Eur Biophys J (1987) 14: 403–408

European Biophysics Journal
© Springer-Verlag 1987

Anaesthetic – phospholipid interaction

The effect of chlorpromazine on phospholipid monolayers

G. Beurer and H.-J. Galla

Department of Biochemistry, Technical University Darmstadt, Petersenstrasse 22, D-6100 Darmstadt, Federal Republic of Germany

Received July 18, 1986/Accepted in revised form December 23, 1986

Abstract. Binding of the positively charged drug chlorpromazine to phospholipid monolayers was investigated. A preferential uptake was observed near the phase transition of the corresponding lipid. Cholesterol considerably diminishes the chlorpromazine uptake, again particularly near a lipid phase transition. The binding properties depend on the chlorpromazine concentration in the subphase. A critical concentration is $5 \cdot 10^{-5}$ M, where higher uptake occurs in the liquid condensed than in the liquid expanded state of the monolayer at pressures of about 10 mN/m. Dipalmitoylphosphatidylcholine monolayers spread on a subphase containing chlorpromazine are comparable to monolayers at higher temperature but in the absence of chlorpromazine. These data are in agreement with previous fluorescence and electron paramagnetic resonance experiments on lipid bilayer membranes (Luxnat and Galla 1986).

Key words: Phospholipids, anaesthetics, monolayer, membrane solubility of amphipaths

Introduction

The mechanism of anaesthetic action is not yet completely understood. Although it is known that these drugs are involved in the metabolism of the organism, one of the main sites of action is phospholipid membranes. It is generally accepted that drugs and other amphiphilic molecules can be incorporated into lipid membranes, which may cause a dramatic change in the thermodynamic properties of the lipid membrane, for example, an expansion (Lee 1976; Roth 1979), an increase of fluidity (Lee 1976) or a dispersion of lipid-protein-complexes (Galla and Trudell 1981). There may be also a direct interaction between some drugs and the membrane proteins (Seeman 1972).

Model membranes are in widespread use to investigate lipid-drug interaction. In recent years these studies became doubtful in the light of investigations by Conrad and Singer (1979, 1981) who reported a considerably lower solubility of amphipaths in biological membranes compared to artificial bilayer systems. Earlier identical partition coefficients observed by a centrifugation technique were interpreted as an artifact due to external CPZ micelle formation. However, micelles at the bilayer surface could not be detected experimentally (Forrest et al. 1984). Partition coefficients of chlorpromazine (CPZ) and fatty acids were investigated by Pjura et al. (1984). In contrast to Conrad and Singer they found a very similar behaviour of artificial and natural membranes. Very recently, a set of equivalent experiments has been carried out (Luxnat and Galla 1986), and partition coefficients in biological membranes were found to be comparable to the values of artificial bilayers. Obviously, liposomes are still a useful tool to get more information about biological systems.

In order to learn more about the anaestethic-lipid interaction, the uptake of CPZ from the aqueous subphase into phosphatidylcholine monolayers was investigated. In this paper it will be demonstrated that an incorporation of CPZ in monolayers occurs as well as in bilayers. The influence of cholesterol within the monolayer will also be considered. CPZ incorporated into the membrane lowers the phase transition and causes a fluidisation of the lipid monolayer, which will be discussed in analogy to a temperature decrease in the presence of drug.

Abbreviations: CPZ, chlorpromazine; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; LE, liquid expanded; LC, liquid condensed

Experimental

Materials

Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) were obtained from Fluka and used without further purification. Cholesterol and CPZ were purchased from Serva. Water used for the monolayer experiments was twice distilled in a full-quartz still. All experiments were performed on pure aqueous subphases containing the given amount of CPZ at a constant pH of 5.6 ± 0.2. CPZ stock solutions were stored in the dark at 4 °C and were used within three days. At CPZ concentrations below 1 m*M* the change in the surface tension due to the surface activity of the drug is negligible (Scholtan 1955).

Film balance

The surface tension was determined with a Langmuir film balance equipped with a Wilhelmy system. A piece of filter paper of 20 mm width was dipped in the subphase, and the forces on this plate were compensated by two strips of refined steel. The position of the plate was taken up by an inductive displacement transducer from Burster. After a set of measurements with increasing CPZ concentrations the filter paper was exchanged. For pressure calibration, the transition point of the palmitic acid monolayer was set to 22.4 mN/m (Albrecht and Sackmann 1980).

Monolayers

Monolayers of pure DMPC, DPPC or DPPC/cholesterol mixtures were spread from a solution of about 0.5 mg lipid/ml chloroform. For spreading, a 100 µl Hamilton syringe was slowly compressed to obtain an efflux rate of about 1.5 µl/s. The solution was guided to the water surface by a thin teflon tube without formation of droplets. This spreading procedure avoided disturbance of the surface. Pressure-area diagrams were taken exclusively by compression. The monolayer was equilibrated for at least 10 min. In that time, complete evaporation of the organic solvent was obtained. The compression rate was 0.053 Å^2/s for DMPC- and 0.043 Å^2/s for DPPC-monolayers. All experiments were performed at 21 °C. Each measurement was repeated at least 3–4 times. Areas are given as mean value per molecule in lipid-cholesterol mixtures.

Results

Typical pressure-area isotherms of DPPC (curve 1) and mixed DPPC-cholesterol monolayers (curve 2) spread on pure water and on a $3.2 \cdot 10^{-4}$ M solution of chlorpromazine (CPZ) as subphase (curve 3 and 4, respectively) are shown in Fig. 1. Curve 1 allows the comparison to a van der Waals transition with a coexistence region between the liquid expanded (LE) and the liquid condensed (LC) phase of the monolayer. The onset of the LE-LC transition is marked by the point P. The transition is less pronounced in cholesterol containing monolayers (e.g. Albrecht et al. 1981). In the presence of chlorpromazine the phase transition becomes less cooperative and an increase of the mean lipid area is clearly observable at a given pressure. The area increase is larger in pure DPPC-monolayers compared to the cholesterol containing monolayer.

In Fig. 2 this area increase caused by the incorporation of CPZ into the monolayer is given as function of the monolayer surface pressure. Again pure DPPC (curve 1) and cholesterol containing DPPC-monolayers (curve 2 and 3) are compared. The mole fraction of cholesterol was $X_{chol} = 0.17$ (curve 2) and $X_{chol} = 0.23$ (curve 3). At the given temperature of $T = 21$ °C and a CPZ-concentration of $3.2 \cdot 10^{-4}$ M in the subphase the area increase in pure DPPC-monolayers is maximal at a surface pressure of about 8 to 10 mN/m which is slightly above the LC-LE phase transition (e.g. Fig. 1, curve 1). At all pressures the relative increase is larger in pure than in cholesterol containing membranes. This is clearly demonstrated with curve 4 in Fig. 2 which is

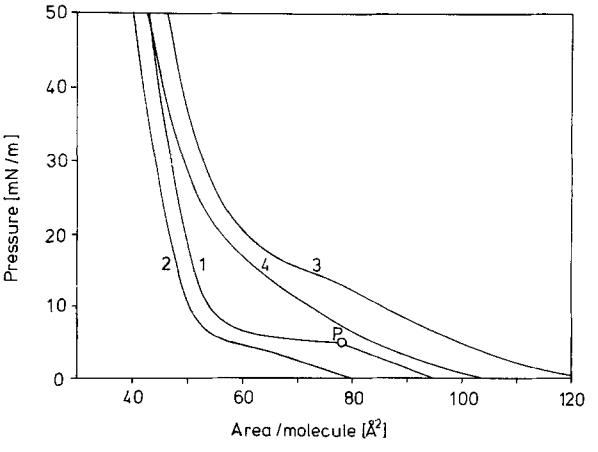

Fig. 1. Pressure-area diagrams of lipid monolayers taken at $T = 21$ °C. 1. Dipalmitoylphosphatidylcholine (DPPC) on pure water; 2. DPPC-cholesterol in a 5:1 Mol/Mol mixture on pure water; 3. DPPC on a solution containing $3.2 \cdot 10^{-4}$ M CPZ; 4. DPPC-cholesterol 5:1 Mol/Mol mixture on $3.2 \cdot 10^{-4}$ M CPZ. The point *P* characterizes the onset of the LC-LE transition

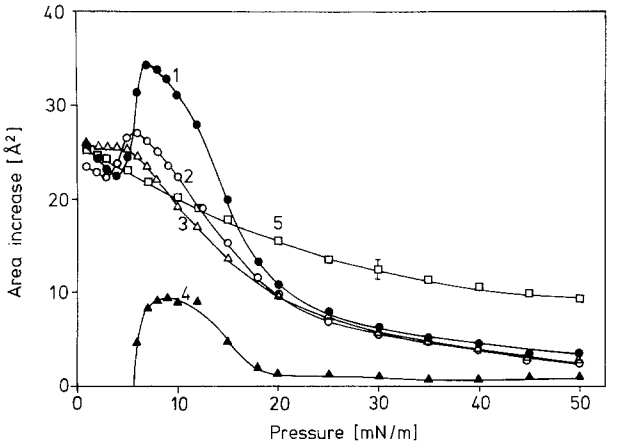

Fig. 2. Lipid area increase as a function of surface pressure in monolayers made of DPPC (curve 1 —●—), DPPC-cholesterol in a 5:1 Mol/Mol mixture (curve 2 —○—) and in a 3.3:1 Mol/Mol mixture (curve 3 —△—). Curve 4 (—▲—) gives the difference between curve 1 and 2 which clearly demonstrates the smaller uptake of chlorpromazine caused by addition of cholesterol. Curve 5 (—□—) stands for a DMPC-monolayer which is in the LE-phase over all the pressure range. The area increase between monolayers spread on a $3.2 \cdot 10^{-4}$ M solution of CPZ was continuously taken from pressure-area diagrams as shown in Fig. 1. All measurements were performed at 21 °C

the difference between curve 1 (pure DPPC) and curve 2. The deviation at pressures ~ 5 mN/m is not significant within the error of the determination and is therefore not included in the difference curve 4. At $X_{chol} = 0.23$ this maximum has nearly disappeared.

Curve 5 in Fig. 2 results from DMPC monolayers again spread on a subphase containing $3.2 \cdot 10^{-4}$ M CPZ. Note that DMPC is also in the LE-Phase at $\pi = 40$ mN/m. The area increase for this lipid with a shorter chain decreases monotonously. The area increase is always higher for the short chain lipid at high pressures ($\pi > 18$ mN/m), but is identical at $\pi \sim 3$ mN/m.

The effect of cholesterol in DPPC monolayers on CPZ-uptake is shown in Fig. 3 at different surface pressures. In the LE-phase, that is at $\pi \sim 5$ mN/m, and similarly in the LC-phase, that is at $\pi \gtrsim 10$ mN/m, a slight maximum uptake occurs at a cholesterol mole fraction of about $X_{chol} = 0.1$. However, the absolute area increase induced by CPZ is small. Near the phase transition ($\pi \sim 10$ mN/m) the large increase in the presence of CPZ in the subphase, as already demonstrated in Fig. 2, is drastically reduced from $\Delta A = 34$ Å^2 to $\Delta A = 20$ Å^2 at $X_{chol} = 0.23$. Control experiments using dimyristoylphosphatidylcholine, which forms monolayers in the LE-phase at 21 °C and the surface pressure used for this investigation, are in agreement with DPPC measurements in the LE-phase. The difference in the area increase

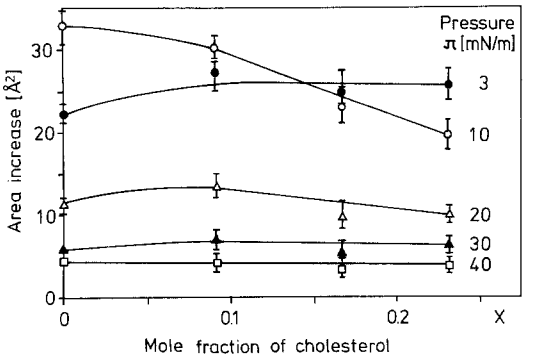

Fig. 3. CPZ-induced area increase of monolayers containing lipid-cholesterol mixtures. The area increase of DPPC-cholesterol monolayers spread on a $3.2 \cdot 10^{-4}$ M CPZ solution with respect to lipid monolayers spread on pure water is shown at different cholesterol concentrations

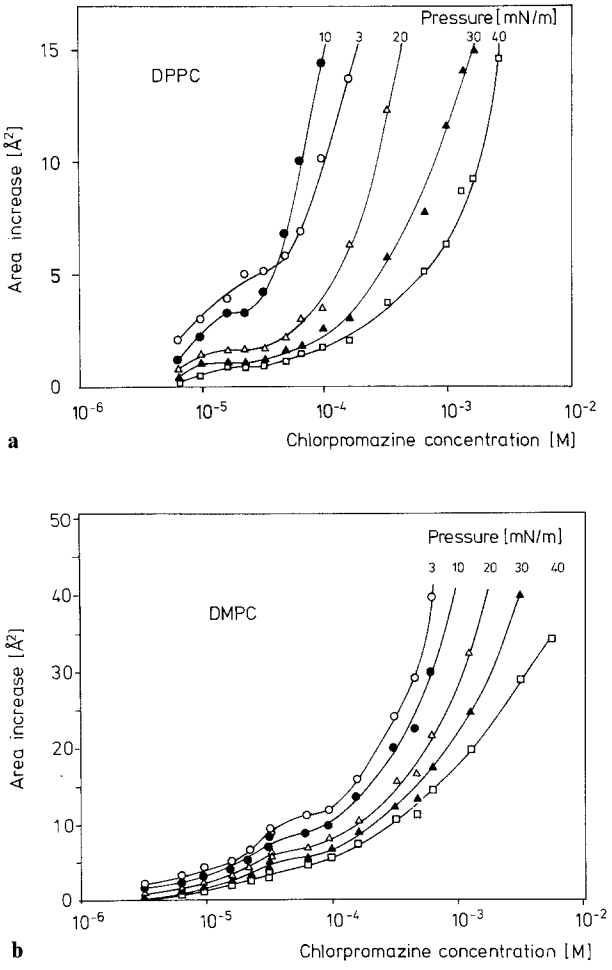

Fig. 4a and b. Area increase of phosphatidylcholine monolayers measured on aqueous subphases containing CPZ in various concentrations and at different surface pressures. **a.** Dipalmitoylphosphatidylcholine (DPPC), **b.** Dimyristoylphosphatidylcholine (DMPC). Note that the 10 mN/m curve in DPPC-monolayers crosses the 3 mN/m curve of the liquid expanded state

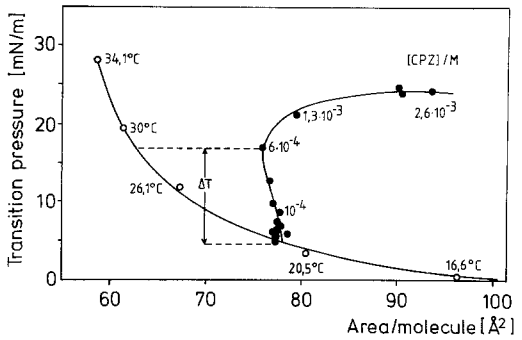

Fig. 5. Pressure-area diagram of the onset point P of the LE-LC phase transition of DPPC-monolayers taken at various temperatures (–○–○–○–) in the absence of CPZ and taken at various CPZ-concentrations at a given temperature of 21 °C. (●–●–●) (e.g. Fig. 1) Filled circles give different CPZ-concentrations, some of them are marked

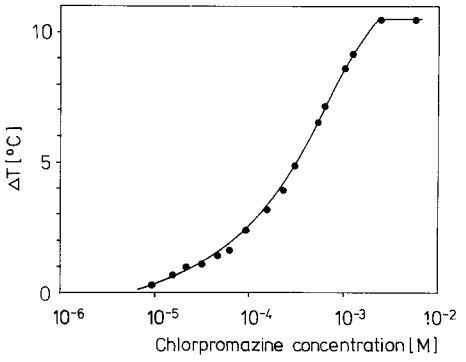

Fig. 6. The temperature difference ΔT taken from Fig. 5 is given as function of the corresponding CPZ-concentration

induced by CPZ in the absence and in the presence of cholesterol is less than 1 Å^2 at all surface pressures up to 40 mN/m (data not shown).

The area increase induced by CPZ at given surface pressures is shown in Fig. 4a for DPPC and Fig. 4b for DMPC-monolayers. In each diagram particular attention should be paid to the concentration range around $5 \cdot 10^{-5}$ M CPZ. At this concentration the slopes of all curves exhibit a plateau which is most clearly visible at low pressure. The strongest effect of CPZ in both types of phosphatidylcholine occurs at low pressure. In DPPC-membranes, however, this expansion is maximal around the phase transition (~ 10 mN/m) at CPZ-concentrations $> 5 \cdot 10^{-5}$ M.

The question arises whether the expansion of PC-monolayers is correlated with a temperature effect in the absence of CPZ. The position of the point P marked in the pressure-area diagram in Fig. 1 for onset of the LE-LC phase transition is given in Fig. 5 as function of temperature in the absence of CPZ and as function of CPZ-concentration at 21 °C. With increasing temperature the point P moves to higher pressure and lower area values (e.g. Albrecht et al. 1978). With increasing chlorpromazine concentration, P moves to higher pressure values and less pronounced to lower area values up to about 6×10^{-4} M of CPZ. Further increase of the CPZ-concentration exhibits an almost constant pressure but a drastic area increase for point P. Each black dot given in Fig. 5 stands for a point P determined at a different CPZ-concentration. Only a few concentrations are marked. We correlated each point with a temperature difference, ΔT, as demonstrated in Fig. 5. For example, a $6 \cdot 10^{-4}$ M CPZ-concentration corresponds to a temperature shift $\Delta T = 7$ °C. Figure 6 gives the complete curve of the ΔT-CPZ-concentration-correlation and clearly demonstrates that an increased CPZ-concentration is comparable to an increased temperature in the absence of the drug. A maximal corresponding temperature shift of $\Delta T = 10$ ° was obtained.

Discussion

The aim of the present paper was to study the influence of chlorpromazine on lipid monolayers. This is a continuation of our recent work on lipid bilayers (Luxnat and Galla 1986; Müller et al. 1986). In the first paper we reported a chlorpromazine partition which is highly dependent on the physical state and the composition of the bilayer membrane. The partition coefficient k_p of CPZ in DPPC-bilayer vesicles, for example, increases from about $k_p = 500$ to $k_p = 1{,}000$ at the pretransition and to $k_p = 3{,}500$ at the main transition. Equivalent results were obtained for DMPC bilayers, but the partition coefficient was considerably higher ($k_p = 5{,}400$ at 37 °C). Above the main phase transition k_p decreases with increasing temperature. Cholesterol considerably decreases k_p at all temperatures.

We were able to work out some physical parameters that determine amphipath solubility in membranes. Our model membrane study, in comparison with biological membranes, clearly disproved the recently established assumption of Singer and coworkers (Conrad and Singer 1979, 1981; Maher and Singer 1984) that biological membranes exhibit a totally different solubility behaviour for amphipaths. Our present monolayer study is in excellent agreement with our bilayer data. In principle it illuminates the anaesthetic-lipid interaction, which will now be discussed.

Chlorpromazine in the aqueous phase expands phosphatidylcholine monolayers and suppresses the lipid phase transition by broadening and reducing the cooperativity (e.g. Fig. 1). The DPPC-monolayer

expansion demonstrated in Fig. 2 is mainly marked at the lipid phase transition which occurs around $\pi = 6$ mN/m. This is in agreement with earlier data published by Albrecht et al. (1978). Below and above this pressure we observed a reduced expansion. This is not the case in DMPC-monolayers which are in the LE-phase in this pressure range at 21 °C. The area increase is monotonously decreasing with increasing pressure. The effect of a phase transition on amphipath incorporation is comparable to the drastically increased penetration of glucagon or melittin into lipid monolayers at such a phase transition (Hendrickson et al. 1983). In this paper as well as others using lipid bilayers (Papahadjopoulos et al. 1973; Galla et al. 1985; Marsh et al. 1976) the phase transition region is characterized by a maximal permeability. Here we reported the maximal incorporation of a drug into a lipid monolayer at the phase transition pressure. This is in agreement with the maximal incorporation of chlorpromazine at the thermotropic phase transition in lipid bilayers (Luxnat and Galla 1986).

Cholesterol is known to disturb defect structure in bilayer membranes (Sackmann et al. 1980). Moreover, it increases the order of fluid membranes and decreases the order of crystalline lipid phases (Marsh and Smith 1973). The effect of cholesterol on lipid monolayers has been investigated carefully by Albrecht et al. (1981). As expected from these results cholesterol drastically decreases the CPZ-uptake mainly at the phase transition. At a 3.3:1 molar ratio of lipid to cholesterol the maximum in the expansion has vanished. A summary of the effect of cholesterol on the CPZ-induced area increase is given in Fig. 3. Chlorpromazine incorporation is strongly antagonized by cholesterol only at the lipid phase transition.

We tried to correlate the effect of chlorpromazine with the effect of temperature on pure DPPC-membranes. The pressure/area correlation for the point P marked in Fig. 1 is plotted in Fig. 5 at different temperatures in the absence of chlorpromazine and at different chlorpromazine concentrations at 21 °C. It is interesting to follow the course of the point P at the phase transition. In DPPC-monolayers spread on a water subphase it is comparable to a van der Waals gas. P moves to higher pressures and lower areas with increasing temperature. This is not the case with increasing CPZ-concentration. P moves upward almost vertically but not along the coexistence boundary of pure DPPC. The almost constant area at the transition pressure may be due to the space in the monolayer taken up by the drug. Now the amount of CPZ in the monolayer can be estimated from the area occupied by a CPZ-molecule. Assuming an orientation vertical to the monolayer we obtain an area of about 30 Å^2 for its projection. Then the area difference of 15 Å^2 determined at 30 °C (e.g. Fig. 5) corresponds to a concentration of about 30 Mol % CPZ with respect to the lipids of the monolayer.

At CPZ-concentrations $C > 10^{-3}$ M, P moves horizontally only to higher areas. This may be an effect of the observed lowering of the surface tension at chlorpromazine concentrations above 10^{-3} M (Scholtan 1955). On the other hand, up to $6 \cdot 10^{-4}$ M chlorpromazine seems to be incorporated into the monolayer inducing a fluidisaton observable by the shift in P.

A difference in CPZ-concentration can now be attributed to a temperature difference ΔT which is given in Fig. 6 as function of CPZ-concentration. A 10^{-3} M CPZ-concentration in the subphase is equivalent to a temperature shift of 10 °C. Again a good correlation is found with bilayer vesicles where we observed a decrease of the lipid phase-transition $\Delta T = 7$ °C caused by $2.2 \cdot 10^{-3}$ M CPZ. However, it should be noted that the chlorpromazine concentration is not comparable in both types of experiments because of the completely different lipid/water ratio in monolayer and bilayer experiments.

The last point to discuss is the area increase as function of CPZ-concentration determined at different surface pressures (Fig. 4). As was expected, the expansion induced by the CPZ-uptake increases with CPZ-concentration and decreases with increasing surface pressure. This is true for DPPC as well as for DMPC-monolayers. Note the higher uptake at 10 mN/m in the phase transition region.

Interesting is the concentration region between $3 \cdot 10^{-5}$ and 10^{-4} M. The area increase flattens but a further increase was observed at $C > 10^{-4}$ M. This anomaly may be correlated with recent fluorescence data where we observed the formation of CPZ-aggregates at concentrations above $5 \cdot 10^{-5}$ M in salt containing solution (Luxnat and Galla 1986). In pure water this value is slightly shifted to $4 \cdot 10^{-5}$ M (Luxnat, private communication). The break in the expansion curves of Fig. 4 may also be explained by the formation of CPZ-micelles in the subphase leading to the plateaus in Fig. 4a and b. If aggregates are embedded in the monolayer this would explain the area increase demonstrated in Fig. 5 without changing the pressure at high CPZ-concentrations.

To conclude: from chlorpromazine partition coefficients in bilayer membranes we know that chlorpromazine is able to penetrate into artificial bilayer systems as well as into biological membranes. Now, comparable results are obtained from Langmuir film balance experiments. In both cases CPZ induces

a fluidisation of phospholipid layers. The highest CPZ uptake was observed near the phase transition of the lipid. Cholesterol diminishes the CPZ uptake. At a CPZ-concentration of $5 \cdot 10^{-5}$ M aggregation occurs which should be taken into account in every discussion concerning amphipath-lipid interaction.

Acknowledgements. We wish to thank Mrs. A. Minde for her assistance in the text preparation and Mrs. J. Bendel for the drawings.

This work was supported by the Deutsche Forschungsgemeinschaft under contract Ga 233/9.

References

Albrecht O, Sackmann E (1980) A precision Langmuir film balance measuring system. J Phys E (Sci Instrum) 13:512–515

Albrecht O, Gruler H, Sackmann E (1978) Polymorphism of phospholipid monolayers. J Phys (Paris) 39:301–313

Albrecht O, Gruler H, Sackmann E (1981) Pressure-composition phase diagrams of cholesterol/lecithin, cholesterol/phosphatidic acid and lecithin/phosphatidic acid mixed monolayers: A Langmuir film balance study. J Colloid Interface Sci 79:319–338

Conrad MJ, Singer SJ (1979) Evidence for a large internal pressure in biological membranes. Proc Natl Acad Sci USA 76:5202–5206

Conrad MJ, Singer SJ (1981) The solubility of amphipathic molecules in biological membranes and lipid bilayers and its implication for membrane structure. Biochemistry 20: 808–818

Forrest BJ, Linehan PTP, Mattai J (1984) Interactions of a phenothiazine tranquilizer with phosphatidylcholine and phosphatidylcholine/cholesterol membranes. Biochemistry 23:2288–2293

Galla HJ, Trudell JR (1981) Perturbation of peptide-induced lateral phase separations in phosphatidic acid bilayers by the inhalation anesthetic methoxyflurane. Mol Pharmacol 19:432–437

Galla HJ, Warncke M, Scheit KH (1985) Incorporation of the antimicrobial protein seminalplasmin into bilayer membranes. Eur Biophys J 12:211–216

Hendrickson HS, Fan PC, Kaufman DK, Kleiner DE (1983) The effect of a phase transition on penetration of phospholipid monolayers by melittin and glucagon. Arch Biochem Biophys 227:242–247

Lee AG (1976) Model for action of anaesthetics. Nature 262:545–548

Luxnat M, Galla HJ (1986) Partition of chlorpromazine into lipid bilayer membranes: the effect of membrane structure and composition. Biochim Biophys Acta 856:274–282

Maher P, Singer SJ (1984) Structural changes in membranes produced by the binding of small amphipathic molecules. Biochemistry 23:232–240

Marsh D, Smith ICP (1973) An interacting spin label study of the fluidizing and condensing effects of cholesterol and lecithin bilayers. Biochim Biophys Acta 298:133–144

Marsh D, Watts A, Knowles PF (1976) Evidence for phase boundary lipid-permeability of tempocholine into DML-vesicles at the phase transition temperature. Biochemistry 15:3570–3578

Müller HJ, Luxnat M, Galla HJ (1986) Lateral diffusion of small solutes and partition of amphipaths in defect structures of lipid bilayers. Biochim Biophys Acta 856:283–289

Papahadjopoulos D, Jacobson K, Nir S, Isac T (1973) Phase transitions in phospholipid vesicles. Fluorescence polarisation and permeability measurements concerning the effect of temperature and cholesterol. Biochim Biophys Acta 311:330–348

Pjura WJ, Kleinfeld AM, Karnovsky MJ (1984) Partition of fatty acids and fluorescent fatty acids into membranes. Biochemistry 23:2039–2043

Roth SH (1979) Physical mechanisms of anaesthesia. Annu Rev Pharmacol Toxicol 19:159–178

Sackmann E, Rüppel D, Gebhardt C (1980) Defect structure and texture of isolated bilayers of phospholipids and phospholipid mixtures. In: Helfrich W, Heppke G (eds) Liquid crystals of one- and two-dimensional order. Springer, Berlin Heidelberg New York, pp 309–326

Scholtan W (1955) Kolloidchemische Eigenschaften von Phenothiazinderivaten. Kolloid-Z 142:84–104

Seeman P (1972) The membrane actions of anaesthetics and tranquilizers. Pharmacol Rev 24:583–655