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Interaction of Quinidine with Phospholipids. I. Effect on Fluidity and Permeability of Phosphatidylcholine Vesicles

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The effect of quinidine and its isomer, quinine, on the thermotropic behavior of phosphatidylcholine vesicles was studied by differential scanning calorimetry (DSC) and fluorescence polarization measurement. The effect on the release rates of carboxyfluorescein and acrinol was also investigated. Quinidine and quinine lowered the lipid transition temperatures and increased the release of the drugs contained in the lipid vesicles. This effect seems to be due to a fluidizing action on the membrane.

Keywords——quinidine; phosphatidylcholine; bilayer; membrane fluidity; membrane permeability

It is well known that anesthetics and tranquilizers perturb membrane structure.[1-6] Quinidine, a potent antiarrhythmic agent, exhibits a local anesthetic action on various membranes and is believed to act by a mechanism similar to that of local anesthetics.[7] It was reported on the basis of electron spin resonance (ESR) experiments that quinidine strongly perturbs the molecular organization of lipid bilayers of acidic phospholipids.[8] In this report we present the results of differential scanning calorimetry (DSC) and fluorescence polarization measurements of the effect of quinidine and its isomer, quinine, on the thermotropic behavior of phosphatidylcholine vesicles. The effect of these drugs on the release rates of carboxyfluorescein and acrinol from lipid bilayer vesicles was also studied. The transition temperatures of distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) bilayers were considerably lowered by adding quinidine or quinine and the release rates of carboxyfluorescein and acrinol from the lipid vesicles were increased.

Experimental

Materials——DSPC, DPPC and DMPC were obtained from Sigma Chemical Co. Carboxyfluorescein was from Eastman Kodak Co. All other reagents and solvents were of the highest commercial grade. In all experiments quinidine sulfate, quinine hydrochloride and chlorpromazine hydrochloride were used.

DSC——DSC measurements were performed on a Daini Seikosha SSC/560 calorimeter at a scan rate of 1 K/min. Transition temperature was determined from the position of the transition peak. The lipids (2 mg) were weighed directly in the pans, hydrated with 30 μl of distilled water or drug solution and mixed well.

Fluorescence Polarization——Fluorescence polarization measurements were done with perylene or diphenylhexatriene (DPH) as a fluorescent probe. Lipid vesicles were prepared as follows. Lipid (5 μmol) and a fluorescent probe were mixed with chloroform, which was then removed by vacuum evaporation. Molar ratios of lipids and fluorescent probe were 1000/1 for perylene and 500/1 for DPH. An aqueous solution of quinidine or quinine (5 ml) was added and vesicles were obtained by vortexing and sonicating the sample for 12 min above the transition temperature, using a Ohtake Works 5202 sonicator. The degree of polarization $P=(I_{\parallel}-I_{\perp})/(I_{\parallel}+I_{\perp})$ of a probe was determined by using a Shimadzu RF-500 spectrofluorophotometer equipped with polarizers. $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities measured with the emission analyzer parallel and perpendicular, respectively, to the polarization of the excitation beam. The excitation and the emission wavelengths were 380 and 470 nm for perylene and 360 and 430 nm

for DPH.

Release from Lipid Bilayer Vesicles——DPPC vesicles were prepared as follows. Chloroform solution of DPPC (80 μmol) was dried by vaccum evaporation. Then 10 mM carboxyfluorescein or 100 mM acrinol (5 ml) was added and vesicles were prepared by vortexing and sonicating the sample by the method described above. The vesicles were separated from the free drug by gel filtration through a Sephadex G-50 column. Collection of the effluent was done at the signs of turbidity. An aqueous solution of quinidine or quinine was added to the suspension and the mixture was incubated. Samples were taken at 30 min and filtered through an Amicon MPS system. The filtrate was used for assay of the released drug. Fluorescence of the filtrate was measured with a Shimadzu RF-500 spectrofluorophotometer. The excitation and the emission wavelengths were 490 and 510 nm for carboxyfluorescein (after alkalizing the sample) and 410 and 500 nm for acrinol. The total drug concentration was determined by adding Triton X-100 to the lipid suspension.

Results and Discussion

DSC

Figures 1 and 2 show the effects of quinidine and quinine on the transition temperatures of DSPC, DPPC and DMPC. The effect of chlorpromazine was also examined for comparison. The transition temperatures of the phospholipids before adding the drugs were 55 °C for DSPC, 42 °C for DPPC and 24 °C for DMPC, and agreed well with the literature values. The lowering with DPPC and DMPC was as large as that by chlorpromazine (Fig. 2), which is extensively studied to perturb the membrane structure.[1,9-11] In the DSPC system the lowering by quinidine and quinine was significant (Fig. 1), but at concentrations higher than 10 mM the effect of chlorpromazine was stronger. As chlorpromazine is known to be a strong membrane perturber, the results in this experiment suggest that quinidine and quinine significantly perturb the membrane structure.

Fluorescence Polarization Measurements

Fluorescence polarization of perylene and DPH in phospholipid vesicles was measured to

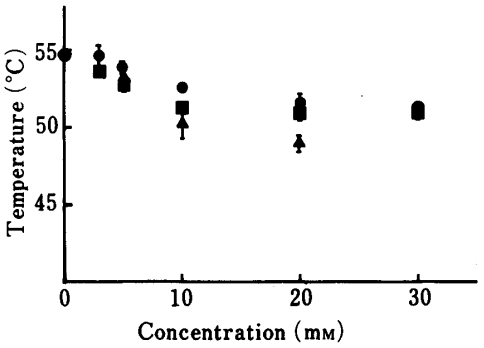

Fig. 1. Effect of Drugs on the Transition Temperature of DSPC Determined by DSC

■, quinidine; ●, quinine; ▲, chlorpromazine.
Each point represents the mean±S.D. of three experiments.

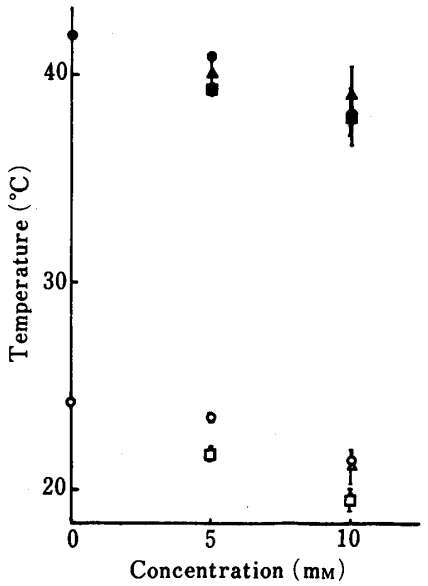

Fig. 2. Effect of Drugs on the Transition Temperatures of DPPC and DMPC Determined by DSC

Effect on DPPC: ■, quinidine; ●, quinine; ▲, chlorpromazine.
Effect on DMPC: □, quinidine; ○, quinine; △, chlorpromazine.
Each point represents the mean±S.D. of three experiments.

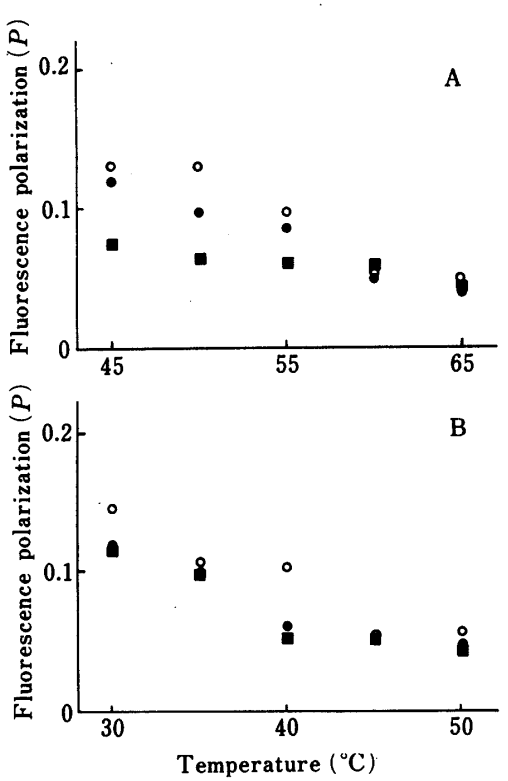

Fig. 3. Effect of Drugs on the Fluorescence Polarization of Perylene in Phosphatidylcholine Vesicles

A (DSPC): ○, control; ■, 10 mM quinidine; ●, 10 mM quinine.
B (DPPC): ○, control; ■, 10 mM quinidine; ●, 10 mM quinine.

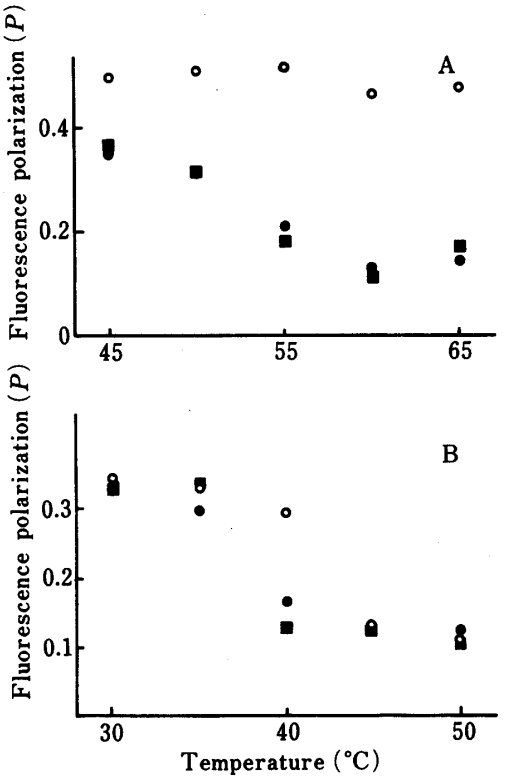

Fig. 4. Effect of Drugs on the Fluorescence Polarization of DPH in Phosphatidylcholine Vesicles

A (DSPC); ○, control; ■, 10 mM quinidine; ●, 10 mM quinine.
B (DPPC): ○, control; ■, 10 mM quinidine; ●, 10 mM quinine.

study further the thermotropic behavior of lipids. Figures 3 and 4 show the *P* values of perylene or DPH at various temperatures in the absence and in the presence of 10 mM quinidine and quinine. In DSPC vesicles (Fig. 3A, Fig. 4A) the *P* values were markedly depressed by quinidine and quinine, which suggests that the two drugs increased the fluidity of the membrane. In DPPC vesicles (Fig. 3B, Fig. 4B) the *P* values were greatly decreased at 40 °C. This result shows that the transition temperature of the lipid was lowered by the two drugs, and the finding agrees well with the result of DSC.

From the measurements of fluorescence polarization, quinidine and quinine are thought to have a fluidizing effect on the membrane. Therefore the effect of these drugs on the permeability of the DPPC membrane was investigated next.

Release from DPPC Vesicles

Table I shows the effect of quinine and quinidine on the release rate of carboxyfluorescein from DPPC vesicles at 45 °C. The residual percentage in vesicles at 30 min was 43% in the control experiment. Quinine (5 mM) slightly increased the release and quinidine (5 mM) markedly enhanced it (only 16% of the drug remained at 30 min). This effect seems consistent with a fluidizing effect of the drugs because increase of the fluidity is expected to cause enhancement of the permeability. A similar experiment was performed with quinidine at 25 °C, which is below the transition temperature of DPPC (Table II). Quinidine also increased the release at this temperature. The residual percentage decreased from 78% in the control experiment to 53% in the presence of 5 mM quinidine. These results show that quinidine enhances the release rate of carboxyfluorescein both below and above the transition

TABLE I. Effect of Drugs on the Release Rate of Carboxyfluorescein from DPPC Vesicles

	Residual %±S.D.[a] at 30 min
Control	43± 7
Quinine 5 mM	37±21
Quinidine 5 mM	16±10

a) Each value is the mean±S.D. of three experiments.

TABLE II. Effect of Quinidine on the Release Rate of Carboxyfluorescein at 25 °C

	Residual %±S.D.[a] at 30 min
Control	78±3
Quinidine (5 mM)	53±8

a) Each value is the mean±S.D. of three experiments.

TABLE III. Effect of Quinidine on the Release Rate of Acrinol from DPPC Vesicles

	Residual %±S.D.[a] at 30 min
Control	59±1
Quinidine (5 mM)	25±5

a) Each value is the mean±S.D. of three experiments.

temperature of the lipid.

Because carboxyfluorescein is an acidic drug and quinidine is a basic one, the effect of quinidine might be related to the charge of the compound. Therefore the effect of quinidine was further investigated with vesicles containing a basic drug, acrinol. Table III shows the effect of quinidine on the release rate of acrinol from DPPC vesicles at 45 °C. The residual percentage at 30 min was 59% in the control sample and 25% in the presence of 5 mM quinidine, which shows that quinidine also enhances the release of a basic drug. Thus, quinidine is thought to increase the release of drugs not because of its charge but by fluidizing the membrane and enhancing its permeability.

Surewicz studied the perturbation of the membrane by acidic phospholipids.[8] In this report we investigated neutral phospholipids, and found that quinidine also interacts with phosphatidylcholines. The results obtained with lipid vesicles having other constitutents will be reported elsewhere.

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