J. Pharm. Pharmac., 1975, **27**, 806–810 *Received March* 14, 1975

The interaction of antihistamines with lecithin monolayers

D. ATTWOOD AND O. K. UDEALA

Pharmacy Department, University of Manchester, Manchester M13 9PL, U.K.

The interaction of a series of antihistamines with monolayers of L-α-dipalmitoyl lecithin has been examined. An increase in the monolayer surface pressure was noted for monolayers spread on the antihistamine solutions, suggesting penetration of the film by drug molecules. At high surface pressures there was an apparent ejection of drug molecules from the film. The ability of the antihistamines to increase surface pressure was correlated with their surface activity at the air-solution interface. The effect of drug concentration on the magnitude of the surface pressure was examined for diphenhydramine hydrochloride. Application of the Gibbs adsorption equation at low surface compressions indicated an approximate area per molecule for diphenhydramine in the film which was in good agreement with the value previously obtained at the air-solution interface. Preliminary measurements showed that the surface pressure increase was larger in the presence of phosphate buffer at pH 6·8. It was not clear whether this effect was caused by the buffer components or was a pH effect.

The interaction of insoluble monolayers with soluble surface-active drugs has been reported by many workers and has been extensively reviewed (Florence, 1968; Felmeister, 1972). In a series of papers, Skou (1954a, b, c,) and later Hersh (1967) established that local anaesthetics increased the surface pressure of stearic acid and lipid films and correlated the penetrating ability with the minimum concentration required to block nerve excitability. A number of authors have studied the penetration of biological membranes by phenothiazines and other psychoactive drugs. Zografi, Auslander & Lytell (1964) and Zografi & Auslander (1965) reported the effect of pH, ionic strength and buffer components on the properties of mixed phenothiazine-phospholipid films. Van Deenen & Demel (1965) studied the interaction of some psychoactive drugs with cholesterol, synthetic phosphoglycerides, sphingomyelin, and preparations of cerebrosides and gangliosides from beef brain. The above workers have interpreted their results in terms of the penetration of the drug molecules into the insoluble monolayer. An alternative explanation has been proposed by Sears & Brandes (1969) who concluded that the phenothiazines acted immediately below the lipid monolayer.

With the exception of the phenothiazine derivatives, many of which have antihistamine properties, little attention has been given to the interaction of the antihistamines with lipid films. Bangham, Rees & Shotlander (1962) studied a wide variety of compounds known to prevent liver necrosis in rats, including the antihistamine diphenhydramine hydrochloride. The protective activity of these compounds was correlated with their ability to interact with lipid films. In a previous paper, (Attwood & Udeala, 1975), we have reported the surface activity of a series of antihistamines at the air-solution interface. The surface activitites were correlated with the nature of the hydrophilic and hydrophobic regions of the molecules. We now report an investigation of the

interaction of these drugs with monolayers of L-α-dipalmitoyl lecithin which provides a suitable approximation to the properties of cell mebranes.

MATERIALS AND METHODS

Materials. The following antihistamines were used: tripelennamine hydrochloride (Ciba), thenyldiamine hydrochloride (Winthrop) pheniramine maleate (Hoechst), chlorcyclizine and cyclizine hydrochloride (Burroughs Wellcome), diphenhydramine and bromodiphenhydramine hydrochloride (Parke-Davis), dimenhydrinate and mepyramine maleate (May and Baker). The pure synthetic lecithin was L-α-dipalmitoyl-phosphatidylcholine (Fluka A. G. Switzerland) and water was distilled twice from alkaline permanganate in an all-glass still. Buffer components and organic solvents were reagent grade.

Surface pressure measurements. Surface pressures were measured by the Wilhelmy plate method using a roughened platinum plate suspended from a microtorsion balance. The edges of the Langmuir trough and the barriers were Teflon coated to prevent wetting. The trough had a capacity of approximately 900 ml. Measurements were at $298 \pm 0{\cdot}5$ K. Before spreading a monolayer, the water surface was repeatedly swept until no change in surface tension was detectable on compression of the surface.

The importance of selecting a suitable spreading solvent for dipalmitoyl lecithin has been recently stressed (Munden & Swarbrick, 1973; Cadenhead & Kellner, 1974). It has been concluded that a hexane–ethanol mixture produces the least solvent effect on the lecithin film. This solvent mixture was used in the present investigation in the ratio 3:1 v/v hexane–ethanol. Solvents were checked for the presence of surface active impurities by compressing the water surface after solvent addition. No surface pressure could be detected with either of the solvents.

Lipid solutions were added to the surface of the water or drug solution using an Agla syringe. 30 min was allowed before compression for the attainment of equilibrium conditions. Films were compressed slowly and, to ensure equilibrium after each change of area, a period of 3–5 min was allowed before measurement. The area per molecule was reduced until monolayer collapse or film leakage.

RESULTS AND DISCUSSION

Data are presented as plots of the surface pressure, π, (surface tension of pure water—surface tension of monolayer covered surface) against area per molecule of lecithin (Figs 1, 2). The curves for dipalmitoyl lecithin on water and buffer solution are similar to recently reported curves (Phillips & Chapman, 1968; Cadenhead & Kellner, 1974). They show a clearly defined phase change from liquid condensed to liquid expanded film which is generally recognized as an indication of purity.

The increase in π of monolayers spread on drug solutions suggests the penetration of the monolayer by antihistamine molecules. In general, such penetration does not alter the behaviour of the film on compression since the mixed films still show the phase transition noted for lecithin alone. With the exception of dimenhydrinate, a displacement of the π—a curves was not detectable at high compression, although there may be a small effect which is obscured by the scatter in the experimental results. Similar behaviour has been shown, for example, by the local anaesthetics (Skou 1954a, b, c), the phenothiazines (Zografi & Auslander, 1965) and progesterone

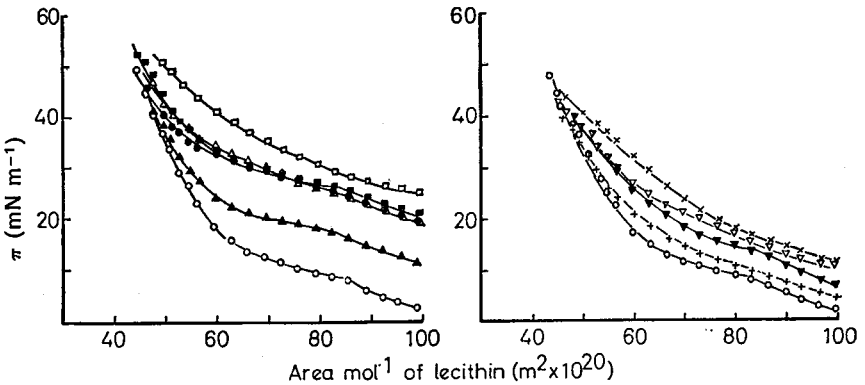

FIG. 1. Plot of surface pressure, π, against area per molecule of dipalmitoyl lecithin for lecithin films on -○- H_2O, and 5×10^{-3} mol kg^{-1} solutions of -□- dimenhydrinate; -■- chlorcyclizine HCl; -●- bromodiphenhydramine HCl; -△- cyclizine HCl; -▲- diphenhydramine HCl; -×- tripelennamine HCl; -▽- mepyramine maleate; -▼- thenyldiamine HCl; -+- pheniramine maleate.

(Taylor & Haydon, 1965), when interacting with lipid films. It is generally interpreted as implying ejection of the drug molecules from the film. It is possible, however, that the surface area of the monolayer occupied by the small hydrophobic groups of the antihistamine molecules at high compression may not be sufficiently large to produce a measureable change in π.

An increase in surface pressure was noted (Fig. 2A) when diphenhydramine and tripelennamine solutions were buffered at pH 6·8 using a Sorensen's phosphate buffer. Zografi & Zarenda (1966) and Patel & Zografi (1966) have shown that the surface pressure of phenothiazines at the air-solution interface was influenced by the pH of the solution and by the components of buffer solutions. Thus, an appreciable increase in π was noted for chlorpromazine (pKa 9·3) at pH 7·1 compared to that at pH's below 5·0. This was attributed to the presence of the nonprotonated form of the drug. A similar pKa—pH difference occurs in the diphenhydramine (pKa 8·98) and tripelennamine (pKa 8·95) solutions studied here, the drugs being approximately 99% ionized. Similarly it was shown that addition of buffers, including a phosphate buffer, resulted in an increased surface pressure of chlorpromazine due to the effect of buffer components.

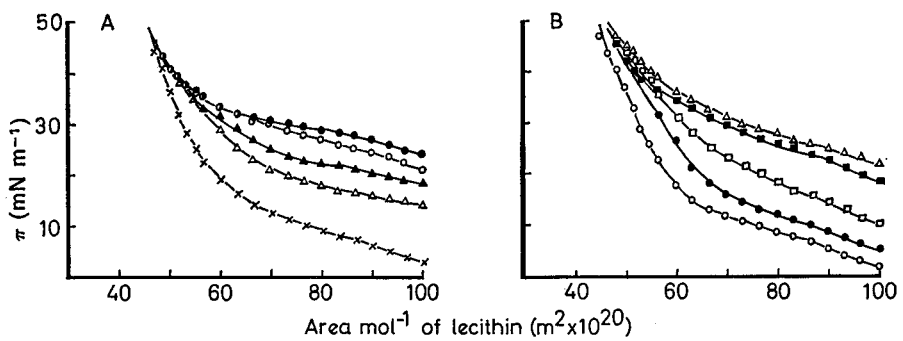

FIG. 2A. Effect of phosphate buffer (pH 6·8) on the interaction of antihistamines with dipalmitoyl lecithin films. Key: Lecithin spread on (-×-) buffer solutions and on 1×10^{-2} mol kg^{-1} solutions of diphenhydramine HCl in -○-, H_2O and -●- buffer; and tripelennamine HCl in -△-, H_2O and -▲-, buffer.

B. Effect of antihistamine concentration of the surface pressure, π, of dipalmitoyl lecithin films spread on aqueous solutions of diphenhydramine HCl. Concentration of diphenhydramine (mol $kg^{-1} \times 10^3$) -○-, 0; -●-, 0·25; -□-, 1·0; -■-, 5·0; -△-, 10·0.

In this respect it is of interest that the surface activity of the antihistamines was previously shown to be significantly increased by organic counterions. It is not clear from these preliminary measurements which of these two effects is primarily responsible for the increased π values of the antihistamines.

Fig. 2B shows the effect of diphenhydramine concentration on the magnitude of the surface pressure. π *vs* concentration graphs of Fig 3 are similar in shape to those reported by Skou (1954c) for the penetration of lipid films by local anaesthetics. Skou (1954c) has shown that the Gibbs adsorption isotherm, equation 1, may be used to estimate the area per molecule of a soluble surface active drug at a film covered surface. It is assumed that the change of free energy of the insoluble monolayer is negligible and that the usual substitution of concentration for activity is valid.

$$\Gamma = -\frac{1}{x2{\cdot}303\ RT}\left(\frac{d\pi}{d\log c}\right) \qquad .. \quad .. \quad .. \quad (1)$$

Γ is the apparent surface excess of antihistamine, and c is the concentration of antihistamine in the subsolution; x is assumed to be unity (See Attwood & Udeala, 1975).

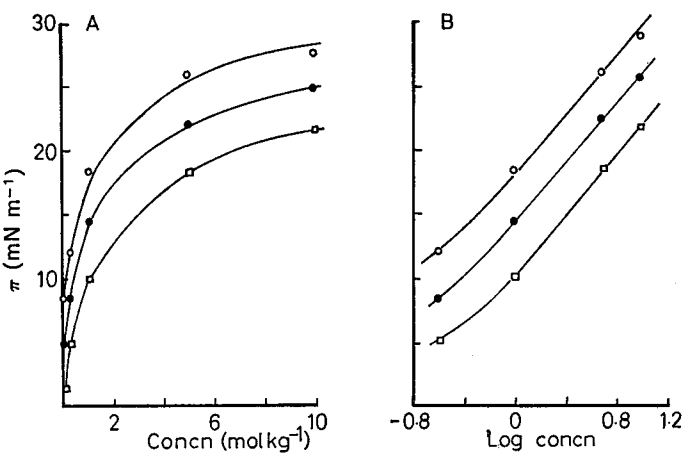

FIG. 3. Surface pressure, π, against (A) concentration and (B) log concentration for diphenhydramine HCl - dipalmitoyl lecithin films at lecithin areas per molecule of -○-, 80×10^{-20} m²; -●-, 90×10^{-20} m² and -□-, 100×10^{-20} m².

Fig. 3B shows that, apart from an initial curvature at low concentrations, graphs of π *vs* log c at low film compression were linear and parallel. The mean area per molecule calculated over this compression range from equation 1 was 83×10^{-20} m² molecule^{-1}, in excellent agreement with the value of 84×10^{-20} m² molecule^{-1} previously calculated at the air-solution interface (Attwood & Udeala, 1975).

A comparison of the relative abilities of the antihistamines to increase the surface pressure of the lecithin film is given Table 1. The results are expressed as $\Delta\pi$ the difference in surface pressure between the lecithin film at an area per molecule of 100×10^{-20} m² molecule^{-1} on water and on 5×10^{-3} mol kg^{-1} drug solutions. Included in the Table are values of the concentrations of the antihistamines required to produce a surface tension lowering of 10 mN m^{-1} at the air-solution interface, as determined in a previous investigation (Attwood & Udeala, 1975). In general, there is

Table 1. *Comparison of effect of antihistamines on surface pressure of lecithin monolayers and their surface activity at air-solution interface.*

	$\triangle\pi$ mN m^{-1}	Concn required for surface tension decrease of 10mN m^{-1} mol $kg^{-1} \times 10^3$
Dimenhydrinate	22·76	1·3
Chlorcyclizine HCl	18·14	1·0
Bromodiphenhydramine HCl	16·83	2·3
Diphenhydramine HCl	16·47	3·0
Cyclizine HCl	9·04	0·14
Tripelennamine HCl	8·52	10
Mepyramine maleate	8·41	—
Thenyldiamine HCl	5·40	20
Pheniramine maleate	2·81	35

a good correlation between the surface activity at the film covered and film free surface. The relative surface activities of the drugs were previously correlated with the nature of their hydrophilic and hydrophobic groups. The surface activity of mepyramine maleate was not previously determined. This compound is similar in structure to tripelennamine, differing only by the possession of a $-OCH_3$ group on the phenyl ring, and would be expected to possess a similar surface activity. Two anomalies in the table are dimenhydrinate, which has a very much more pronounced effect on π than might be expected from its relative surface activity at the air-solution interface, and cyclizine, of which the reverse is true. Dimenhydrinate is the 8-chlorotheophyllinate salt of diphenhydramine and it is possible that the considerable disrupting effect which this compound has on the film is a consequence of the large counterion associated with it. It is not clear why cyclizine shows such apparently anomalous behaviour.

Acknowledgement

O. K. Udeala wishes to acknowledge financial support from a British Technical Aid award to the University of Nigeria.

REFERENCES

ATTWOOD, D. & UDEALA, O. K. (1975). *J. Pharm. Pharmac.*, **27**, 754–758.
BANGHAM, A. D., REES, K. R. & SHOTLANDER, V. (1962). *Nature*, **193**, 754–756.
CADENHEAD, D. A. & KELLNER, B. M. J. (1974). *J. Colloid Interface Sci.*, **49**, 143–145.
FELMEISTER, G. (1972). *J. pharm. Sci.*, **61**, 151–164.
FLORENCE, A. T. (1968). *Adv. in Colloid Interface Sci.*, **2**, 115–149.
HERSH, L. (1967). *Mol. Pharmac.*, **3**, 581–585.
MUNDEN, J. W. & SWARBRICK, J. (1973). *J. Colloid Interface Sci.*, **42**, 657–659.
PATEL, R. M. & ZOGRAFI, G. (1966). *J. pharm. Sci.*, **55**, 1345–1349.
PHILLIPS, M. C. & CHAPMAN, D. (1968). *Biochim. biophys. Acta*, **74**, 678–687.
SEARS, D. F. & BRANDES, K. K. (1969). *Agents Actions*, **1**, 28 through FELMEISTER, (1972).
SKOU, J. C. (1954a). *Acta pharmac. tox.*, **10**, 305–316.
SKOU, J. C. (1954b). *Ibid.*, **10**, 317–324.
SKOU, J. C. (1954c). *Ibid.*, **10**, 325–337.
TAYLOR, J. L. & HAYDON, D. A. (1965). *Biochim. biophys. Acta*, **94**, 488–493.
VAN DEENEN, L. L. M. & DEMEL, R. A. (1965). *Ibid.*, **94**, 314–316.
ZOGRAFI, G., AUSLANDER, D. E. & LYTELL, P. L. (1964). *J. pharm. Sci.*, **53**, 573–576.
ZOGRAFI, G. & AUSLANDER, D. E. (1965). *Ibid.*, **54**, 1313–1318.
ZOGRAFI, G. & ZARENDA, I. (1966). *Biochem. Pharmac.*, **15**, 591–598.