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Biophysical Chemistry 119 (2006) 247 – 255

Biophysical Chemistry

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Hydroxyzine, promethazine and thioridazine interaction with phospholipid monomolecular layers at the air–water interface

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Received 14 June 2005; received in revised form 9 September 2005; accepted 9 September 2005
Available online 17 October 2005

Abstract

In this work the interaction of Hydroxyzine, Promethazine and Thioridazine with Langmuir films of dipalmitoylphosphatidylcholine (dpPC) and dipalmitoylphosphatidic acid (dpPA), is studied. Temporal variations in lateral surface pressure (π) were measured at different initial π (π_i), subphase pH and drug-concentration.

Drugs with the smallest (PRO) and largest (HYD) molecular size exhibited the lowest adsorption (k_a) and the highest desorption (k_d) rate constant values, respectively. The affinity binding constants (K_b) obtained in monolayers followed the same profile ($K_{b,PRO}<K_{b,HYD}<K_{b,THI}$) of the egg-PC/water partition coefficients (P) determined in bilayers. The drug concentration required to reach the half-maximal $\Delta\pi$ at $\pi_i=14$ mN/m ($K_{0.5}$), was very sensitive to pH. The maximal increment in π upon drug incorporation into the monolayer ($\Delta\pi_{max}$) will depend on the phospholipid collapse pressure (π_c), the monolayers's compressibility and drug's size, shape and charge. The higher π_c of dpPC lead to higher $\pi_{cut\text{-}off}$ values (maximal π allowing drug penetration), if compared with dpPA. In dpPC and dpPA $\pi_{cut\text{-}off}$ decreased as a function of the molecular size of the uncharged drugs. In dpPA, protonated drugs became electrostatically trapped at the monolayer surface hence drug penetration, monolayer deformation and π increase were impaired and the correlation between $\pi_{cut\text{-}off}$ and drug molecular size was lost.

Keywords: Hydroxyzine; Promethazine; Thioridazine; Langmuir films; Lateral surface pressure; Phospholipids

1. Introduction

It is well known that the ability of a compound to interact with biological membranes can influence important pharmacologic parameters such as absorption, biotransformation, half-life, excretion, as well as its biological activity. This is of particular importance when evaluating compounds that act on the central nervous system (CNS) where the biological barriers formed by endothelial cells or membranes from the choroid plexus have the function to protect this tissue from the action of exogenous substances [1].

Some authors [1,2] mention three physicochemical properties that are determinant for the interaction of drugs with the CNS: hydrophobicity, the ionization state and the molecular weight. This last property gives a gross approximation of the

Abbreviations: cmc, critical micelle concentration; CNS, central nervous system; dpPC, dipalmitoylphosphatidylcholine; dpPA, dipalmitoylphosphatydic acid; EPC, egg phosphatidylcholine; HYD, Hydroxyzine; K_b, association binding constant; k_d, dissociation rate constant; k_a, association rate constant; $K_{0.5}$, drug concentration required to reach half maximal $\Delta\pi$; π, lateral surface pressure; π_c, collapse pressure; π_i, initial lateral surface pressure; $\pi_{cut\text{-}off}$, maximal pressure allowing drug penetration in the monolayer; π_{tmax}, πvalue at the plateau level; $\Delta\pi$, difference between π at time t (π_t) and the initial π (π_i); $\Delta\pi_{max}$, maximal $\Delta\pi$; NMDA, *N*-methyl-D-aspartate; P, partition coefficient; PRO, Promethazine; THI, Thioridazine.

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doi:10.1016/j.bpc.2005.09.006

molecule's size while hydrophobicity and ionization strongly affects the membrane partition coefficient of the drug.

Drug-membrane interaction is a dynamic phenomenon, bilaterally controlled not only by the drug chemical structure but also by the membrane organization. The latter can affect the internalization of the drug [3], its adsorption at the membrane–water interface [4] and, in turn, can be modified as a consequence of drug incorporation within its structure [5–7 and references therein]. Due to the nonlinear and complex behavior of membrane lipid dynamics, the effect of drugs on membrane organization, even if they were initially subtle and local, might be amplified and spread within the membrane surface and volume [8,9]. Taking embedded-protein for example, several non-specific mechanisms are known to affect the conformation and activity of membrane receptors, like: (i) the coupling between hydrophobic mismatch and curvature stress [10], (ii) changes in the lateral stresses profile (the depth-dependent distribution of lateral stresses within the membrane) which affect the conformation equilibrium and the activity of intrinsic proteins, the function of which involves a structural change accompanied by a depth-dependent variation in its cross-sectional area in the transmembrane domain [11], and (iii) the dipolar arrangement of the membrane which was shown to affect significantly the insertion, folding, conformation and activity of membrane proteins [12–14]. Studies on natural membranes support the hypothesis that the allosteric modulation of monoterpenes on $GABA_A$ receptor [14–16] as well as the mechanosensitivity of NMDA receptors [17] comprise effects caused by drug insertion or other sources of mechanical tension on the supramolecular organization of the receptor environment, through the mechanisms described above. The effects of monoterpenes on the dipolar potential of model membranes, has also been demonstrated [18 and references therein].

Hydroxyzine (HYD), Promethazine (PRO) and Thioridazine (THI) are neurotrophic drugs (Fig. 1). They are all CNS active molecules with different physico-chemical features.

The piperazine HYD is an antihistaminic drug, antagonist of the H_1 receptor. It is an anxiolytic medicine used in pre and post surgery and also for the treatment of skin allergies. It could also be associated to opioids, in the control of cancer pain [19]. PRO is a phenotiazine which exerts anti-histaminic and sedative effects [20]. It is also an antagonist of H_1 receptors, with a potent inhibition of the muscarinic activity and is one of the most effective agents to treat kinesia. The third drug tested was THI, which has an anti-psychotic activity similar to that of Chlorpromazine and that is used to manage squizophrenia, maniac, severe anxiety and behavior disturbs [19].

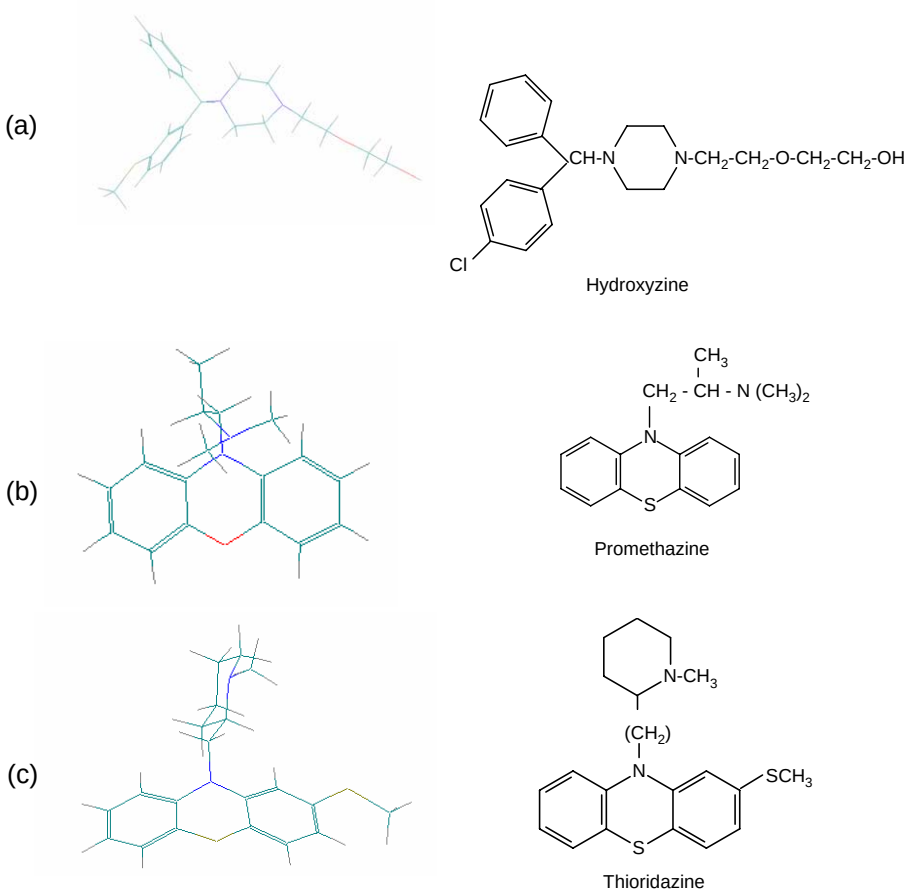

Fig. 1. Chemical structures of (a) Hydroxyzine, (b) Promethazine, and (c) Thioridazine.

Because these 3 compounds act in the CNS they must be able to go throw the complex membrane arrangement formed by blood brain barrier, so that studying the drug-membrane interactions in biomimetic systems play an important role in the understanding of their bioactivity properties. Several membrane properties define its organization in a combined manner, mainly the packing and the mobility of its molecular components and the topology of its surface. In a tri-dimensional system like the liposomes, typically used as a biomembrane model, these properties are mutually affected. Conversely, the use of phospholipid monolayers at the air–water interface allows to maintain a constant planar topology and to control the molecular packing. So, we have chosen this experimental model – measuring the lateral surface pressure of monolayers – for the analysis of the interaction between these three CNS active compounds (Hydroxyzine, Promethazine and Thioridazine) and biomembranes. Moreover, the use as monolayer components of a zwitterionic (dpPC) and a charged (dpPA) phospholipid having well known surface behaviors, allowed the inspection of drug-surface electrostatic interactions at a constant hydrocarbon chain composition.

2. Materials and methods

HYD, PRO and THI were purchased from Sigma Chemical Co (St. Louis, MO). Dipalmitoyl phosphatidylcholine (dpPC) and dipalmitoyl phosphatidic acid (dpPA) were from Avanti Polar Lipids (Alabaster AL, USA). Water was bi-distilled in an all-glass apparatus. The other solvents used were of analytical grade.

2.1. Phospholipid monolayer

Phospholipid monomolecular layers at the air–water interface were prepared as described previously [4,21] using a Minitrough II from KSV Instruments Ltd. (Helsinki, Finland). A chloroform solution (5–30 μL) of the phospholipid (dpPC or dpPA) was spread on a buffered aqueous solution in a circular Teflon trough (4.5 cm diameter and 0.5 cm depth). After 5 min, to allow the evaporation of chloroform, we have started the continuous and automatic measurement of the lateral surface pressure (π) of the formed monolayer, by the Wilhelmy plate method, using a platinized Pt foil (5 mm wide × 20 mm long × 0.025 mm thick). Reproducibility was within ±0.001 mN/m surface pressure. The pH of the subphase was kept constant at pH 5.5, 7.4 or 10.5 with 100 mM acetate, 50 mM phosphate or 50 mM carbonate buffers, respectively.

Penetration experiments were performed at constant surface areas, but at different initial lateral surface pressures, π_i. After the injection of a specific concentration of the drug in the subphase, the temporal dependence of $\dot{\pi}_t$ was recorded (Fig. 2a). The values of $\Delta\pi(\Delta\pi = \pi_{max} - \pi_i)$ were plotted against π_i and adjusted to a straight line. The intersection of the line at the abscise axis gave the maximum π allowing drug penetration ($\pi_{cut-off}$) (Fig. 2b).

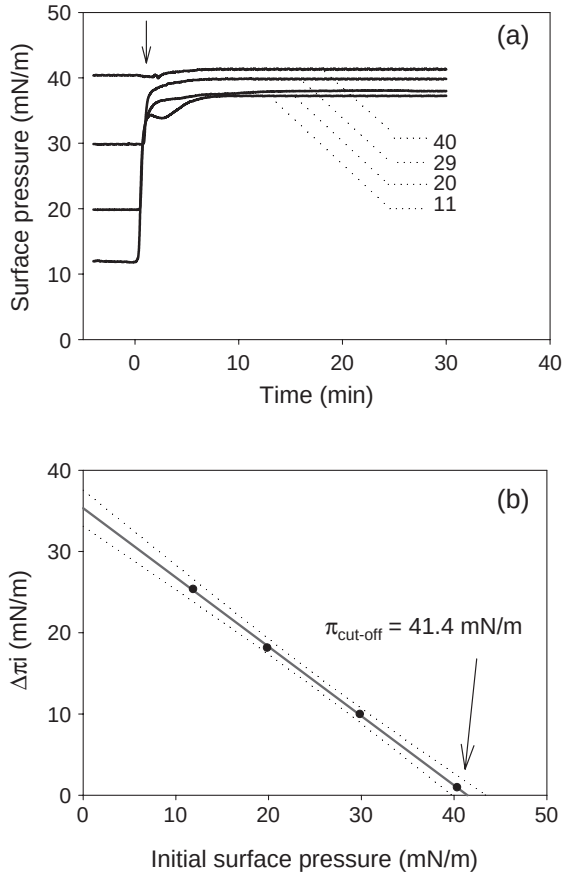

Fig. 2. Effect of the molecular packing on drug penetration in monomolecular layers, at the air–water interface. A typical experiment, with 0.08 mM Promethazine in 200 mM carbonate buffer, pH 10.5 in the subphase, under dpPA monolayers is shown as an example. (a): Variation in surface pressure as a function of time; the numbers refer to the initial surface pressure (π_i) and the arrow points to the injection time of the drug in the subphase. (b): Changes in lateral surface pressure ($\Delta\pi = \pi_t - \pi_i$) as a function of π_i. The $\pi_{cut-off}$ value, representing the maximal surface pressure that allows drug penetration in the monolayer, is indicated. The 99% confidence intervals are shown.

2.2. Partition coefficient (P) — determination by phase separation in bilayers

A known amount of drug was incubated with EPC multilamellar vesicles; four freeze-thawing cycles were performed resulting in incubation time around to 30 min. After incubation P was determined according to Malheiros et al. [22], using 100 mM acetate buffer, pH 5.5 or 50 mM phosphate buffer, pH 7.4 or 50 mM carbonate buffer, pH 10.5.

2.3. Determination of critical micelle concentration (cmc)

CMC values were determined with a K12 Krüss tensiometer. The surface tension of solutions having increasing drug concentrations was measured using 200 mM acetate buffer at pH 5.5 or 50 mM phosphate buffer at pH 7.4.

2.4. Computer calculations

The software used to design the starting points molecules was HyperChem 7.0 (Hyper Cube, Co.). Optimization and van

Table 1
Adsorption and desorption rate constants and association binding constants of drugs injected under a dpPC monomolecular layer

Parameter	Drug		
	Hydroxyzine	Promethazine	Thioridazine
pH	7.4	5.5	5.5
π_i (mN/m)	14	14.5	17
Concentration range (mM)	0.3–3	1–10	0.1–1
k_d (min^{-1})	0.1865	0.1147	0.1139
k_a (M^{-1} min^{-1})	0.1026	0.0105	0.1203
K_b (M^{-1})	0.55	0.091	1.056

Experiments performed at 21 °C and at the indicated subphase pH and initial surface pressure. Drug concentration in the subphase changed within the range indicated. Firstly, individual τ values were determined by fitting Eq. (1) to the temporal variation of π_t after drug injection in the subphase (e.g. Fig. 2a) at each drug concentration. Then, Eq. (2) was fitted to $1/\tau$ vs. drug concentration to obtain k_d and k_a values. Finally, K_b (binding constant) was calculated from Eq. (3).

der Waals volumes calculations were performed using molecular mechanics with MM+ Forced Field to minimize them in vacuum.

3. Results

3.1. Kinetics and thermodynamics of drug adsorption and desorption

The kinetics of drug incorporation in dpPA monolayers was studied by recording the changes in π vs. time (Fig. 2a), monitored as described in the Materials and methods section. These experiments were performed within a concentration range below the cmc of each drug. The subphase pH and the π_i of the dpPC monolayer were maintained constant for the whole set of concentrations corresponding to each drug (see Table 1 for details). The rate-constants for the adsorption and desorption processes at the monolayer–water interface were determined in order to infer about the differences of these compounds in their interaction kinetics with the phospholipid monolayers. Hence, each plot of $\Delta\pi(\pi_t - \pi_i$, calculated as shown in Fig. 2a) vs. t (the time elapsed after the drug injection in the subphase) was analyzed on the basis of a single-exponential model represented by Eq. (1):

$$\Delta\pi \cong [1 - \exp(-t/\tau)] \quad (1)$$

and the time constant (π) values could be determined for each individual drug and drug concentration in the subphase. Then, the rate constants for the adsorption (k_a) and desorption (k_d) processes were obtained by fitting Eq. (2) to the plots of π vs. drug concentration.

$$\frac{1}{\tau} = \frac{1}{k_d + k_a[\text{drug}]} \quad (2)$$

Finally, Eq. (3) allowed calculation of the association binding constant (K_b) [23], as follows:

$$K_b = \frac{k_a}{k_b}. \quad (3)$$

The rate constant values as well as the association-binding constant obtained are shown in Table 1. PRO exhibited the lowest value for k_a while THI exhibited the highest k_a. The highest k_d value corresponded to HYD. These differences seem to determine somehow the resulting value of the binding constant, K_b. The K_b values obtained in monomolecular layers at the air–water interface ($K_{b,PRO} < K_{b,HYD} < K_{b,THI}$) followed the same profile as the EPC/water partition coefficients in an aqueous vesicle dispersion — compare K_b (Table 1) and P_{EPC} values (Table 3) at the corresponding pH.

3.2. Effect of drug concentration in the subphase on the increase in lateral surface pressure

The values of $\Delta\pi$ measured as a function of drug concentration at different pH of the subphase solution seemed to follow a hyperbolic-like behavior and showed, in most cases, a tendency to reach a plateau level within the concentration range assayed (Fig. 3). In order to find the value of $\Delta\pi$ at the plateau ($\Delta\pi_{max}$) as well as to define a parameter that reflected the capacity of the drug to induce $\Delta\pi$, the plots of $\Delta\pi$ vs. [drug] were transformed to their double reciprocal ($1/\Delta\pi$ vs. $1/$[drug]) as shown in the insets of Fig. 3. Then, $\Delta\pi_{max}$ and $K_{0.5}$ were determined from the x and y axis intercept, respectively, according to Eq. (4).

$$\frac{1}{\Delta\pi} = \frac{1}{\Delta\pi_{max}} + \frac{K_{0.5}}{\Delta\pi_{max}} \frac{1}{[\text{drug}]} \quad (4)$$

Here, $K_{0.5}$ represents the drug concentration required to reach the half maximal increment in $\Delta\pi$ ($\Delta\pi_{max}/2$) and is a measure of the drug ability to induce a change in the molecular packing of the monolayer. The values of $K_{0.5}$ and $\Delta\pi_{max}$ so determined were summarized in Table 2. The values of $\Delta\pi_{max}$ for HYD showed a tendency to change as a function of pH and, in the cases of PRO and THI, they seemed to remain roughly

Table 2
Kinetic parameters for the concentration-dependent drug penetration in the dpPC monomolecular layer at a fixed initial surface pressure

Drug	π_i^a (mN/m)	$\Delta\pi_{max}$ (mN/m)[b]			$K_{0.5}$ (mM) [c]		
		pH=5.5	pH=7.4	pH=10.5	pH=5.5	pH=7.4	pH=10.5
Hydroxyzine	14	15.6	28.6	25.6	0.22	0.21	0.06
Promethazine	14.5	22.0	nd	19.0	2.97	nd	0.063
Thioridazine	17	14.7	nd	14.3	0.076	nd	0.00054

[a] π_i: initial surface pressure; [b] $\Delta\pi_{max}$ is the maximal increase in surface pressure; [c] $K_{0.5}$ is the drug concentration in the subphase required to reach the half-maximal increase in π. $\Delta\pi_{max}$ and $K_{0.5}$ were calculated by the fitting of a straight line to the double reciprocal plots of $\Delta\pi$ vs. drug concentration in the subphase, as shown in Fig. 3 (inset). nd=not determined.

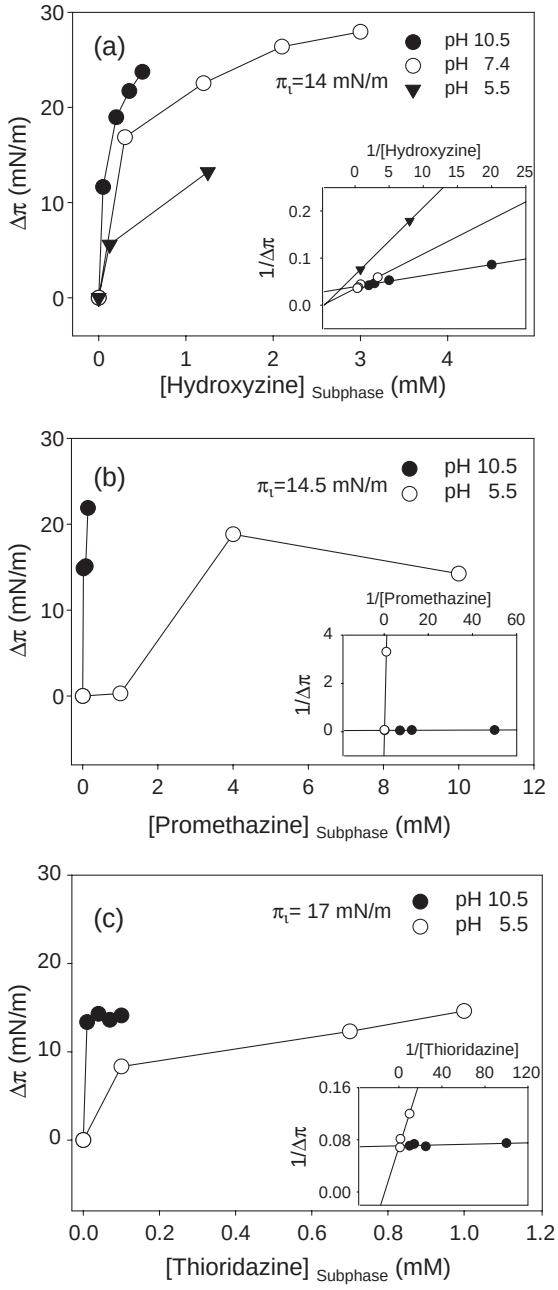

Fig. 3. Effect of drug concentration in the subphase under a dpPC monolayer on the changes in surface pressure ($\Delta\pi$). These experiments were performed at a fixed initial pressure ($\pi_i \cong 14$ mN/m) and at different pH of the aqueous subphase. The change in surface pressure ($\Delta\pi$) between π_i and the π value reached at the plateau level (see Fig. 1a) was determined as a function of Hydroxyzine (a), Prometazine (b) or Thyoridazine (c) concentration in the subphase. The inserts show the corresponding double reciprocal plots that allowed determination of the maximal $\Delta\pi$ obtained by varying the drug concentration in the subphase ($\Delta\pi_{max}$) as well as the drug concentration required to reach the half maximal $\Delta\pi_{max}$ ($K_{0.5}$) at the present π_i (see Table 2).

constant. Taking into account their respective pK_a values, it was expected that at pH=5.5 the three drugs were protonated and that at pH 10.5 they all exhibit a zero net charge. The later condition notably lowers the concentration of drug required to reach $\Delta\pi_{max}/2$ ($K_{0.5}$ decreases) for the three compounds.

3.3. Maximal surface pressure allowing drug penetration in the monolayer

Drug penetration in neutral and charged monolayers was studied at different pH of the subphases. The monolayer-forming lipids used were dpPC and dpPA. The former has an ionizable group at the phosphate moiety (pK_a<1) and a permanent positive charge at the choline quaternary amine group. So, it remained as a zwitterionic compound within the whole pH range covered in the experiments. dpPA possess two ionizable groups (both at the phosphate moiety) with interfacial pK_{a1}=4 and pK_{a2}=9.5, respectively [24]. Within the pH range studied dpPA showed a net charge of −1 (at pH=5.5 and 7.4) or −2 (at pH=10.5). This indicates that, while the molecular packing and resistance to compression of the monolayers formed by dpPC will remain roughly constant as a function of pH, they are expected to decrease in the dpPA monolayers.

The maximal surface pressure that allowed drug penetration ($\pi_{cut\text{-}off}$) was determined as explained in the Materials and methods section. A typical experiment (Fig. 2a) could be analyzed as shown in Fig. 2b. The values of $\pi_{cut\text{-}off}$ in dpPC as well as in dpPA tended to decrease as a function of pH for the three drugs studied (Fig. 4a and b). It is important to recall that $\pi_{cut\text{-}off}$ reflects: (a) the ability of the drug to overcome the resistance of the monolayer to expansion, as well as (b) the stability of the lipid-drug mixed-monolayer formed. The former is related with the compressibility of the monolayer (the more condensed the film, the lower the values of $\Delta\pi$ at the same drug concentration in the subphase). The latter is related with the collapse pressure (π_c) of the lipid (the lower the π_c the higher the probability that it can be reached due to drug incorporation into the lipid film). The decreasing tendency of $\pi_{cut\text{-}off}$ with increasing pH may be related with a decrease in film stability. This should be confirmed through π-area isotherm experiments. The absolute values of $\pi_{cut\text{-}off}$ were higher for PRO in dpPC (Fig. 4a) (ranging from 69 to 49 mN/m) compared with HYD and THI in the same lipid (ranging between 49 and 41 mN/m). In dpPA the three drugs exhibit similar values, ranging from 49 to 37 mN/m (Fig. 4b). The higher $\pi_{cut\text{-}off}$ values reached with dpPC may be related

Table 3
Physicochemical properties of the phenothiazine compounds studied

Drug	Hydroxyzine	Promethazine	Thioridazine
S_w (M)[a]	>2	2	0.3
pK[b]	7.1	9.1	9.5
Van der Waals volume (Å^3)[c]	1179.3	818.0	1004.5
P_{EPC} (pH 5.5)[d]	56±15	125±38	479±95
P_{EPC} (pH 7.4)[e]	257±57	729±120	2.577±594
P_{EPC} (pH 10.5)[f]	1239±355	10 007±2000	–
cmc (pH 5.5)[g]	30–40 mM	37–55 mM	5–10 mM
cmc (pH 7.4)[g]	2–30 mM	1.1–2.8 mM	0.3 mM

[a]Water solubility, according to [28], [b]Ionization constant, according to [28]; [c]van der Waals' volume in vacuum, calculated as described in methods; [d]EPC-water partition coefficient, determined at 100 mM acetate buffer, pH 5.5 or at [e]50 mM PBS, pH 7.4 or at [f]50 mM carbonate buffer, pH 10.5; [g]Determined by the surface tension technique, as described in Materials and methods.

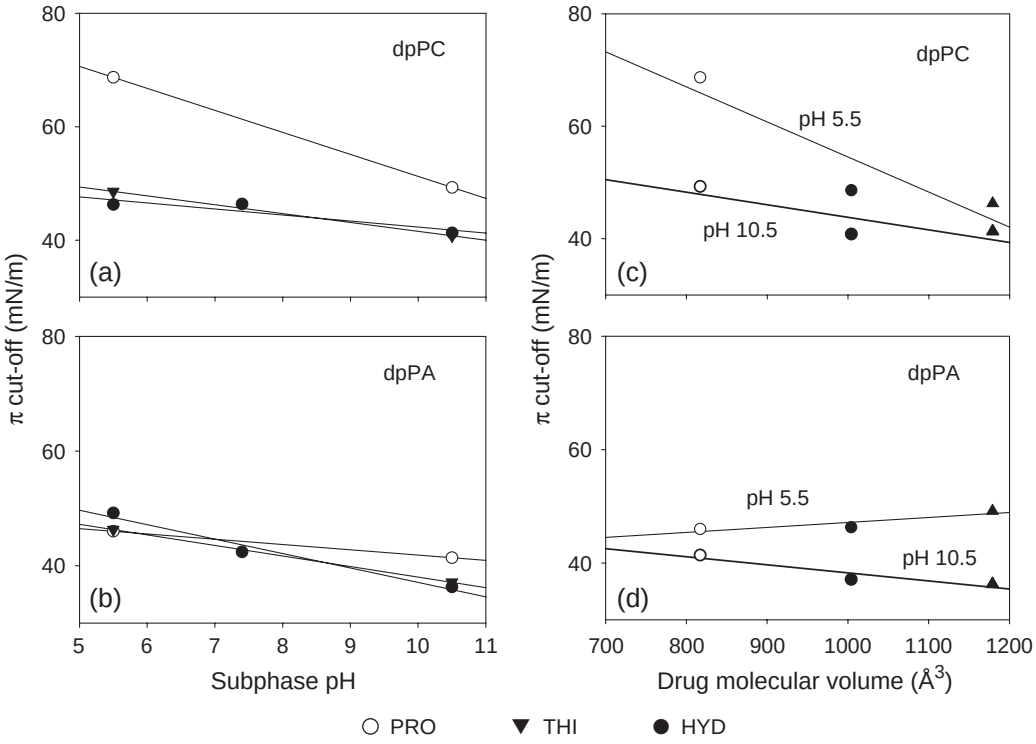

Fig. 4. Effect of subphase pH and molecular size on $\pi_{cut\text{-}off}$ values. Drugs were injected under monolayers of dpPC (a,c) or dpPA (b,d) settled at different values of π_i, and at a fixed concentration in the subphase. The $\pi_{cut\text{-}off}$ values were determined as shown in Fig. 2b, at the indicated subphase pH. The effect of pH (a,b) and molecular size (c,d) on the $\pi_{cut\text{-}off}$ values for each drug were analyzed drug molecular volumes (c,d) correspond to the van der Waal's volumes of the molecules (Table 3). Other experimental conditions were as described in the Materials and methods section. Symbols represent: >, PRO; ▼, THI; ⋆, HYD, in all the graphs.

with the higher surface stability (higher collapse pressure) of this lipid compared with dpPA [25], and also reflect a more favorable interaction with PRO, compared with the other two drugs. The latter might be explained by the smaller size of PRO molecules. This concept is better illustrated in Fig. 4c and d where a decrease in $\pi_{cut\text{-}off}$ as a function of molecular size is observed, particularly for the zwitterionic dpPC–water interface (Fig. 4c) and also with the charged interface of dpPA–water, when interacting with uncharged drug forms (pH=10.5) (Fig. 4d). At pH=5.5, the three drugs are in their protonated form and they are able to establish an electrostatic interaction with the negatively charged dpPA monolayer. In this condition drug molecules became electrostatically trapped at the monolayer surface so that drug penetration and the resulting monolayer deformation and increase in the lateral surface pressure were impaired ($\pi_{cut\text{-}off}$ in dpPA at pH 5.5 is lower than in dpPC at the same pH) and the correlation between $\pi_{cut\text{-}off}$ and drug molecular size disappeared (Fig. 4d). This result can also be interpreted in terms of a molecular condensation of the interface due to the attracting drug-surface electrostatic interactions, at pH 5.5.

4. Discussion

The study of drug-membrane interactions in biomimetic systems plays an important role in the understanding of the pharmacologic properties of drugs. From this point of view and considering that Hydroxyzine, Promethazine and Thioridazine are CNS active compounds, we have used monolayers of two typical phospholipids to measure temporal changes in the lateral surface pressure induced by these drugs, in order to determine features of their particular interaction with biomembranes.

4.1. The binding models

At least three types of parameters can be used to evaluate drug-membrane interaction: (a) $P_{m/w}$, (b) K_b and (c) $K_{0.5}$. It is important to take into account the topological features of the model membranes used (monolayers and bilayers, see below) as well as the thermodynamic definitions for each parameter.

(i) The membrane/water partition coefficient (P) is a ratio between the concentration in the membranous phase (C_m) and in the water phase (C_w): $P=C_m/C_w$. The values of P are generally defined in bilayers using a dispersion of membrane vesicles. This was done for PRO, HYD and THI in EPC large multilamellar vesicles. Because, in the present work, the amount of drug in the monolayer was not measured, the value of $P_{monolayer/water}$ could not be determined in a direct manner. However, it could be inferred from the values of the other two parameters determined (K_a and $K_{0.5}$).

(ii) The binding affinity, calculated from the ratio between association and dissociation rate constants (Eq. (4))

assumes drug binding to a finite number of one-type independent binding sites through a process that follows the mass action law:

$$\text{Drug} + N_f \underset{k_d}{\overset{k_a}{\rightleftarrows}} \text{N.Drug}$$

where N_f and N.Drug represent the free and bound sites, respectively.

(iii) The binding affinity, defined as the drug concentration necessary to reach half maximal binding ($K_{0.5}$). This constant was stated on an empirical basis. The model assumed the existence of a finite number of binding sites without any other constraint such as different site types, allosteric modulations, etc.

In models (ii) and (iii), binding was assumed to be expressed through measurable increments in the lateral surface pressure. In this sense, the binding constants summarize several properties of the system: drug–solvent interaction, drug–monolayer interaction, monolayer organizational state as well as drug charge, size and shape. These properties could be synthesized in the concept of "drug ability to modify the monolayer's molecular packing", which described more precisely the physical phenomenon that was actually being measured.

4.2. Kinetics and thermodynamics of drug adsorption and desorption in monolayers

The time dependence of the surface pressure of a penetration system during the adsorption of a solute is still far from comprehensive. Publications on this subject are rather scarce and are mainly related with the behavior of surfactants and proteins (see Ref. 29 for a review).

In the experimental conditions assayed, PRO exhibited the lowest and THI exhibited the highest value for k_a, the highest k_d value corresponded to HYD. The whole process of drug transference into the monolayer is the sum of two contributions: (a) the change in the interactions of the solute with its surroundings, reflecting the change in the chemical microenvironment of the solute and (b) the work required to create a cavity to incorporate the solute in-between the lipids, which can be described as a pressure-area type work [26]. Mitragotri and coworkers, demonstrated that the dependence on the surface molecular packing of the interfacial partition coefficient ($K_b^{int}(r)$) relative to that in an isotropic solvent $K_0(r)$, is stronger for solutes having larger radii (r) and suggested that, physically this indicates that the enhancement of the probability of cavity formation due to an increase in surface molecular density is larger for the larger solutes [26]. If we take into account that probability and kinetics are directly related, it can be concluded that at constant π_i and drug concentration in the subphase, the initial rate of drug penetration is expected to be faster with the biggest molecules. On the other hand, if the bigger molecules are assumed to penetrate less deep in lipid–water interface, they will be more prone to leave the interface.

In spite of the significant differences between the topology and number of degrees of freedom of bilayers and monolayers as well as the thermodynamic definitions of K_b and P, it was interesting that the K_b values obtained in monomolecular layers at the air–water interface ($K_{b,PRO} < K_{b,HYD} < K_{b,THI}$) had followed the same sequence of the EPC/water partition coefficients of these drugs in a vesicle aqueous dispersion (compare K_b and P_{EPC} values in Tables 1 and 3, at the corresponding pH). Then, K_b values determination and therefore the temporal dependence of π, may be suggested as a predicting method of P.

4.3. Effect of drug concentration in the subphase on the increase in lateral surface pressure

$K_{0.5}$ resulted a parameter very sensitive to measure the effect of pH on drug–membrane interaction, exerted through a modification in the charge state of the drug. The free energy for drug transference (ΔG_T) from the aqueous phase to the monolayer phase involves the intrinsic binding step in addition to the simultaneous dehydration step of both interacting surfaces (drug and monolayer). The latter process would be easier (less energy consuming) when the drug is in its non-charged form. In this condition ΔG_T will be lower and consequently more favorable from the thermodynamic point of view. This fact will be expressed as a lower drug concentration required to observe the same effect (lower $K_{0.5}$). The absolute $\Delta\pi_{max}$ value will depend on the compressibility modulus of the monolayer and the drug structural properties (size, shape and charge) and its upper limit will be determined by the π_c of the lipid.

4.4. Maximal surface pressure allowing drug penetration in the monolayer

The solute P values in bilayer systems exhibit a strong dependence on the local lipid microstructure, a spatial variation with a minimum near the lipid–water interface and a maximum near the bilayer core and a size selectivity which depends on lipid density and the closed-packed lipid area (two parameters that cannot be varied independently). So, they should be thought of as the average of local values, the difference of which depends on the lateral and interlamellar asymmetry of the bilayer. The bilayer is a tension-free state [27]. This condition is lost by drug partitioning which generates instabilities that can be relieved by a lipid molecular rearrangement, through the translocation of the drug molecules to the other membrane leaflet, lipid flip-flop, curvature change, budding, vesiculation, vesicle size change, etc. On the other hand, in a monolayer the equilibrium state is reached by the application of an external lateral surface pressure. There, the lipid molecular-packing can be controlled by working at a fixed π_i, but the mechanisms above mentioned for a bilayer, which involve a topological change, will not work. In their place, the collapse of the monolayer might occur if the experiment is performed at a constant total area of the monolayer (like in

the present work) and the amount of drug accumulated in it is high enough. In order for the drug to enter the monolayer the latter should be submitted to a mechanical deformation which will depend on the monolayer's π_i and on the drug's molecular properties. The higher $\pi_{cut\text{-}off}$ values reached with dpPC may be related with the higher surface stability (higher collapse pressure) of this lipid compared with dpPA. $\pi_{cut\text{-}off}$ in dpPC and dpPA decreased as a function of molecular size for the neutral form of the drugs. In dpPA the protonated drugs become electrostatically trapped at the monolayer surface so that drug penetration, monolayer deformation and lateral surface pressure increase were impaired and the correlation between $\pi_{cut\text{-}off}$ and drug molecular size disappeared.

5. Conclusions

Concluding, the monomolecular layer at the air–water interface resulted a very useful tool to obtain information about the kinetics and thermodynamics of drug–membrane interactions. Drug molecular size, interface mechanical properties and thermodynamic stability seemed to be the most important parameters that ruled the whole process. The smallest (PRO) and the largest (HYD) molecules exhibited the lowest k_a and highest k_d values, respectively. K_b values obtained in monolayers ($K_{b,PRO}<K_{b,HYD}<K_{b,THI}$) followed the same profile of EPC/water partition coefficients (P) in bilayers, at the corresponding pH. So, K_b may be suggested as a predicting method for P determination. $K_{0.5}$ resulted a parameter very sensitive to measure the effect of pH on the affinity of the drug–membrane interaction. The upper limit of the $\Delta\pi_{max}$ value will depend on the collapse pressure of the lipid, the compressibility modulus of the monolayer and the drug structural properties (size, shape and charge). Hence, the higher $\pi_{cut\text{-}off}$ values reached with dpPC may be related with the higher surface stability (higher collapse pressure) of this lipid compared with dpPA. $\pi_{cut\text{-}off}$ in dpPC and dpPA decreased as a function of molecular size for the drugs' neutral species. In dpPA the protonated drugs become electrostatically trapped at the monolayer surface so that drug penetration, monolayer deformation and lateral surface pressure increase were impaired and the correlation between $\pi_{cut\text{-}off}$ and drug molecular size disappeared.

Acknowledgements

This work was supported by CAPES, Agencia Córdoba Ciencia SE, SeCyT-UNC, CONICET. S.V.P.M. was the recipient of a post-doc fellowship from FAPESP, E.P. received a research fellowship from CNPq and M.A.P. is a Career Investigator from CONICET, Argentina.

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