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Biochemical Pharmacology 61 (2001) 817–825

Biochemical Pharmacology

Chorpromazine-induced increase in dipalmitoylphosphatidylserine surface area in monolayers at room temperature

Armelle Varnier Agasøsler [a,*], Laila M. Tungodden[a], Daniel Čejka[a], Einar Bakstad[b], Leiv K. Sydnes[b], Holm Holmsen[a]

[a]*Department of Biochemistry and Molecular Biology, University of Bergen, Arstadveien 19, N-5009 Bergen, Norway*
[b]*Department of Chemistry, University of Bergen, N-5009 Bergen, Norway*

Received 19 July 2000; accepted 27 September 2000

Abstract

The Langmuir technique revealed that the surface area of acidic glycerophospholipids (dipalmitoylphosphatidylserine, -glycerol, and dipalmitoylphosphatidic acid) in monolayers increased dramatically when micromolar concentrations of the antipsychotic drug chlorpromazine (CPZ) were present in the subphase. Monolayers of neutral glycerophospholipids (dipalmitoylphosphatidylcholine and -ethanolamine) did not show such a large effect with CPZ. Compared to CPZ, millimolar concentrations of the monovalent cations Li^+, K^+, Na^+, Rb^+, and Cs^+ did not appear to influence the dipalmitoylphosphatidylserine monolayer, suggesting that the effect of CPZ, a monovalent cationic amphophile, was due to an interaction with the acyl chains of the lipids. In addition, the effect of CPZ was reduced by 150 mM Na^+, suggesting that the sodium cations might screen the negatively charged headgroups from an electrostatic interaction with the positively charged drug molecule. Two CPZ analogs, chlorpromazine sulfoxide and CPZ with 2 carbons in the side chain, were also studied. These observations suggest that part of the biological effects of CPZ, being antipsychotic and/or side effects, may be due to CPZ's action on the acidic glycerophospholipids in nerve cell membranes.

Keywords: Chlorpromazine; Dipalmitoylphosphatidylserine; Langmuir; Membrane; Monolayer

1. Introduction

CPZ, a cationic, amphophilic phenothiazine derivative, is widely used as an antipsychotic drug and has many diverse biological effects. The antipsychotic effect has been ascribed to CPZ's powerful antagonism of dopaminergic receptors [1], without taking into account the effect of the phenothiazine on numerous other well-known cellular responses to CPZ (see below). A serious concern in the experimental delineation of the action of CPZ on intact cells is that the drug damages the plasma membrane of cells at higher concentrations. Thus, in concentrations of 100 μM and higher, CPZ makes 14 Å holes in red cell membranes [2] and damages myocardial cells in culture [3]. Studies with human platelets have shown that CPZ [4,5] and the structurally related trifluoperazine [6] in concentrations above 40 μM render the cells permeable to low-molecular-weight substances such as ATP, ADP, glucose 6-phosphate, and fructose 1,6-bisphosphate, but not to lactate dehydrogenase, which is commonly used to monitor cell lysis. Unfortunately, a large proportion of studies on the effects of CPZ on intact cells has employed concentrations of CPZ above 40 μM. This causes ATP depletion and cessation of ATP turnover [5], which well may have been causing the large number of cellular responses reported; these studies are not referred to herein.

In addition to binding to the D_2 dopamine receptor in striatal membranes [7], non-permeabilizing concentrations of CPZ were shown to induce a decrease in serotonin level in the central nervous system of the pond snail *Lymnaea stagnalis* [8]. At low concentrations, CPZ increases the activity of the *N*-methyl-D-aspartate (NMDA) receptors. Experiments in rat striatal slices indicated a maximal enhance-

* Corresponding author. Tel.: +47-55-58-64-42; fax: +47-55-58-64-00.

E-mail address: armelle.varnier@pki.uib.no (A. Varnier).

Abbreviations: CPZ, chlorpromazine; CPZ SO, chlorpromazine sulfoxide; CPZ 2C, chlorpromazine with a 2-carbon side chain; DPPA, dipalmitoylphosphatidic acid; DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DPPG, dipalmitoylphosphatidylglycerol; DPPS, dipalmitoylphosphatidylserine; and MMA, mean molecular area.

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PII: S0006-2952(01)00542-1

ment of this activity at a CPZ concentration of 0.1 μM. Interestingly, the influence on NMDA receptors was shared with other antipsychotic agents, but not with drugs with high affinity for D_2 dopamine receptors that lack antipsychotic effects [9]. The mechanisms by which CPZ acts as an antipsychotic remain to be clarified. In addition, a wide range of effects of CPZ has been reported. Thus, CPZ was shown to be a calmodulin antagonist [10,11]. In insuloma cells, CPZ and related phenothiazines at 10-μM concentration were reported to inhibit the ATP-sensitive K^+ channels [12,13], and micromolar concentrations of CPZ inhibited the voltage-dependent sodium current in rat hippocampal pyramidal neurones [14]. CPZ increased choline uptake across the plasma membranes of myocytes [3] and interfered with polyphosphoinositide metabolism in platelets [15,16]. In addition, non-permeabilizing concentrations of CPZ have been reported to act as an antineoplastic agent [17] and to delay mitosis in human lymphocytes [18]. Interestingly, the protective effect of CPZ on osmotic hemolysis of red cells was dependent on the proportion of membrane linoleate [19], sustaining the idea of a close relationship between the drug's action and the fatty acid composition of the plasma membrane. In contrast, chlorpromazine sulfoxide, a major metabolite in CPZ detoxification [20], is pharmacologically inactive. In rat hepatocytes, in contrast to CPZ, its sulfoxide did not inhibit the Na^+-K^+-ATPase cation pumping [21]. Common structural features among the antipsychotic phenothiazines are the length of the side chain (three-carbon chain) between the ring and chain nitrogen [22].

The various effects of the amphophilic CPZ on processes taking place in biological membranes (above) have prompted many physico-chemical studies of the interaction between CPZ and glycerophospholipids. Thus, ESR studies by Luxnat and Galla [23] established that the lipid bilayer structure in diacylphosphatidylcholine liposomes was not altered below 30 μM CPZ in the bulk phase, while mixed micelles formed above this concentration. Pharmacokinetic data [22] reported a CPZ concentration in the plasma of treated patients between 30 and 350 ng/mL, with 95–98% bound to plasma proteins. Thus, the free concentration of chlorpromazine found in patients was lower than 0.05 μM; however, the amount of CPZ bound to cell membranes was not determined, and possibly varies from tissue to tissue. Luxnat and Galla also reported that the partition coefficient K_p for CPZ between the aqueous and lipid phases was dependent on the type of acyl groups in the phospholipids and more generally that K_p was influenced by the physical state of the membrane. Zachowski and Durand [24] elucidated this further by showing that CPZ bound to biological and artificial membranes in a biphasic manner: high- and low-affinity binding with the dissociation constant K_d in the 8–28 and 50–240 μM range, respectively, with the high-affinity binding being caused by simple partitioning (the amount of drug bound to the membrane is proportional to the amount added) and the variation in K_d being dependent on the type of lipid used, among other factors. Since the membranes used in these studies were either composed of several phospholipid classes or neutral phospholipids, the variation in K_d may be explained by the findings that CPZ forms tight 1:1 complexes with phosphatidic acid and phosphatidyl inositol [25], but not with neutral glycerophospholipids such as phosphatidyl choline. Other authors have observed a greater affinity of CPZ for acidic as compared to neutral phospholipids [26]. ESR studies on liposomes have shown that CPZ caused pronounced perturbation of membranes (high dynamics and/or low order) at 0.1–1 CPZ/lipid ratios, and that the degree of perturbation depended strongly on the lipid composition of the liposomes [27]. The action of CPZ was proposed to eliminate the phospholipid headgroup influence on the lipid phase transition temperature, in accordance with the finding that the induced CPZ phase transition T_t of CPZ–phospholipids was the same for neutral as for acidic phospholipids, and dependent on the drug molecule used [28].

The present study was undertaken to further explore the interaction between CPZ and glycerophospholipids in monolayers with the Langmuir–Blodgelt technique to measure the surface pressure as a function of the lipid's apparent molecular surface area. We employed glycerophospholipids with the same acyl groups but with different headgroups. Then, we focused our efforts on the DPPS monolayer to define the role of the electrostatic interactions in the effect of the drug molecule on the lipids. The DPPS monolayer was studied with increasing size alkaline monovalent cations Li^+, K^+, Na^+, Rb^+, and Cs^+ present in the monolayer's subphase, simultaneously with CPZ and Na^+. The last part of this study compares different CPZ analogs in their action on a DPPS monolayer.

2. Materials and methods

2.1. Lipids and chemicals

DPPC, DPPE, and DPPA were purchased from Sigma, DPPS was obtained from Avanti Polar Lipid, and DPPG was from Matreya and used without further purification. The lipids were dissolved at 1 mg/mL in chloroform (>99%) purchased from Merck. CPZ–HCl was from Sigma, while CPZ-SO and CPZ-2C were synthesized by ourselves. The salts LiCl and RbCl were purchased from Sigma, and NaCl, KCl, and CsCl were from Merck. Milli-Q water was used throughout this study. It was cleansed from inorganic ions and from dissolved aromatic contaminants until the water resistance achieved 18 MΩ/cm at room temperature.

2.2. Surface pressure/molecular area isotherms

Surface pressure/molecular area isotherms were obtained at room temperature with a Langmuir–Blodgelt minitrough

1 from KSV Instruments Ltd. using the manufacturer's software connected to an IBM AT-compatible PC. The Teflon trough (75 mm × 364 mm × 5 mm) was filled with milli-Q water with or without various drugs and alkaline monovalent cations. The surface was swept and the possible impurities removed from the air/water interface with a Pasteur pipette. Twenty microliters of glycerophospholipid in chloroform was carefully spread at the surface with a Hamilton syringe, and the chloroform was allowed to evaporate before the measurements started.

2.3. Surface pressure measurement

Compressions of the lipid monolayers were done at 5 and 10 mm/min while an electrobalance recorded the surface tension with a Wilhelmy plate. The surface tension of the film-free solution was taken as a reference. For each system, three surface pressure/area curves were recorded, and the most representative shown in the figures. Penetration of CPZ and its analogs into the monolayer kept at constant surface pressure (20 mN/m) was followed by measuring the lipid's apparent molecular area with time. Three parallels were done and the percent area increase of the lipid molecule was plotted as a function of the drug (or its analogs) concentration.

2.4. Organic synthesis

2-Chloro-10-(3-dimethylaminopropyl)phenothiazine 5-oxide (CPZ SO) hydrochloride was prepared by oxidation of chlorpromazine with sodium perborate in acetic acid [29]. The product was obtained pure in 90% yield and m.p. 211–214° (m.p. 213–215° [30]).

2-Chloro-10-(2-dimethylaminoethyl)phenothiazine (CPZ 2C) hydrochloride was synthesized from 2-chlorophenothiazine. Treatment with ethylmagnesium bromide in dry tetrahydrofuran followed by 2-chloro-1-dimethylaminoethane gave CPZ 2C, which was extracted with diethyl ether. The final product was obtained pure in 95% yield and m.p. 217–220° (m.p. 220–221° [31]).

3. Results

3.1. Effect of the glycerophospholipid headgroup on the increase in surface area caused by CPZ

The surface pressure isotherms for DPPC and DPPE monolayers appeared to be slightly dependent on the CPZ concentration in the subphase (Fig. 1). Due to the presence of 100 μM CPZ in the subphase, DPPC isotherms exhibited a shift in the surface pressure liftoff from the basal line from a molecular surface area of 74 Å2 to 92 Å2. With the same amount of CPZ, the onset area of the phase transition plateau increased from 58 to 64 Å2, and the surface pressure at this onset was raised from 7 to 10 mN/m. The collapse of the monolayer occurred at a similar surface pressure (around 60 mN/m). Finally, we observed that all DPPC isotherms had an identical limiting area that was independent of the drug concentration. In contrast to DPPC, none of the curves obtained with DPPE revealed any phase transition plateau. A difference in the surface pressure/area curves was noticed for the liftoff area that increased from 50 Å2 to 90 Å2 with 100 μM CPZ.

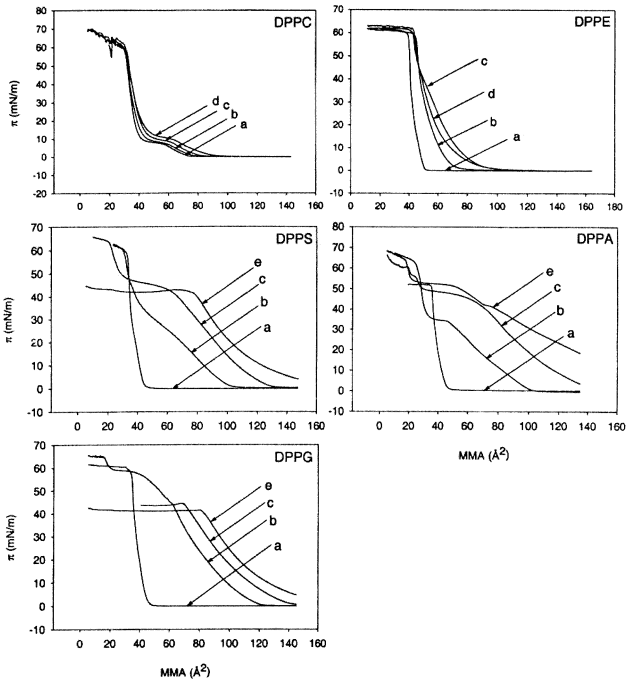

Fig. 1. Surface pressure π versus apparent surface molecular area (MMA) at room temperature for various phospholipids with 0 (a), 1 (b), 10 (c), 100 (d), and 1000 μM (e) subphase CPZ concentrations.

For the anionic glycerophospholipids DPPS, DPPA, and DPPG, CPZ induced very large effects as was also observed by Hanpft *et al*. with liposomes of DPPA and DPPG [28]. Moreover, the antipsychotic drug induced a phase transition plateau in the surface pressure isotherms with DPPS and DPPA. At 30 mN/m, only the negatively charged glycerophospholipids exhibited a large and similar CPZ-induced monolayer expansion (Fig. 2). A 1-μM CPZ concentration was enough to cause a DPPS and DPPA area increase of around 40% and a 90% increase for DPPG, but penetration of CPZ into the monolayer kept at constant surface pressure (20 mN/m) shows that these values might not correspond to equilibrium values (Fig. 7). With 10 μM CPZ, the expansion was 120% for all three acidic lipid classes. Here also, these values can be compared only with the other data measured in compression experiments. At larger concentrations, an apparent saturation effect was seen (Fig. 2).

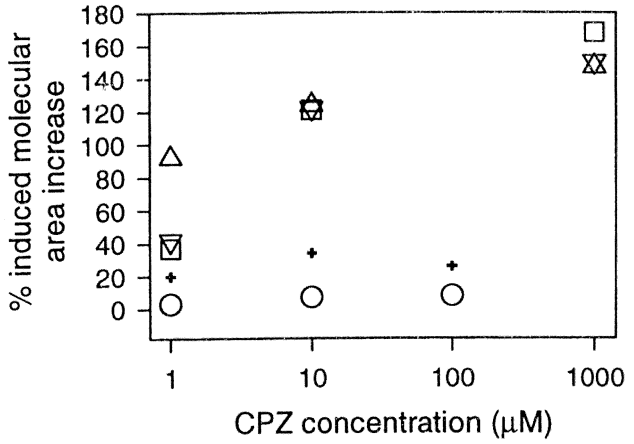

Fig. 2. Percentage of induced apparent molecular area increase in DPPC (○), DPPE (+), DPPS (▽), DPPA (□), and DPPG (△) at 30 mN/m versus CPZ concentration.

3.2. Effect of alkaline monovalent anions on surface pressure/area curves for DPPS

The effect on the DPPS surface area, as induced by different-sized monovalent cations such as chloride salt: Li^+, Na^+, K^+, Rb^+, and Cs^+ at 2- and 150-mM concentrations, was small compared to the increase caused by micromolar concentrations of CPZ, and no phase transition plateau was observed. Fig. 3 shows the surface pressure plotted versus the surface area for a monolayer spread on NaCl and LiCl solutions. Surface pressure/area curves with K^+, Rb^+, and Cs^+ are not shown here: they were identical to the isotherms obtained with Na^+. All cations, except Li^+, induced an increase in the area at the liftoff from 46 Å2 to 50 and 54 Å2 with 2- and 150-mM cation concentrations, respectively. LiCl (2 mM) did not show any differences in the effect on the monolayer compared with the other ions. With 150 mM LiCl, the area increase at the liftoff was slightly less than with the other ions at the same concentration. Here, the limiting DPPS areas were not influenced by the alkaline monovalent cations. The size of monovalent cations we used did not influence the monolayer in a detectable manner. The area increase at 30 mN/m appeared to be dependent only on the ion concentration. This expansion was smaller than 3% with 2 mM ions and gave an increase with 150 mM between 5 and 10%.

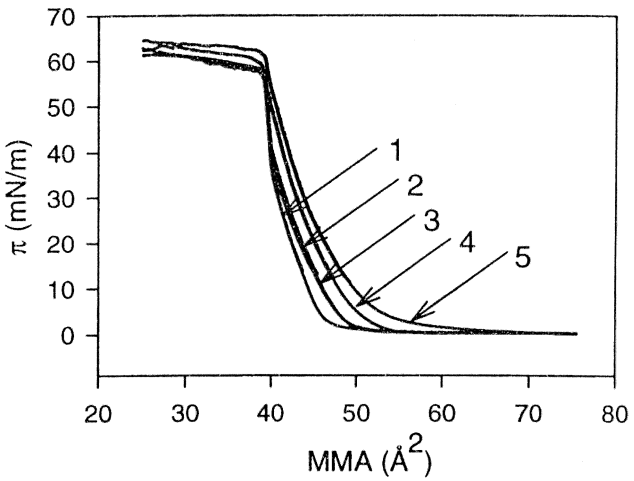

Fig. 3. Surface pressure π versus dipalmitoylphosphatidylserine apparent molecular surface area (MMA) at room temperature with 0 mM cations (1), 2 mM NaCl (2), 2 mM LiCl (3), 150 mM LiCl (4), and 150 mM NaCl (5).

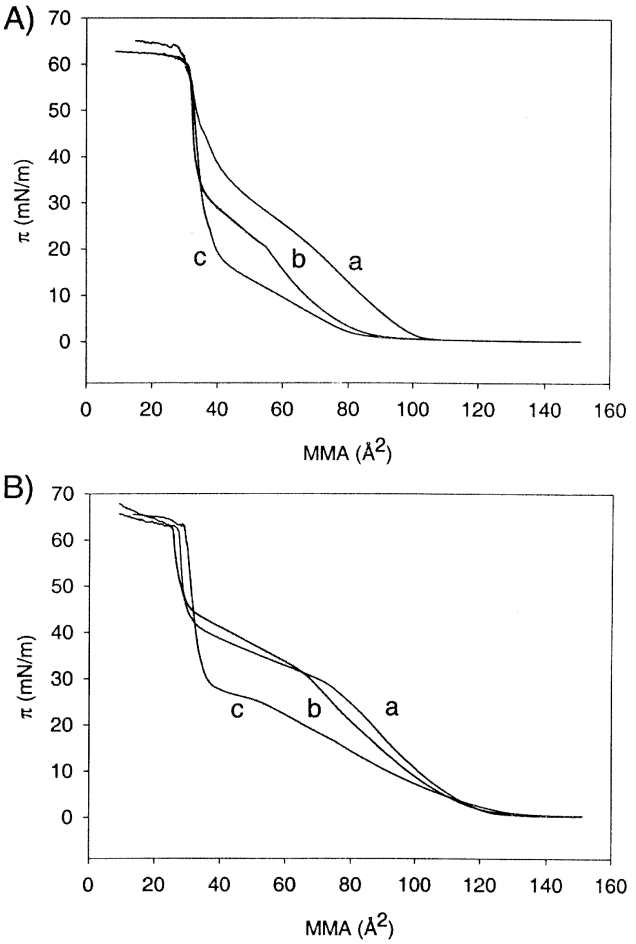

Fig. 4. Surface pressure isotherm for a DPPS monolayer spread at room temperature on (A) 1 μM CPZ and (B) 20 μM CPZ subphase with 0 (a), 2 (b), and 150 (c) mM NaCl.

3.3. Modulations of the CPZ-induced increase in the DPPS area as a function of subphase ionic strength

In order to observe if the adsorption and the effect of CPZ on a DPPS monolayer were affected by the presence of monovalent cations in the subphase, surface pressure isotherms were run for the acidic phospholipids spread on aqueous subphase with different CPZ/NaCl composition (Fig. 4). Except for the subphase containing 1 μM CPZ and 150 mM NaCl, all DPPS isotherms gave a phase transition plateau. The limiting areas obtained with 1 μM CPZ were independent of the NaCl concentration. This was not the

case for the higher CPZ concentration (Fig. 4B). Common to the two different CPZ concentrations was the shift toward a smaller molecular area induced by NaCl. The percentage of the increase in the area of DPPS compared to its area on pure milli-Q water was plotted for the isotherm's liftoff and for a surface pressure of 30 mN/m (Table 1). At low surface pressure, the increase in the apparent molecular area appeared to be largely governed by CPZ, and was relatively independent of the NaCl concentration. At 30 mN/m, this was no longer the case. Though there were no variations between the area increase with the systems containing 1 μM CPZ, the larger concentration of CPZ revealed the importance of the NaCl concentration on the effect of the drug on the DPPS monolayer. At this surface pressure, the molecular area increase induced by 20 μM CPZ (100% over 0 μM CPZ) vanished when 150 mM NaCl was also present in the monolayer's subphase. The onset pressure of the expanded to condensed phase transition plateau was dependent on the subphase composition. For 20 μM CPZ, 150 mM NaCl induced a lowering of the surface pressure at the transition onset from 30 to 25 mN/m. For 1 μM CPZ, the same Na^+ concentration caused this transition plateau to vanish.

3.4. Effect of chemical modification of CPZ on DPPS isotherms

DPPS surface pressure isotherms were recorded with two CPZ analogs shown in Fig. 5. One had a 2-carbon side chain, instead of 3 carbons, and the other molecule was the sulfoxide form of CPZ. The surface pressure/area curves were largely dependent on the drug used and on its concentration (Figs. 6 and 7). It appeared that the drug's potential to induce an increase in the apparent DPPS molecular area decreased in the order CPZ > CPZ 2C > CPZ SO. Although all the drugs induced a transition plateau at a 10-μM concentration, the surface pressures at which the transition plateau occurred were also dependent on the nature of the drug, and their values decreased in the order CPZ > CPZ 2C > CPZ SO. In addition, the limiting areas with 10 μM CPZ and CPZ 2C were significantly smaller than with pure milli-Q water, and the induced decrease was largest with CPZ. In addition, the percentage of the induced increase in DPPS area at the liftoff and at 30 mN/m was influenced by the molecular characteristics and the concentration of the drug used (Table 1). At 30 mN/m, the drug-induced increase in the molecular area was dependent on the drug concentration. The apparent expansion was larger with CPZ than with CPZ 2C, while with CPZ SO no significant change was seen (Fig. 6 and Table 1). This was not the case for the liftoff point. The behavior of the area increase was dependent on the drug concentration: at a 1-μM concentration, the drug's potential to increase the DPPS area was again strongest for CPZ and weakest for CPZ SO. Surprisingly, however, this tendency was reversed at the 10-μM concentration, where CPZ SO induced a larger area increase compared to CPZ.

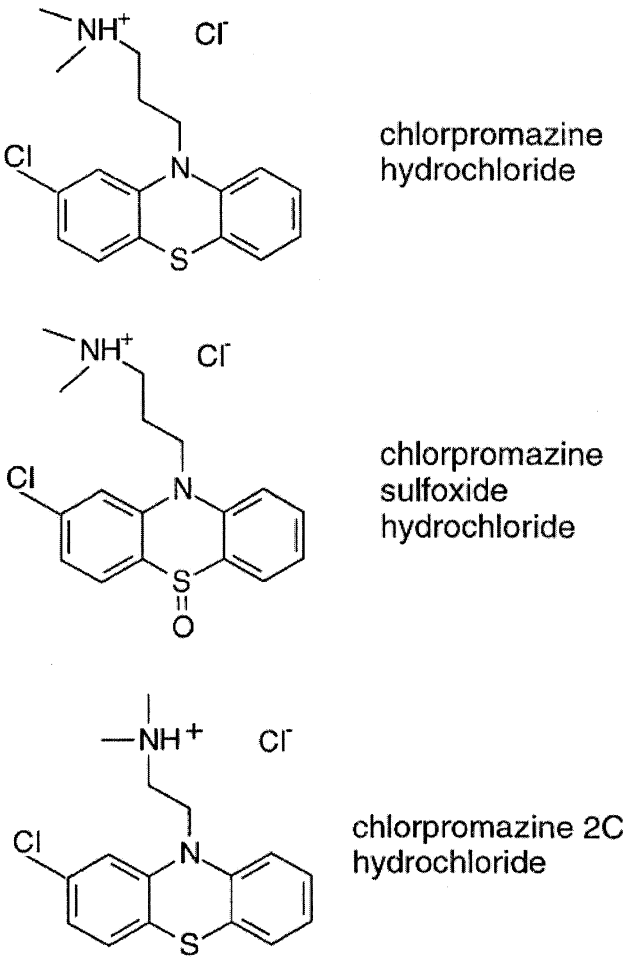

Fig. 5. The different drugs solubilized in the subphase of a DPPS monolayer.

Though the equilibrium areas were probably not reached in the previous compression experiments, penetration of CPZ and its analogs into DPPS monolayer at constant surface pressure confirms the larger effect of CPZ compared to CPZ 2C, while CPZ SO did not induce any increase in the lipid surface molecular area (Fig. 7).

4. Discussion

4.1. Influence of the phospholipid headgroup in the interaction with CPZ with monolayers on pure water

The melting temperatures for the lipids used in this study have been previously reported to be 42° for DPPC, 63.5° for DPPE at pH 8 [32], 53° for DPPS at neutral pH [33], 63° for DPPA, and 42° for DPPG in bilayers [28]. In monolayers, at room temperature and at surface densities equivalent to those found in vesicles, the monolayers of the studied lipids

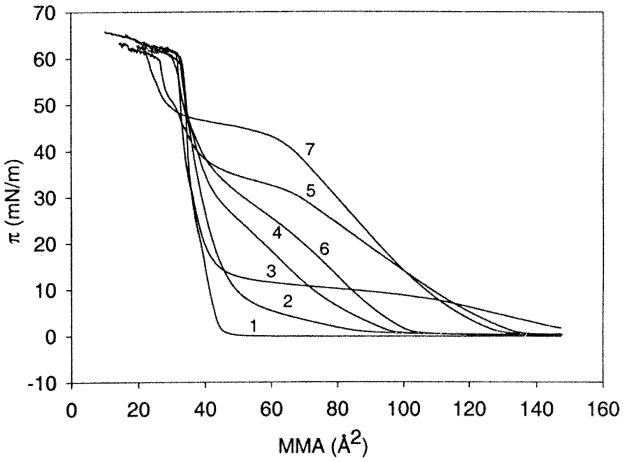

Fig. 6. Surface pressure/area curve for a DPPS monolayer spread on milli-Q water (1), 1 μM CPZ SO (2), 10 μM CPZ SO (3), 1 μM CPZ 2C (4), 10 μM CPZ 2C (5), 1 μM CPZ (6), and 10 μM CPZ (7).

Table 1
Apparent molecular surface area increase for DPPS

Subphase composition	% DPPS area increase	
	At liftoff	At 30 mN/m
1 μM CPZ	131	42
10 μM CPZ	183	122
20 μM CPZ	188	20
2 mM NaCl	9	1
150 mM NaCl	54	7
1 μM CPZ + 2 mM NaCl	102	9
1 μM CPZ + 150 mM NaCl	95	3
20 μM CPZ + 2 mM NaCl	188	91
20 μM CPZ + 150 mM NaCl	223	3
1 μM CPZ SO	91	8
1 μM CPZ 2C	116	19
10 μM CPZ SO	244	0
10 μM CPZ 2C	200	92

The apparent molecular surface area increase (%) for a DPPS monolayer spread on different subphases was compared with its molecular area on pure milli-Q water at the liftoff point and the surface pressure 30 mN/m.

can be considered to be under their phase transition temperature. The charge of the phospholipids depends on a large set of various and related parameters such as temperature, surface density, the packing of the monolayer, and the ionic strength of the subphase [34]. In addition, these experiments were done on pure water. Therefore, no control of the charge state of the monolayer at different compression stages was possible. However, monolayers of lipid species with small intrinsic pK values such as DPPS, DPPA, and DPPG were markedly fluidized by micromolar concentrations of CPZ compared to DPPC and DPPE monolayers. Thus, it might be concluded that the negative charge on the phospholipid headgroup is decisive for the large CPZ-induced monolayer fluidization. This result is supported by the studies of Dachary-Prigent *et al*. [26], who proposed that electrostatic interactions with the positively charged CPZ favor the dispersion in negatively charged membranes, although partition of CPZ was also observed in DPPC [35]. Surprisingly and contrary to the expected effect of repulsion forces between anionic lipids, DPPS, DPPA, and DPPG monolayers appeared to be in a condensed phase at low surface pressures, while the uncharged DPPC was in an expanded phase. The supposed presence of the negative charge on the headgroup may cause a higher degree of hydration and/or induce stronger headgroup interaction [33].

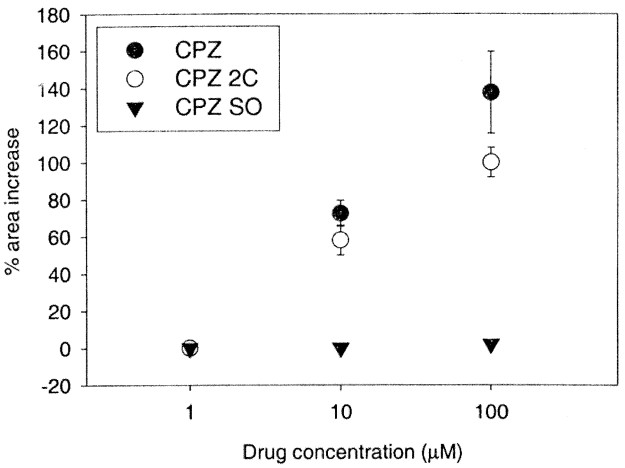

Fig. 7. % surface area increase in DPPS in monolayers at 20 mN/m, induced by different drug concentrations.

4.2. Interaction of DPPS with alkaline monovalent cations

It has been assumed that the salt-induced effect on glycerophospholipid packing was principally due to electrostatic screening [36]. Though the monovalent cations did not appear to cause a drastic effect on the monolayer measured by the Langmuir technique, the increase in the surface pressure at collapse onset as the salt concentration increased supports the idea of a stabilizing effect of the salts on DPPS monolayer. Our findings that no difference could be observed with the Langmuir technique between the inducing effect of Na^+, K^+, Rb^+, and Cs^+ is in opposition to the different intrinsic association constants measured by Eisenberg *et al*. [37] for Li^+, Na^+, K^+, Rb^+, and Cs^+ with multilamellar phosphatidylserine vesicles that were estimated to 0.8, 0.6, 0.15, 0.08, and 0.03, respectively. This might be due to a lower potential of the Langmuir technique to reveal the difference in association constants. However, as revealed by molecular dynamics simulations of DPPS bilayer in the presence of water [38], the degrees of diffusion and orientation of water molecules at the polar interface are also dependent on the nature of the cation [38]. The slight difference noticed for the Li^+ cation, compared with the isotherms recorded with Na^+, K^+, Rb^+, and Cs^+, is

supported by Hauser *et al*. [39]. They suggested that the characteristic action of Li^+ on a phospholipid membrane was to dehydrate the polar region of the lipids, inducing a closer packing of the lipids than with the other monovalent cations. Then, the difference observed with Langmuir between Li^+ and the other cations would be due to a lower hydration state and/or a change in the orientation of the DPPS polar headgroups. Li^+ is also a widely used medication in the treatment of manic depression. Therefore, it might be possible that the action of Li^+ is mediated by changes in the membrane packing. In addition, Mattai *et al*. [40] showed that a small reduction in the area of DPPS resulted from the presence of Li^+. In the present study, the isotherms shifted slightly toward larger molecular areas in the presence of monovalent cations compared to pure water. This might be the result of the change in the ionization state of the DPPS monolayer. The shapes of the isotherms might be compared with the DPPS isotherms spread on buffers with different pH. DPPS isotherms on pure water and on 150 mM salt were close to the isotherms reported by Demel *et al*. [41] at low and neutral pH, respectively. Since no counterions are available in milli-Q water, the ionization equilibrium may be displaced toward a higher pK, so that more DPPS molecules are protonized. Accordingly, fewer electrostatic repulsions in the interface plane would occur, resulting in a closer packing.

4.3. Interaction of DPPS with CPZ in the presence of NaCl

The reduction of the CPZ effect due to the presence of a large NaCl concentration may be explained by a competitive electrostatic interaction with the negatively charged DPPS headgroup. Since the effect on the DPPS pressure/area curve was mainly dependent on CPZ concentration, CPZ might have a higher affinity for the monolayer than did Na^+. In addition, this effect was much more drastic than that obtained with NaCl alone at millimolar concentration. This suggests that the interaction of CPZ with DPPS is not only electrostatic in nature, and that the possible hydrophobic interaction between the aromatic rings of CPZ and the acyl chains of DPPS is of importance. As for the phase transition in DPPC [42], condensed domains could be formed at the beginning of the plateau in the DPPS surface pressure/area curve. Their size could increase until the totality of the monolayer is in a condensed state. The formation of CPZ-rich domains in DPPA bilayers studied with differential scanning calorimetry has already been proposed by Hanpft and Mohr [28], where the CPZ-rich domains would be in a more fluid state than the bulk monolayer. A similar mechanism could also occur with DPPS. Upon monolayer compression, the domains without CPZ would condense first, followed by the domain richest in CPZ. The screening effect of a large concentration of NaCl on CPZ interaction with the DPPS monolayer might also explain the disappearance of the transition plateau.

4.4. The role of drug structure in drug interaction with DPPS

The three drugs studied probably have different hydrophobic interactions with the monolayer, corresponding to a variation in the surface pressure/area curve. The surface pressure at which the phase transition plateau occurs, the area increase at 30 mN/m, and the limiting area at high drug concentration seemed to be related to each other. The degree of penetration of the drug into the monolayer probably depends on the analog used. CPZ might interact more strongly with the lipid hydrophobic part, while CPZ 2C might have a reduced hydrophobicity due to a shorter distance between the positive charge and the aromatic rings. CPZ SO would have very weak interactions with the acyl chains, due to a relatively polar ring system [43]. High surface pressure possibly contributes to discriminating between the drugs interacting with the monolayer by pressing out the less hydrophobic analogs. At 30 mN/m, for example, all CPZ SO might be squeezed out of the lipid monolayer, with CPZ remaining. At higher surface pressure, the lipids might be squeezed out with CPZ in mixed micelles, decreasing the apparent limiting area of DPPS. Explaining the results showing an inversion of the effects at the isotherm liftoff with 10-μM drug concentration is more speculative. The orientation of the drug at the interface might depend on its hydrophobicity. CPZ SO may lie horizontal at the surface, and a larger surface would then be covered at a concentration close to saturation than with CPZ, which might be more perpendicularly oriented due to a more hydrophobic ring system. This phenomenon would be noticeable only at low surface pressure. The fine balance between the high affinity of CPZ for the DPPS monolayer and the small surface area on the one hand and the large CPZ SO surface area but small affinity for the lipids on the other possibly causes the inversion observed for the liftoff at a specific drug concentration. It cannot be ruled out that the different effects observed with the CPZ analogs can be related to the formation of micelles and that the critical micelle concentration varies for the different analogs.

4.5. Conclusion

Oxidation of the chlorpromazine sulphur atom abolishes the effect of CPZ on DPPS monolayers, and the reduction in the length of its side group from three to two carbons also diminishes CPZ action. Though it has been shown that CPZ binds to the D_2 dopamine receptor, we show here that the drug also has a significant effect on monolayers of acidic phospholipids. Since many proteins involved in signal transduction are embedded in the plasma membrane of the cells, the indirect action of CPZ on these proteins via a

change in phospholipid bilayer cannot be ruled out. This might confirm a supplementary way of action of CPZ to the direct interaction with receptor proteins.

Acknowledgment

This work was supported by EC BIOMED-2 Grant BMH4-CT-97-2609.

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