Chem. Phys. Lipids 1 (1966) 68–82 © North-Holland Publ. Co., Amsterdam

PENETRATION OF LIPID MONOLAYERS BY PSYCHOACTIVE DRUGS

R. A. DEMEL and L. L. M. VAN DEENEN

Laboratory of Organic Chemistry, Department of Biochemistry, University of Utrecht, Utrecht, The Netherlands

The ability of a number of psychoactive drugs to penetrate lipid monolayers of varying composition was examined, and the following observations were made: (1) The increase in surface pressure of a monomolecular film appeared to depend on the chemical nature of the lipid as well as on the initial film pressure. (2) Several psychoactive compounds showed a significant interaction with anionic lipid films at initial surface pressure above 18 dynes/cm. (3) The results support the view that coulombic attractions between cationic drugs and negatively charged lipids are involved. (4) Significant differences in the magnitude of the effect of different drugs on films of gangliosides are demonstrated.

The concept that psychoactive drugs and local anaesthetics act on biological membranes was supported by studies on the physico-chemical properties of such compounds and their action on various model systems consisting of lipids[1–3]). The surface activity of a great number of tranquilizers was studied by Seeman and Bialy[4]) and the surface activity of neuroleptic drugs was found to correlate with clinical potencies. Investigations with fluorescence polarization methods demonstrated that several cationic molecules can interact with lipids[5]) or proteins[6]) containing sialic acid. The ability of local anaesthetics to penetrate monomolecular films of lipids extracted from peripheral nerves could be related to their blocking potency[3]). Bangham has recently found that local anaesthetics alter the permeability of concentric lamellae of lecithin[7]). Rogeness and Abood[8]) made a comparison between the action of calcium and of psychotomimetic agents on the surface of interfacial layers of stearic acid. Zografi and Auslander[9]) studied mixed monomolecular films of chlorpromazine and chlorpromazine sulfoxide with cholesterol and lecithins. Chlorpromazine was found to be more surface active than its sulfoxide.

The use of monomolecular or bimolecular films of defined lipids has been proved to be useful tool in the study of the selective action of drugs[10–12]). Experiments on the interaction of psychoactive compounds with different lipid classes may provide some clue to the understanding of the site of binding of these compounds.

Experimental materials

Cholesterol was purchased from Fluka A.G. Switzerland. Most of the phospholipids were synthesized in our laboratory by methods described in detail[13]). The purity of the compounds was determined by thin-layer chromatography. The following synthetic compounds were used: (1-stearoyl-2-oleoyl)-2-phosphatidylcholine, (1,2-didecanoyl)-3-phosphatidylcholine; (1,2-dipalmitoyl)-3-phosphatidylethanol-amine; disodium, 3,1-bis (2′-oleoyl-1′-stearoyl-3′-glycerylphosphoryl)glycerol; (1-oleoyl-2-palmitoyl)-3-phosphatidylglycerol; (1-palmitoyl-2-oleoyl)-3-phosphatidylserine. Preparations of cerebrosides and mixtures of gangliosides isolated from beef brain were kindly provided by Dr. R. M. Burton (Dept. of Pharmacology, Washington University, School of Medicine, St. Louis). Monosialogangliosides were generously donated by Dr. L. Svennerholm (Institute of Medical Biochemistry, University of Gothenburg, Gothenburg). Glycolipids were dissolved in 15 percent methanol chloroform, the other lipids in chloroform (p.a.). The psychoactive drugs were obtained through the good services of the research department N.V. Koninklijke Pharmaceutische Fabrieken v/h Brocades-Stheeman en Pharmacia, Amsterdam. Freshly distilled dimethylformamide was used to dissolve the psychoactive drugs. The solutions were used within 2 hr after preparation.

Trough and surface measuring apparatus

Force area measurements and experiments on the penetration of monolayers were made at the air–water interface at room temperature (20 °C) in a glass trough 58 cm long × 14 cm wide, having a capacity of 700 ml. Surface pressures were recorded with a conventional Langmuir–Adam surface balance. The trough was filled with unbuffered water which had been distilled from alkaline permanganate and then redistilled in a quartz still.

Penetration measurements of lipid monolayers at constant area

The term monolayer penetration is used to describe the interaction of an insoluble monolayer (lipid) spread at a phase boundary with a soluble surface active species (psychoactive drug) in one phase[14]). The lipids were spread on the area of 560 cm^2 and compressed to approximately 140 cm^2 so as to give surface pressures between 2 and 30 dynes/cm. The psychoactive drugs were injected carefully underneath the monolayer with an Agla micrometer syringe. A stable increase in the surface pressure was obtained within 6 min after injection. For every lipid compound the pressure increase was measured at seven different initial pressures.

Monolayer penetration at constant pressure

The procedure was essentially the same as above except that after injection of the psychoactive drugs, the increase in area necessary to maintain the initial surface pressure was determined.

Results

The following psychoactive drugs have been studied: (a) diphenylmethanes: orphenadrine HCl, nor-orphenadrine HCl, meclizine di-HCl; (b) phenothiazines: chlorpromazine HCl *, ethopropazine HCl; (c) dibenzocycloheptenes: deptropine citrate; (d) the rauwolfia alkaloid reserpine; (e) meprobamate; (f) pentobarbital sodium.

Increase in pressure at constant area

Diphenylmethanes. The psychoactive compound extensively studied in the present investigation was orphenadrine HCl. Several investigators have reported that this compound may affect the permeability of biological mem-

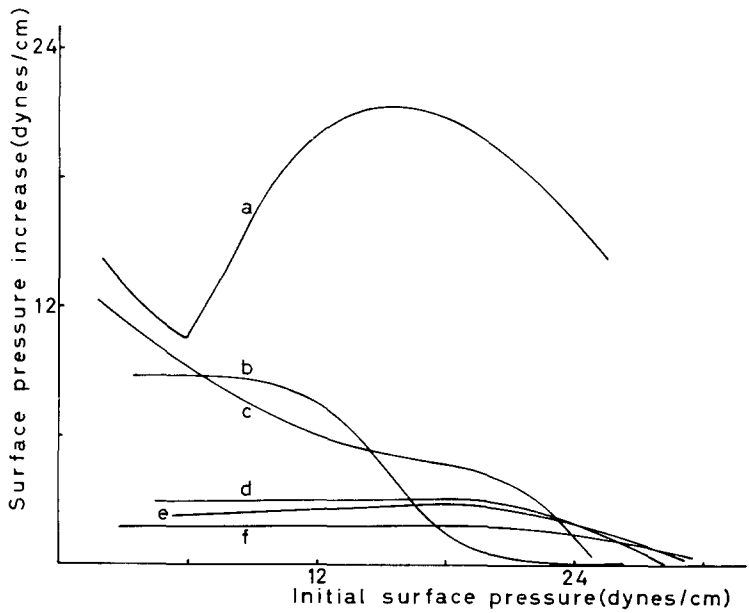

Fig. 1. Increase in surface pressure of lipid monolayers by orphenadrine HCl; monomolecular films of (a) gangliosides, (b) cholesterol, (c) cerebrosides, (d) phosphatidylethanolamine, (e) sphingomyelin, (f) lecithin were compressed to give the initial pressure indicated on the abscissa and orphenadrine HCl (5.3 μM) was injected underneath.

* WHO proposed non-proprietary name tofenacine HCl.

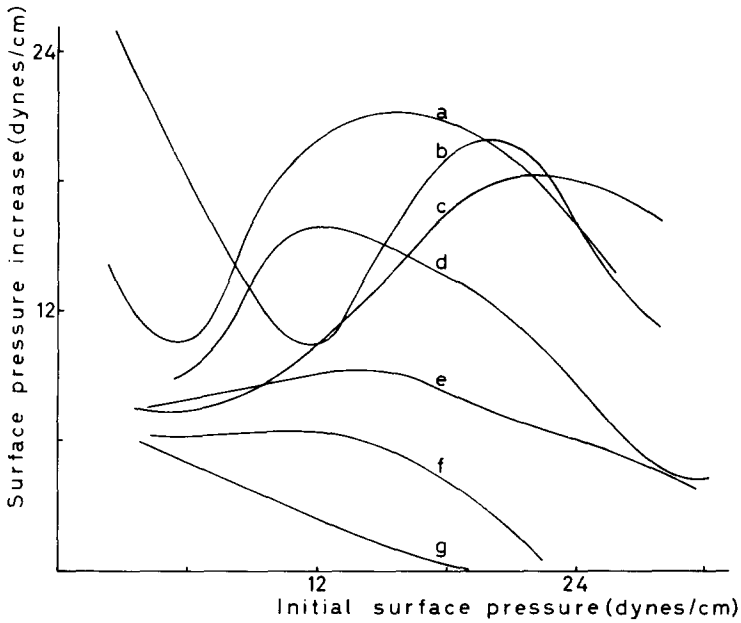

Fig. 2. Increase in surface pressure of anionic lipid monolayers by orphenadrine HCl; monomolecular films of (a) gangliosides, (b) cardiolipin, (c) monosialoganglioside, (d) phosphatidylglycerol, (e) phosphatidylserine, (f) oleic acid, (g) stearic acid were compressed to give the initial pressure indicated on the abscissa and orphenadrine HCl (5.3 μM) was injected underneath.

branes[15–18]). The increase of surface pressure of films of the phospholipids: lecithin, phosphatidylethanolamine and sphingomyelin after injection of orphenadrine HCl was very limited (fig. 1). Films of cholesterol and cerebroside revealed a notable increase in surface pressure at least at low initial film pressures. On the other hand films of gangliosides and cardiolipin (figs. 1 and 2) exhibited a very significant increase in film pressure also at high initial pressures, the effect on the film of a monosialoganglioside being somewhat different from that obtained with films containing a mixture of gangliosides. It is worth noting that the effect of orphenadrine HCl (fig. 3) was strongly reduced after hydrolysis of monosialoganglioside with neuraminidase [19]) which treatment is known to remove apart of the sialic acid groups[20]).

In addition to the glycolipids and phospholipids mentioned also films of other anionic phospholipids viz. phosphatidylglycerol and phosphatidylserine revealed an increase in surface pressure (fig. 2).

The effects of orphenadrine HCl on monomolecular films of fatty acids were rather limited particularly at higher film pressures (fig. 2). It has to be emphasized that the foregoing experiments were carried out with a subphase

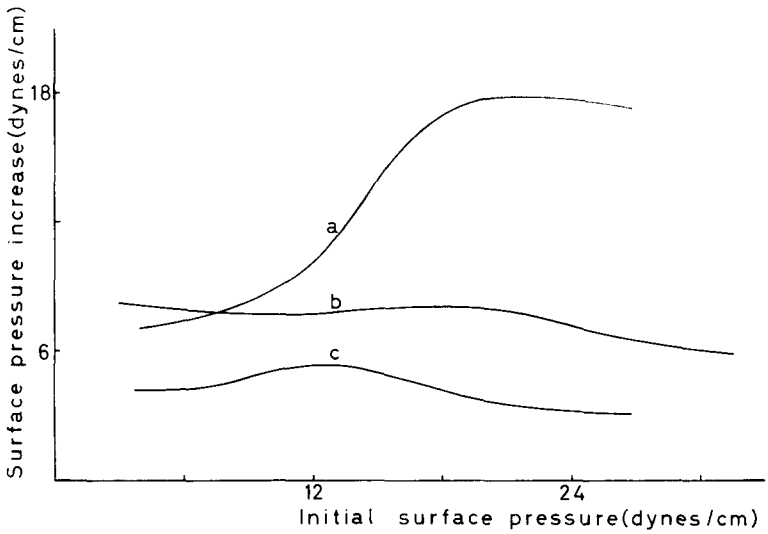

Fig. 3. Increase in surface pressure of lipid monolayers by orphenadrine HCl; monomolecular films of (a) monosialoganglioside, (b) neurominidase treated monosialoganglioside, (c) monosialoganglioside on a subphase of 0,1 M calciumchloride were compressed to give the initial pressure indicated on the abscissa and orphenadrine HCl (5.3 μM) was injected underneath.

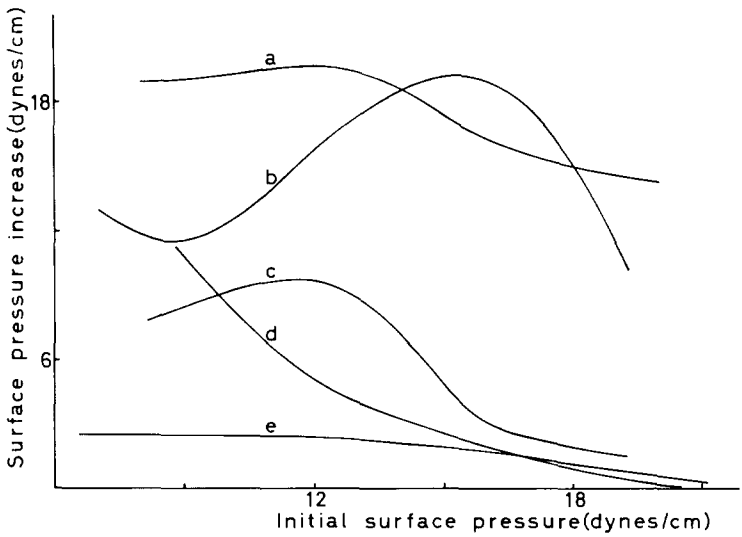

Fig. 4. Increase in surface pressure of lipid monolayers by nor-orphenadrine HCl; monomolecular films of (a) cardiolipin, (b) gangliosides, (c) cerebrosides, (d) cholesterol, (e) lecithin were compressed to give the initial pressure indicated on the abscissa and nor-orphenadrine HCl (4.9 μM) was injected underneath.

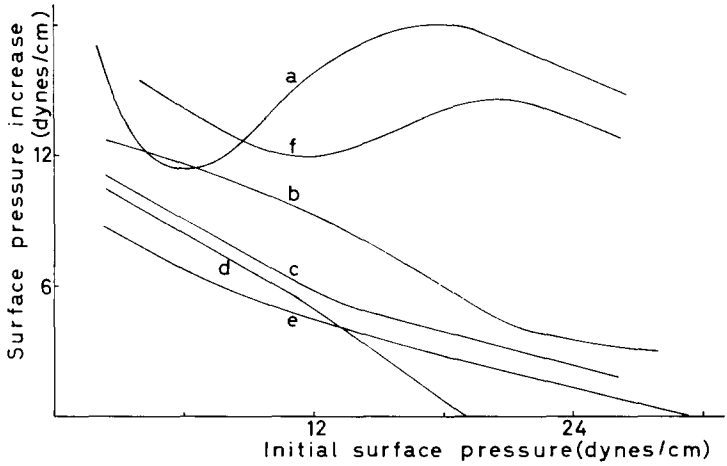

Fig. 5. Increase in surface pressure of lipid monolayers by meclizine-di-HCl; monomolecular films of (a) gangliosides, (b) cerebrosides, (c) lecithin, (d) cholesterol, (e) sphingomyelin, (f) cardiolipin were compressed to give the initial pressure indicated on the abscissa and meclizine di-HCl (4.2 μM) was injected underneath.

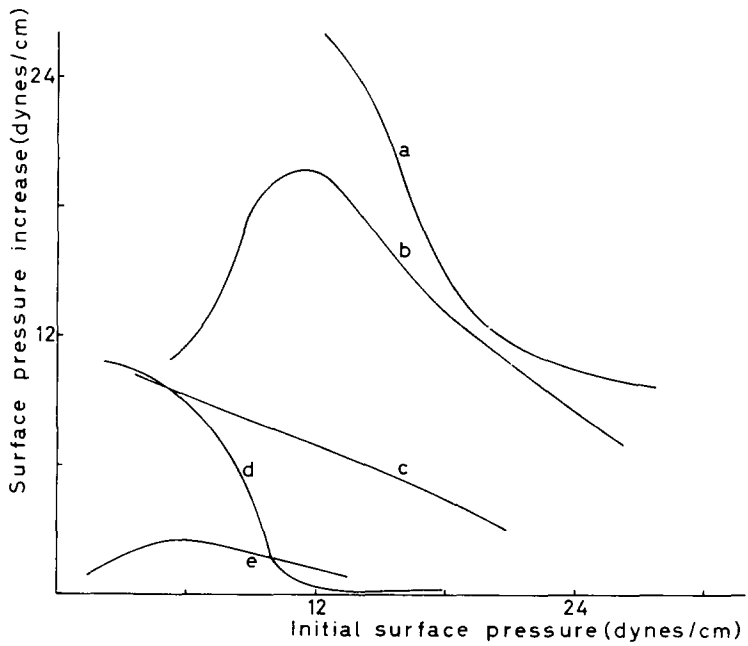

Fig. 6. Increase in surface pressure of lipid monolayers by chlorpromazine HCl; monomolecular films of (a) cardiolipin, (b) gangliosides, (c) cerebrosides, (d) cholesterol, (e) lecithin were compressed to give the initial pressure indicated on the abscissa and chlorpromazine HCl (5.8 μM) was injected underneath.

consisting of water only. After spreading of anionic lipids such as gangliosides or cardiolipin on a substrate of 0.1 M $CaCl_2$ (fig. 3), the increase of surface pressure due to the injection of orphenadrine HCl takes only a small fraction of the values obtained without $CaCl_2$ present.

The behaviour of nor-orphenadrine HCl was fairly similar to that of orphenadrine HCl films consisting of gangliosides or cardiolipin giving the highest increase in surface pressure (fig. 4). This was true also for meclizine di-HCl, though the penetration of films of non-anionic lipids was more obvious when compared with orphenadrine HCl (fig. 5).

Phenothiazines. The action of chlorpromazine HCl on various lipid monolayers is illustrated by fig. 6. This compound was found to give hardly any interaction with lecithin, but at low initial pressures there was some penetration of cholesterol films, while also cerebrosides exhibited some interaction. However, an extremely strong interaction was observed between chlorpromazine HCl and anionic lipids as gangliosides and cardiolipin. Also at high initial pressure of the anionic film the pressure increase due to this

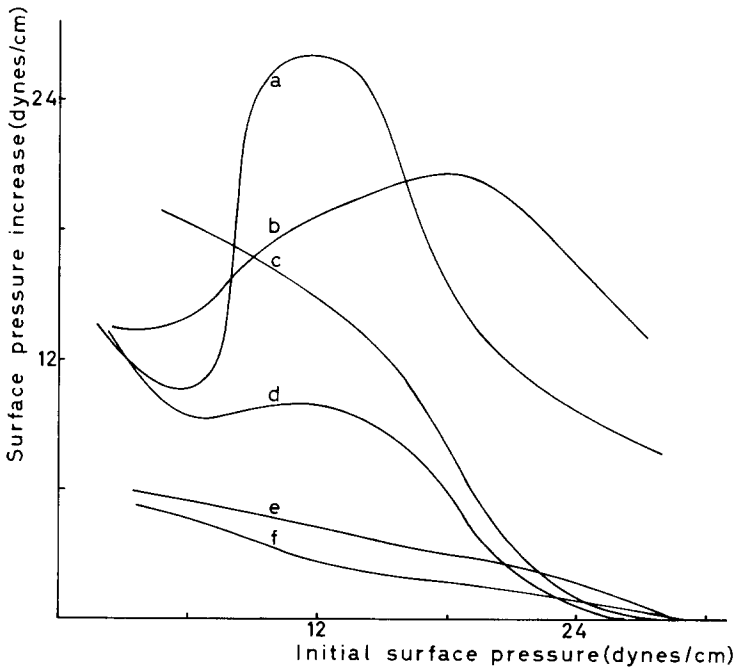

Fig. 7. Increase in surface pressure of lipid monolayers by deptropine citrate; monomolecular films of (a) gangliosides, (b) cardiolipin, (c) cholesterol, (d) cerebrosides, (e) lecithin, (f) sphingomyelin were compressed to abscissa and deptropine citrate (3.7 μM) was injected underneath.

drug was most significant. With films of gangliosides and cardiolipin even ethopropazine HCl showed a greater pressure increase than chlorpromazine HCl did.

Dibenzocycloheptenes. Deptropine citrate (fig. 7) revealed an interaction with anionic glycolipids and phospholipids, but the increase in surface pressure of cholesterol films was also highly significant.

Reserpine. Reserpine was found to give a notable effect on monolayers of cholesterol and cerebrosides at initial pressures below 24 dynes/cm, but at higher pressures the effect on gangliosides and cardiolipin again was conspicuous (fig. 8). On a quantitative basis the effects of reserpine on films of anionic lipids even exceeded that of all drugs assayed to date (compare fig. 15). The curve describing the interaction between reserpine and cholesterol (fig. 8) has been drawn partly as a dotted line so as to indicate that the values recorded of the increase in surface pressure dropped rapidly. This observation suggested that at low initial film pressures the cholesterol present in the monolayer was to some extent dissolved into the underphase by reserpine.

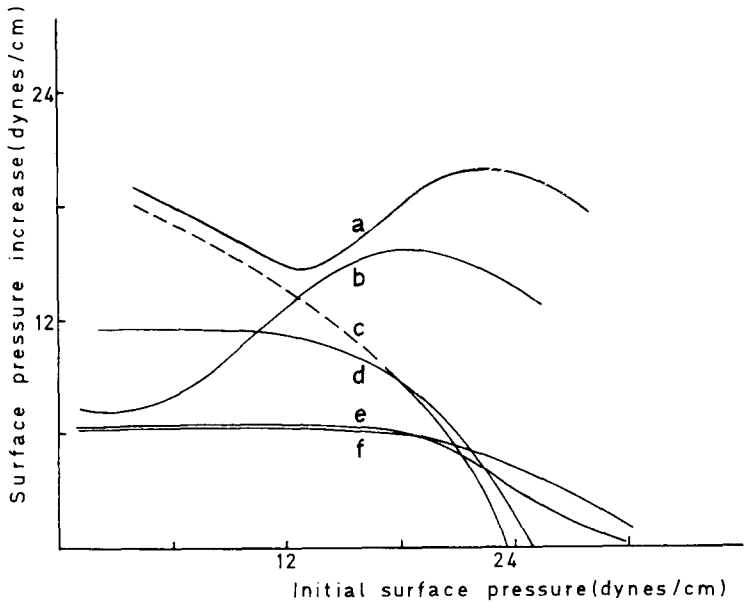

Fig. 8. Increase in surface pressure of lipid monolayers by reserpine; monomolecular films of (a) cardiolipin, (b) gangliosides, (c) cholesterol, (d) cerebrosides, (e) lecithin (saturated), (f) lecithin (unsaturated) were compressed to give the initial pressure indicated on the abscissa and reserpine (0.75 μM) was injected underneath.

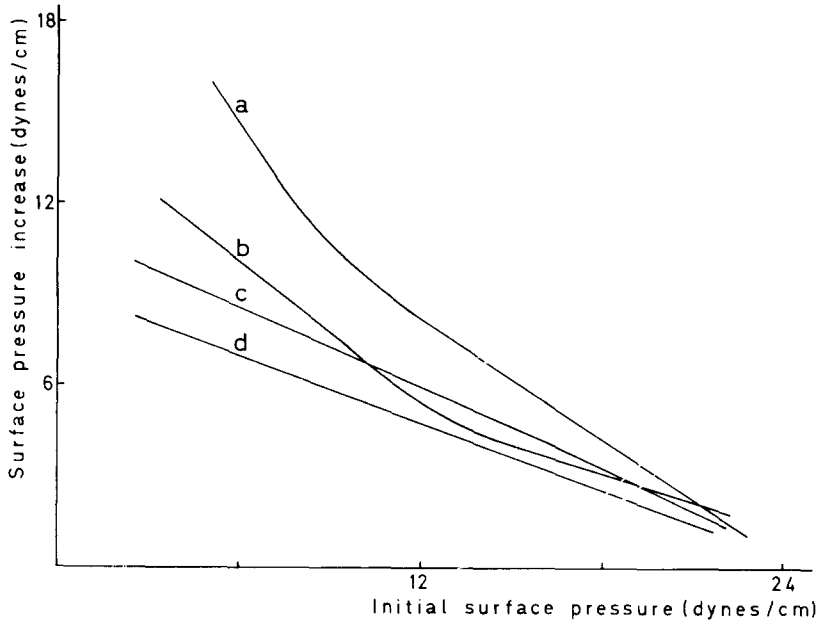

Fig. 9. Increase in surface pressure of lipid monolayers by meprobamate; monomolecular films of (a) cholesterol, (b) cardiolipin, (c) gangliosides, (d) sphingomyelin were compressed to give the initial pressure indicated on the abscissa and meprobamate (6.9 μM) was injected underneath.

Meprobamate. Meprobamate caused some increase in the pressure of films of the four lipids investigated (fig. 9). The effect of this compound on films of gangliosides and cardiolipin was very limited when compared with the alterations induced by the psychoactive compounds dealt with above. The pressure increase caused by meprobamate at low initial surface pressure of lipid films may be due to its own surface activity[4]) and is considered to be rather unspecific.

Pentobarbital sodium. A small increase of film pressure was observed after injection of pentobarbital sodium underneath the films of cholesterol, at least at initial surface pressures below 18 dynes/cm (fig. 10). The effects on films of other lipids, including gangliosides and cardiolipin, were negligible. Similar results were obtained with diethyl barbituric acid.

INCREASE IN AREA AT CONSTANT PRESSURE

In the foregoing experiments the interaction between lipids and psychoactive drugs was studied by measuring the pressure increase at constant area. In addition to this approach one can determine the increase in area, keeping

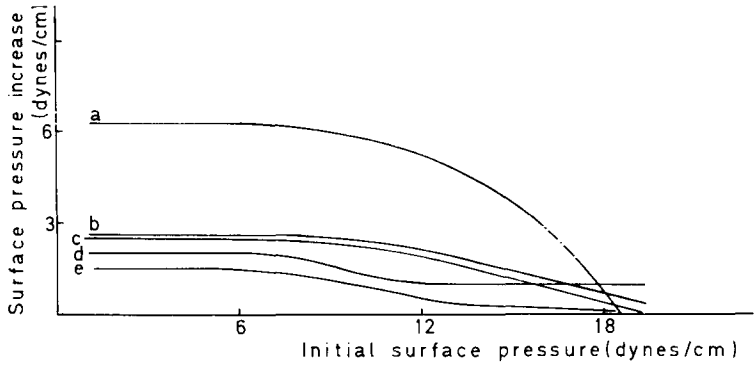

Fig. 10. Increase in surface pressure of lipid monolayers by pentobarbital sodium; monomolecular films of (a) cholesterol, (b) cerebrosides, (c) cardiolipin, (d) gangliosides, (e) sphingomyelin were compressed to give the initial pressure indicated on the abscissa and pentobarbital sodium (4.3 μM) was injected underneath.

the surface pressure at a constant value. Nearly all the psychoactive drugs mentioned in the foregoing section have been assayed by this method of utilizing films of the lipids included in figs. 1–10. Only a number of examples illustrating the major results obtained with this procedure are given in detail.

Diphenylmethanes. The percentage increase in area of the lipid film caused by the injection of orphenadrine HCl under the monolayer is presented in fig. 11. Most significant effects were recorded with films of gangliosides cardiolipin and phosphatidylserine. Films of monosialoganglioside and phosphatidylglycerol exhibited a similar behaviour. A film prepared of monosialogangliosides treated before with neuraminidase revealed upon injection of the drug a much lower increase of area. The effects on films of cerebrosides sphingomyelin, phosphatidylethanolamine, cholesterol, lecithin (fig. 11) and fatty acids were very limited and disappeared at higher initial surface pressures. In general the results obtained with orphenadrine HCl on the increase of area of lipid films kept at constant pressure are in very good agreement with the observations on the increase of surface pressure at constant area. This was true also for nor-orphenadrine HCl which compound induced a significant increase in area of a ganglioside film, but failed to give an appreciable effect on films of cholesterol, lecithin and cerebrosides. On the other hand, meclizine di-HCl brought about a conspicuous increase in area of films of these three lipids, at least at initial film pressures below 12 dynes/cm (fig. 12). Under these conditions meclizine di-HCl caused also an appreciable increase of surface pressure of these lipid films (constant area) (compare fig. 5). However, other compounds e.g. deptropine-citrate (fig. 7) and reserpine (fig. 8), which gave also a notable increase of surface pressure of

films of cholesterol and cerebrosides, failed to induce such a pronounced increase of surface area of these lipid films (compare fig. 14). At high film pressure meclizine di-HCl gave an increase of film area with anionic lipids such as cardiolipin and gangliosides only.

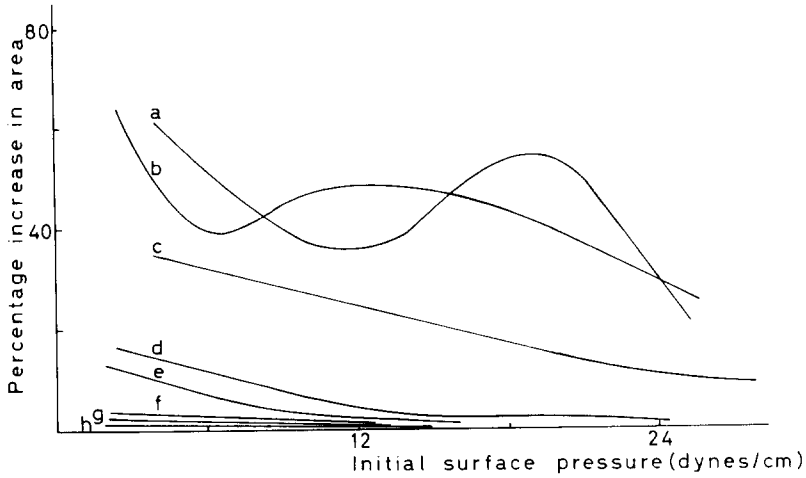

Fig. 11. Increase in surface area of lipid monolayers at constant pressure by orphenadrine HCl; monomolecular films of (a) cardiolipin, (b) gangliosides, (c) phosphatidylserine, (d) cerebrosides, (e) sphingomyelin, (f) phosphatidylethanolamine, (g) cholesterol, (h) lecithin were compressed to give the initial pressure indicated on the abscissa and orphenadrine HCl (5.3 μM) was injected underneath.

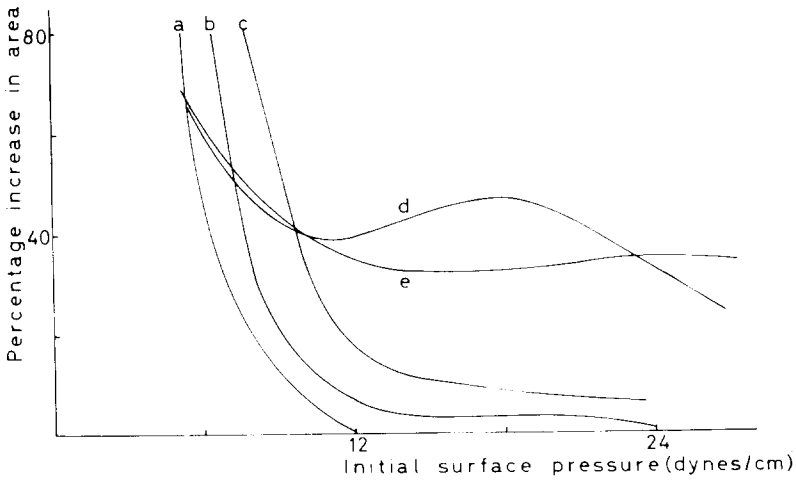

Fig. 12. Increase in surface area of lipid monolayers at constant pressure by meclizine di-HCl; monomolecular films of (a) cholesterol, (b) cerebrosides, (c) lecithin, (d) gangliosides, (e) cardiolipin were compressed to give the initial pressure indicated on the abscissa and meclizine di-HCl (4.2 μM) was injected underneath.

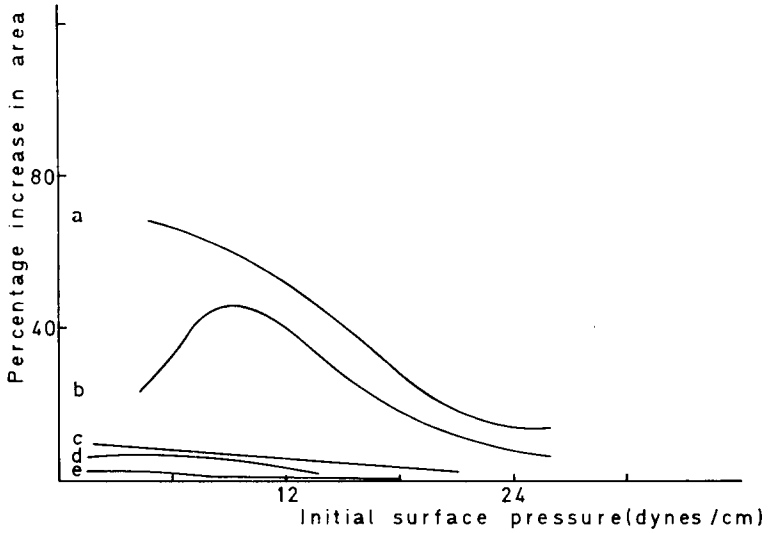

Fig. 13. Increase in surface area of lipid monolayers at constant pressure by chlorpromazine HCl; monomolecular films of (a) cardiolipin, (b) gangliosides, (c) cerebrosides, (d) lecithin, (e) cholesterol were compressed to give the initial pressure indicated on the abscissa and chlorpromazine HCl (5.8 μM) was injected underneath.

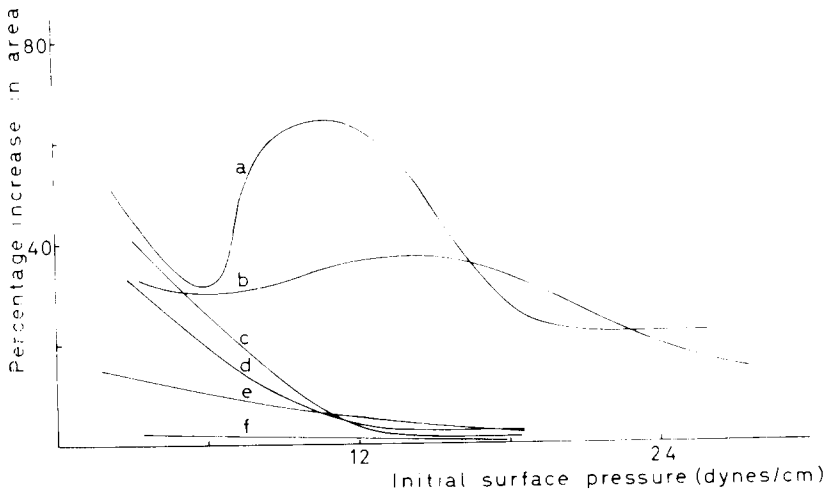

Fig. 14. Increase in surface area of lipid monolayers at constant pressure by deptropine citrate; monomolecular films of (a) gangliosides, (b) cardiolipin, (c) sphingomyelin, (d) lecithin, (e) cerebrosides, (f) cholesterol were compressed to give the initial pressure indicated on the abscissa and deptropine citrate (3.7 μM) was injected underneath.

Phenothiazines. Chlorpromazine HCl caused an increase in area of films of anionic lipids (gangliosides and cardiolipin) varying from 20–70%, depending on the initial surface pressure. The effect on films consisting of lecithin, cerebrosides and cholesterol was very small even at low initial film pressures (fig. 13).

Dibenzocycloheptenes. At film pressures above 12 dynes/cm the greatest increase in an area caused by the injection of deptropine citrate was observed with films of gangliosides and cardiolipin (fig. 14). At low film pressures some differences in the increase of film area are to be noted between films of different classes of lipids.

Reserpine. The behaviour of reserpine was fairly similar to that recorded already for deptropine-citrate.

Meprobamate. This compound did not cause any significant increase in

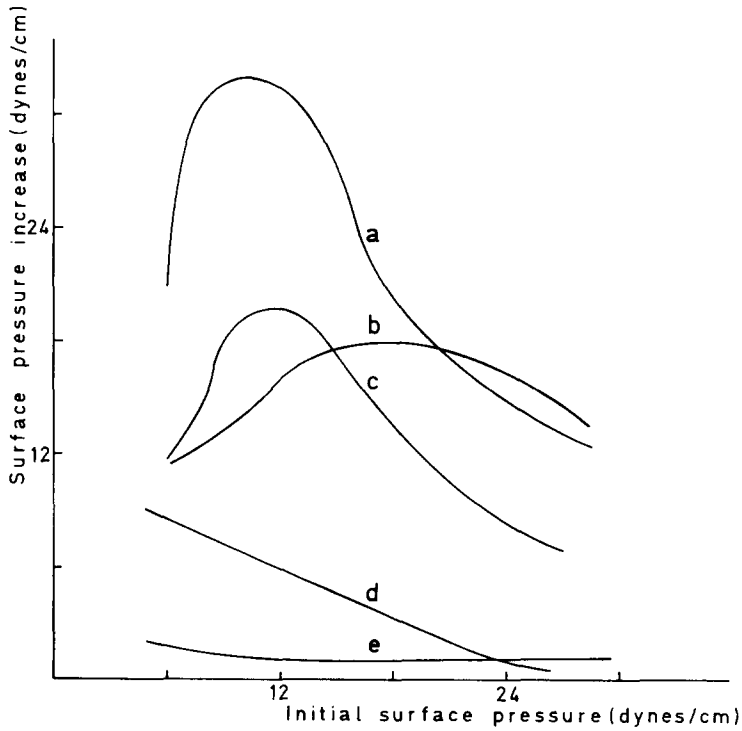

Fig. 15. Penetration of monomolecular films of gangliosides by (a) reserpine (1.2 μM), (b) meclizine-di-HCl (4.2 μM), (c) chlorpromazine-HCl (5.8 μM), (d) meprobamate (6.9 μM), (e) pentobarbital sodium (4.3 μM).

area of films of gangliosides, sphingomyeline, cholesterol or cardiolipin kept at a pressure above 12 dynes/cm. At film pressures of 6 dynes/cm a considerable increase in area (80–100%) was observed with all lipid films investigated.

Pentobarbital sodium. Pentobarbital sodium and diethyl barbituric acid did not evoke any area increase of films of gangliosides, sphingomyeline, cholesterol and cardiolipin at film pressures between 6 dynes/cm and 30 dynes/cm.

Discussion

The monolayer experiments indicate that a number of psychoactive drugs are capable of penetrating lipid films. The increase in pressure of a monomolecular film after injection of a given psychoactive drug appears to depend on the chemical nature of the lipid as well as on the initial surface pressure of the lipid film. At low film pressures e.g. below 12 dynes/cm several psychoactive drugs appeared to give an interaction with e.g. cholesterol. However, when the psychoactive compounds were injected underneath a cholesterol film kept at or above 18 dynes/cm the effects were very small or absent. It is difficult to evaluate which conditions of the simple monolayer system can best be compared with the complex system of a biological interface. However, it is worth noting that polyene antibiotics which cause a specific lysis of cell membranes contain a considerable quantity of sterols that are capable of giving a significant effect on monolayers of sterols at film pressure above 18 dynes/cm[10]). On the other hand, several psychoactive compounds gave also a significant interaction with lipid films at surface pressure above 18 dynes/cm at least when the film consisted of anionic lipids. Orphenadrine HCl interacted with sialic acid containing glycolipids and with anionic phospholipids such as cardiolipin, phosphatidylglycerol and phosphatidylserine. This compound and several other drugs (nor-orphenadrine HCl, meclizine di-HCl, chlorpromazine HCl, deptropine citrate and reserpine) caused an increase in pressure of films of gangliosides and cardiolipin at initial film pressures of 18–24 dynes/cm. The results obtained to date appear to support the view that Coulombic attractions between the cationic drugs and the negatively charged lipids stabilize the interaction at high film pressures. Feinstein[21]) demonstrated that local anaesthetics react with acidic phospholipids, and more recently it has been concluded that these drugs interacted specifically with phosphodiester groups occurring in phospholipids, phosphoproteins, ribonucleic acid or synthetic phosphodiesters. The importance of the anionic moiety of the lipid for interaction films of psychoactive drugs, maintained at a high pressure is indicated by (a) a decrease of the magnitude of the effect of orphenadrine HCl with phospholipids demonstrating a decrease in negative charge (cardiolipin, phosphatidylglycerol, phosphatidyl-

serine and phosphatidylethanolamine), (b) a strong reduction of the interaction with gangliosides after treatment of the lipids with neuraminidase which removes part of the sialic acid residues. Furthermore, spreading of a ganglioside film on a substrate containing calcium ions greatly reduced the interaction with orphenadrine HCl. The experiments on the increase of surface area of lipids indicate that the apolar residues of the psychoactive drugs may interact with the paraffinic chains of the lipids concerned. A comparison of the interaction of various psychoactive compounds with monomolecular films of gangliosides demonstrates that significant differences exist in the magnitude of the effect caused by different drugs (fig. 13). Perhaps some of these differences are related to the molecular sizes and/or pK values of the drugs. Although it is not permitted to extrapolate from the monolayer system to complex biological structures the present experiments are believed to favour the view that psychoactive compounds may be bound to lipids of natural membranes. The observations made on the interaction of psychoactive drugs with films of anionic lipids such as gangliosides may be of interest with regard to the work of Woolley and Gommi[22,23]) who concluded that particular gangliosides may function as serotonin receptors.

References

1) A. M. Shanes, Pharmacol. Rev. **10** (1958) 59.
2) R. Straub, Arch. Intern. Pharmacodyn. **107** (1956) 414.
3) J. Chr. Skou, J. Pharmacol. **13** (1961) 204.
4) P. M. Seeman and H. S. Bialy, Biochem. Pharmacol **12** (1963) 1181.
5) R. W. Albers and G. J. Koval, Biochim. Biophys. Acta **60** (1962) 359.
6) E. G. Trams and C. J. Lauter, Biochim. Biophys. Acta **83** (1964) 296.
7) A. D. Bangham, M. M. Standish and N. Miller, Nature **208** (1965) 1295.
8) G. Rogeness and L. G. Abood, Arch. Biochem. Biophys. **106** (1964) 483.
9) G. Zografi and D. E. Auslander, J. Pharm. Sci. **54** (1965) 1313.
10) R. A. Demel, S. C. Kinsky and L. L. M. van Deenen, J. Biol. Chem. **240** (1965) 2749.
11) L. L. M. van Deenen and R. A. Demel, Biochim. Biophys. Acta **94** (1965) 314.
12) H. van Zutphen, L. L. M. van Deenen and S. C. Kinsky, Biochim. Biophys. Res. Commun. **22** (1966) 393.
13) L. L. M. van Deenen and G. H. de Haas, Advan. Lipid Res. **2** (1964) 167.
14) B. A. Pethica, Trans. Faraday Soc. **51** (1955) 1402.
15) G. Quadbeck and W. Schmitt, Arch. Exptl. Pathol. Pharmacol. **237** (1959) 94.
16) G. Liebaldt, Med. Welt **36** (1960) 1876.
17) M. J. E. Ernsting, W. F. Kafou, W. Th. Nauta, H. K. Oosterhuis and P. A. Roukema, in: J. T. Holden, ed., Amino acid pools, Elsevier, Amsterdam 1962, p. 493.
18) R. C. Roozemond, Abstracts 6th Intern. Congr. Biochem., New York 1964, vol. 5, p. 417.
19) R. M. Burton, J. Neurochem. **10** (1963) 503.
20) M. C. Dodd, Nature **204** (1964) 549.
21) M. B. Feinstein and M. Paimre, Biochim. Biophys. Acta **115** (1966) 33.
22) D. W. Woolley and B. W. Gommi, Nature **202** (1964) 1074.
23) D. W. Woolley and B. W. Gommi, Proc. Nat. Acad. Sci. **53** (1965) 959.