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Interaction of antiarrhythmic drugs with model membranes

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Abstract

Several hypotheses link the molecular mechanism of action of the antiarrhythmic drugs (AAD) that belong to class I to nonspecific interactions with phospholipids sited in the neighborhood of the sodium channels in the membrane of the myocard. The interactions of asocainol (ASOC), procainamide (PROC) and quinidine (QUIN) with: (a) multibilayers of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE), in both a hydrophobic and a hydrophilic medium, and (b) DMPC vesicles, were studied, respectively, by X-ray diffraction and fluorescence spectroscopy. It was found that the three AAD interacted with the lipid bilayers. However, the extension of these interactions depended on the nature and concentration of the lipids and AAD as well as on the medium where the interactions were performed. The different capacity of ASOC and PROC to perturb the bilayer structures, mainly that of DMPC, indicated that the interactions were strongly dependent on the lipophilicity of these drugs. The fact that QUIN did not completely interact in accordance to its lipophilicity suggested that other factors also play a role in these interactions. It is concluded that it may be valid the suggested molecular mechanisms of action of class I AAD involving their interaction with the membrane phospholipids.

Keywords: Antiarrhythmic drug; Phospholipid bilayer; Drug–membrane interaction

1. Introduction

The antiarrhythmic drugs (AAD) that belong to class I exert their action in the myocard cell membrane by blocking the sodium channels [1,2]. However, it is unclear the molecular mechanism implied in these interactions. In fact, five possible mechanisms by which AAD may modify membrane channel functions have been suggested [3]: (a) AAD may directly enter the channel to block the passage of ions across the membrane; (b) given the AAD amphiphilic nature, they can become inserted into the membrane phospholipid bilayer expanding it in a lateral direction; this would result in a compression of the channel which will hinder the proper permeation of ions [4]; (c) AAD can incorporate into the hydrophobic core of the lipid bilayer increasing the thickness of the membrane; consequently, there is a change of the membrane potential gradient; (d) membrane-bound calcium ions can be released by a change of the phospholipids head-group arrangement, affecting the channel function, and (e) by interacting with the hydrophobic region of the channel proteins imbedded in the hydrophobic core of the phospholipid bilayer that surrounds the channel; thus, AAD can alter the lipid environment of the protein and perturb the functional partnership between these membrane constituents [4]. It is noteworthy that three out of the five suggested mechanisms involve nonspecific interactions of AAD with the membrane phospholipids. On the other hand, it has been reported that structural perturbations induced to phospholipids in the neighborhood of ion channels affect their activity [5–8]. For these reasons we thought of interest to study the binding affinity of class I AAD with phospholipid bilayers and their perturbing effects upon the lipid structures.

This paper describes the results of our studies on three AAD with different degrees of lipophilicity: asocainol (ASOC), procainamide (PROC) and quinidine (QUIN), whose structural formulas are shown in Fig. 1. Their respective octanol/water partition coefficients, expressed

Abbreviations: AAD, antiarrhythmic drugs; ASOC, asocainol; PROC, procainamide; QUIN, quinidine; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; GP, general polarization.

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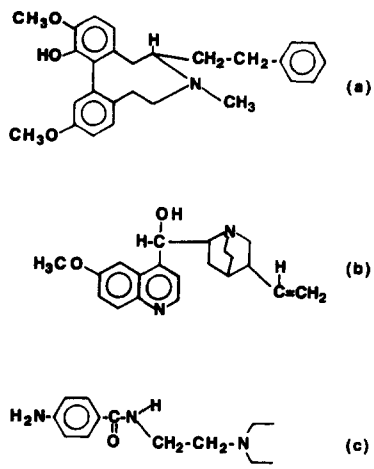

Fig. 1. Structural formulas of (a) ASOC, (b) QUIN and (c) PROC.

as log P, are 4.85, 0.82 and 3.71 being, therefore, ASOC the most lipophilic and PROC the least lipophilic drug of this group. The relative lipophilicity of these three drugs was also confirmed by other experimental and calculating methods [9,10]. The interactions of the AAD with phospholipid bilayers were studied by two different methods. In the first, the AAD were made to interact with multibilayers built-up of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE). They are the type of phospholipids which are found, respectively, in high proportions in the outer and inner monolayers of the erythrocyte membrane [11]. The interactions of the AAD with DMPC and DMPE multibilayers were performed in a hydrophobic and a hydrophilic medium given their amphiphilic nature. The structural perturbation induced to both phospholipids was determined by X-ray diffraction techniques. This method has been previously used in our laboratory to study how other therapeutical drugs affect the bilayer structure of DMPC and DMPE [12–15].

Fluorescence steady-state anisotropy of DPH (1,6-diphenyl-1,3,5-hexatriene) and Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) general polarization were determined to study the influence of the AAD in the physical properties of DMPC large unilamellar vesicles. DPH, an all-*trans* polyene with a rod-like shape, is one of the most commonly used probe for hydrophobic regions of phospholipid bilayers. DPH fluorescence steady-state anisotropy measurements are useful to investigate structural and dynamic properties of lipid bilayers; it provides a measure of the rotational diffusion of the fluorophore restricted within a certain region, such as a cone, due to the phospholipid acyl chains packing order. Laurdan, an amphipathic probe, has a high sensitivity of excitation and emission spectra to the physical state of membranes. With the fluorophore in a shallow position of the bilayer normal, Laurdan provides information of dynamic properties in this zone of the bilayer. The quantification of Laurdan fluorescence spectra intensity data at different wavelengths is done using general polarization (GP) concept [16].

2. Materials and methods

2.1. X-ray diffraction analysis of phospholipid multibilayers

Synthetic DMPC (lots 57F-8365 and 80H-8371, A Grade, MW 677.9) and DMPE (lot 67F-8350, A Grade, MW 653.9) were from Sigma. Asocainol hydrochloride, MW 454.0, procainamide hydrochloride, MW 271.8 and quinidine sulfate, MW 783.0 of the highest purity were gifts from Drs. R. Mannhold and W. Voigt of the University of Düsseldorf and Gödecke Laboratories (Germany). Powder mixtures of DMPC and of DMPE with each AAD were prepared in the molar ratios of 10:1, 5:1 and 1:1. Each one was dissolved in chloroform/methanol (3:1, v/v) and left to dry very carefully. The resulting samples, in the form of crystalline powders, were introduced in special glass capillaries of 0.7 mm diameter. They were diffracted in Debye–Scherrer cameras of 114.6 mm diameter and in flat-plate cameras with 0.25 mm diameter glass collimators [17] provided with rotating and cooling devices. The same procedure was followed with single samples of each phospholipid and AAD.

The interactions in a hydrophilic medium were attained in 1.5 mm diameter glass capillaries. To each capillary, which contained about 2–3 mg of DMPC or DMPE, was respectively added about 100 μl of (a) distilled water; (b) 0.01 mM, (c) 0.1 mM, (d) 1 mM and (e) 10 mM aqueous solution of each AAD, and then they were sealed. The samples thus prepared were X-ray diffracted 2 and 14 days after preparation in flat-plate cameras. Specimen-to-film distances were either 8 or 14 cm, standardized by sprinkling calcite powder on the capillaries surface. Ni-filtered Cu K_α radiation from a Philips PW 1140 X-ray generator was used. The relative intensities of the reflections were measured from films by peak integration in a Joyce-Loebl MK III CS microdensitometer interfaced to an ACER 915 microcomputer. No correction factors were applied. All experiments with aqueous solutions were carried out at 17 ± 2°C, which is below the main transition temperature of each phospholipid under study.

2.2. Fluorescence studies on vesicles

Large DMPC unilamellar vesicles suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspension through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Costar), employing nitrogen pressure at a temperature of 10°C over the transition temperature, to a final lipid concentration of 500 μM. Fluorescent probes (DPH and Laurdan, from Molecular Probe) were incorporated into the vesicles

preparation by injecting small aliquots of a concentrated solution of the probe in ethanol and gently shaken for ca. 30 min. The probe to phospholipid ratio was in all cases 1:1000. Fluorescence spectra and anisotropy measurements were performed on a Spex Fluorolog (Spex Industries) and on a phase shift and modulation Gregg-200, steady-state and time resolved spectrofluorometer (I.S.S.) respectively, both interfaced to personal computers. Software from I.S.S. were used for data collection and analysis. All measurements were made at 18°C using 1-cm path length square quartz cuvettes. The sample temperature was controlled using an external bath circulator (Cole-Parmer). The actual temperature was measured at the sample cell prior and after each measurement using a digital thermometer (Omega). A xenon arc lamp was utilized as light source, and the wavelength of excitation was set at 360 nm. Anisotropy measurements were done in the 'L' configuration using Glan Thompson prism polarizers in both exciting and emitting beams. The emission was measured using a WG-420 Schott high pass filter, which showed negligible fluorescence. Generalized polarization (GP) [16] was evaluated by $GP = (I_B - I_R)/(I_B + I_R)$, where I_B and I_R are the emission intensities at the blue and red edges of the emission spectrum, respectively. These intensities have been measured at the emission wavelength of 440 and 500 nm, which correspond to the emission maxima of Laurdan in gel and liquid-crystalline phases, respectively [18]. Blank subtraction was performed in all measurements using unlabeled samples. Each AAD was incorporated to DMPC vesicle suspensions by addition of small aliquots of a concentrated solution of the drug in ethanol, except ASOC that was dissolved in water, and incubated at 40°C for ca. 15 min. Addition of the same amounts of pure ethanol did not produce any change in both fluorescent parameters.

3. Results and discussion

3.1. X-ray studies on lipid multibilayers

The molecular interactions of ASOC, PROC and QUIN with multibilayers of the phospholipids DMPC and DMPE were studied in both a hydrophobic and a hydrophilic medium. Towards this end, X-ray diffraction patterns were taken of the following specimens: (a) samples of DMPC and of each AAD in the molar ratios of 10:1, 5:1 and 1:1 recrystallized from chloroform/methanol (3:1, v/v); (b) samples of DMPE and of each AAD in the same molar ratios and recrystallized from the same solution, and (c) mixtures of each phospholipid in their crystalline phases with 0.01 mM, 0.1 mM, 1 mM and 10 mM aqueous solutions of ASOC, PROC and QUIN. All these patterns were compared with those of the corresponding phospholipid and AAD obtained under the same physicochemical conditions. The results are presented in Figs. 2 to 13. Fig. 2 shows the diffractograms of recrystallized specimens of

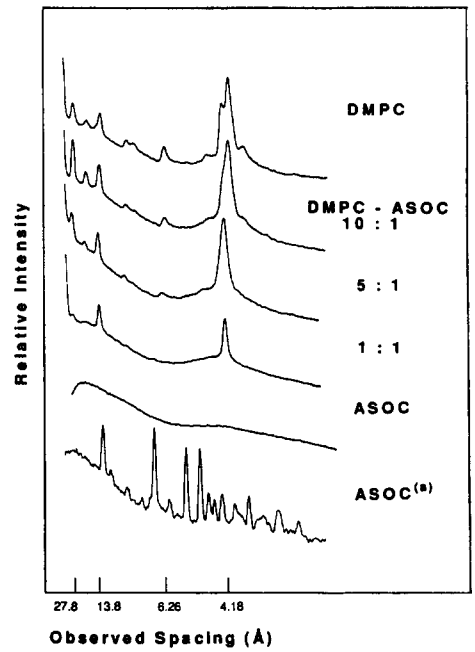

Fig. 2. Microdensitograms from X-ray diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm). (a) Not recrystallized.

DMPC, ASOC and of their molar mixtures. The analysis of these results indicated that the bilayer structure of DMPC was increasingly perturbed by the higher ratios of ASOC in their mixtures. In fact, at such a small DMPC/ASOC 10:1 molar ratio, the strong 4.29 Å reflection due to DMPC was considerably weakened while that of 4.0 Å disappeared. At the 1:1 ratio, most of the reflections from the lipid were absent. However, a new and relatively strong reflection of 4.18 Å showed up. The appearance of this reflection was indicative of the fluid state reached by the hydrocarbon chains of the lipid. In fact, this reflection corresponds to the average separation of the fully extended chains organized with rotational disorder in a hexagonal lattice [19,20]. This result is noteworthy as it was observed in the absence of water. Similar effects on DMPC have been previously reported for the amphiphilic antibiotics chlortetracycline [13] and gentamicin [15]. This, together with the fact that the bilayer width of about 55 Å remained practically unchanged despite the gradual incorporation of ASOC implied a deep penetration of ASOC into the hydrophobic core of DMPC. It was also observed that no reflections from ASOC were present in any of its recrystallized mixtures with DMPC neither with those of DMPE. This was due to the fact that recrystallized ASOC gave only amorphous patterns. The same effect was observed in PROC (Fig. 3) but not in QUIN (Fig. 4).

Fig. 3 exhibits the diffractograms of recrystallized specimens of DMPC, PROC and of their molar mixtures. As it can be observed, the effects of PROC upon the bilayer structure of DMPC were very similar to those produced by

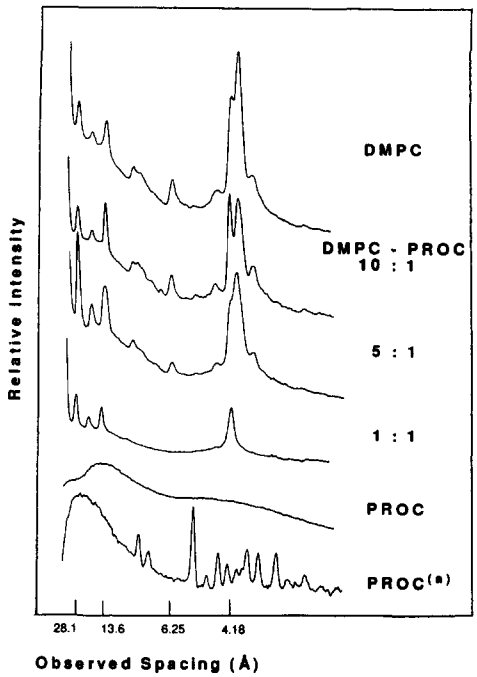

Fig. 3. Microdensitograms from X-ray diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm). (a) Not recrystallized.

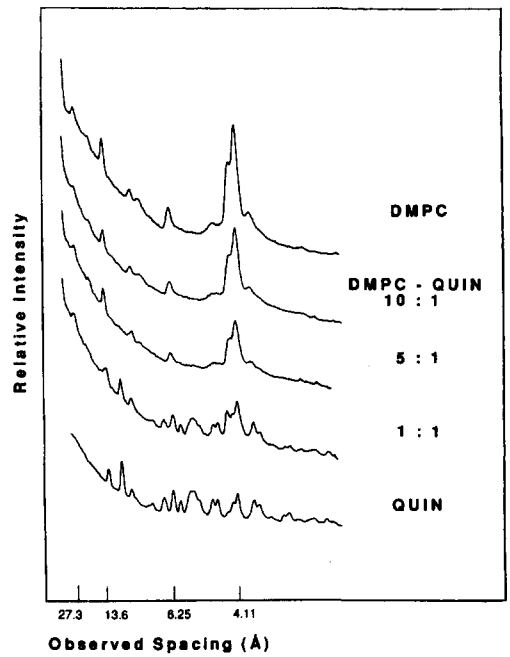

Fig. 4. Microdensitograms from X-ray diffraction diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm).

ASOC. The only significant difference consisted in that the bilayer width of DMPC increased from 54.5 Å up to 57.4 Å in its 1:1 mixture and some reflections were more intense than in the case of ASOC. This indicated that the PROC penetration into DMPC bilayers was not as deep as that of ASOC.

Fig. 4 presents the results obtained when QUIN was made to interact with DMPC in a hydrophobic medium. As its lipophilicity lies between those of ASOC and PROC, it was expected that the structural perturbation induced by QUIN to DMPC bilayers would also lie between those produced by ASOC and PROC. However, this was not the case. As the concentration of QUIN increased DMPC reflections became weakened until they were almost absent in the 1:1 mixture, where most of the observed reflections were those of QUIN. On the other hand, it was not observed the 4.18 Å reflection induced to DMPC by ASOC and PROC. These results indicated that whereas ASOC and PROC changed the bilayer structure of DMPC into a more fluid one, characterized by an hexagonal packing of its hydrocarbon chains, QUIN gradually perturbed the lipid organization towards a more random state.

The results obtained when the AAD were made to interact with DMPE in a hydrophobic medium are presented in Figs. 5–7. Fig. 5 shows the microdensitograms of recrystallized DMPE, ASOC and of their 1:1 mixture. As it has been reported elsewhere [21], DMPE presents two polymorphic forms when it is recrystallized from chloroform/methanol. One phase, L_{c1}, is obtained when these solvents are in a 3:1 ratio. The bilayer width of about 52 Å indicates that the hydrocarbon chains are extended and parallel to the bilayer normal. The other phase, L_{c2}, is obtained by its recrystallization from chloroform/methanol in a 1:3 ratio. In this case, the bilayer width is of about 44 Å because the hydrocarbon chains are tilted by nearly 30°. The results obtained in the present study confirmed that DMPE recrystallized from chloroform/methanol (3:1,

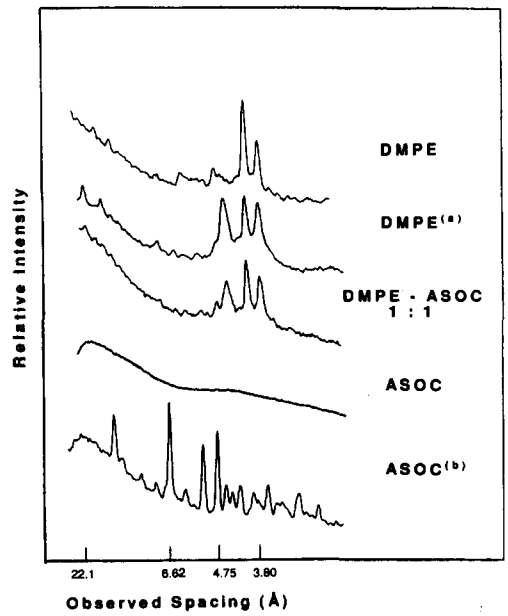

Fig. 5. Microdensitograms from X-ray diffraction diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm). (a) Recrystallized from chloroform/methanol (1:3, v/v); (b) Not recrystallized.

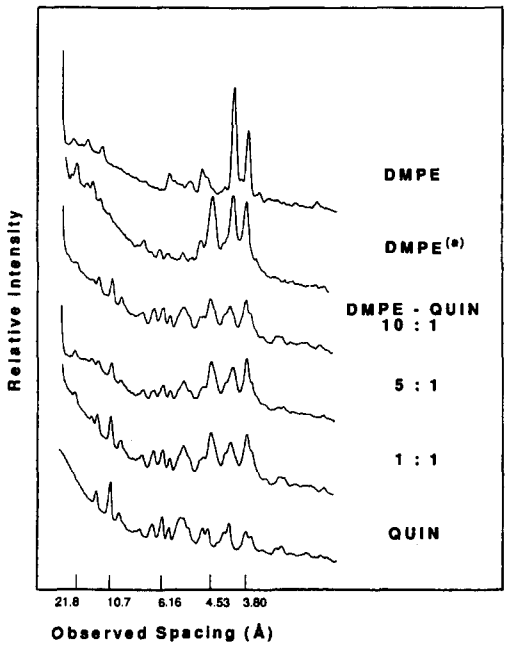

Fig. 6. Microdensitograms from X-ray diffraction diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm). (a) Not recrystallized.

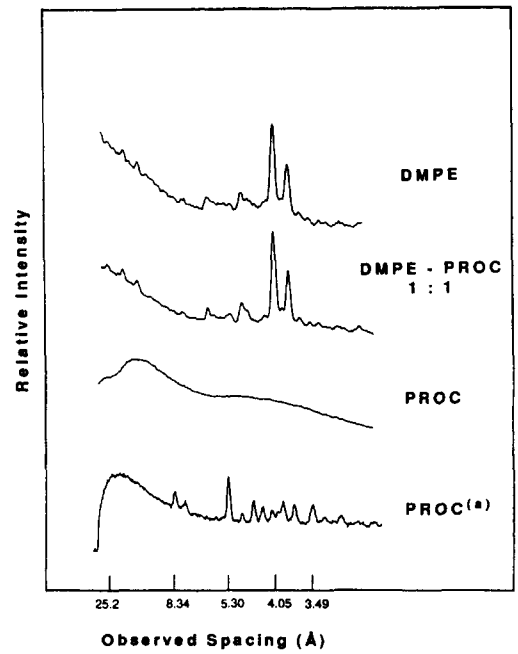

Fig. 7. Microdensitograms from X-ray diffraction diagrams of specimens recrystallized from chloroform/methanol (3:1, v/v). Flat-plate cameras ($D = 8$ cm). (a) Recrystallized from chloroform/methanol (1:3, v/v).

v/v) was in the L_{c1} form given its 51.4 Å bilayer width. However, the X-ray pattern of DMPE:ASOC 1:1 showed that DMPE changed to the inclined L_{c2} form as its bilayer width was reduced to 44.6 Å. The comparison of this pattern with that of pure DMPE in the L_{c2} form showed that they were very similar. This result implied that ASOC molecules did not interact with those of DMPE. Otherwise, the X-ray pattern of DMPE in the mixture would have differed from that of its single phase. The observed phase transition of DMPE most likely was due to a change in the physicochemical characteristics of the solvent after the dissolution of ASOC. The interactions of QUIN with DMPE under the same conditions (Fig. 6) produced the following results: (a) DMPE also underwent the L_{c1} to L_{c2} phase transition; (b) a continuous weakening of DMPE reflections with the increasing proportions of QUIN in their mixtures; (c) QUIN reflections were present in all its mixtures, even at that of 10:1, and (d) there was only a slight increase in the bilayer width of DMPE. These effects clearly indicated that part of QUIN indeed interacted with DMPE. This result is surprising given the lower lipophilicity of QUIN compared to that of ASOC, and the high stability of DMPE bilayers [17]. On the other hand, PROC did not produce a phase transition neither a structural perturbation to DMPE (Fig. 7), which is not surprising given its low lipophilicity.

Figs. 8–10 show the X-ray patterns obtained after DMPC in its crystalline phase was mixed and allowed to interact with water and aqueous solutions of each AAD. They were obtained 2 and 14 days after preparation without showing any significant change with time. DMPC expanded its bilayer width from about 55 Å to 64.4 Å when exposed to pure water. The observed reflections were reduced to only the first three orders of the 64.4 Å bilayer width and one of 4.2 Å. In the case of ASOC (Fig. 8), a concentration as low as 0.01 mM changed somewhat the X-ray pattern of DMPC, which completely disappeared at 10 mM. These results clearly indicated that ASOC produced a deep perturbation to the DMPC bilayer structure. On the other hand PROC, even at a concentration as

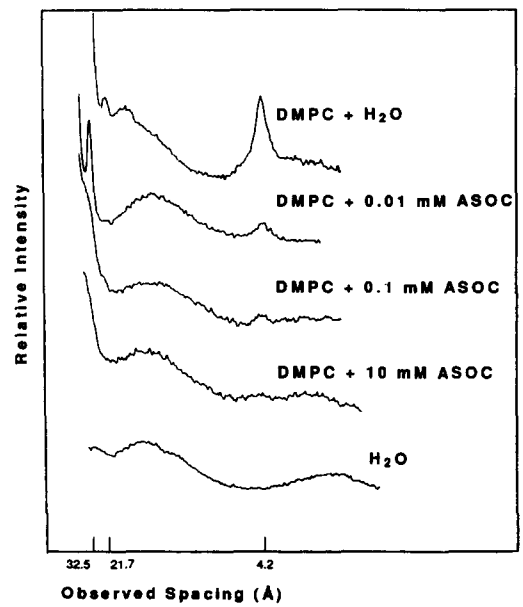

Fig. 8. Microdensitograms of DMPC with aqueous solutions of ASOC. Flat-plate cameras ($D = 8$ cm).

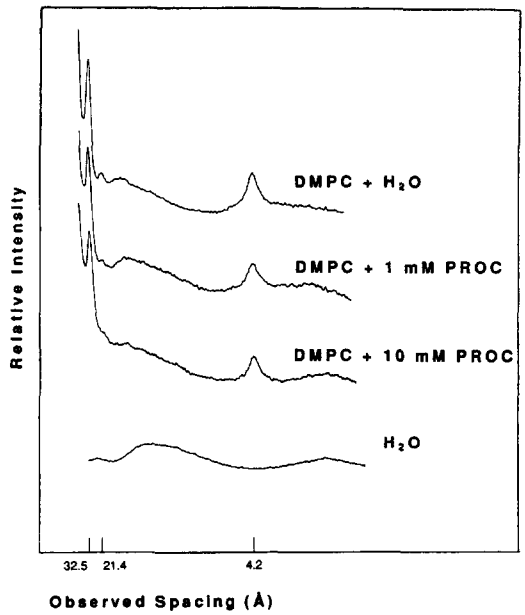

Fig. 9. Microdensitograms of DMPC with aqueous solutions of PROC. Flat-plate cameras ($D = 8$ cm).

high as 10 mM, did not produce any significant change to the lipid structure (Fig. 9). QUIN (Fig. 10) interacted with DMPC in a similar way as ASOC, although its effects were slightly milder. Finally, Figs. 11–13 present the results obtained when water and the AAD aqueous solutions were allowed to interact with DMPE in the same conditions as those described for DMPC. It can be noticed that the X-ray pattern of DMPE in the presence of water remained essentially the same L_{c1} form observed in the

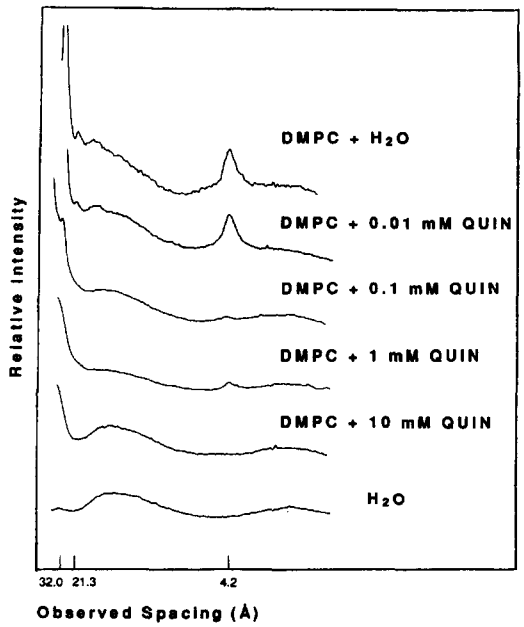

Fig. 10. Microdensitograms of DMPC with aqueous solutions of QUIN. Flat-plate cameras ($D = 8$ cm).

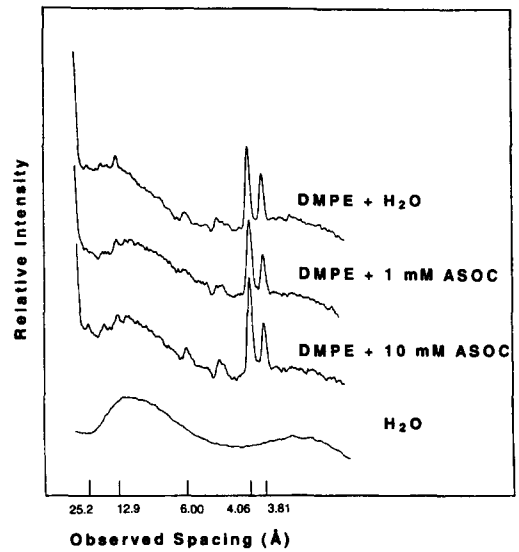

Fig. 11. Microdensitograms of DMPE with aqueous solutions of ASOC. Flat-plate cameras ($D = 8$ cm).

recrystallized state. On the other hand, none of the three AAD affected, in a significant extent, the X-ray pattern of DMPE even in their most concentrate solutions.

The difference in the extent of the structural perturbation induced to DMPC and DMPE in an aqueous medium by both ASOC and QUIN can be related to the different packing arrangement of the lipid bilayers. In fact, DMPE molecules are so tightly packed that neither water nor AAD were able to penetrate and interact with them. DMPC, on the contrary, presents large interbilayer spaces whose separation increases as water fills in [17]. This allowed ASOC and QUIN molecules to interact with the polar and hydrocarbon chains of the lipid resulting in the complete disruption of DMPC molecular organization.

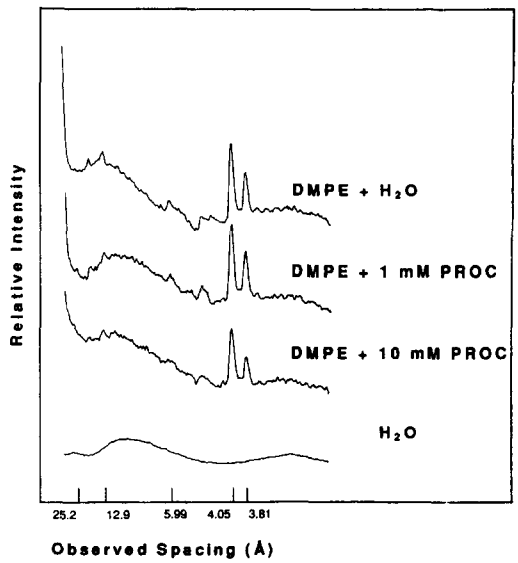

Fig. 12. Microdensitograms of DMPE with aqueous solutions of PROC. Flat-plate cameras ($D = 8$ cm).

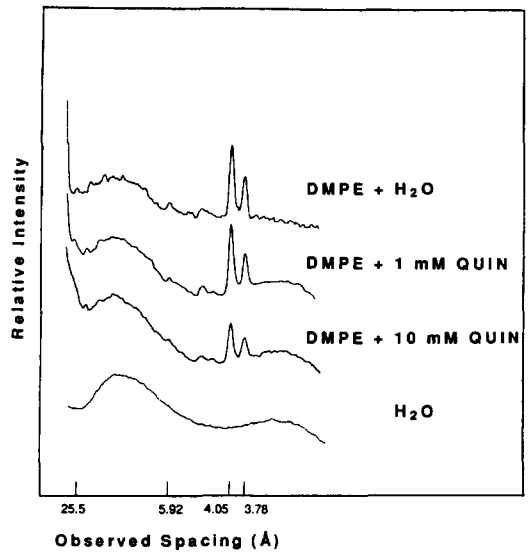

Fig. 13. Microdensitograms of DMPE with aqueous solutions of QUIN. Flat-plate cameras ($D = 8$ cm).

3.2. Fluorescence measurements

DPH emission steady-state anisotropy and Laurdan general polarization were studied in DMPC large unilamellar liposomes. The presence of increasing concentrations of PROC up to 10 mM did not produce any change in the fluorescence anisotropy of DPH nor in Laurdan GP, as shown in Table 1. The increasing presence of QUIN produced a monotonous decrease in the DPH fluorescence anisotropy and only a slight decrease in Laurdan GP at its highest concentration (Table 2). On the other hand, ASOC addition in about the same concentration range as the other two AAD produced a decrease in the fluorescence parameters of both probes (Table 3).

The rotational behavior of DPH in an anisotropic system as a bilayer is described by the hindered rotational model [22,23]. In this model the steady-state anisotropy can be related primarily to the restriction of the rotational motion [24] due to the hydrocarbon chain packing order. On the other hand, Laurdan has been reported to be located at the hydrophobic/hydrophilic interface of the lamella [25,26] with the lauric acid anchored in the phospholipid acyl chains. The spectral sensitivity to the phospholipid phase state has been attributed to a dipolar relaxation phenomenon originating from the sensitivity of the probe to the polarity of its environment. This is due to the large charge separation that the fluorophore exhibits upon excitation, i.e., a large excited state dipole [27]. In a phospholipid bilayer the relaxation process depends on the dynamics of the dipolar moieties surrounding the Laurdan excited state molecules at this interface [18].

Table 2
Effect of QUIN on the anisotropy (r) of DPH and the general polarization (GP) of Laurdan embedded in large unilamellar DMPC vesicles

QUIN. concn	r (DPH)	GP (Laurdan)
0 mM	0.31	0.54
0.04 mM	0.27	0.55
0.5 mM	0.12	0.55
8.5 mM	0.01	0.49

The null effect of PROC on the DPH steady-state fluorescence anisotropy and on Laurdan general polarization indicated that this drug exerted no perturbation to the acyl chain packing organization nor in the molecular dynamics at the hydrophilic/hydrophobic interface. The monotonous decrease that QUIN produced to the fluorescence anisotropy of DPH and practically the null effect on Laurdan general polarization indicated that it modified the structural order in the hydrophobic domain but not in the dynamic characteristics of the hydrophilic/hydrophobic interface. These results did not contradict those observed by X-ray diffraction, which are related to structural modifications that might not produce changes in the mobility of dipolar molecules at the lipid polar group environs. On the other hand, the observed decrease induced by ASOC to the fluorescence parameters of both probes can be rationalized as a general fluidity increase of the bilayer, both in terms of a disorder in the hydrocarbon chain organization and a greater molecular mobility in DMPC polar group environments. Therefore, there was a general agreement on the results observed by X-ray diffraction on DMPC in a hydrophilic medium and those obtained by fluorescence methods in DMPC liposomes.

The results obtained in this study allow to arrive to the following conclusions: (a) the three AAD are able to interact with phospholipid bilayers (however, the extension of the interactions depend on the nature and concentration of the lipids and AAD as well as on the medium where the

Table 1
Effect of PROC on the anisotropy (r) of DPH and the general polarization (GP) of Laurdan embedded in large unilamellar DMPC vesicles

PROC concn.	r (DPH)	GP (Laurdan)
0 mM	0.31	0.56
0.02 mM	0.31	0.57
0.1 mM	0.31	0.56
1 mM	0.31	0.57
10 mM	0.31	0.57

Table 3
Effect of ASOC on the anisotropy (r) of DPH and the general polarization (GP) of Laurdan embedded in large unilamelar vesicles

ASOC concn.	r (DPH)	GP (Laurdan)
0 mM	0.32	0.52
0.01 mM	0.32	0.53
0.1 mM	0.31	0.52
1 mM	0.27	0.43
10 mM	0.22	0.32

interactions were performed); (b) the different capacity of ASOC and PROC to perturb the bilayer structures, mainly that of DMPC, indicates that these interactions are strongly dependent on the lipophilicity of the AAD; (c) the fact that the perturbing ability of QUIN did not lie, as expected, between those of ASOC and PROC shows that other factors besides lipophilicity play a role in these interactions, and (d) the suggested molecular mechanisms of action of class I AAD involving their interaction with the membrane phospholipids might be valid.

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