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QUINIDINE IS A STRONG PERTURBER OF ACIDIC PHOSPHOLIPID BILAYER ORDER AND FLUIDITY

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The effect of an antiarrhythmic drug, quinidine, on the organization of model phospholipid membranes was studied by the spin-labeling technique. Quinidine strongly perturbs the molecular organization of lipid bilayers prepared from acidic phospholipids (phosphatidylserine, phosphatidic acid) and has only a slight effect on neutral phosphatidylcholine membranes. The interaction of the drug with acidic phospholipids manifests itself in a pronounced increase in the order parameter of the region close to the polar surface of the bilayer and in some decrease in its inner hydrocarbon core fluidity. It is suggested that the perturbation in the organization of membrane lipids may contribute to the mechanisms by which quinidine exerts its pharmacological effects.

The alkaloid drug quinidine is well known as a potent antiarrhythmic agent. Physiological and biochemical experiments have shown that the drug affects various membrane-mediated events [1–8]. It is thought that the pharmacological action of quinidine derives ultimately from its ability to perturb the lipoprotein structure of biological membranes [6]. However, the molecular mechanisms of drug-membrane interaction are not well known. As an attempt to elucidate the molecular basis of membrane perturbations by quinidine, model studies with artificial lipid membranes have been undertaken. The results presented in this paper give evidence that quinidine induces pronounced changes in the molecular organization of membrane lipids. This evidence has been obtained by electron spin resonance (ESR) experiments using two stearic acid spin-label probes sounding the region close to the polar headgroups of phospholipids and the hydrophobic core of the bilayer.

Egg yolk phosphatidylcholine, phosphatidic acid, bovine brain phosphatidylserine and quinidine-HCl were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Lipids were chromatographically pure. Spin labels, 5-doxylstearic acid and 16-doxylstearic acid, were from Syva (Palo Alto, CA, U.S.A.). For preparation of phospholipid suspensions used in ESR measurements, appropriate amounts of phospholipid, spin label and drug were dissolved in a chloroform/ethanol mixture (90 : 10, v/v). After evaporation of the solvent under argon, Tris-HCl buffer (30 mM, pH 7.4) was added and the mixture was shaken by means of a vortex rotamixer for 5 min. The final phospholipid concentration was 20 mg per ml buffer. Spin label/phospholipid molar ratio was 1 : 100. The resulting suspension of multilamellar liposomes containing quinidine was equilibrated, before spectroscopic measurements, for at least 3 h at room temperature. ESR spectra were obtained at 20°C with an SE/X-28 ESR spectrometer (Breslau Technical University) operating at 9.5 GHz.

ESR spectra of 5-doxylstearic acid, incorporated by spin probe into phosphatidic acid, phosphatidylserine and phosphatidylcholine liposomes,

are qualitatively very similar. They reflect a relatively high degree of order in the region close to the polar surface of the bilayer [9,10]. Spectra of this type can be characterized by the order parameter, S, being a measure of the motion amplitude of the molecular long axis about the average orientation of the fatty acid chains in the lipid bilayer. The order parameter, S, is calculated from the anisotropic hyperfine splittings $A_{\parallel}$ and $A_{\perp}$, measured as shown in Fig. 1, using the equation [9]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - \frac{1}{2}(A_{xx} + A_{yy})} \cdot \frac{a}{a'}$$

In this equation A_{xx}, A_{yy} and A_{zz} are components of the hyperfine splitting tensor obtained from single crystal spectra [11], $a = (A_{xx} + A_{yy} + A_{zz})/3$ and $a' = (A_{\parallel} + 2A_{\perp})/3$.

Typical spectra of phosphatidic acid multibilayers spin-labeled with 5-doxylstearic acid without and in the presence of quinidine are shown in Fig. 1. The spectral changes observed in the presence of quinidine indicate that the drug produces a pronounced increase in the molecular order of the outer part of the hydrocarbon chains. A similar strong ordering effect was also observed upon addition of the drug to bilayers prepared from another acidic phospholipid, phosphatidylserine. In contrast, the effect of quinidine on the organization of neutral phosphatidylcholine membranes

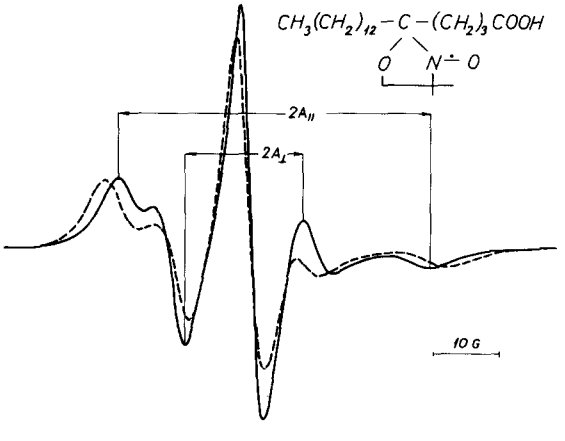

Fig. 1. Typical ESR spectra of 5-doxylstearic acid spin probe incorporated into phosphatidic acid liposomes without and in the presence of quinidine. ———, phosphatidic acid alone; — — —, phosphatidic acid/quinidine (10:4 molar ratio).

was much smaller. In this case only slight changes in ESR spectra could be detected.

Quantitative analysis of the changes in the order parameter produced by quinidine in bilayers prepared from different phospholipids is given in Fig. 2.

The paramagnetic group of the spin probe 16-doxylstearic acid is located in the inner hydrocarbon core of the lipid bilayer [12]. This part of the membrane is much more fluid than regions close to polar groups of phospholipids and, consequently, the ESR spectrum (Fig. 3) is characteristic for fast, nearly isotropic motion. From such a spectrum an empirical motion parameter, τ, can be derived using the equation [13,14]:

$$\tau = 6.5 \cdot 10^{-10} W_0 \left[(h_0/h_{-1})^{1/2} - 1 \right] \text{s}$$

where W_0 is the width of the central line (in gauss) and h_0 and h_{-1} are the heights of the central and high-field lines, respectively.

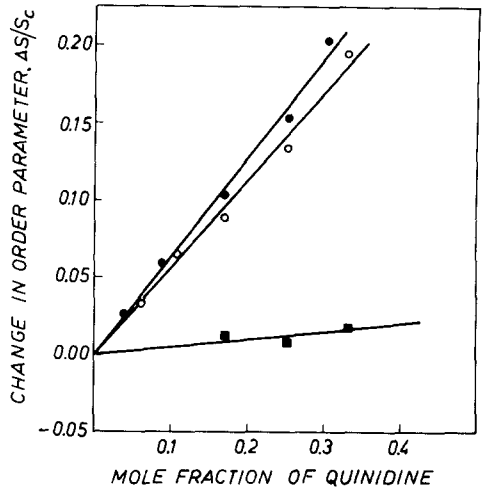

Fig. 2. The effect of quinidine on the relative change in order parameter, $\Delta S/S_c$, of 5-doxylstearic acid spin-labeled liposomes prepared from phosphatidic acid (●), phosphatidylserine (○) and phosphatidylcholine (■). Lipid/drug mixtures were dispersed in 30 mM Tris-HCl buffer, pH 7.4. ΔS is the absolute change in order parameter, S_c is the value of the order parameter for liposomes without drug. Quinidine mol fraction was calculated as quinidine/(phospholipid + quinidine). The mean order parameters for control liposomes, S_c, were 0.590, 0.578 and 0.615 for phosphatidic acid, phosphatidylserine and phosphatidylcholine, respectively. Each point represents the mean of three or four experiments.

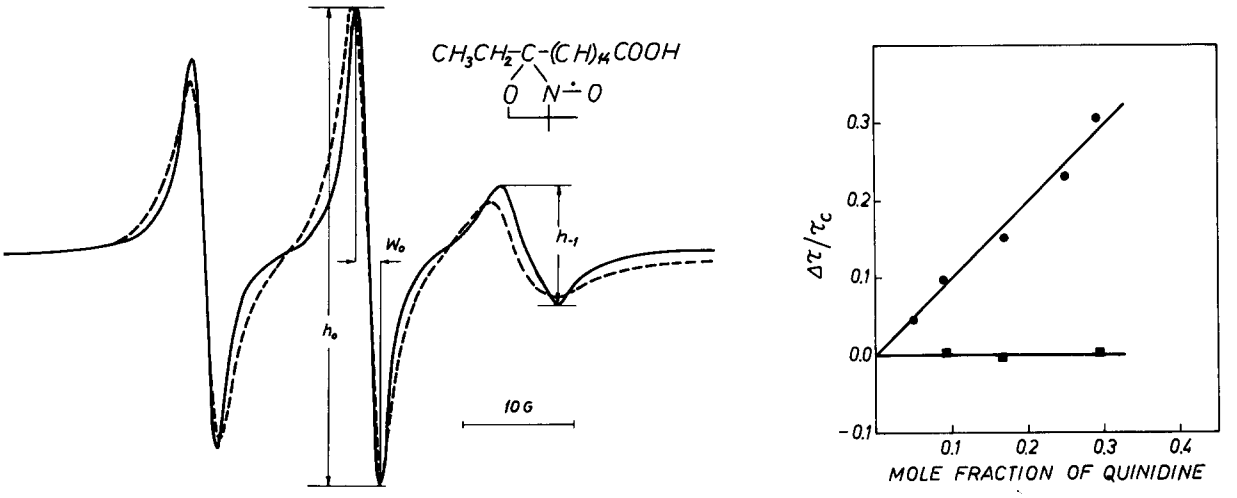

Fig. 3. Typical ESR spectra of 16-doxylstearic acid spin probe incorporated into phosphatidic acid liposomes without and in the presence of quinidine. ———, phosphatidic acid alone; — — —, phosphatidic acid/quinidine (10 : 4 molar ratio).

Fig. 4. The effect of quinidine on the relative change in motion parameter, $\Delta\tau/\tau_c$, of 16-doxylstearic acid spin-labeled liposomes prepared from phosphatidic acid (●) and phosphatidylcholine (■). Lipid/drug mixtures were dispersed in 30 mM Tris-HCl buffer, pH 7.4. $\Delta\tau$ is the absolute change in motion parameter, τ_c is the value of motion parameter for liposomes without drug. The mean values of the motion parameter for control liposomes, τ_c, were $1.22 \cdot 10^{-9}$ s and $1.30 \cdot 10^{-9}$ s for phosphatidic acid and phosphatidylcholine, respectively. Each point represents the mean of three or four experiments.

Fig. 4 shows the effect of quinidine on the above defined motion parameter τ. It is seen that the drug produces a significant increase in τ in the case of phosphatidic acid liposomes, but not in the case of those of phosphatidylcholine. These results indicate that the membrane-perturbing action of quinidine is not confined to the polar regions of the bilayer only. The tendency to a more rigid disposition of the lipid moieties around the probe, caused by quinidine, exists also in the inner hydrophobic core of the bilayers prepared from acidic phospholipids.

Quinidine has pK_d values of about 4.2 and 8.6 [1,15], so that at an applied pH of 7.4 the majority of the drug (about 94%) is present in a single-protonated, cationic form. This, combined with the demonstrated preferential effect of quinidine on acidic phospholipids, is indicative of the importance of charge-charge interaction in the membrane action of the drug. Relatively very high ionic strength is, however, required to weaken the interaction between quinidine and acidic phospholipids (Table I). The effects produced by the drug are only slightly decreased when the ionic strength is raised from 30 mM up to physiological values. It suggests that the quinidine-acidic phospholipids interaction is not of a purely electrostatic nature. Apparently some combination of electrostatic and hydrophobic interactions accounts for the observed perturbations in the organization of lipids in model membranes.

TABLE I

QUINIDINE-INDUCED INCREASE IN ORDER PARAMETER OF 5-DOXYLSTEARIC ACID SPIN-LABELED LIPOSOMES AT VARIOUS IONIC STRENGTHS

The mol fraction of quinidine was 0.25. NaCl was used to adjust the ionic strength of the buffer (30 mM Tris-HCl, pH 7.4).

Ionic strength (M)	% Increase in order parameter	
	Phosphatidic acid	Phosphatidyl-serine
0.03	15.2	13.5
0.15	13.2	11.7
0.25	11.8	10.7
0.45	10.3	9.0

The perturbations produced by quinidine in acidic phospholipid membranes are very marked, especially in the region close to the polar surface of the bilayer. The increase in order parameter of membrane-incorporated 5-doxylstearic acid spin label produced by the highest concentrations of quinidine used is of the same order of magnitude as its change observed at the phase transition of phospholipids from liquid-crystalline to the gel-like state [9]. Such a strong increase in the molecular order of acidic phospholipids might trigger a chain of structural and functional perturbations in biological membranes. For example, formation of relatively rigid domains of acidic phospholipid-quinidine complexes separated from the fluid regions of the membranes may be suggested as a likely consequence of the action of the drug.

The effect of other small lipophilic molecules of pharmacological importance on the degree of order and fluidity of phospholipid bilayers has been extensively studied during the last years. Spin-labeling experiments have shown that some substances may increase the degree of molecular order in cholesterol-free phospholipid membranes [16–19]. The ordering effect of the drugs was, however, usually significantly smaller than the effect of cholesterol [19]. To compare the ordering potency of quinidine with other membrane perturbers, slopes of $\Delta S/S_c$ vs. quinidine molar fraction plots were calculated. They amount to 0.63 and 0.57 for phosphatidic acid and phosphatidylserine, respectively. Comparison of these values with the respective values of 0.45 and 0.52 obtained for cholesterol in phosphatidylcholine multibilayers [19] and phosphatidic acid liposomes (Surewicz, W.K., unpublished data) shows that on the mole basis the effect of quinidine on the order of polar headgroup regions of acidic phospholipid membranes is even stronger than that of such a potent membrane orderer as cholesterol.

The relevance of quinidine-induced changes in the molecular organization of model membranes to the pharmacological effects of the drug remains to be elucidated. Nevertheless it should be noted here that structural changes produced in model membranes by quinidine are, at least qualitatively, very similar to those induced by propranolol [16]. Similar membrane effects of these two, structurally unrelated, antiarrhythmic compounds suggests that perturbations in the organization of membrane lipids may contribute to the molecular mechanisms by which antiarrhythmic drugs modify the functional properties of excitable tissues.

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