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Lipid monolayer interaction behavior of sympathomimetic amines

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Summary

The interactions of phenmetrazine hydrochloride, levarterenol bitartrate, D-amphetamine sulfate and fenfluramine hydrochloride with a spread monolayer of lecithin, cephalin and cholesterol have been measured by surface pressure and surface potential. The surface pressure data indicate that penetration is a function of the relative hydrophobicities of the compounds. However, the effect of drug concentration in the subphase and the surface potential data demonstrate that polar head group interactions play an important role in determining the structure of the membrane. The expansion of the monolayer at constant pressure follows in the same order as the hydrophobic properties of the drugs but only fenfluramine-contributed expansion was sensitive to the lateral surface pressure of the monolayer. Use of the multicomponent lipid monolayer for interaction studies provides a highly structured model of a membrane which may be warranted in some instances.

Introduction

In a recent review on the structure–activity relationships of phenylethylamine hallucinogens, it was pointed out that very little is known about their molecular mechanism of action although a vast number of studies have been reported on their empirical structure–activity relationships (Nichols, 1981). When it is difficult to obtain quantitative data on drug–receptor interactions from biological experiments, in vitro studies on model systems often shed light on important physicochemical factors which control the intensities of drug action in a comparison of particular sets

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of congeners. A widely employed model membrane system for such a purpose is the spread phospholipid monolayer (Goddard, 1975). With regard to the activity of phenylethylamine derivatives at interfaces, it is surprising to find that only a few studies of the surface activity of a few compounds have been reported (Conine, 1965; Malspeis et al., 1965) but none involving penetration of monolayers by subphase concentrations of phenylethylamines.

Acknowledging the multiplicity of actions which the many phenylethylamines exert on the biological system, it was nevertheless considered worthwhile to determine if any significant changes could be observed in the physical properties of a generalized model membrane by a select group of these agents. Thus, the objectives of this study were: (1) to determine whether 4 sympathomimetic amines employed at subphase concentrations approaching those found in biological fluids demonstrate any significant differences in their interaction with a spread monolayer consisting of components commonly found in biological membranes (Herzog and Swarbrick, 1970); and (2) to measure the effect of these drugs on a condensed model membrane, if any, since a biological membrane generally exists in a state which is more condensed than that represented by a monolayer of a single phospholipid. The properties of the monolayer were monitored by surface pressure and surface potential measurements using a conventional surface balance following injections of various concentrations of the sympathomimetic amines beneath the monolayer.

Experimental

Materials

Cholesterol (ICN Pharmaceuticals) was recrystallized twice from acetone (m.p. 149.2°C). β-γ-dipalmitoyl-L-α-lecithin (99+% pure), phosphatidyl-L-serine (bovine brain, 92% pure) and phosphatidylethanolamine (ICN Pharmaceuticals) were used as received. The sympathomimetic amines investigated included D-amphetamine sulfate (Smith, Kline & French), phenmetrazine hydrochloride (Ciba), levarterenol bitartrate (Winthrop) and fenfluramine hydrochloride (Robins). Chloroform (BDH, reagent grade) and ethanol (95%) were double-distilled prior to use. Aqueous buffer solutions were prepared at pH 7.4 using distilled deionized water and reagent grade monobasic potassium phosphate, dibasic sodium phosphate and sodium acetate ($\mu = 0.26$). All glassware was pretreated with chromic acid soaking solution, thoroughly rinsed with water and dried before use.

Methods

Preparation of monolayers

The aqueous buffer solution was placed as subphase in a cleaned surface trough (65 × 14 × 1.5 cm, Central Scientific) the edges of which had been made hydrophobic with purified paraffin. Surface tensions were measured by the Wilhelmy plate method employing a roughened platinum blade (1 × 2.5 cm) suspended from a torsion balance (Rosano Surface Tensiometer). The aqueous surface was swept clean

prior to measurements with 3 paraffin-coated brass bars then the working area of the trough was marked off by a stationary and a moveable Teflon rod to provide a surface area of 350 cm^2. The trough was enclosed in an inner rectangular stainless steel box and an outer stainless steel water-bath. The actual temperature of the aqueous subphase was monitored with a submerged thermometer and was maintained at 21 ± 1°C. A clear, plastic dust cover (Perspex) in which an aperture had been cut was placed on top of the complete assembly.

A stock solution of lecithin, cephalin (85% phosphatidyl-L-serine and 15% phosphatidylethanolamine, Tobias et al., 1962) and cholesterol (1 : 1 : 2 mole ratio) was prepared in ethanol–chloroform (1 : 10) and a suitable volume was delivered to the surface of the aqueous subphase using an Agla micrometer syringe (B & W) to provide a mean initial area per molecule of 60×10^{-2} nm^2. A period of 30 min following spreading was allowed to ensure that the spreading solvent had evaporated and to permit equilibration of the monolayer with the aqueous surface. Surface potential measurements were made employing an air-ionizing electrode of ^{241}Am (French Atomic Energy Commission, Centre D'etudes Nucleaires de Saclay, France) connected to a high impedance electrometer (Keithley Instruments, Model 610C). The air-ionizing electrode was reproducibly positioned 2 mm above the liquid surface and a platinum reference electrode (Fisher Scientific) was immersed in the aqueous subphase outside of the film-covered area. The surface tensions and surface potentials of the clean aqueous surface and film-covered surface were measured at the beginning of each run. Equilibration of the monolayers was verified from the observation that for replicate determination the measured surface tension remained constant.

Drug–membrane interaction

Following establishment of an equilibrated monolayer, surface pressure, π, and change in surface potential, ΔV, were recorded according to:

$$\pi = \gamma_0 - \gamma \tag{1}$$

and

$$\Delta V = V_M - V_0 \tag{2}$$

where γ and γ_0 are the surface tensions and V_M and V_0 are the surface potentials of the film-covered and clean surfaces, respectively. The films were then compressed by driving the moveable barrier 1 cm along the trough at a rate of 2.9 cm · min^{-1}, equilibrating for 10 min at each stage, then π and ΔV were measured and recorded. The rate of compression can affect the stability of the monolayer. The compression rate used here has also been found to be satisfactory by others (e.g. Sears and Brandes, 1969; Cleary and Zatz, 1973). This procedure was repeated until the film collapsed or the minimum area of the surface was achieved. Surface tension measurements of the subphase outside of the film-covered area after compression confirmed that film material had not leaked past the two Teflon barriers.

Stock solutions of the sympathomimetic amines were prepared in aqueous buffer. After equilibration of the film at maximum area a volume of either 0.4 ml, 0.8 ml or 1.6 ml of stock solution were injected deep into the aqueous subphase outside of the film-covered area but towards the film area. Fifteen minutes were determined to be sufficient to allow for re-equilibration, measurements of π and ΔV were made, then compression was commenced as before. A change in surface area after injection was avoided by prior removal of an equivalent volume of subphase. The mean area per molecule at the surface was computed by dividing the surface area by the total number of molecules on the surface. This treatment ignores the relative differences in molecular sizes in the mixed film and, therefore, the true surface areas occupied by each kind of molecule. Thus, the individual changes in molecular area which occur upon compression of the monolayer are averaged and the net change in area occupied by the molecules on the surface is observed (Sears and Brandes, 1969).

Results

The surface characteristics of the spread monolayer on phosphate buffer are seen in curve 1 of Figs. 1 and 2. The shape of the π-A curve is similar to previously

Fig. 1.

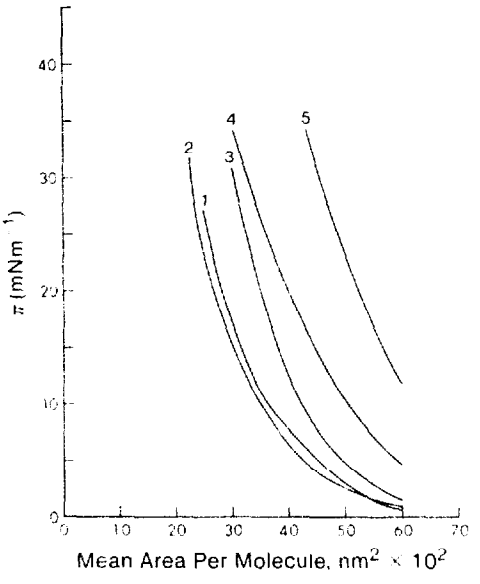

Fig. 2.

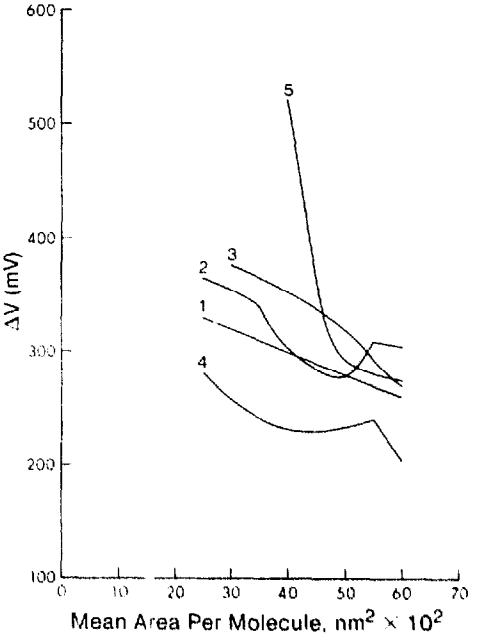

Fig. 1. Surface pressure-area curves of a spread monolayer of lecithin : cephalin : cholesterol (1 : 1 : 2 mole ratio) on an aqueous phosphate-buffered (pH 7.4) subphase into which equimolar concentrations (0.18 ± 0.04 mM) of drug have been injected. Curves: 1 = no drug; 2 = phenmetrazine HCl; 3 = levarterenol bitartrate; 4 = D-amphetamine sulfate; 5 = fenfluramine HCl.

Fig. 2. Surface potential-area curves of a spread monolayer of lecithin : cephalin : cholesterol (1 : 1 : 2 mole ratio) on an aqueous phosphate-buffered (pH 7.4) subphase into which equimolar concentrations (0.18 ± 0.04 mM) of drug have been injected. Curves: 1 = no drug; 2 = phenmetrazine HCl; 3 = levarterenol bitartrate; 4 = D-amphetamine sulfate; 5 = fenfluramine HCl.

reported curves for cholesterol–phosholipid monolayers (Van Deenen et al., 1962; Demel and Joos, 1968; Chapman et al., 1969; Zatz and Cleary, 1975) except that this multiple-component monolayer is more expanded at large areas and exhibits greater compressibility before collapse occurs. The ΔV increases in a uniform manner as compression of the monolayer is increased indicating a gradual straightening of the molecules as a result of packing.

Interactions of the sympathomimetic amines with the model membrane at equimolar subphase concentrations (0.18 ± 0.04 mM) are described in Figs. 1 and 2 and Table 1. The results of initial surface pressure following injection of drug in the subphase and the variation of π with mean molecular area (curve 2) suggest that at this concentration, there is very little penetration of the monolayer by phenmetrazine. However, by examining the changes in ΔV of the monolayer due to the presence of the drug, it becomes evident that the drug is interacting with the monolayer in a manner which causes a shift in orientation of the surface lipid molecules. This is demonstrated by a 15% increase in the initial surface potential, followed by a reduction, then again an increase in ΔV as the film undergoes various stages of compression. This suggests that phenmetrazine is interacting with the polar regions

TABLE 1

INITIAL SURFACE PRESSURES, INITIAL SURFACE POTENTIALS, COLLAPSE PRESSURES AND MINIMUM AREAS OF A LECITHIN : CEPHALIN : CHOLESTEROL (1 : 1 : 2) MONOLAYER ON pH 7.4 PHOSPHATE BUFFER SOLUTIONS OF SYMPATHOMIMETIC AMINES

Compound	Subphase concentration (mM)	Initial surface pressure ($mN \cdot m^{-1}$)	Initial surface potential (mV)	Collapse pressure ($mN \cdot m^{-1}$)	Minimum in mean area per molecule ($nm^2 \times 10^2$)
No drug		0.60	265	30	22
Phenmetrazine HCl	0.16	0.98	305	–	–
	0.32	1.18	310	31	27
	0.65	4.71	520	33	39
Levarterenol bitartrate	0.10	1.61	295	33	24
	0.21	1.57	270	34	27
	0.42	0.75	320	–	–
D-Amphetamine sulfate	0.09	1.33	260	33	34
	0.19	4.67	205	36	27
	0.38	0.94	295	–	–
Fenfluramine HCl	0.04	1.77	300	–	–
	0.07	3.92	200	–	–
	0.14	12.32	275	38	38

of the monolayer (since penetration does not occur) to alter the orientation of lipid molecules such that their alignments in the electrical field are somewhat altered from their original positions after compression and from their positions normally obtained in the absence of drug. This evidence, however, is unable to differentiate whether the interaction is directly with the polar groups of the lipid molecules extending downward into the aqueous phase, or whether the interaction takes place mainly with the water structure associated with the monolayer, or both.

In contrast to phenmetrazine, levarterenol produces a substantial increase in the initial surface pressure and in π (curve 3) as the film becomes more compressed. This is accompanied by an increase in the collapse pressure and minimum mean area per molecule of the monolayer. Initial surface potential in this case is essentially unchanged but ΔV rapidly increases upon compression. These results are indicative of penetration of the monolayer by levarterenol in which each molecule of drug which penetrates joins the lipid molecules in the same plane contributing to the surface potential of the monolayer but decreasing its compressibility.

D-Amphetamine sulfate injected into the subphase produces an even larger increase in the initial surface pressure of the monolayer, an upward shift of the π-A curve (curve 4), and an increased collapse pressure. Although this π data may resemble that of levarterenol and suggest a similar type of interaction, ΔV results indicate quite dramatically that such is not the case. The decrease in ΔV at all pressures compared to the drug-free subphase is indicative of a major structural change within the monolayer which is responsible for a substantial change in the dipole moments of the molecules in the plane of the interface. This could be due to coulombic interaction between the cationic drug and the anionic cephalin component (phosphatidylserine) of the monolayer to reduce the measurable charge (Demel and Van Deenen, 1966). In comparison, a monolayer of stearic acid has a ΔV of about +400 mV whereas sodium stearate has a value of about −50 mV (Sears and Schulman, 1964).

Penetration of the monolayer by fenfluramine molecules was the most pronounced among the 4 amines. The initial surface pressure as well as π at various stages of compression (curve 5) were more than twice the values found in the presence of D-amphetamine. Increases in the collapse pressure and minimum area per molecule were also observed as seen in Table 1. On the other hand, the initial surface potential was unchanged and ΔV did not change significantly until several stages of compression had occurred. The $-CF_3$ group on the aromatic ring and the N-ethyl group confer considerable lipid solubility to the molecule and, therefore, promote penetration of the monolayer. The lower compressibility of the monolayer and the rapid increase in ΔV suggest a horizontal configuration of fenfluramine at the surface initially which is then followed by an increasing orientation towards the vertical as the molecules are forced closer together to pack within a smaller area, although the monolayer is more expanded in the presence of fenfluramine than with any of the other drugs.

Effects of drug concentrations on the monolayer

A comparison of the effects of varying the concentration of drug in the subphase

on the surface pressure and surface potential of the monolayer can be made from the results in Table 1 and Fig. 3. Approximately the same range of concentrations were studied for each drug except fenfluramine which, due to its limited aqueous solubility, necessitated the use of lower concentrations. It can be seen that the initial surface pressure before compression and π at a constant area of 0.45 nm^2 follow the same general trend when either levarterenol or D-amphetamine are present in the subphase. This is characterized by a slight to moderate increase in π up to 0.2 mM followed by a sharp decrease up to about 0.4 mM concentration. A reverse trend in the initial surface potential is found (Table 1) but under a state of compression this property of the monolayer is little influenced by varying the concentration of levarterenol. The decreased π but increased ΔV of the monolayer a 0.4 mM D-amphetamine in the subphase suggests that the dominating influence o this drug on the monolayer is concentration dependent, changing from polar head group interaction at lower concentrations to hydrocarbon chain interaction at the higher concentrations.

Both phenmetrazine and fenfluramine cause a nearly uniform increase in the initial surface pressure and π at 0.45 nm^2 with increasing concentration indicating simple mixing of the drug molecules with the lipid molecules in the monolayer and with minimal polar group interaction. The surface potential results further reflect this behavior although the range of concentrations of these two drugs under

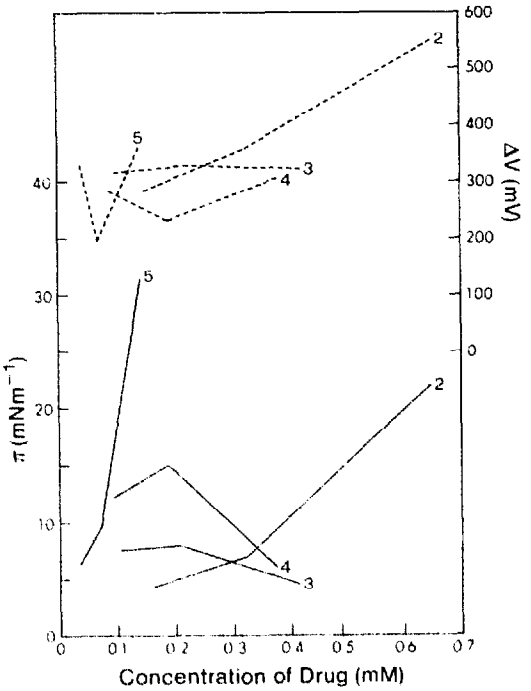

Fig. 3. Effect of drug concentration on the surface pressure (————) and surface potential (— — —) of a spread monolayer of lecithin:cephalin:cholesterol (1:1:2 mole ratio) on an aqueous phosphate-buffered (pH 7.4) subphase at a constant mean molecular area per molecule of 0.45 nm^2. Curves: 2 = phenmetrazine HCl; 3 = levarterenol bitartrate; 4 = D-amphetamine sulfate; 5 = fenfluramine HCl.

observation are quite different. At lower concentrations, fenfluramine undergoes considerably less penetration but disrupts the dipole–dipole polar head group interactions of the monolayer sufficiently to cause changes in the packing orientation of the molecules.

Discussion

The monolayer penetration technique has been employed to gain some insight into the qualitative and quantitative differences among 4 sympathomimetic amines to interact with biological membranes. Although the compounds are closely structurally related, it is clear from these results that each drug exerts its own characteristic interaction with the monolayer to yield different degrees of packing and orientation of the model membrane molecules. By comparing the surface pressures and the surface potentials of the monolayer in the presence of various concentrations of the drugs, it is possible to make certain assumptions regarding the types of interactions involved. Thus, phenmetrazine appears not to penetrate the monolayer at about 2×10^{-4} M but interacts with the polar groups of the lipids and disrupts the water structure which participates in the stability of the monolayer (Cleary and Zatz, 1973; Sears and Brandes, 1969). Penetration takes place at higher concentrations, however, as π and ΔV both increase proportionally and appear to be controlled by hydrophobic interactions. The possibility exists of an electrostatic attraction between the cationic amine and the anionic carboxyl and phosphoryl groups of cephalin and lecithin in the monolayer. However, this does not occur to any significant degree with phenmetrazine or fenfluramine probably because the cationic amine group is sterically hindered in both cases. On the other hand, levarterenol and D-amphetamine show evidence of a strong polar group interaction with the monolayer, particularly at higher concentrations, with D-amphetamine exerting the most pronounced effect. D-Amphetamine, being less polar than levarterenol, penetrates the monolayer more readily and enhances it position through polar head-group interaction even under maximum compression of the monolayer.

The comparative effects of the sympathomimetic amines on the expansion of membranes can be obtained from results of the mean molecular areas at the surface at different surface pressures. Table 2 makes this comparison at $\pi = 15$ mN · m^{-1} and $\pi = 25$ mN · m^{-1} for equimolar subphase concentrations of the drugs. It is obvious that the greatest expansion occurs in the presence of the less polar compounds, namely D-amphetamine and fenfluramine and is probably an activity related phenomenon since the solubilities of these compounds follow in the same order. On the other hand, the penetration of the sympathomimetic agents has little apparent dependency on basicity since the pKs are not in the same order. Monolayer expansion by each drug appears to be the same at these two pressures except fenfluramine which, in this case, is considerably greater at the higher pressure. Thus, penetration of the monolayer is driven mainly by hydrophobic interactions but it is offset to varying extents by polar group interactions which, in turn, are modulated by orientation and steric hindrance effects at the boundary of the monolayer and the aqueous substrate.

Studies involving a similar group of compounds, namely the phenothiazines, also lend support to the contention that drug–membrane interaction is a complex synthesis of physical, chemical and electrical events occurring between functional groups of the drug molecule and the molecular components of a membrane. Phenothiazines have been found to expand monolayers of lecithin and cholesterol, become expelled from the monolayer at high surface pressures, to decrease surface potentials (Sears and Brandes, 1969) and to have intrinsically different perturbing abilities in phospholiid bilayers (Jain and Wu, 1978). The present results with the sympathomimetic amines also indicate that equimolar concentrations of drug in the aqueous substrate do not yield equal response in a monolayer and should not be expected to do so in a bilayer.

Penetration of mixed lipid films cannot be predicted by the penetration characteristics of the pure components (Schwinke et al., 1983). In the application of model membrane systems to predict drug behavior, a more highly structured membrane rather than the simple, single component bulk oil phase or spread monolayer might be warranted. An attempt has been made here to test this approach with 4 derivatives of phenylethylamine using a multicomponent structure of a model membrane as a spread monolayer. It is not suggested that the monolayer used here mimics a biological membrane but that this three-component monolayer may possess more realistic characteristics as a model membrane than a single phospholipid. The results obtained have demonstrated both qualitative and quantitative differences in behavior of these drugs with the monolayer even though drug concentrations in the subphase were well below surface-active concentrations and the monolayer exists in a relatively condensed state. If these sympathomimetic amines should interact with biological membranes in a similar fashion, then it may be speculated that differences in biological activities among this group of pharmacological agents are at least partly related to their abilities to induce structural changes which, perhaps, leads to alteration of the permeabilities of selective cell membranes to various ions and solutes.

TABLE 2

THE EFFECT OF SYMPATHOMIMETIC AMINES ON THE EXPANSION OF A LECITHIN:CEPHALIN:CHOLESTEROL (1:1:2) MONOLAYER

Compound	Mean area per molecule ($nm^2 \times 10^2$)	
	$\pi = 15$ mN·m^{-1}	$\pi = 25$ mN·m^{-1}
None	31.8	26.0
Phenmetrazine HCl	30.1 (−5%)	24.7 (−5%)
Levarterenol bitartrate	38.0 (24.5%)	32.8 (26.2%)
D-Amphetamine sulfate	44.8 (40.9%)	36.1 (38.8%)
Fenfluramine HCl	51.8 (62.9%)	48.9 (88.1%)

Numbers in brackets are the percentage changes in mean area per molecule for each drug relative to the drug-free subphase. The effects of the drugs are compared at a concentration of 0.18 ± 0.04 mM.

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