the present study, a cellulose nitrate microfiltration membrane/water interface has been deliberately chosen as the site for formation of the liquid membrane so that the specific and active interaction of the drug with the components of the biological membranes is totally ruled out and data on passive transport alone are obtained.

## 2. Experimental

### 2.1. Materials

Chlorpromazine hydrochloride (May and Baker (India) Ltd.), dopamine chlorhydrate, adrenaline hydrogen tartrate (Loba Chemie), L-noradrenaline (Fluka A.G.), 5-hydroxytryptamine creatinine sulfate (Koch Light Laboratories Ltd.), L-glutamic acid, γ-aminobutyric acid (B.D.H.), NaCl, KCl, glucose (all Analar grade) and distilled water, distilled once over potassium permanganate in an all-pyrex glass still, were used in the present experiments.

### 2.2. Methods

The CMC of aqueous chlorpromazine hydrochloride was determined from the variation of surface tension with concentration. The surface tensions were measured by a surface tensiomat (Fisher Tensiomat Model 21). The CMC of aqueous solution of chlorpromazine hydrochloride thus determined was $4.5 \times 10^{-5}$ M.

The all-glass cell described earlier [6,9] was used for transport studies. A Sartorious cellulose nitrate Millipore filter (Catalog No. 11307 of thickness $1 \times 10^{-4}$ m and area $5.373 \times 10^{-5}$ m$^2$) acted as a support for the liquid membrane and separated the transport cell into two compartments, C and D (fig. 1 of refs. 6 and 9).

For measurements of hydraulic permeability, aqueous solutions of chlorpromazine of various concentrations, ranging from 0 to $1.8 \times 10^{-4}$ M, were placed in compartment C of the transport cell (fig. 1 of refs. 6 and 9) and compartment D was filled with water. The concentration range $0-1.8 \times 10^{-4}$ M of the drug was purposely chosen to obtain data both above and below the CMC of the drug. The method of measurement has been described earlier [6,9,10].

For measurement of the permeability ($\omega$) of biogenic amines, amino acids, cations and glucose two sets of experiments were performed. In the first set of experiments, compartment C of the transport cell (fig. 1 of refs. 6 and 9) was filled with solutions of the respective permeable substances prepared in a $1.8 \times 10^{-4}$ M aqueous solution of chlorpromazine and compartment D was filled with distilled water. In the second set of experiments compartment D was filled with a $1.8 \times 10^{-4}$ M aqueous solution of chlorpromazine and compartment C was filled with aqueous solutions of known concentrations of permeable substances. In control experiments, however, no chlorpromazine was used. Since chlorpromazine is a surface-active substance, it has both hydrophilic and hydrophobic groups in its structure. In the first set of experiments, therefore, the liquid membrane generated by chlorpromazine would present a hydrophilic surface to the approaching permeable substances because the hydrophobic ends of the drug molecules will be preferentially oriented towards the hydrophobic supporting membrane – the cellulose nitrate microfiltration membrane. In the second set of experiments, however, the permeable substances present in compartment C will face the hydrophobic surface of the liquid membrane generated by the drug present in compartment D.

The values of solute permeability ($\omega$) were measured using the definition [11,12].

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_v=0} = \omega \qquad (1)$$

where $\Delta\pi$ is the osmotic pressure difference, and $J_s$ and $J_v$, respectively, the solute flux and volume flux per unit area of the membrane. The method of measurement has been described earlier [6–8]. For solute permeability measurements the concentration $1.8 \times 10^{-4}$ M of chlorpromazine, which is well above its CMC, was chosen to make sure that the supporting membrane becomes fully covered with the liquid membrane generated by the drug.

To safeguard against the reported [13] photo-oxidation of chlorpromazine, the portion of the

transport cell containing the drug was painted black.

All measurements including the CMC determination were carried out at 37 ± 0.1°C.

### 2.3. Estimation

The amounts of adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine transported were estimated in the presence of chlorpromazine. Since chlorpromazine was observed to quench the fluorescence of the biogenic amines, their amounts transported to other compartments were estimated using a Cary 17-D spectrophotometer by measuring the absorbance at their absorption maxima (282.4 nm).

For this, calibration curves were constructed by noting the absorbance of solutions of varying concentrations of biogenic amines prepared in a solution of a fixed concentration of chlorpromazine which was equal to the concentration of chlorpromazine used in solute permeability experiments. The calibration curves in all cases were found to be linear in accordance with Beer's law.

The amounts of glutamic acid and γ-aminobutyric acid were estimated by spectrophotometric determination of their reaction products with ninhydrin [14] at 570 nm.

The amounts of $Na^+$ and $K^+$ were estimated using a Systronics flame photometer and that of glucose by spectrophotometric determination of its reaction product with dinitrosalicylic acid [15].

## 3. Results and discussion

The hydraulic permeability data at various concentrations of chlorpromazine (fig. 1) are in accordance with the linear relationship

$$J_v = L\Delta P \quad (2)$$

where $J_v$ represents the volume flux per unit area of membrane, $\Delta P$ the applied pressure difference and $L$ the hydraulic conductivity coefficient. The values of $L$ (table 1) computed from the slopes of the curves in fig. 1 show a progressive decrease with increase in concentration of chlorpromazine. The values of $L$ continue to decrease up to the CMC of chlorpromazine and thereafter become more or less constant. This trend is in keeping with Kesting's liquid membrane hypothesis [5] according to which as the concentration of surfactant is increased, the supporting membrane becomes progressively covered with the surfactant layer liquid membrane and at the CMC coverage is complete.

Analysis of the flow data (fig. 1 and table 1) in the light of the mosaic membrane model [16–18] furnishes additional support in favor of liquid membrane formation in series with the supporting cellulose nitrate membrane. Following the arguments given earlier [6,7,9], it can be shown that if the concentration of the surfactant is $n$-times its CMC, $n$ being less than or equal to 1, the value of $L$ would be equal to $[(1-n)L^c + nL^s]$ where $L^c$ and $L^s$, respectively, are the values of $L$ at 0 and the CMC of the surfactant. The values of $L$ thus

Table 1

Values of $L$ at various concentrations of chlorpromazine

| | [Chlorpromazine] (M) ($\times 10^5$) | | | | |
|---|---|---|---|---|---|
| | 0 | 2.25 (0.5CMC) | 3.775 (0.75CMC) | 4.5 (1CMC) | 18.0 (4CMC) |
| $L^a$ ($m^3\ s^{-1}\ N^{-1}$) ($\times 10^9$) | 3.960 ±0.112 | 3.621 ±0.168 | 3.341 ±0.089 | 3.102 ±0.286 | 3.305 ±0.134 |
| $L^b$ ($m^3\ s^{-1}\ N^{-1}$) ($\times 10^9$) | – | 3.632 ±0.148 | 3.468 ±0.166 | – | – |

[a] Experimental values.

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

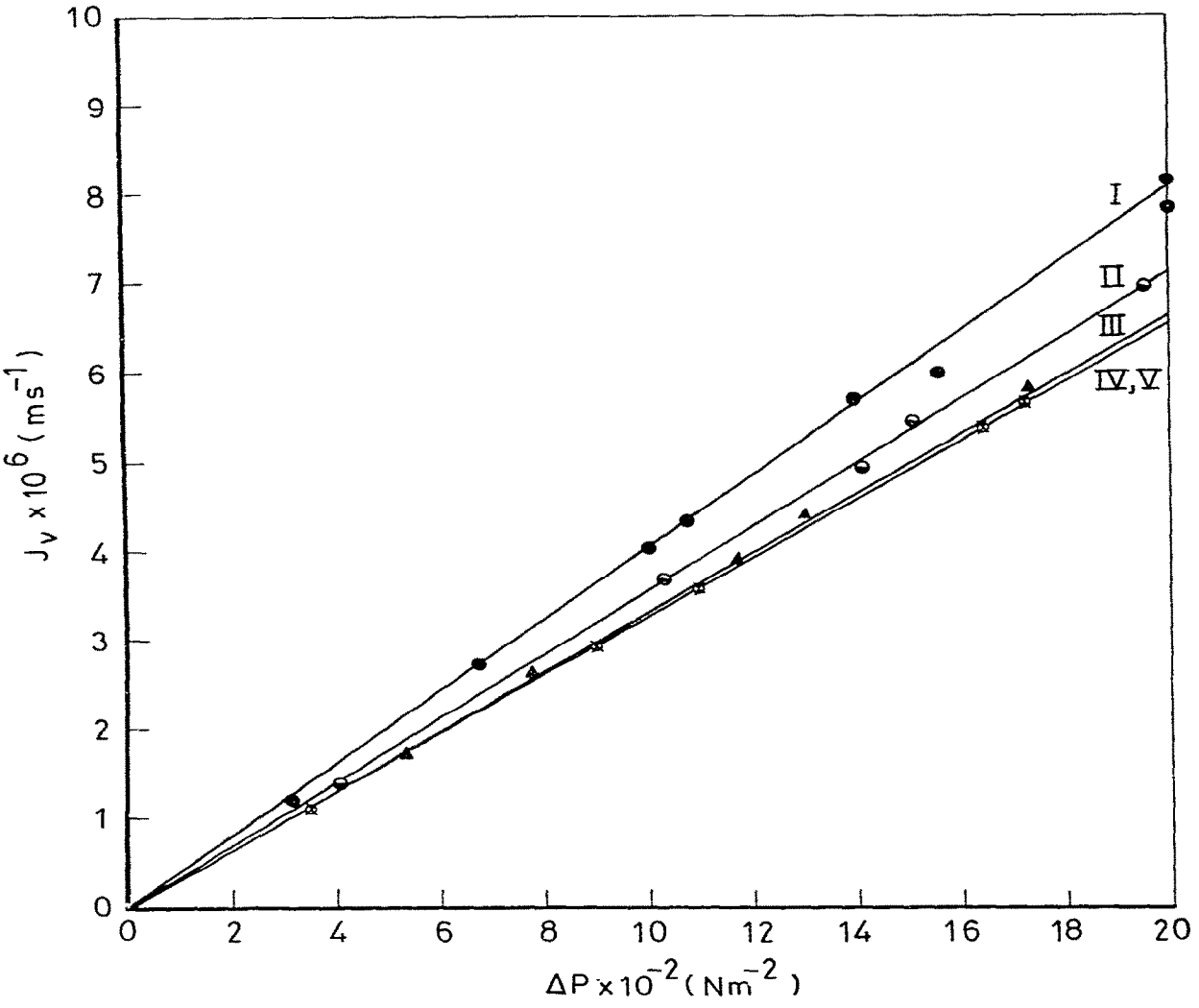



Fig. 1. The hydraulic permeability data. Curves I, II, III, IV and V are for 0, $2.25 \times 10^{-5}$, $3.775 \times 10^{-5}$, $4.5 \times 10^{-5}$ and $1.8 \times 10^{-4}$ M concentrations of chlorpromazine hydrochloride, respectively.

computed for 0.5CMC and 0.75CMC of chlorpromazine are in good agreement with the experimentally determined values (table 1).

The data on solute permeability ($\omega$) recorded in table 2 clearly indicate the ability of the chlorpromazine liquid membrane to reduce the permeability of biogenic amines and amino acids. The data further indicate that the reduction in permeability is maximum when the approaching permeable substances face the hydrophobic surface of the liquid membrane – the second set of experiments. Since chlorpromazine is known to act by

Table 2

Solute permeability ($\omega$) of biogenic amines, cations, glucose and amino acids in the presence of $1.8 \times 10^{-4}$ M chlorpromazine hydrochloride

$\omega_1$, control value – when no chlorpromazine was used; $\omega_2$, chlorpramazine in compartment D of the transport cell and permeable substance in compartment C; $\omega_3$, chlorpromazine in compartment C of the transport cell and permeable substance in compartment C; $\omega_4$, chlorpromazine and γ-aminobutyric acid in compartment D and permeable substance in compartment C.

| | Solute permeability ($\omega$) (mol $s^{-1}$ $N^{-1}$) ($\times 10^{12}$) | | | |
|---|---|---|---|---|
| | $\omega_1$ | $\omega_2$ | $\omega_3$ | $\omega_4$ |
| Dopamine [a] | 1015.0 | 344.9 | 531.5 | 70.98 |
| Noradrenaline [a] | 778.7 | 166.3 | 609.0 | |
| Adrenaline [a] | 2535.0 | 301.5 | 2000.0 | |
| 5-Hydroxytryptamine [a] | 842.8 | 164.1 | 334.2 | |
| Glutamic acid [b] | 426.0 | 325.1 | 366.0 | |
| γ-Aminobutyric acid [c] | 784.7 | 608.3 | 624.1 | |
| Sodium (chloride) [d] | 37.0 | 29.8 | 27.6 | |
| Potassium (chloride) [e] | 62.1 | 36.0 | 51.8 | |
| Glucose [f] | 74.8 | 57.1 | 51.9 | |

[a] Initial concentration 10 μg/ml.
[b] Initial concentration 500 μg/ml (pH 3.2).
[c] Initial concentration 200 μg/ml (pH 7.0).
[d] Initial concentration 5.382 mg/ml.
[e] Initial concentration 10.430 mg/ml.
[f] Initial concentration 20.000 mg/ml.

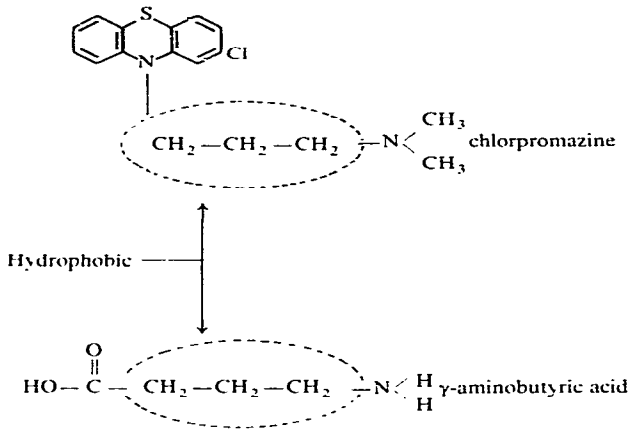
reducing the permeability of biogenic amines [19,20] and amino acids, it appears that the specific orientation of chlorpromazine with the hydrophobic ends of the molecule facing the permeable substances may be necessary even in biological cells. This implies that the receptor should have hydrophilic moieties projected outwards to which the hydrophilic ends of the drug become attached. Such an orientation can be rationalised if one examines the nature of receptors, in general, in relation to the lipid bilayer part of the biomembranes.

The receptors generally are membrane proteins and hence have to be surface active in nature. Thus, they will have both hydrophilic and hydrophobic moieties in their structure. Since the exterior environment of biological cells is aqueous in nature, it is logical to expect that the hydrophobic part of these membrane proteins will be associated with the hydrophobic core of the lipid bilayers and that only the hydrophilic part will face the exterior. Thus, the hydrophilic part of the drugs will interact preferentially with the hydrophilic part of the receptor protein, leaving the hydrophobic part to face the permeable substances. Predictions about similar orientations of receptor proteins in general have been made recently [21].

The effects of chlorpromazine have been noted with membrane-containing units like mitochondria [22], nerve-ending particles [23], platelets [24], adrenomedullary particles [25] and muscle fibers [26]. The influence of phenothiazines on the uptake and release of various neurotransmitters [19,20] seems to be of much significance to its action. In order to investigate the role of accumulation of the drug in biomembranes in the mechanism of its action, studies on the interaction of the drug with synthetic monolayers were undertaken by various authors [27,28]. To what extent the permeability of biogenic amines and amino acids is modified as a result of this interaction has not been reported. The present experiments provide evidence that the liquid membrane generated by chlorpromazine itself offers resistance to the flow of biogenic amines and neurotransmitter amino acids. Although this resistance is passive in nature, it is likely to be accompanied by reduction in their active transport as well. This is because the liquid membrane generated by the drug is likely to reduce access of the permeable substances to the active site located on the biomembrane.

The present experiments also show that the chlorpromazine liquid membrane reduces the permeability of γ-aminobutyric acid and glutamic acid (table 2).The major factor in the antipsychotic action of chlorpromazine is the reduction in permeability to dopamine [29] which is under the control of the glutamic acid-γ-aminobutyric acid system [30]. To determine whether a similar trend is also observed on a nonspecific membrane, the dopamine permeability was measured in the presence of γ-aminobutyric acid. It is interesting to note that addition of γ-aminobutyric acid further reduces

the permeability of dopamine through the chlorpromazine liquid membrane. This can be explained by considering that the hydrophobic core of the chlorpromazine liquid membrane is strengthened by γ-aminobutyric acid. This is evident from the structural similarity of the hydrophobic components of their structures as given below.




In order to assess the specificity of the liquid membrane generated by chlorpromazine to the permeability of neurotransmitters, the permeabilities of ions such as $Na^+$ and $K^+$ and neutral molecules like glucose through the liquid membrane have also been estimated (table 2). The data (table 2) indicate that the resistance offered by the liquid membrane to the transport of cations and glucose is much less in comparison to that offered to catecholamines.

Thus, the increased resistance to the flow of dopamine in the presence of γ-aminobutyric acid, coupled with the resistance to the flow of glutamic acid offered by the liquid membrane generated by chlorpromazine, appears to make a significant contribution to the antipsychotic action of the drug.

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