*International Journal of Pharmaceutics*, 24 (1985) 297–305
Elsevier



IJP 00829

# Liquid membrane phenomenon in antiepileptic drugs

S.B. Bhise, C.V.S. Subrahmanyam, A.K. Malhotra and R.C. Srivastava

*Birla Institute of Technology and Science, Pilani - 333031 (India)*

(Received August 16th, 1984)
(Modified version received December 20th, 1984)
(Accepted January 4th, 1985)

*Key words*: antiepileptic drugs – liquid membrane phenomena – GABA transport – permeability

**Summary**

Antiepileptic drugs have been shown to generate liquid membrane at the interface. Transport of γ-aminobutyric acid (GABA) has been studied in presence of the antiepileptic drugs. It is observed that when hydrophilic moieties of the drug molecules in the liquid membrane face the permeating molecules of GABA, the permeability of the latter is enhanced. This enhancement in the permeability of GABA is discussed in the light of mechanism of action of the drugs. The antiepileptic drugs chosen for the present investigation are diphenylhydantoin, carbamazepine and valproate sodium.

## Introduction

Recent studies (Bhise et al., 1982; 1983a, b and c; Srivastava et al., 1982; 1983a and b; 1984) on a variety of surface-active drugs belonging to different chemical and pharmacological categories, have revealed that modification in the transport of relevant permeants by the liquid membranes likely to be generated by them at the respective sites of action, may be an important step common to the mechanism of action of all surface-active drugs. In the present communication, studies have been extended to antiepileptic drugs to gain information on the role of liquid membrane generated by them in the mechanism of their action.

*Correspondence:* R.C. Srivastava, Birla Institute of Technology and Science, Pilani-333031, India.

Antiepileptic drugs are known to stabilize biological membranes (Bowman and Rand, 1980) after interacting with them. They are known to contain both hydrophilic and hydrophobic moieties in their structure (Isaacson and Delgado, 1980). The antiepileptic drugs therefore, are expected to be surface active in nature and hence capable of generating liquid membrane at the interface in accordance with Kesting's hypothesis (Kesting et al., 1968). Depressant drugs, in general, are reported to populate at the air–solution interface (Buchanan et al., 1969). In the present studies, existence of liquid membranes generated by the antiepileptic drugs, at a cellulosic microfiltration membrane/aqueous interface has been demonstrated. Data on the modification in the transport of γ-aminobutyric acid (GABA) by the liquid membranes have been obtained and discussed in the light of the mechanism of action of the drugs. Three structurally dissimilar antiepileptic drugs, namely diphenylhydantoin, carbamazepine and valproate sodium, have been chosen for the present study. A Sartorius cellulose acetate microfiltration membrane was deliberately chosen as supporting membrane for the liquid membranes so that active interaction of the drugs with constituents of biological membranes as a cause for modification in the permeabilities is eliminated and the role of passive transport through the liquid membrane is highlighted.

## Materials and Methods

Diphenylhydantoin (Sigma, U.S.A.), carbamazepine (S.G. Pharmaceuticals, India), valproate sodium (Reckitt Colman (India)), γ-aminobutyric acid (B.D.H.) and triple-distilled water, distilled in an all pyrex–glass still, were used in the present experiments.

For spectrophotometric estimation of GABA, ninhydrin (Loba Chemie), hydrindantin A.R. (Koch-Ligh Laboratories, U.K.) and ethylene glycol monomethyl ether (E. Merck (India)) were used.

The critical micelle concentrations (CMC) of aqueous drugs were determined from the variation of surface tension with concentration. Aqueous solutions of diphenylhydantoin and carbamazepine of desired concentration were prepared by adding the necessary volumes of stock solution of known strength, prepared in acetone, to the aqueous phase with constant stirring. The amount of acetone in the final aqueous solutions never exceeded 0.025% v/v and it was shown by a control experiment that 0.025% solution of acetone does not lower the surface tension of water to any measurable extent. The surface tensions were measured using a surface tensiomat (Fisher Tensiomat Model 21). The CMC values are recorded in Table 1.

The all-glass two-compartment cell described earlier (Bhise et al., 1982) was used for transport studies. A Sartorius cellulose acetate microfiltration membrane (Cat. No. 11107), of thickness $1 \times 10^{-4}$ m and area $5.373 \times 10^{-5}$ m$^2$ which acted as a supporting membrane for the liquid membrane separated the transport cell into two compartments.

The hydraulic permeability data, at various concentrations of the drugs, which were utilized to demonstrate the existence of a liquid membrane in series with the

TABLE 1

CRITICAL MICELLE CONCENTRATIONS (CMC) OF ANTIEPILEPTIC DRUGS

| Drugs | CMC (M) |
|---|---|
| Diphenylhydantoin | $4.00 \times 10^{-7}$ |
| Carbamazepine | $8.56 \times 10^{-8}$ |
| Valproate sodium | $7.97 \times 10^{-5}$ |

supporting membrane were obtained using the procedure described earlier (Bhise et al., 1982). The concentration ranges chosen were such that hydraulic permeability data were obtained both below and above the CMCs of the drugs.

For the measurement of solute permeability ($\omega$) of GABA, two sets of experiments were performed using the method described earlier (Bhise et al., 1982). In the first set of experiments, a mixture of the aqueous solutions of GABA and one of the antiepileptic drugs under investigation was filled in one of the compartments while the other compartment was filled with distilled water. In the second set of experiments, an aqueous solution of GABA was filled in the first compartment and the second compartment was filled with an aqueous solution of antiepileptic drug. In control experiments, however, no antiepileptic drug was used.

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

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

where $\Delta\pi$ is the osmotic pressure difference, and $J_s$ and $J_v$, respectively, are the solute flux and volume flux per unit area of the membrane. The method of measurement has been described earlier (Bhise et al., 1982; 1983a, b and c; Srivastava et al., 1982; 1983a and b; 1984). For solute permeability measurements, the concentrations of the antiepileptic drugs taken were always higher than their CMCs. This was done to make sure that the supporting membrane was completely covered with the liquid membrane generated by the drugs — according to the liquid membrane hypothesis (Kesting et al., 1968), the interface is completely covered with liquid membrane at surfactant concentrations equal to or higher than their CMCs.

All measurements including the CMC determination were carried out at $37 \pm 0.1$°C.

*Estimations*

The amount of GABA transported to the second cell compartment was estimated by spectrophotometric determination of its reaction product with ninhydrin (Moore and Stein, 1954) at 570 nm. The antiepileptic drugs were found not to interfere with the estimation of GABA. A spectronic 20 Bausch and Lomb spectrophotometer was used for estimations.

## Results and Discussion

The hydraulic permeability data at various concentrations of the drugs in case of all the three drugs were found to be in accordance with the linear relationship:

$$J_v = L \cdot \Delta P \tag{2}$$

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 data for valproate sodium are presented in Fig. 1. The values of L show a progressive decrease with increase in concentration of the drug up to its CMC, beyond which they become more or less constant. Similar trends in the values of L were found in case of the other two drugs also, viz. diphenylhydantoin and carbamazepine. The normalized values of the hydraulic conductivity coefficient — the values of $(L/L^\circ)$ where $L^\circ$ is the value of L when no drug was used — are plotted against drug concentrations in Fig. 2, for all the three drugs.

The progressive decrease in the values of hydraulic conductivity with increasing concentrations of the drugs up to their CMCs (Fig. 2) is indicative of the progressive

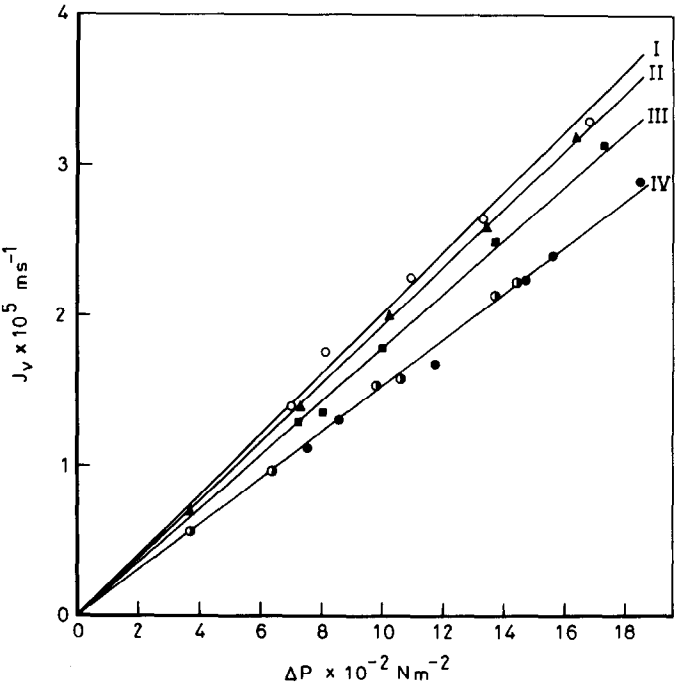


Fig. 1. Hydraulic permeability data at various concentrations of valproate sodium. Curves I, II and III are for 0, $1.992 \times 10^{-5}$ M and $3.984 \times 10^{-5}$ M concentration of the drug, respectively. Curve IV represents data for concentrations equal to and higher than the CMC of the drug, viz. $7.968 \times 10^{-5}$ M and $15.936 \times 10^{-5}$ M.

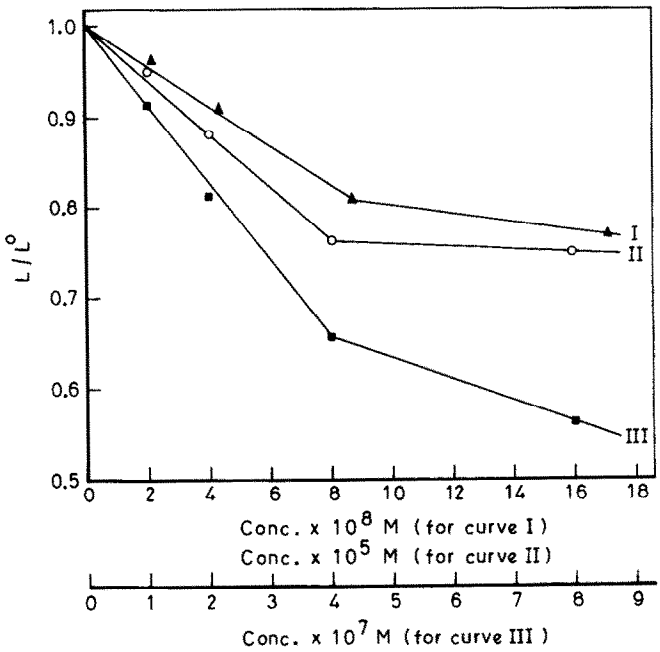


Fig. 2. Variation of (L/L°) with concentration of the drugs. Curves I, II and III represent data in presence of carbamazepine, valproate sodium and diphenylhydantoin, respectively.

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 liquid membrane is complete. The slight decrease in the values of (L/L°) beyond the CMCs particularly in the case of diphenylhydantoin and carbamazepine may be due to densing of the liquid membrane which is completely developed at the CMC of the drugs, as postulated by Kesting in the liquid membrane hypothesis (Kesting et al., 1968).

TABLE 2

VALUES OF L AT VARIOUS CONCENTRATIONS OF VALPROATE SODIUM

| | Valproate sodium concentration $\times 10^5$ M | | | | |
|---|---|---|---|---|---|
| | 0 | 1.992 (0.25 CMC) | 3.984 (0.5 CMC) | 7.968 | 15.936 |
| $L^a \times 10^8$ $(m^3 \cdot s^{-1} \cdot N^{-1})$ | 2.0299 ± 0.0605 | 1.9312 ± 0.0237 | 1.7878 ± 0.0525 | 1.5494 ± 0.0374 | 1.5189 ± 0.0743 |
| $L^b \times 10^8$ $(m^3 \cdot s^{-1} \cdot N^{-1})$ | – | 1.9098 ± 0.0548 | 1.7897 ± 0.0500 | | |

[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) furnishes additional evidence in favour of the existence of the liquid membrane in series with the supporting membrane. Following the arguments given earlier (Bhise et al., 1982; 1983a, b and c; Srivastava et al., 1982; 1983a and b; 1984), it can be shown that, if the concentration of the surfactant is n times its CMC, $n \leqslant 1$, the values of L would be equal to $[(1 - n)L^{\circ} + nL^{c}]$ where $L^{c}$ is the value of L at the CMC of the surfactant. The values of L thus computed at 0.25 CMC and 0.5 CMC in case of sodium valproate are in agreement with the experimentally determined values (Table 2). Similar agreement was found in case of other two drugs also, viz. diphenylhydantoin and carbamazepine.

*Role of liquid membranes in antiepileptic action*

The values of solute permeability ($\omega$) of GABA in presence of the antiepileptic drugs, in the two sets of experiments, are recorded in Table 3. These drugs which were found to be surface active in nature have both hydrophilic and hydrophobic moieties in their structure (Isaacson and Delgado, 1980). In the first set of experiments, therefore, the permeant molecules, molecules of GABA, will face the hydrophilic surface of the liquid membrane generated by the drug, because the hydrophobic ends of the drug molecules will be preferentially oriented towards the hydrophobic supporting membrane – the cellulose acetate microfiltration membrane. In the second set of experiments, however, the permeant present in the first cell compartment will face the hydrophobic surface of the liquid membrane generated by the drug present in the second compartment. The gross picture of the orientation of the drug liquid membrane vis-a-vis permeant in the two sets of experiments for the measurement of solute permeability ($\omega$) is depicted in Fig. 3.

A perusal of Table 3 reveals that in the first set of experiments, where the permeant, GABA, faces the hydrophilic surface of the drug liquid membrane, the permeability of GABA is enhanced considerably in case of all the three drugs. In the

TABLE 3

PERMEABILITY OF GABA [a] ($\omega$) * IN PRESENCE OF ANTIEPILEPTIC DRUGS [b]

| Drugs | $\omega_1 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$) | $\omega_2 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$) | $\omega_3 \times 10^{10}$ ($mol \cdot s^{-1} \cdot N^{-1}$) |
|---|---|---|---|
| Diphenylhydantoin | $1.1682 \pm 0.1532$ | $6.8487 \pm 0.6815$ | $1.1501 \pm 0.1078$ |
| Carbamazepine | $1.1682 \pm 0.1532$ | $4.8175 \pm 0.4424$ | $1.0680 \pm 0.1545$ |
| Valproate sodium | $1.1682 \pm 0.1532$ | $2.0039 \pm 0.3782$ | $1.6624 \pm 0.1534$ |

$\omega_1$ = control value — when no drug was used. $\omega_2$ = drug and GABA in first compartment and water in second compartment. $\omega_3$ = GABA in first compartment and drug in second compartment.

[a] Initial concentration of GABA is 200 $\mu$g/ml.

[b] The concentrations of diphenylhydantoin, carbamazepine and valproate sodium are $8 \times 10^{-7}$ M, $1.6 \times 10^{-7}$ M and $1.6 \times 10^{-4}$ M, respectively.

* Values of $\omega$ are reported as arithmetic mean of 10 repeats $\pm$ standard deviation.

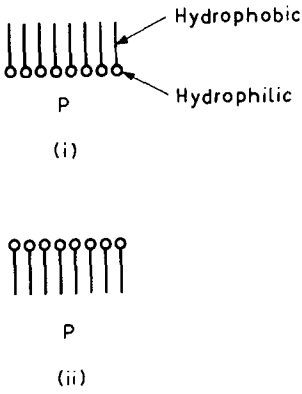


Fig. 3. The gross picture of the orientation of the drug liquid membrane on the supporting membrane vis-a-vis permeant (P) in the two sets of experiments for solute permeability measurement: (i) drug and permeant in the same compartment; (ii) drug and permeant in different compartments.

second set of experiments, however, there is a distinct reduction in the permeability of GABA, except in case of sodium valproate, where a marginal increase in the permeability is observed (Table 3). Even in the case of sodium valproate, the increase in the permeability of GABA is much more in the first set of experiments than in the second set. The present observations on the increase in the permeability of GABA appear relevant to the antiepileptic action.

The antiepileptic drugs which, when administered, exert stabilizing effect (Bowman and Rand, 1980) on excitable cell membranes, are known to increase the concentration of GABA in brain. The present experiments appear to indicate that increased permeability of GABA in presence of the drug liquid membranes which are likely to be formed at the site of action, may be responsible for the increased concentration of GABA in brain. Since enhancement in the permeability of GABA was observed to be maximum in the first set of experiments, it appears that the specific orientation of the drug molecules in the liquid membrane, with their hydrophilic ends facing the permeant may be necessary even at the actual site of action. To substantiate this conjecture, detailed investigations of the nature of the site of action are called for.

Another indication of the possible role of the liquid membrane phenomenon in antiepileptic action is obtained from the gradation in values of the CMCs of the drugs (Table 1) vis-a-vis the gradation in the concentrations of these drugs in plasma. The CMC values of the three drugs are in the following order (Table 1):

valproate > diphenylhydantoin > carbamazepine

which also is the gradation in their concentrations in plasma (Rall and Schleifer, 1980). Concentrations of the drugs in plasma can be taken to be a measure of their concentrations at the site of action. The reported concentrations of these drugs in plasma (Rall and Schleifer, 1980) are far higher than their respective CMCs. Hence, complete liquid membranes can be generated by the drugs at the site of action. Since modification in the permeability of GABA due to the presence of the drug liquid

membranes is responsible for the antiepileptic action, the concentrations of the drugs required to produce maximum biological response may be related to their CMCs. CMC is the concentration at which the interface is completely covered by the liquid membrane and therefore, modification in permeability of biomembrane to GABA will be maximum at this concentration. Hence agreement between the gradation in the concentration of the drugs in plasma and the gradation in their CMCs is also indicative of the contribution of the liquid membrane generated by these drugs, to their antiepileptic action.

Thus the present study indicates that the formation of liquid membrane at the site of action, by the drugs, modifying the transport of GABA, may be an important step common to the mechanism of action of all the three drugs, namely diphenylhydantoin, carbamazepine and valproate sodium.

## Acknowledgements

Thanks are due to the Council of Scientific and Industrial Research, New Delhi, for financial assistance and to Professor S.S. Mathur for his active interest in this work. We also record our gratitude to Messrs. S.G. Pharmaceuticals and Messrs. Reckitt and Colman for gifts of carbamazepine and valproate sodium, respectively.

## References

Bhise, S.B., Marwadi, P.R., Mathur, S.S. and Srivastava, R.C., Liquid membrane phenomena in haloperidol action. J. Pharm. Sci. 71 (1982) 526–529.

Bhise, S.B., Marwadi, P.R., Mathur, S.S. and Srivastava, R.C., Liquid membrane phenomenon in reserpine action. J. Pharm. Sci. 72 (1983a) 599–601.

Bhise, S.B., Marwadi, P.R., Mathur, S.S. and Srivastava, R.C., Liquid membrane phenomena in chlorpromazine action. Biophys. Chem., 17 (1983b) 187–192.

Bhise, S.B., Subrahmanyam, C.V.S. and Srivastava, R.C., Liquid membrane phenomenon in antihistamines. Int. J. Pharm., 17 (1983c) 263–272.

Bowman, W.C., and Rand, M.J., Text Book of Pharmacology, Blackwell Scientific, Oxford, 1980, pp. 18·30.

Buchanan, A.S., Glenda, M.L. and Shulman, A., Action of central nervous system stimulant and depressant drugs in the intact animal IV. Surface activity of drugs with central stimulant, depressant or dual stimulant-depressant action. Eur. J. Pharmacol., 7 (1969) 60–65.

Harris, F.L., Humphreys, G.B. and Spiegler, K.S., In Meares, P. (Ed.), Membrane Separation Processes, Elsevier, Amsterdam, 1976, p. 126.

Isaacson, E.I. and Delgado, J.N., In Burger's Medicinal Chemistry, Part III, Wolff, M.E. (Ed.), John Wiley and Sons, New York, 1980, p. 854.

Katchalsky, A. and Kedem, O., Thermodynamics of flow processes in biological systems. Biophys. J., 2 (1962) 53–78.

Katchalsky, A. and Curran, P.F., Non-Equilibrium Thermodynamics in Biophysics, Harvard University Press, Cambridge, MA, 1967, pp. 113–116.

Kesting, R.E., Subcasky, W.J. and Paton, J.D., Liquid membrane at the cellulose acetate membrane/saline solution interface in reverse osmosis. J. Colloid Interface Sci., 28 (1968) 156–160.

Moore, S. and Stein, W.H., A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 211 (1954) 907–913.

Rall, T.W. and Schleifer, L.S., In The Pharmacological Basis of Therapeutics, Gilman, A.G., Goodman, L.S. and Gilman, A. (Eds.), MacMillan Press, New York, 1980, pp. 455, 460, 463.

Sherwood, T.K., Brian, P.L.T. and Fischer, R.E., Desalination by reverse osmosis. Ind. Eng. Chem. Fund., 6 (1967) 2–10.

Spiegler, K.S. and Kedem, O., Thermodynamics of hyperfiltration, criteria for efficient membranes. Desalination, 1 (1966) 311–326.

Srivastava, R.C., Jakhar, R.P.S. and Bhise, S.B., Liquid membrane phenomena in imipramine action. J. Colloid Interface Sci., 87 (1982) 56–61.

Srivastava, R.C., Sharma, R.K. and Bhise, S.B., Liquid membrane phenomena in diazepam action. J. Colloid Interface Sci., 93 (1983a) 72–77.

Srivastava, R.C., Sharma, R.K., Srinivasan, R. and Bhise, S.B., Liquid membrane phenomena in local anesthetics. J. Colloid Interface Sci., 94 (1983b) 456–462.

Srivastava, R.C., Bhise, S.B. and Mathur, S.S., Liquid membrane phenomena and drug action. Adv. Colloid Interface Sci., 20 (1984) 131–161.