X-Ray Studies on Phospholipid Bilayers. XIV. Interactions with the Antiarrhytmic Asocainol

M. Suwalsky, I. Sánchez, and F. Neira

Faculty of Chemical Sciences, University of Concepción, Casilla 3-C, Concepción, Chile

Z. Naturforsch. **48c,** 930–938 (1993); received June 30/September 1, 1993

X-Ray Diffraction, Phospholipid Bilayer, Antiarrhytmic, Asocainol

Asocainol (ASOC) belongs to class I of the antiarrhytmic drugs, *i.e.*, those that exert their action at the level of the sodium channels of the myocardial cell membrane. It has been suggested that their molecular mechanism of action might be through nonspecific interactions with phospholipids that surround the channel proteins. In order to test this hypothesis, ASOC was made to interact with two multibilayer systems, one built-up of dimyristoylphosphatidylcholine (DMPC) and the other of dimyristoylphosphatidylethanolamine (DMPE). These are the type of lipids that are respectively found in the outer and inner monolayers of human erythrocytes. The experiments were carried out in a hydrophobic as well as in a hydrophilic medium below the phospholipid main transition temperatures. The perturbing effect of ASOC upon the bilayer structures was determined by X-ray diffraction. It was found that ASOC was able to fluidize DMPC in both media but not to DMPE.

Introduction

Although the mode of action of the antiarrhytmic drugs (AAD) is not yet fully understood, there is consensus that those that belong to class I exert their action in the myocardial cell membrane by blocking the sodium channels [1, 2]. The molecular mechanism is still controversial. AAD may directly enter the channel to block the passage of ions across the membrane. However, many AAD exert a variety of effects on different aspects of membrane function, which suggests that the channel blocking effects may be due to nonspecific interactions with phospholipids that surround and functionally modulate ion transport by channel proteins [3]. In fact, most AAD are amphiphilic compounds and so they can insert into the phospholipid structure causing severe structural alterations. The drug-induced changes in the physical state of the membrane phospholipids surrounding an ion channel may modify its function by altering the conformation and mobility of the protein within the membrane. Thus, the AAD may expand the membrane by their incorporation into the bilayer and by disordering the hydrocarbon chains of the bilayer. Such expansion has been postulated to inhibit the ability of sodium ions to pass through the sodium channel [3]. Evidence has been obtained with studies on the interaction of local anesthetics (most AAD possess local anesthetic activity) with monomolecular films of membrane lipids extracted from nerve cells [4]. These experiments indicated that the drugs penetrate the monolayer and increase the surface pressure. The drugs, therefore, would tend to compress the channels and hinder their enlargement at times when an increased permeability is required [1]. A second possible effect is the incorporation of the amphiphilic drug into the hydrophobic core of the membrane. The resulting increase in thickness of the bilayer may also inhibit the channel function. This hypothesis lies in the proposition that the channel gating sensor is sited within the membrane and responds to changes in the membrane field, *i.e.* the gradient of potential across the membrane. If the membrane thickness is increased, the field will be reduced affecting the gating state of the channel [5]. The incorporation of amphiphiles into membranes can also displace calcium ions from negatively charged binding sites by altering the arrangement of the phospholipid head groups such as that the distance between anionic sites becomes energetically unfavorable for calcium binding. The release of Ca^{2+} has been proposed to influence membrane protein function [3]. Finally, by inserting the drug at the phospholipid-channel boundary (annulus), it can perturb the functional partnership between these membrane constituents [3]. It has been

Reprint requests to Dr. M. Suwalsky.

Verlag der Zeitschrift für Naturforschung,
D-72072 Tübingen
0939–5075/93/1100–0930 $ 01.30/0

found, indeed, that the structural perturbation of the phospholipid arrangement in the neighborhood of ion channels in biological membranes affect the protein conformation and, therefore, their activity [5–9].

In order to test the binding affinity of AAD to phospholipid bilayers, Mannhold and Voigt [10, 11] measured and calculated the lipophilicity of 15 class I antiarrhytmics and quantified their binding to phosphatidylcholine (PC) membranes by fluorescence spectroscopy. They found that the AAD exhibit extreme variations in lipophilicity, being this the main factor that determined the extent of the AAD binding to PC bilayers.

For all these reasons, it was thought of interest to study the perturbing effects of AAD upon the structure of phospholipid bilayers. With this aim, three of those AAD studied by Mannhold and Voigt with different degrees of lipophilicity were made to interact with a membrane model system. This consisted of multibilayers built-up of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE). These phospholipids are respectively representative of lecithins and cephalins, which are mostly and respectively found in the outer and inner monolayer of the erythrocyte membrane [12]. Chemically, they only differ in their terminal amino groups, being $^{+}NH_3$ in DMPE and $^{+}N(CH_3)_3$ in DMPC. Their structures under different hydrations below their main transition temperatures have been reported, being very similar in their dry Lc crystalline phases [13]. In fact, both have their hydrocarbon chains mostly parallel and extended with the polar groups perpendicular to them. However, DMPE molecules pack tighter than those of DMPC. This effect, due to the smaller polar group and higher effective charge of DMPE, results in a very stable bilayer structure, which is not significatively affected by the addition of water [14]. However, the gradual hydration of DMPC under the same conditions results in water molecules occupying the highly polar interbilayer spaces. As a consequence, there is an increase in its bilayer separation [15], undergoing the phospholipid the reversible phase transition $Lc \rightleftharpoons L\beta' \rightleftharpoons P\beta' \rightleftharpoons L\alpha$. Lc denotes the crystalline phase, $L\beta'$ the gel phase, $P\beta'$ the rippled gel phase, and $L\alpha$ the liquid crystalline phase, being this present at high temperatures [16].

These bilayer systems have already being used in this laboratory to study mainly by X-ray diffraction how they interact with several therapeutical drugs such as chlorpromazine [17], chlortetracycline [18], chloramphenicol [19] and gentamicin

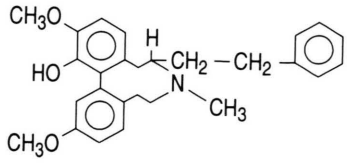

Fig. 1. Structural formulae of ASOC.

[20]. In this paper are presented the results obtained from the interaction of asocainol (ASOC) with DMPC and DMPE bilayers. ASOC, whose structural formulae is shown in Fig. 1, is the most hydrophobic of the three AAD studied in this laboratory. Its molecular structure corresponds to most nonspecific AAD, which consist of three moities which seem necessary for their activity: a benzene ring or condensed aromatic portion connected to a basic amino group, usually tertiary or secondary, by way of an ester, ether, amide or hydroxyalkyl group capable of becoming involved in H-bonding [1]. The experiments were carried out in a hydrophobic as well as in a hydrophilic medium given the amphiphilic character of ASOC, DMPC and DMPE.

Materials and Methods

Synthetic DMPE from SIGMA (Lot 67F-8350 A Grade, MW 635.9), DMPC from SIGMA (Lots 57F and 88F 8365 A Grade, MW 677.9) and asocainol hydrochloride of MW 454.0 (a gift of Drs. R. Mannhold and W. Voigt, of the Department of Clinical Physiology, University of Düsseldorf, Germany) were used without further purification. Powder mixtures of DMPC:ASOC and DMPE:ASOC were prepared in the molar ratios of 10:1, 5:1 and 1:1. Each mixture was dissolved in chloroform:methanol 3:1 and left to dry very slowly and carefully. The resulting samples, in the form of crystalline powders, were introduced in special glass capillaries of 0.7 mm diameter. They were X-ray diffracted in Debye-Scherrer cameras of 114.6 mm diameter and flat-plate cameras with 0.25 mm diameter glass collimators [13], provided

with rotating and cooling devices. The same procedure was followed with pure samples of each phospholipid and ASOC.

Hydrated samples were prepared in 1.5 mm diameter glass capillaries, each containing about 3 mg of DMPC or DMPE. To each capillary it was added about 100 µl of: a) pure water, b) 10^{-5} M ASOC; c) 10^{-4} M ASOC; d) 10^{-3} M ASOC and e) 10^{-2} M ASOC, and then sealed. They 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 Kα 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 connected to an Acer 915 microcomputer. No correction factors were applied. All the experiments with aqueous solutions were carried out at about 17 ± 2 °C, which is below the main transition temperature of each phospholipid under study.

Results

The molecular interactions of ASOC with multibilayers of the phospholipids DMPC and DMPE were studied by X-ray diffraction techniques. The patterns were obtained from the following specimens: a) dry samples of DMPC with ASOC in the molar ratios of 10:1, 5:1 and 1:1 recrystallized from an hydrophobic solution: b) dry samples of DMPE:ASOC in the same molar ratios, also recrystallized from an hydrophobic solution, and c) mixtures of each phospholipid in their crystalline phases with 10^{-5} M, 10^{-4} M, 10^{-3} M, and 10^{-2} M aqueous solutions of ASOC. All these patterns were compared with those of the pure ASOC and the corresponding phospholipid obtained under the same physicochemical conditions. The results are presented in Tables I to IV and Fig. 2 to 5. Table I shows the interplanar spacings and the relative intensities of the reflections produced by dry samples of DMPC and its mixtures with ASOC, while their diffractograms are compared in Fig. 2. The analysis of these results indicated that the X-ray pattern of DMPC was perturbed by increasing concentrations of ASOC. In fact, at such a small DMPC:ASOC molar ratio as of 10:1, the strong 4.29 Å reflection observed in the DMPC pattern was considerably weakened while that of 4.05 Å was absent. At the molar ratio of 1:1 most of the reflections produced by the lipid disappeared. On the other hand new although weak reflections showed up such as that of about 35 Å in the 10:1 mixture and another one of about 40 Å, observed in the three mixtures. Despite these changes, the four first orders of a period of about 55 Å were present in all these patterns without significant changes except in their relative intensities. In any X-ray diagram of these mixtures were observed reflections from ASOC. This most likely is due to the fact that recrystallized ASOC only produced amorphous patterns (see Fig. 2).

Table II and Fig. 3 show the results obtained from dry samples of DMPE, ASOC and of their 1:1 molar mixture. As it has been previously reported, this phospholipid presents two polymorphic forms when it is recrystallized from chloroform:methanol mixtures [18]. One phase (Lc 1)

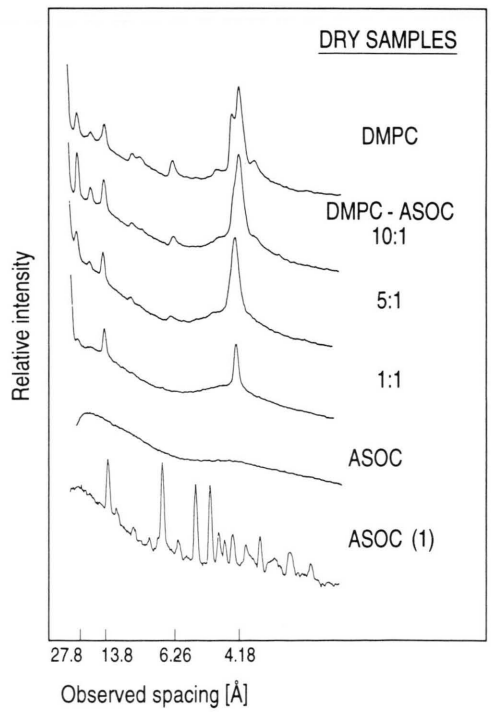

Fig. 2. Microdensitograms from X-ray diffraction diagrams of dry specimens recrystallized from chloroform:methanol 3:1. Flat-plate cameras (D = 8 cm). (1) Not recrystallized.

Table I. Comparison of observed interplanar spacings (do) and relative intensities (Io rel.) of DMPC, ASOC and of their 10:1, 5:1 and 1:1 molar mixtures (a, b, c).

DMPC		DMPC:ASOC 10:1		DMPC:ASOC 5:1		DMPC:ASOC 1:1		ASOC (d)	
do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.
55*	504*	55*	525*	55*	532*	55*	496*	–	–
–	–	42.1	1	40.2	2	39.4	6	–	–
–	–	35.3	2	–	–	–	–	–	–
28.1	9	28.1	19	27.4	7	27.6	1	–	–
18.4	4	18.6	6	18.6	3	18.6	1	–	–
13.6	12	13.8	13	13.8	12	13.8	12	–	–
–	–	–	–	–	–	–	–	12.6	31
–	–	–	–	–	–	–	–	11.1	6
9.21	5	9.17	3	9.23	2	–	–	–	–
–	–	–	–	–	–	–	–	8.74	8
8.38	4	8.28	2	8.33	3	–	–	–	–
–	–	–	–	–	–	–	–	7.56	8
–	–	–	–	–	–	–	–	6.99	3
–	–	–	–	–	–	–	–	6.62	67
6.25	9	6.26	8	6.27	1	–	–	6.27	1
–	–	–	–	–	–	–	–	5.88	7
5.61	1	–	–	–	–	–	–	5.53	1
5.32	1	–	–	–	–	–	–	5.19	67
–	–	–	–	–	–	–	–	4.96	1
–	–	–	–	–	–	–	–	4.75	48
4.66	3	4.63	2	4.67	1	–	–	–	–
–	–	–	–	–	–	–	–	4.57	16
–	–	–	–	–	–	–	–	4.40	10
4.29	28	4.26	3	4.27	9	–	–	4.23	21
4.11	87	4.12	100	4.17	80	4.18	25	–	–
4.05	11	–	–	–	–	–	–	3.97	18
3.85	9	3.85	2	3.88	1	–	–	3.86	6
3.70	1	–	–	–	–	–	–	3.72	21
–	–	–	–	–	–	–	–	3.60	10
–	–	–	–	–	–	–	–	3.53	18
3.48	1	3.46	1	–	–	–	–	3.46	7
–	–	–	–	–	–	–	–	3.32	36
–	–	–	–	–	–	–	–	3.24	6
3.16	1	–	–	–	–	–	–	3.17	4
–	–	–	–	–	–	–	–	3.10	14
–	–	–	–	–	–	–	–	3.03	3

a) All the specimens were recrystallized from $CHCl_3$:CH_3OH 3:1.
b) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14* cm.
c) Additional reflections with spacings below 3.0 Å were also observed.
d) Recrystallized ASOC gave an amorphous X-ray pattern (see Fig. 2). This data corresponds to unrecrystallized ASOC.

is obtained when these solvents are in a 3:1 v/v ratio. Its bilayer repeat of about 52 Å indicates that the hydrocarbon chains are extended and parallel to the bilayer normal [14]. The other phase of DMPE (Lc2) can be obtained by its recrystallization from chloroform:methanol 1:3. In this case, the bilayer repeat is of about 44 Å because the hydrocarbon chains are tilted by nearly 30°. The experimental results observed in this study confirmed that DMPE, when it was recrystallized from chloroform:methanol 3:1, showed the Lc1 form, proved by its 51.4 Å bilayer repeat. However, the X-ray pattern of DMPE:ASOC 1:1 showed that DMPE changed from the Lc1 form to the inclined Lc2. In fact, its bilayer repeat was reduced from 51.4 Å to 44.6 Å. A comparison in Table II of the spacings and intensities of the reflections obtained from DMPE in its Lc2 form with

Table II. Comparison of observed interplanar spacings (do) and relative intensities (Io rel.) of DMPE, DMPE:ASOC 1:1 and ASOC obtained from dry powder samples (a, b, c).

DMPE (1)		DMPE (2)		DMPE:ASOC 1:1		ASOC (3)	
do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.
51.4*	495*	–	–	–	–	–	–
–	–	44.6	614	44.6	414	–	–
25.2	2	–	–	–	–	–	–
–	–	22.1	8	22.1	7	–	–
17.1	5	17.3	1	17.5	1	–	–
14.7	2	14.7	5	14.7	4	–	–
12.7	7	12.7	2	12.6	3	12.6	31
11.3	2	11.1	2	11.1	1	11.1	6
–	–	8.84	1	8.84	1	8.74	8
7.89	1	7.72	1	7.65	1	7.56	8
7.30	7	7.28	5	7.27	6	–	–
–	–	–	–	–	–	6.99	3
6.80	1	6.80	1	6.83	1	–	–
–	–	–	–	–	–	6.62	67
6.37	1	6.32	7	6.32	1	–	–
5.94	14	5.86	4	5.94	3	5.88	7
5.69	1	–	–	–	–	–	–
5.21	1	–	–	–	–	5.19	67
–	–	5.30	6	5.32	1	–	–
5.07	9	–	–	–	–	–	–
4.77	15	4.79	8	4.79	11	4.75	48
4.66	5	–	–	–	–	–	–
4.50	4	4.50	100	4.49	56	4.57	16
–	–	4.40	7	–	–	4.40	10
4.25	4	4.19	7	4.19	1	4.23	21
4.05	98	4.04	46	4.07	47	3.97	18
3.80	51	3.80	32	3.81	35	3.86	6
–	–	3.69	1	–	–	3.72	21
3.64	4	3.63	1	3.64	3	3.60	10
3.52	1	3.52	1	3.52	2	3.53	18
3.40	3	3.39	1	3.40	2	3.46	7
–	–	–	–	–	–	3.32	36
–	–	3.27	1	3.27	3	3.24	6
–	–			3.16	3	3.17	4
3.10	2	3.13	1	–	–	3.10	14
–	–	–	–	–	–	3.03	3

a) DMPE and DMPE:ASOC 1:1 were recrystallized from $CHCl_3$:CH_3OH 3:1.
b) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14* cm.
c) Additional reflections with spacings below 3.0 Å were also observed.
(1) This phase (Lc_1) is normally obtained when DMPE is recrystallized from $CHCl_3$:CH_3OH 3:1.
(2) This phase (Lc_2) can be obtained when DMPE is recrystallized from $CHCl_3$:CH_3OH 1:3.
(3) Unrecrystallized ASOC.

those observed in the DMPE:ASOC 1:1 molar mixture showed that they are very similar. This result implies that ASOC molecules did not interact with those of DMPE. Otherwise, the X-ray pattern of the mixture would have differed from that of the pure lipid.

Fig. 4 and Table III respectively show the X-ray patterns and the interplanar spacings obtained after DMPC in its crystalline phase was mixed and allow to interact with pure water and ASOC aqueous solutions. They were obtained 2 and 14 days after preparation without showing any signif-

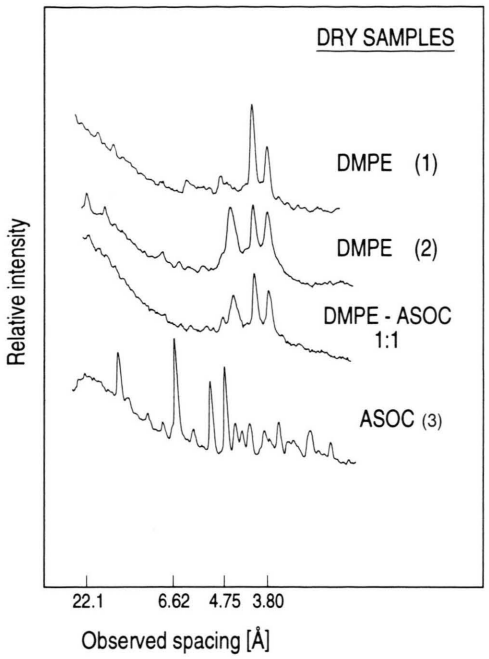

Fig. 3. Microdensitograms from X-ray diffraction diagrams of dry specimens. Flat-plate cameras (D = 8 cm).
(1) Recrystallized from chloroform : methanol 3 : 1.
(2) Recrystallized from chloroform : methanol 1 : 3.
(3) Not recrystallized.

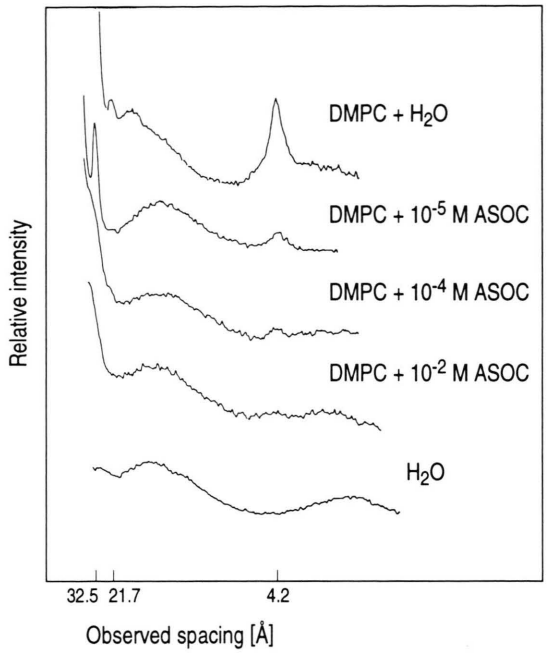

Fig. 4. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPC. Flat-plate cameras (D = 8 cm).

Table III. Comparison of observed interplanar spacings (do) and relative intensities (Io rel.) of DMPC specimens with water and ASOC aqueous solutions (a, b).

PC + H_2O		DMPC + 10^{-5} M ASOC		DMPC + 10^{-4} M ASOC		DMPC + 10^{-3} M ASOC		DMPC + 10^{-2} M ASOC	
Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.	do [AÅ]	Io rel.
4*	225*	65.1*	50*	65.0*	33*	–	–	–	–
2	200	32.5	43	32.5	31	–	–	–	–
2	16	21.7	3	21.7	2	–	–	–	–
20	100	4.23	14	4.21	14	4.22	10	4.19	2

a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14* cm.
b) The samples were X-ray diffracted 2 and 14 days after preparation. No differences were observed.

icant change with time. It was observed that DMPC expanded its bilayer period from about 55 Å when dry to 64.4 Å when it was in contact with water changing, at the same time, from the Lc phase to the lamellar Lβ′. The observed reflections were reduced to only the first three orders of the 64.4 Å repeat in the low angle region and one of 4.2 Å in the high angle. About the same pattern was observed when DMPC was made to interact with 10^{-5} M ASOC, although the intensities of the reflections became considerably weakened. The 10^{-4} M ASOC solution produced a further reduction of the reflection intensities, and the appearance of a central diffuse scattering in the low angle

region. When the ASOC concentration was raised to 10^{-3} M, all the low angle reflections vanished and were completely replaced by the diffuse scattering, remaining only a very weakened 4.2 Å reflection. At an ASOC concentration of 10^{-2} M, practically no reflections from DMPC were observed. The central diffuse scattering made the only difference of this pattern with respect to that of pure water. These results clearly indicated that ASOC in aqueous solutions produced a deep perturbation to the DMPC bilayer structure.

Finally, Fig. 5 and Table IV present the results obtained when water and ASOC aqueous solutions were allowed to interact with DMPE in the same conditions as described for DMPC. First, it can be noticed that the X-ray pattern of DMPE in the presence of water remained essentially the same Lc1 form observed in the dry state. It was observed that only the weakest reflections were absent in the humid sample. On the other hand, ASOC did not affect in any significant extent the X-ray pattern of DMPE, even in its most concentrated solutions.

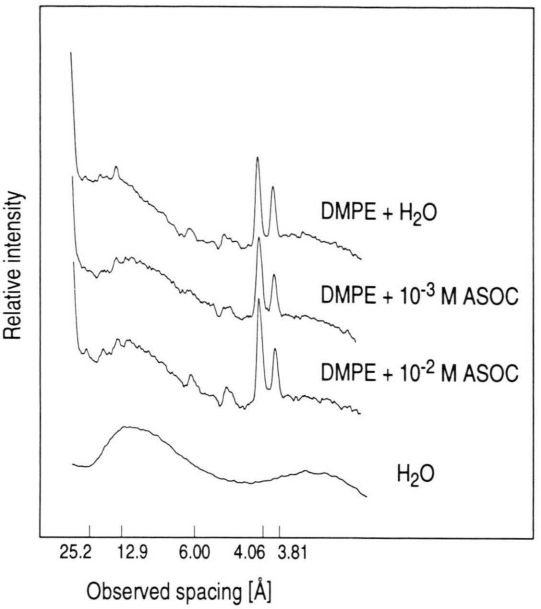

Fig. 5. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPE. Flat-plate cameras (D = 8 cm).

Table IV. Comparison of observed interplanar spacings (do) and relative intensities (Io rel.) of DMPE specimens with water and ASOC aqueous solutions (a, b).

DMPE + H_2O		DMPE + 10^{-3} M ASOC		DMPE + 10^{-2} M ASOC	
do [Å]	Io rel.	do [Å]	Io rel.	do [Å]	Io rel.
50.8*	1187*	50.8*	819*	50.8*	822*
25.2	3	25.4	5	25.2	4
17.1	3	16.9	7	16.9	8
12.8	10	12.9	7	13.0	10
11.3	2	11.3	5	11.3	4
7.34	3	7.36	2	7.32	5
6.00	12	5.97	10	6.00	10
5.09	3	5.07	4	5.08	4
4.82	3	4.82	12	4.80	16
4.66	17	4.65	4	4.61	4
4.52	10	4.53	12	4.54	14
4.23	8	4.25	2	4.24	2
4.06	78	4.06	76	4.05	100
3.93	1	3.93	1	3.92	2
3.81	45	3.82	33	3.80	38
3.65	3	3.67	2	3.64	2
3.52	3	3.55	2	3.50	2
3.41	4	3.41	2	3.39	2
3.18	4	3.18	2	3.16	2

a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. D = 8 and 14* cm.
b) The samples were diffracted 2 and 14 days after preparation. No differences were observed.

Discussion

Model systems consisting of pure phospholipids have been a valuable tool for obtaining information about the structure and physicochemical characteristics of the lipidic moiety of cell membranes. They are also been used to study how therapeutical drugs and other chemicals of biological interest might affect their integrity, fluidity and functionality. This information can help to understand the mechanisms of action of these compounds as well as their possible toxicity. In the present study three AAD with different degrees of lipophilicity were made to interact with multibilayer systems of the phospholipids DMPC and DMPE. They were asocainol (ASOC), procainamide (PROC), and quinidine (QUIN). Given the amphiphilic character of these drugs, their interactions with both phospholipids were carried out in a hydrophobic as well as in a hydrophilic medium. The aim of this study was to determine whether there is a direct relationship between the hydrophobicity of these drugs and their capacity to perturb the bilayer structure of the phospholipids involved in this research. As explained in the introduction, a possible molecular mechanism of action of this group of AAD is related to their ability to change the physical state of the membrane lipids that surround the sodium channels. In this way, the functionality of the proteins might be hindered affecting, therefore, the potential gradient across the membrane.

In this paper are presented the results obtained with ASOC, the most lipophilic of the three AAD included in this study, whereas those from PROC and QUIN will be published elsewhere [21]. They indicate that ASOC was able to perturb to different degrees the bilayer arrangements of both phospholipids. In the case of DMPE, the produced effects were very mild. In fact, it was only observed that DMPE, in the presence of ASOC in a 1:1 molar ratio, recrystallized from a hydrophobic solution in the tilted Lc2 form instead of the Lc1 extended one. This phase transition of DMPE is most likely due to a change in the physicochemical characteristics of the solvent after the dissolution of the amphiphilic ASOC rather than to a molecular interaction between ASOC and DMPE. Otherwise, the X-ray pattern of DMPE in its mixture with ASOC would have differed from that of pure DMPE in the same form, which is not the case. On the other hand, it should be mentioned that nearly similar effects on DMPE have been previously reported for the amphiphilic antibiotics chlortetracycline [18], chloramphenicol [19] and gentamicin [20].

The structural changes induced by ASOC to DMPC bilayers in the same hydrophobic medium were much more profound than those described for DMPE. As it is shown in Fig. 2 and Table I, only one molecule of the antiarrhytmic in ten molecules of DMPC was enough to perturb the phospholipid bilayer structure. This structural alteration, which increased with higher concentrations of ASOC in its mixtures with DMPC, lead to a phase transition of the lipid from its crystalline Lc form to more fluid phases. The fact, that the bilayer repeat of about 55 Å remained practically unchanged despite the gradual incorporation of ASOC points to a deep penetration into the hydrophobic core of DMPC. The appearance of the nearly 4.2 Å reflection in the 5:1 and 1:1 DMPC:ASOC mixtures proves that the hydrocarbon chains of the lipid became hexagonally arranged. This reflection, which is present in lecithin:water mixtures below their main transition temperatures (β and β′ phases), arises from the stiff and fully extended hydrocarbon chains organized with rotational disorder in a hexagonal lattice [16, 22]. These results are noteworthy as they were observed in the abscence of water. Somewhat similar effects on DMPC were previously reported for the amphipilic antibiotics chlortetracycline [18] and gentamicin [20]. The weak reflections of about 35 Å and 40 Å observed in the DMPC:ASOC X-ray diagrams obviously cannot be assigned to the 55 Å bilayer repeat. Most likely, they are orders of the ripple period of the lipid in the Pβ′ phase [16, 22].

The interaction of ASOC with DMPE and DMPC in a hydrophilic medium, in this case water, also showed different results for both phospholipids. As it can be observed in Table IV and Fig. 5, ASOC in a concentration as high as 10^{-2} M did not significatively alter the bilayer structure of DMPE. However, in the case of DMPC:ASOC in a concentration as low as 10^{-5} M produced structural perturbations to the lipid, which increased with higher concentrations of the drug. In fact, ASOC 10^{-2} M completely destroyed the bilayer or-

ganization of DMPC as all the reflections disappeared, included that of 4.2 Å. The resulting diffractogram is similar to that of pure water, except for the presence of the central diffuse scattering.

The different type and degree of perturbation produced by ASOC to DMPE and DMPC can be related to their respective packing arrangements and the effect of water upon them. As explained in the introduction, DMPE molecules are so tightly packed in the crystalline and gel phases that neither water nor ASOC are able to disrupt them. DMPC, on the contrary, presents large interbilayer spaces whose separation increases from about 55 Å up to 64.4 Å as water fills in. This allows the incorporation of ASOC molecules into DMPC interbilayer spaces and their interaction with the lipid polar groups. As a consequence, DMPC multibilayer arrangement becomes further disordered. This effect explains the replacement of the low angle reflections by the central diffuse scattering observed at 10^{-3} M concentration of ASOC. The increase of ASOC concentration to 10^{-2} M implied a further molecular penetration reaching the hydrophobic core and the complete disruption of the lipid molecular organization. These results agree with those reported by Mannhold and Voigt [10, 11]. In fact, they found a direct relationship between ASOC lipophilicity and its binding to lecithin membranes.

The experimental results described for ASOC with DMPC in a aqueous medium might be relevant to explain the molecular mechanism of action of this drug. In fact, it is very likely that ASOC can also interact with lecithins present in cell membranes. Therefore, the structural perturbation of those sited in the neighborhood of the sodium channels might affect their functional integrity [23].

Acknowledgements

Research grants from FONDECYT (1 930 504) and University of Concepción (92.13.94-1), as well as the gift of AAD from Drs. R. Mannhold and W. Voigt of the University of Düsseldorf, are gratefully acknowledged.

[1] P. H. Morgan, I. W. Mathison, J. Pharm. Sci. **65,** 467–482 (1976).
[2] J. T. Bigger, B. F. Hoffman, in: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed. (A. Goodman Gilman, T. W. Rall, A. S. Nies, P. Taylor, eds.), pp. 840–873, Pergamon, New York 1990.
[3] A. M. Katz, F. C. Messineo, L. Herbette, Circulation **65,** Suppl. I, 12–110 (1982).
[4] D. H. R. Gourley, in: Interactions of Drugs with Cells, chap. 5, p. 79, Thomas, Springfield 1971.
[5] J. R. Elliott, A. A. Elliott, A. A. Harper, J. P. Winpenny, Gen. Pharmac. **23,** 1005–1011 (1992).
[6] O. G. Mouritsen, K. Jorgensen, Bioessays **14,** 129–136 (1992).
[7] P. L. Jorgensen, Biochim. Biophys. Acta **694,** 27–68 (1982).
[8] F. Giraud, M. Claret, R. Bruckdorfer, B. Chailley, Biochim. Biophys. Acta **647,** 249–258 (1981).
[9] B. Martinac, J. Adler, C. Kung, Nature **348,** 261–263 (1990).
[10] W. Voigt, R. Mannhold, J. Limberg, G. Blaschke, J. Pharm. Sci. **77,** 1018–1020 (1988).
[11] R. Mannhold, W. Voigt, K. Dross, Cell Biol. Int. Rep. **14,** 361–368 (1990).
[12] J. A. F. Op den Kamp, Ann. Rev. Biochem. **48,** 47–71 (1979).
[13] M. Suwalsky, in: Physical Properties of Biological Membranes and their Functional Implications (C. Hidalgo, ed.), pp. 3–19, Plenum, U.S.A. 1988.
[14] M. Suwalsky, L. Duk, Makromol. Chem. **188,** 599–606 (1987).
[15] M. Suwalsky, J. Tapia, Bol. Soc. Chil. Quím. **30,** 27–35 (1985).
[16] A. Tardieu, V. Luzzati, F. C. Reman, J. Mol. Biol. **75,** 711–733 (1973).
[17] M. Suwalsky, L. Gimenez, V. Saenger, F. Neira, Z. Naturforsch. **43c,** 567–575 (1985).
[18] M. Suwalsky, F. Neira, I. Sánchez, Z. Naturforsch. **46c,** 133–138 (1991).
[19] M. Suwalsky, M. A. Espinoza, I. Sánchez, F. Villena, Z. Naturforsch. **46c,** 647–655 (1991).
[20] M. Suwalsky, J. Frías, Z. Naturforsch. **48c,** 632–639 (1993).
[21] M. Suwalsky, F. Neira, I. Sánchez (manuscript in preparation).
[22] M. J. Janiak, D. M. Small, G. G. Shipley, Biochemistry **15,** 4575–4580 (1976).
[23] B. L. Barchi, S. A. Cohen, L. E. Murphy, Proc. Natl. Acad. Sci. **77,** 1306–1310 (1980).