Interaction of the Antiarrhythmic Drug Procainamide with Phospholipid Bilayers

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Procainamide, Antiarrhythmic, Drug, Phospholipid Bilayer

Several hypotheses link the molecular mechanism of action of the antiarrhythmic drugs (AAD) that belong to class I to non-specific interactions with phospholipids sited in the neighborhood of sodium channels in the membrane of the myocardium. Procainamide (PROC), one of the least lipophilic drugs of this group, was induced to interact with bilayers of dimyristoylphosphatidylcholine (DMPC) and dimirystoylphosphatidylethanolamine (DMPE), liposomes of DMPC and human erythrocytes. The perturbing effects of PROC upon these systems were respectively determined by X-ray diffraction, fluorescence spectroscopy and scanning electron microscopy. It was found that PROC exerted very little effect upon DMPC and DMPE even at such a high concentration as 10 mM. However, at therapeutical plasma concentrations, PROC induced shape changes *in vitro* to red cells.

Introduction

Procainamide (PROC), whose structural formula is shown in Fig. 1, is an antiarrhythmic drug (AAD) that belongs to class I. These AAD exert their action in the myocard cell membrane by blocking the sodium channels (Morgan and Mathison, 1976; Bigger and Hoffman, 1990). Although the molecular mechanism is still unclear, five types of molecular interactions by which AAD might modify the channel functions have been proposed (Katz *et al.,* 1982). It is of interest to note that three of the suggested mechanisms involve non-specific interactions of AAD with the membrane phospholipids. On the other hand, it has also been reported that structural perturbations induced to phospholipids in the neighborhood of ion channels affect their functions (Mouritsen and Jorgensen, 1992; Madsen *et al.,* 1992; Martinac *et al.,* 1992). For these reasons it was thought of interest to study the interaction of PROC with phospholipid bilayers. These interactions were studied by three different methods. In the first, PROC was made to interact with multilayers built-up of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE). These are the types of phospholipids that are respectively found in high proportions in the outer and inner monolayers of the erythrocyte membrane (Roelofsen, 1991). The interactions of PROC with DMPC multilayers were performed in a hydrophobic and hydrophilic medium given their amphiphilic nature. The structural perturbation induced by PROC to both phospholipids was determined by X-ray diffraction techniques. This method had previously been used in this laboratory to study how other therapeutical drugs affect the bilayer structures of DMPC and DMPE (Suwalsky *et al.,* 1988; Suwalsky *et al.,* 1991; Suwalsky and Frías, 1993).

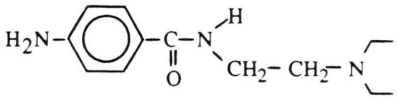

Fig. 1. Structural formula of PROC.

Fluorescence steady-state anisotropy of DPH (1,6-diphenyl-1,3,5-hexatriene) and Laurdan (6-dodecanoyl-2-dimethylaminonaphtalene) general polarization were determined on DMPC large unilamellar vesicles. DPH, an all-*trans* polyene with a rod-like shape, is one of the most commonly used probe for the hydrophobic regions of phospholipid bilayers. DPH fluorescence steady-state anisotropy is useful to investigate structural and dynamic properties of lipid bilayers; it provides a measure of the rotational diffusion of the fluorophor restricted within a certain region due to the

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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 fluorescent moiety within a shallow position in the bilayer, Laurdan provides information of dynamic properties in this zone of the bilayer. The quantitation of Laurdan fluorescence intensity data at different wavelengths is done using the general polarization concept (Parasassi *et al.*, 1990).

Finally, PROC was made to interact *in vitro* with human erythrocytes. They were later observed by scanning electron microscopy (SEM) in order to detect whether PROC was able to induce changes in the shape of these cells.

Materials and Methods

X-ray diffraction analysis of phospholipid multilayers

Synthetic DMPC from Sigma (lots 57F-8365 and 80H-8371, A grade, MW 677.9), DMPE from Sigma (lot 67-8350, A grade, MW 653.9) and procainamide. HCl of the highest purity, MW 271.8 (a gift from Drs. R. Mannhold and W. Voigt, University of Düsseldorf, Germany) were used without further purification. Powder mixtures of DMPC:PROC and of DMPE:PROC were prepared in the molar ratios of 10:1, 5:1 and 1:1. Each mixture was dissolved in chloroform:methanol 3:1 (v/v) and left to dry. 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 in flat-plate cameras with 0.25 mm diameter glass collimators (Suwalsky, 1988) provided with rotating and cooling devices. The same procedure was followed with pure samples of each phospholipid and PROC.

The interactions in a hydrophilic medium were obtained in 1.5 mm diameter glass capillaries, each containing 2–3 mg of DMPC or DMPE. To each capillary was added about 100 µl of: a) distilled water; b) 0.01 mM PROC; c) 0.1 mM PROC; d) 1 mM PROC, and e) 10 mM PROC. The samples thus prepared were X-ray-diffracted 2 and 14 h 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 CuKα 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 computer. 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.

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 Corp.) employing nitrogen pressure at 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 vesicle preparation by injecting small aliquots of a concentrated solution of the probe in ethanol and gently shaked 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 Ind.) and on a phase shift and modulation Gregg-200 steady-state and time-resolved spectrofluorometer (I.S.S.) respectively, both interfaced to 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 Eng.). A Xenon arc lamp was used 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 polarizer in both exciting and emitting beams. The emission was measured using a WG-420 Schott high-pass filter which showed negligible fluorescence. General polarization (GP) was evaluated by GP = IB – IR/IB + IR, where IB and IR 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 the gel and liquid crystalline phases respectively (Parasassi *et al.*, 1991). Blank substraction was incorporated to DMPC vesicle suspensions by addition of small aliquots of a concentrated solution of PROC in ethanol and incubated at 40 °C for *ca.* 15 min.

Morphological observation of PROC-treated erythrocytes

Blood samples were obtained by puncture of the ear lobule from clinically healthy individuals not being treated with any pharmacological agent. One drop was received in a siliconized tube containing 5 ml of phosphate-buffered saline (PBS) (150 mM NaCl/10 mM phosphate, pH 7.4) at 5 °C. This blood stock solution was used to prepare in siliconized tubes the following solutions: a) 1 ml of blood stock solution plus 9 ml of PBS (control), and b) blood stock solution plus PBS-containing PROC in form to obtain final concentrations of 0.01 mM, 0.1 mM and 1 mM. These samples were incubated at 37 °C for 1 h in an oven and then fixed with glutaraldehyde. One drop of each sample was added to a tube containing 1 ml of 2.5% glutaraldehyde in PBS and allowed to rest overnight at 5 °C. Drops of the fixed samples were placed directly on aluminium stubs, air-dried in an oven at 37 °C for 1 h and gold coated in a S150 Edwards sputter coater device for 3 min at 10^{-1} Torr, with a final film width of 35–40 nm. The observation and photographic records were made in an Etec Autoscan scanning electron microscope.

Results

X-ray studies on lipid multilayers

The molecular interaction of PROC with multilayers of 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) dry samples of DMPC:PROC and of DMPE:PROC in the molar ratios of 10:1, 5:1 and 1:1 recrystallized from chloroform:methanol 3:1, v/v, and b) mixtures of each phospholipid in their crystalline phases with 0.01 mM, 0.1 mM, 1 mM and 10 mM aqueous solutions of PROC. All these patterns were compared with those of PROC and the corresponding phospholipid obtained under the same physicochemical conditions. Thus, Table I shows the interplanar spacings and relative intensities of the reflections produced by the recrystallized specimens of DMPC and of its mixtures with PROC, while their diffractograms are compared in Fig. 2. As it can be observed, the X-ray pattern of DMPC increasingly changed with higher ratios of PROC in their mixtures. In the low-angle region there was a consisting decrease in the reflection intensities and, at the same time, an increase in their spacings. In fact, the first order reflection of DMPC, corresponding to the bilayer width, increased from 54.5 Å up to 57.4 Å in its 1:1 mixture. On the other hand, practically all the reflections from DMPC were absent in the wide-angle region of the 1:1 pattern. However, a new and relatively strong reflection of about 4.2 Å showed up. The appearance of this reflection was indica-

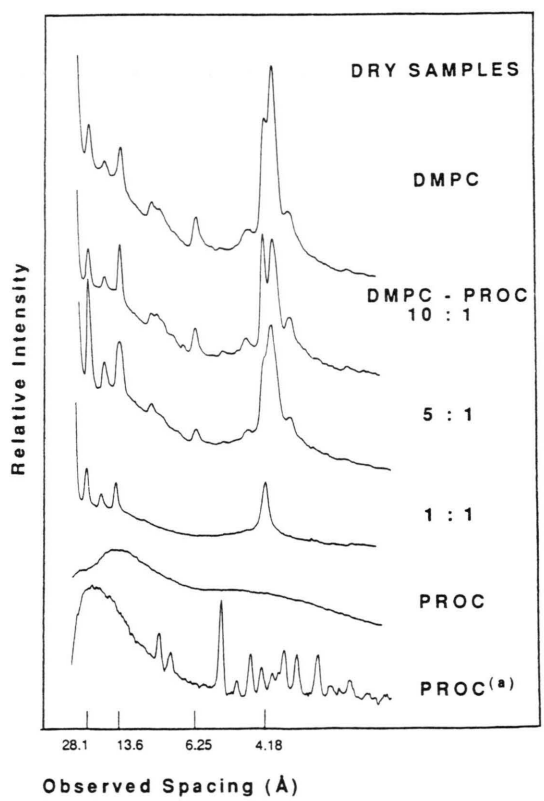

Fig. 2. Microdensitograms from X-ray diagrams of dry specimens recrystallized from chloroform:methanol 3:1 (v/v). Flat-plate cameras. D = 8 cm. [a] Not recrystallized.

Table I. Comparison of observed interplanar spacings (d_o) and relative intensities (I_o rel) of DMPC, PROC and of their 10:1, 5:1 and 1:1 molar mixtures[a,b,c].

DMPC		DMPC:PROC 10:1		DMPC:PROC 5:1		DMPC:PROC 1:1		PROC[d]	
d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel
54.5*	612*	54.5*	380*	55.9*	380*	57.4*	355*	–	–
28.1*	14*	28.3*	14*	28.5*	37*	28.7*	12*	–	–
18.4	5	18.3	5	19.3	11	19.0	6	–	–
13.6	19	13.6	19	14.0	31	14.3	11	–	–
8.93	7	9.12	5	9.29	4	–	–	–	–
8.38	3	8.30	6	8.44	2	–	–	8.30	13
8.19	1	8.19	1	–	–	–	–	–	–
7.43	1	7.43	2	7.43	1	–	–	7.51	8
6.25	19	6.28	14	6.30	7	–	–	–	–
5.61	1	5.64	1	–	–	–	–	5.77	2
5.27	1	5.25	1	5.27	1	–	–	5.27	39
–	–	–	–	–	–	–	–	4.84	6
4.68	7	4.67	7	4.68	3	–	–	–	–
–	–	–	–	–	–	–	–	4.53	17
4.29	82	4.28	61	4.28	41	–	–	4.31	11
4.11	100	4.11	69	4.16	90	4.18	32	4.11	6
4.05	25	4.07	25	4.08	21	–	–	4.01	4
3.85	17	3.83	18	3.85	8	–	–	3.91	20
3.70	1	–	–	–	–	–	–	3.74	17
3.48	1	–	–	–	–	–	–	3.48	21
–	–	–	–	–	–	–	–	3.33	7
3.16	1	3.17	1	3.17	1	–	–	3.14	10
3.03	1	3.03	1	3.04	1	–	–	–	–

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

tive of the fluid state reached by the lipid. In fact, this reflection corresponds to the average separation of the fully extended hydrocarbon chains organized with rotational disorder in an hexagonal lattice (Tardieu *et al.*, 1973; Janiak *et al.*, 1976). It was also noticed that no reflections from PROC were present in any of its dry mixtures with DMPC neither with those of DMPE. This was due to the fact that recrystallized PROC gave amorphous patterns (see Fig. 2 and 3). Fig. 3 shows the X-ray patterns of the recrystallized specimens of DMPE, PROC and of their 1:1 mixture while the spacings and intensities of their reflections are included in Table II. The comparison of the data from DMPE and of its 1:1 mixture with PROC did not show any significant difference. It can therefore be concluded that PROC did not induce structural perturbations to DMPE in a hydrophobic medium.

Table III and Fig. 4 present the results obtained after DMPC was mixed and allowed to interact with water and PROC aqueous solutions. It was observed that DMPC, as expected, expanded its bilayer width from about 55 Å when dry to nearly 65 Å when immersed in water. The observed reflections were reduced to only the first three orders of the bilayer repeat in the low-angle region and one of about 4.2 Å in the wide-angle region. Practically the same patterns were obtained when DMPC was mixed with PROC solutions, even if they were as concentrate as 10 mM. These results indicated that PROC did not interact with DMPC in an aqueous medium. Finally, Table IV and Fig. 5 show the results obtained when DMPE and PROC aqueous solutions were made to interact in conditions analogous to the above reported for DMPC. In the first place, it could be noticed that the X-ray pattern of DMPE remained very similar to that obtained in the dry state except by the absence of its weakest reflections. This difference with respect to DMPC is due to the fact that DMPE molecules pack tighter than those of

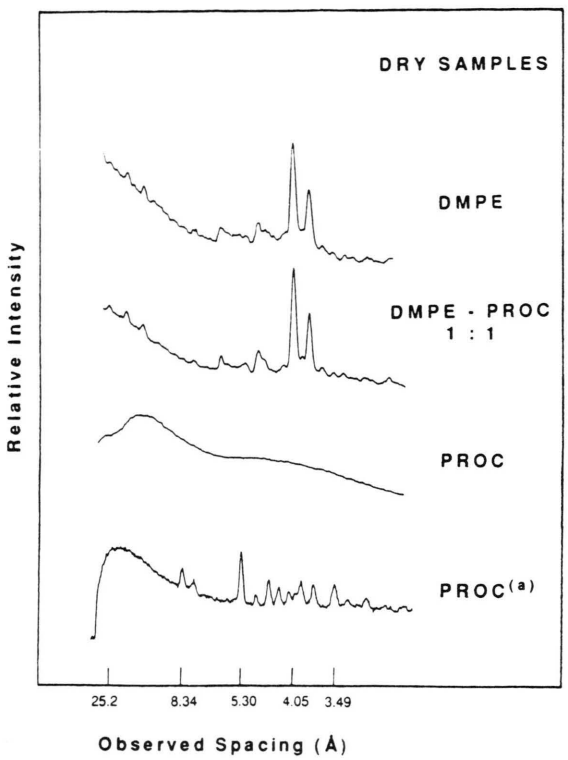

Fig. 3. Microdensitograms from X-ray diagrams of dry specimens recrystallized from chloroform:methanol 3:1 (v/v). Flat-plate cameras. D = 8 cm. (a) Not recrystallized.

DMPC, resulting in a very stable bilayer structure which is not significatively affected by water (Suwalsky, 1988) neither by several amphiphilic drugs (Suwalsky *et al.*, 1988, 1991, 1993). It is not surprising, therefore, that PROC did not affect the X-ray pattern of DMPE even at the highest assayed concentration (10 mM).

Fluorescence measurements

DPH emission steady-state anisotropy and Laurdan general polarization was determined in DMPC large unilamellar vesicles. As it can be observed in Table V, the presence of increasing concentrations of PROC up to 10 mM did not produce any change in the fluorescence anisotropy of DPH (0.31) nor in the general polarization of Laurdan (0.57). These results indicated that PROC had no influence on the rotational mobility of DPH, distributed in the hydrophobic zone of the lamella, nor in Laurdan environment at the lamella hydrophobic-hydrophilic interface. These results agreed with those observed by X-ray diffraction.

Table II. Comparison of observed interplanar spacings (d_o) and relative intensities (I_o rel) of DMPE, DMPE:PROC 1:1 and PROC obtained from dry powder samples[a,b,c].

DMPE		DMPE:PROC 1:1		PROC[d]	
d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel
51.4*	505*	51.4*	414*	–	–
25.2	2	25.4	4	–	–
17.1	5	17.0	7	–	–
14.7	2	14.7	7	–	–
12.7	7	12.7	1	–	–
11.3	2	–	–	–	–
–	–	–	–	8.34	12
7.89	1	7.93	2	7.87	1
–	–	–	–	7.55	9
7.25	7	7.30	3	–	–
6.80	1	–	–	–	–
–	–	–	–	6.53	1
6.37	1	–	–	–	–
5.94	15	5.98	15	–	–
–	–	–	–	5.79	1
5.69	10	5.71	10	–	–
–	–	–	–	5.30	31
5.21	7	5.24	5	–	–
5.07	9	5.07	6	5.03	1
–	–	–	–	4.85	6
4.77	16	4.79	20	–	–
4.66	5	4.63	8	4.69	1
–	–	–	–	4.55	16
4.50	4	4.48	1	–	–
–	–	4.35	1	4.32	10
4.25	4	4.24	4	–	–
–	–	–	–	4.14	8
4.05	100	4.06	95	–	–
–	–	3.93	44	3.92	21
3.80	52	3.80	7	–	–
–	–	–	–	3.75	16
3.64	4	3.65	2	–	–
3.52	1	3.51	1	3.49	19
3.40	3	3.40	6	–	–
3.10	1	–	–	–	–

[a] DMPE and DMPE:PROC 1:1 were recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v).

[b] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from Debye-Scherrer and flat-plate cameras with D = 8 and 14* cm.

[c] Additional reflections with spacings below 3.0 Å were also observed.

[d] Recrystallized PROC gave an amorphous pattern (see Fig. 3). This data corresponds to unrecrystallized PROC.

SEM studies on red cells

The plasma concentration of PROC needed to produce antiarrhythmic effects is about 0.01 mM

Table III. Comparison of observed interplanar spacings (d_o) and relative intensities (I_o rel) of DMPC specimens with water and PROC solutions[a,b].

DMPC + H_2O		DMPC + 10^{-3} M PROC		DMPC + 10^{-2} M PROC	
d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel
64.9*	100*	64.9*	100*	64.9*	80*
32.5	100	32.5	70	32.5	80
21.4	10	21.3	8	21.4	9
4.19	40	4.19	30	4.20	30

[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.

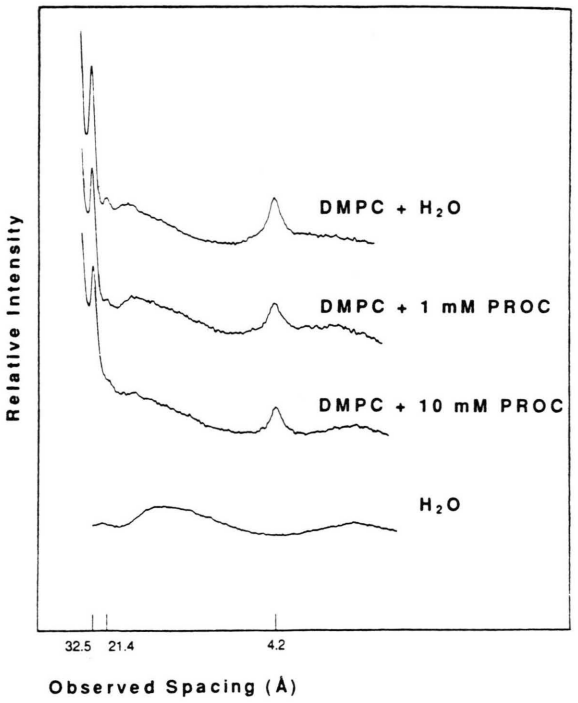

Fig. 4. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPC. Flat-plate cameras. D = 8 cm.

(Bigger and Hoffman, 1990; Mannhold *et al.*, 1990). However, the probability of toxicity becomes greater as its plasma concentration rises above 0.1 mM (Bigger and Hoffman, 1990). For these reasons, most of the reported experiments, particularly those performed on red cells, included 0.01 mM and higher concentrations of PROC.

Table IV. Comparison of observed interplanar spacings (d_o) and relative intensities (I_o rel) of DMPE specimens with water and PROC aqueous solutions[a,b].

DMPE + H_2O		DMPE + 10^{-3} M PROC		DMPE + 10^{-2} M PROC	
d_o [Å]	I_o rel	d_o [Å]	I_o rel	d_o [Å]	I_o rel
50.8*	607*	50.8*	624*	50.8*	619*
25.2	2	25.2	3	25.2	3
17.1	5	16.9	10	16.9	10
12.8	12	13.0	8	12.9	7
11.3	3	11.3	2	11.3	3
7.34	2	7.32	2	7.30	2
5.99	12	5.97	12	5.99	10
5.09	2	5.09	2	5.10	7
4.82	8	4.78	5	4.80	10
4.66	7	4.64	10	4.62	7
4.52	8	–	–	–	–
4.23	2	4.24	2	4.26	2
4.06	97	4.05	78	4.04	100
3.93	3	–	–	–	–
3.81	54	3.79	41	3.81	63
3.65	5	3.63	3	3.65	3
3.57	5	–	–	–	–
3.41	5	3.39	2	3.39	5
3.18	3	3.18	2	3.19	3

[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.

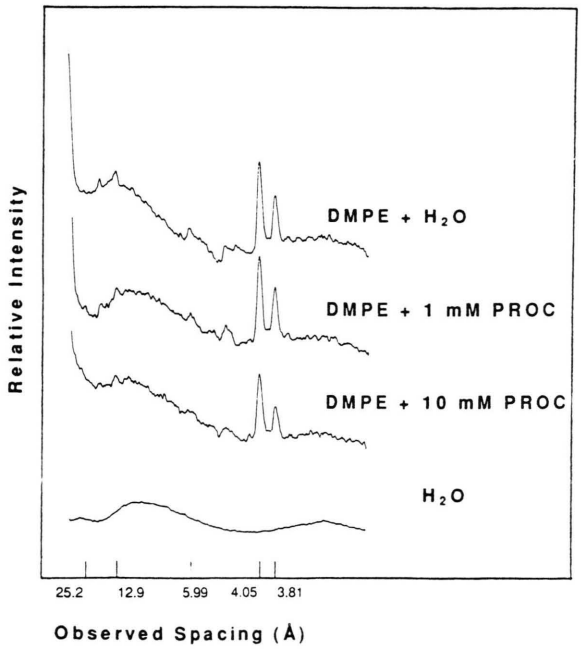

Fig. 5. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPE. Flat-plate cameras. D = 8 cm.

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

PROC [mM]	r (DPH)	GP (Laurdan)
0	0.31	0.56
0.02	0.31	0.57
0.1	0.31	0.56
1.0	0.31	0.57
10.0	0.31	0.57

Human erythrocytes were made to interact *in vitro* with 0.01, 0.1 and 1 mM PROC, whose resulting SEM images are shown in Fig. 6, together with that of PROC-free cells (control). As it can be observed, in the case of PROC-incubated cells the erythrocytes underwent changes in their shapes. At 0.01 mM not all the cells were affected and the alteration consisted mainly in the presence of only 4 to 8 rounded blebs over their surfaces, which is indicative of a comparatively minor crenation phenomena. Higher PROC concentrations induced crenation in the form of echinocytes and spheroechinocytes, a configuration characterized by a great number of blebs or protuberances over their surface. Several studies have shown that amphiphilic drugs induce a transformation from the discoid shape of the erythrocyte to the echinocyte form (Isomaa *et al.*, 1987; Fugii *et al.*, 1979). Sheetz and Singer (1974, 1977) formulated the bilayer couple hypothesis to explain these shape alterations. Accordingly to it they arise from a differential expansion of the two monolayers of the membrane lipid bilayer. Echi-

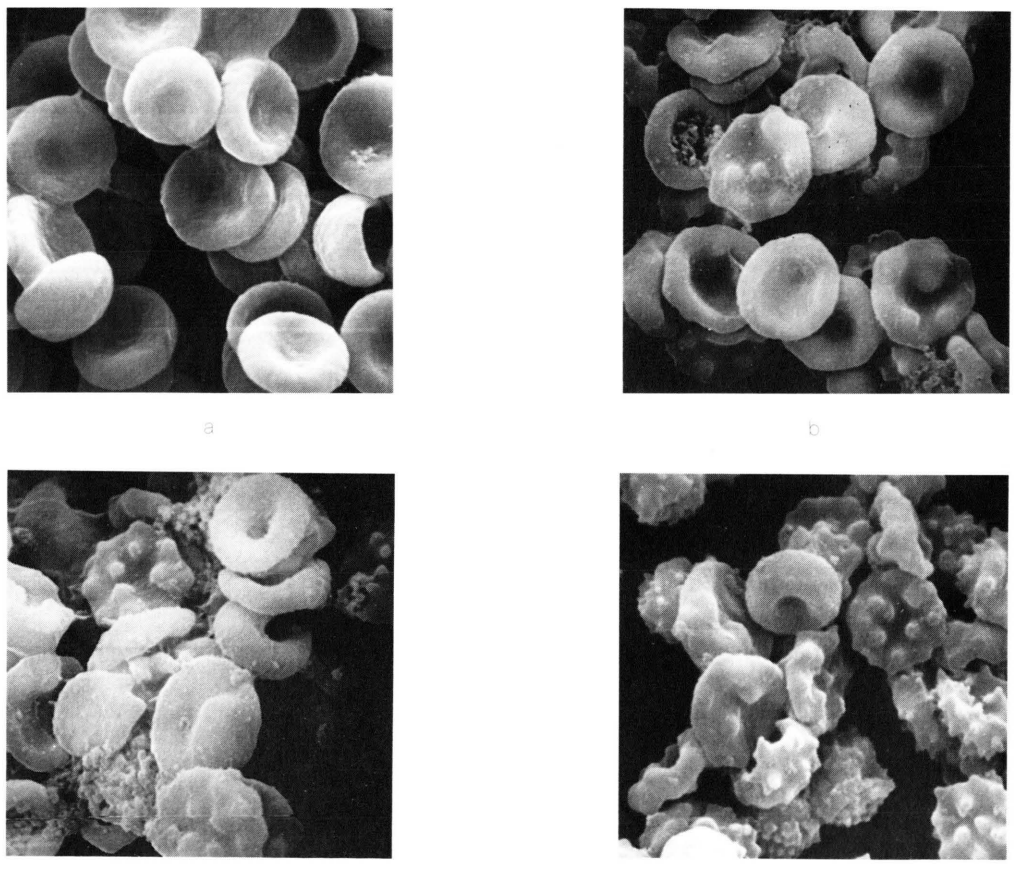

Fig. 6. SEM images of human erythrocytes; 3800×; a) PROC-free cells; b) after incubation with 0.01 mM PROC; c) after incubation with 0.1 mM PROC, and d) after incubation with 1 mM PROC.

nocytogenic amphiphiles would insert mainly in the outer monolayer, thereby expanding it relative to the inner one. The observed results imply, therefore, that PROC indeed interacted with phospholipids located in the outer monolayer of the erythrocytes.

Discussion

The purpose of this research was to determine whether PROC was able to interact with phospholipid bilayers as it has been proposed as a possible molecular mechanism of action of class I AAD. PROC, therefore, was made to interact with phospholipids under the form of DMPC and DMPE multilayers, DMPC liposomes and the membrane of human erythrocytes. The experimental results showed that PROC, in a hydrophilic medium, was able to interact only with the erythrocyte membrane. Given the echinocytic shape adopted by the red cells it is most likely that PROC interacted with phospholipids sited in the membrane external monolayer. The explanation for PROC aqueous solutions not to interact with DMPC multilayers and liposomes might lie in its low lipophilicity; in fact, it is the lowest of class I AAD (Voigt *et al.*, 1988), being 0.82 its octanol/water partition coefficient expressed as log P (Mannhold *et al.*, 1990). Another class I AAD, asocainol, whose lipophilicity is about six times higher than that of PROC, indeed interacted with DMPC multilayers (Suwalsky *et al.*, 1993) and DMPC liposomes (Sotomayor and Bagnara, 1994). On the other hand, PROC interaction with the erythrocyte membrane was facilitated by the incubation at 37 °C. At this temperature the membrane is in a much more fluid state than the multilayers and liposomes, whose interactions with PROC were assayed at about 18 °C.

Acknowledgements

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Bigger J. T. and Hoffman B. F. (1990), Antiarrhythmic drugs. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed. (A. Goodman-Gilman, T. W. Rall, A. S. Nies and P. Taylor, eds.). Pergamon, New York, pp. 840–873.

Fujii T., Sato T., Tamura A., Wakatsuki M. and Kanaho Y. (1979), Shape changes of human erythrocytes induced by various amphipathic drugs acting on the membrane of intact cells. Biochem. Pharmacol. **28,** 613–620.

Isomaa B., Hägerstrand H. and Paatero G. (1987), Shape transformation induced by amphiphiles in erythrocytes. Biochim. Biophys. Acta **899,** 93–103.

Janiak M. J., Small D. M. and Shipley G. G. (1976), Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl and dipalmitoylecithin. Biochemistry **15,** 4575–4580.

Katz A. M., Messineo F. C. and Herbette L. (1982), Ion channels in membranes. Circulation **65,** Suppl. I, I1–I10.

Madsen K. L., Meddings J. B. and Fedorak R. N. (1992), Basolateral membrane lipid dynamics alter Na-K ATPase activity in rabbit small intestine. Can. J. Physiol. Pharmacol. **70,** 1483–1490.

Manhold R., Voigt W. and Dross K. (1990), Phosphatidylcholine: a pharmacologically relevant site for binding of antiarrhythmics? Cell Biol. Int. Rep. **14,** 361–368.

Martinac B., Adler J. and Kung Ch. (1990), Mechanosensitive ion channels of *E. coli* activated by amphipaths. Nature **348,** 261–263.

Morgan P. H. and Mathison I. W. (1976), Arrhythmias and antiarrhythmic drugs: mechanism of action and structure-activity relationships. I. J. Pharm. Sci. **65,** 467–482.

Mouritsen O. G. and Jorgensen K. (1992), Dynamic lipid bilayer heterogeinity: A mesoscopic vehicle for membrane function. Bioassays **14,** 129–136.

Parasassi T., de Stasio G., d'Ubaldo A. and Gratton E. (1990), Phase fluctuation in phospholipid membranes revealed by Laurdan fluorescence. Biophys. J. **57,** 1179–1186.

Parasassi T., de Stasio G. Ravagnan G., Rush T. and Gratton E. (1991), Quantitation of lipid phases in phospholipid vesicles by generalized polarization of Laurdan fluorescence. Biophys. J. **60,** 179–189.

Roelofsen B. (1991), Molecular architecture and dynamics of the plasma membrane lipid bilayer: The

red blood cell as a model. Infection 19, Suppl. **4,** S206–S209.

Sheetz M. P. and Singer S. J. (1974), Biological membranes as bilayer couples. A molecular mechanism of drug-induced interactions. Proc. Natl. Acad. Sci. U.S.A. **71,** 4457–4461.

Sheetz M. P. and Singer S. J. (1977), Equilibrium and kinetic effect of drugs on the shapes of human erythrocytes. J. Cell Biol. **70,** 247–251.

Sotomayor C. P. and Bagnara M. (1994), unpublished data.

Suwalsky M. (1988), in: Physical Properties of Biological Membranes and their Functional Implications (C. Hidalgo, ed.). Plenum, U.S.A., pp. 3–19.

Suwalsky M., Gimenez L., Saenger V. and Neira F. (1988), X-ray studies on phospholipid bilayers. VIII. Interactions with chlorpromazine. HCl. Z. Naturforsch. **43c,** 742–748.

Suwalsky M., Neira F. and Sánchez I. (1991), *ibid.* X. Interactions with chlorotetracycline hydrochloride. Z. Naturforsch. **46c,** 133–138.

Suwalsky M. and Frías J. (1993), *ibid.* XIII. Interactions with gentamicin. Z. Naturforsch. **48c,** 632–639.

Suwalsky M., Sánchez I. and Neira F. (1993), *ibid.* XIV. Interactions with the antiarrhythmic asocainol. Z. Naturforsch. **48c,** 930–938.

Tardieu A., Luzzati V. and Reman F. C. (1973), Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. J. Mol. Biol. **75,** 711–733.

Voigt W., Mannhold R., Limberg J. and Blashke G. (1988), Interactions of antiarrhythmics with artificial phospholipid membranes. J. Pharm. Sc. **77,** 1018–1020.