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EFFECT OF QUINIDINE ON MEMBRANE PROPERTIES

DEPRESSION OF THE LIPID PHASE TRANSITION TEMPERATURE AND CHANGES IN THE PERMEABILITY OF THE LIPID BILAYER

WITOLD K. SUREWICZ and ZBIGNIEW JOZWIAK

Department of Biophysics, Institute of Biochemistry and Biophysics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

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Abstract—The influence of an antiarrhythmic drug, quinidine, on the physical state of membrane phospholipids was investigated using model membranes, liposomes. Turbidimetric measurements on liposomes prepared from neutral (dipalmitoyl phosphatidylcholine) and acidic (dipalmitoyl phosphatidic acid) phospholipids showed that quinidine reduces the temp of the gel to liquid-crystalline phase transition and broadens the temp range of the transition. The effect of quinidine on the thermal behaviour of model membranes depends on both the pH and the type of phospholipids used. It is markedly stronger for acidic than for neutral phospholipids, suggesting the importance of electrostatic effects in drug–membrane interaction. The ability of quinidine to interact with the lipid bilayer was confirmed by permeability measurements with the use of a self-quenched fluorescent compound, calcein. It is suggested that quinidine–phospholipid interaction may contribute to the mechanisms by which the drug exerts its physiological and pharmacological effects.

The role of quinidine as an antiarrhythmic agent is well-recognized. The pharmacological actions of the drug are believed to derive from its ability to interact with biological membranes and much work has been done to understand the nature of this interaction. Most of the studies have been focussed on the effect of the drug on membrane transport and uptake of various ions. Quinidine has been shown to perturb ionic conductances in various membrane systems [1–8]. Some of the physiological effects of the drug have been attributed to its ability to depress membrane uptake of calcium [7–11].

In spite of the relatively large amount of information concerning quinidine-induced functional changes in biological membranes, the molecular mechanism of the drug–membrane interaction remains far from clear. It has been suggested by several authors that the functional effects produced by quinidine may be mediated by changes in membrane structure [12, 13]. It is, however, not clear which membrane components are involved in the interaction with quinidine and what are the details of this interaction on the molecular level.

Although there is no direct experimental evidence, several observations suggest a possible lipid contribution to the drug–membrane interaction: (1) some of the physiological effects of quinidine are very similar to those of local anesthetics [1] whose ability to interact with lipid bilayers is well-recognized [14–17]; (2) another antiarrhythmic drug exhibiting quinidine-like action, propranolol, perturbs the organization of membrane lipids [16, 18–21]; and (3) quinidine affects non-specifically various membrane-bound enzymes which are believed to be influenced by the physical state of surrounding lipids [12, 22]. In view of this, studies have been undertaken towards learning the mode of quinidine interaction with the lipidic part of biological membranes. In the present paper drug–phospholipid interaction is explored with the use of model lipid membranes, liposomes. Such a model has been previously successfully employed for studying the mechanisms of membrane actions of various drugs [14–18, 23, 24].

Phospholipid membranes undergo a quasi-first-order thermotropic phase transition between solid–gel and liquid–crystalline states [25]. The transition may be studied by various methods. One of the simplest ways is to observe the change in turbidity of the lipid suspension in excess water [23, 26, 27]. It is shown here by this method that quinidine depresses the lipid phase transition temp and broadens the transition. In parallel with phase transition studies, the lipid bilayer perturbing action of quinidine was followed by permeability measurements with the use of a self-quenched fluorescent compound, calcein.

MATERIALS AND METHODS

Materials. Dipalmitoyl phosphatidylcholine, egg yolk phosphatidylcholine, bovine brain phosphatidylserine and quinidine–HCl were obtained from Sigma. Dipalmitoyl phophatidic acid (disodium salt) was from Fluka, Sephadex G-25 from Pharmacia, and calcein from POCH (Gliwice, Poland).

Measurement of lipid transition temp. Multilamellar liposomes used in phase transition studies were prepared as follows. Dipalmitoyl phosphatidylcholine was dissolved in chloroform and the solvent was evaporated under a stream of argon. Buffer [0.03 M Tris–HCl (pH 7.4 or 8.5)] was then added to reach the concn of 5 mg of phospholipid/ml of buffer and

the mixture was shaken for 5 min on a Vortex rotamixer at a temp above that of the lipid phase transition. The resulting stock suspension of liposomes was diluted with the same buffer containing appropriate amount of quinidine. This was followed by a 1-hr incubation with intermittent vortexing, during which time the sample was warmed and cooled repeatedly through the transition temp. The final concn of the lipid dispersion was 0.5 mg/ml. Dipalmitoyl phosphatidic acid liposomes were prepared analogously as described earlier for phosphatidylcholine ones, with the exception that the chloroform dissolution step was omitted and buffer was added directly to the powdered lipid. Phase transition temp of the lipid dispersions were determined by measuring the apparent light absorbance of the liposome suspension at 450 nm as a function of temp [23, 27]. These measurements were performed using Carl Zeiss VSU-2P or Unicam SP spectrophotometers equipped with temp programmers. Temp was continously monitored with a thermocouple inserted directly into the cuvette.

Permeability measurements. Leakage from liposomes was examined using the technique of Weinstein *et al.* [28], modified by Allen and Cleland [29]. With this technique liposomes containing a highly quenched fluorescent compound, calcein, are prepared. When present at high concns this fluorescent dye does not exhibit any detectable fluorescence. As calcein leaks from liposomes it becomes diluted and therefore dequenched, and an increase in fluorescence is seen. Liposomes containing entrapped calcein were prepared by the following procedure. Fifteen milligrams of egg yolk phosphatidylcholine or bovine brain phosphatidylserine was dried under argon. To the thin lipid film 1 ml of 0.03 M Tris–HCl buffer (pH 7.4) containing 0.1 M calcein (neutralized with KOH) was added and the dispersion was agitated at room temp on a Vortex mixer. Untrapped calcein was removed by passing the suspension through a column (35 × 1 cm) of Sephadex G-25, using elution at room temp with 0.03 M Tris–HCl buffer (pH 7.4) containing 0.15 M NaCl. A 50-μl aliquot of the gel-filtered liposome suspension was added to 2.5 ml of the elution buffer. The time course of self-quenched, trapped calcein efflux was recorded at room temp using a Jobin Yvon JY3 spectrofluorimeter with excitation and emission wavelengths of 490 and 520 nm, respectively. To calculate the percentage of calcein released, liposomes were disrupted by addition of 50 μl of 10% Triton X-100, and the fluorescence intensity corresponding to maximum calcein release was measured.

RESULTS

Effect of quinidine on the lipid phase transition

The absorbance at 450 nm of the dipalmitoyl phosphatidylcholine liposome suspension in the absence and presence of quinidine is shown in Fig. 1 as a function of temp. A rapid decrease in the absorbance may be observed in these curves. This decrease is due to the lipid phase transition from the gel to the liquid–crystalline state and results from the increase in the volume of the lipid region due to the increased rotational motion. This is reflected in the lowering

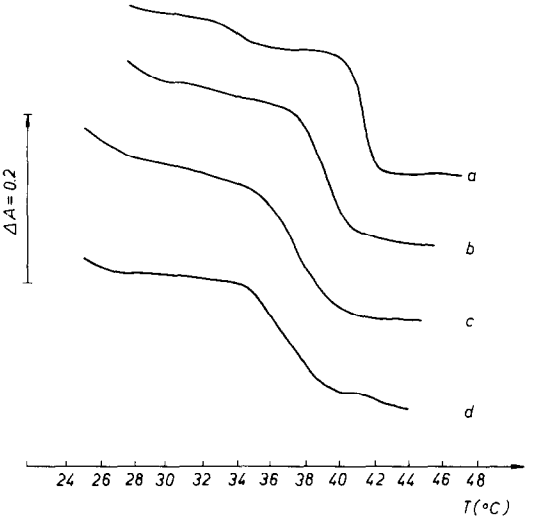

Fig. 1. Influence of quinidine on the apparent light absorbance at 450 nm of a suspension of dipalmitoyl phosphatidylcholine at pH 7.4 as a function of temp; (a) no drug; (b) 1 mM quinidine; (c) 2 mM quinidine; and (d) 3 mM quinidine.

of the refractive index of the liposomes which decreases the turbidity of the suspension [26, 27]. The transition temp, defined as the midpoint of the melting curve, for dipalmitoyl phosphatidylcholine was 41.7°. This value is in reasonable agreement with the transition temp found by other investigators using both the turbidimetric method and other techniques

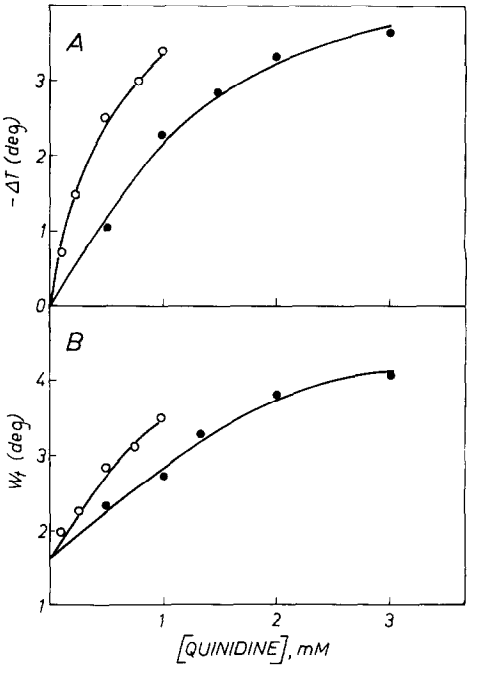

Fig. 2. Effect of quinidine on the thermal behaviour of dipalmitoyl phosphatidylcholine at pH 7.4 (●) and 8.5 (○). (A) Depression of the phase transition temp, ΔT, as a function of quinidine concn. (B) Effect of the drug on the transition width, $W_{\frac{1}{2}}$.

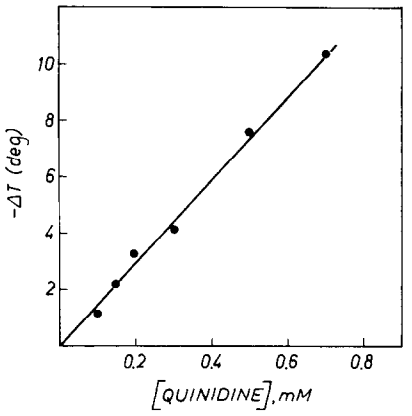

Fig. 3. Depression of the phase transition temp of dipalmitoyl phosphatidic acid at pH 7.4 as a function of quinidine concn.

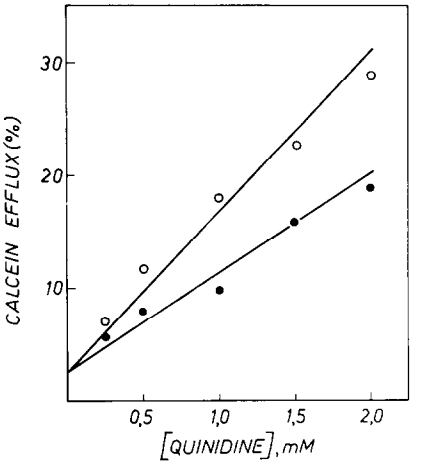

Fig. 5. Effect of quinidine on calcein efflux from liposomes during a 1-hr incubation: (○) bovine brain phosphatidylserine liposomes; and (●) egg yolk phosphatidylcholine liposomes.

[16, 17, 23, 25–27]. As well as the midpoint temp, the actual range of the transition is important. The width of the transition, W_t, may be determined from the intersections of the straight lines drawn through three distinct portions of each curve. For dipalmitoyl phosphatidylcholine it was found to be 1.6°.

Addition of increasing amounts of quinidine to the dipalmitoyl phosphatidylcholine liposome suspension gradually shifts the melting curves towards lower temps and broadens the temp range in which the transition occurs (curves b–d in Fig. 1). These effects are much more pronounced at pH 8.5 compared to pH 7.4 (Fig. 2). At both pH values used the effects are apparently non-linear with respect to the quinidine concn.

Quinidine–lipid interaction was also studied with the use of liposomes prepared from an acidic phospholipid, dipalmitoyl phosphatidic acid. With this lipid the midpoint transition temp obtained from absorbance curves amounted at pH 7.4 to 59°, and the W_t was 9.5°. The influence of quinidine on the thermal behaviour of dipalmitoyl phosphatidic acid is qualitatively similar to that described for dipalmitoyl phosphatidylcholine, i.e. the transition temp is shifted towards lower values (Fig. 3) and the transition is broadened. With dipalmitoyl phosphatidic acid these effects are, however, much more pronounced. As can be seen from comparison of the data presented in Figs. 2A and 3, at pH 7.4 almost 10 times lower quinidine concns are needed to produce the same drop in transition temp for dipalmitoyl phosphatidic acid as for dipalmitoyl phosphatidylcholine liposomes. Moreover, in contrast to the non-linearities observed in the case of neutral phosphatidylcholine membranes, the effect of increasing concns of quinidine on the phase transition temp of negatively charged phosphatidic acid membranes is linear.

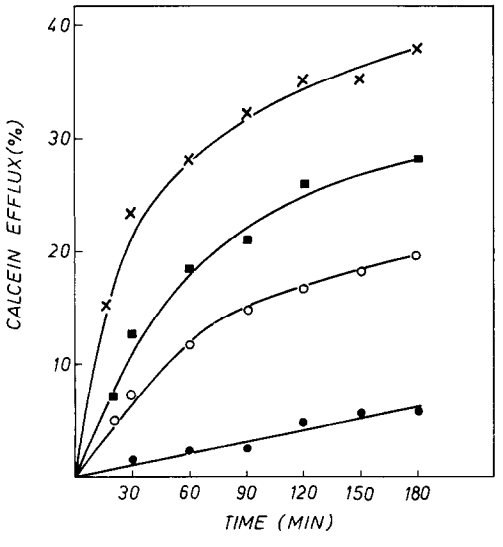

Fig. 4. Time curves of calcein efflux from phosphatidylserine liposomes with and without quinidine: (●) no drug; (○) 0.5 mM quinidine; (■) 1 mM quinidine; and (×) 2 mM quinidine.

Effect of quinidine on trapped calcein efflux from liposomes

Figure 4 illustrates the typical time curves for calcein efflux from phosphatidylserine liposomes. In the absence of quinidine the calcein permeability of these liposomes is very low. Only about 6% of trapped dye is released within a 3-hr incubation period. Addition of quinidine causes a significant increase in the permeability of phosphatidylserine bilayer. Most of the drug-induced efflux of calcein occurs within the first 60–90 min. After this time the release of calcein becomes much slower. The time curves for calcein efflux from phosphatidylcholine liposomes are similar. In this case about 7% of trapped dye is released within 3 hr from control liposomes.

The effect of quinidine on the calcein permeability of phosphatidylserine and phosphatidylcholine liposomes is compared quantitatively in Fig. 5. In Fig. 5 the 1-hr efflux is plotted against drug concn. It is evident from the data shown that the increase in calcein permeability caused by quinidine is markedly

stronger for acidic phosphatidylserine liposomes compared to neutral phosphatidylcholine ones.

DISCUSSION

Changes in the thermotropic behaviour of phospholipids have been widely used as a sensitive indicator of the interaction of various compounds with both model and biological membranes [15–17, 23]. The present results show clearly that the antiarrhythmic drug, quinidine, affects the endothermic transition of phospholipids from the gel-like to the liquid–crystalline state. The phase transition temp is shifted towards lower values and the width of the transition is increased. The latter effect is indicative of the decreased cooperativity of the bilayer transition, probably due to the increased intermolecular spacing.

Quinidine has pK_a values of about 4.2 and 8.6. Thus about 56 and 93% of the drug is present in a cationic, single-protonated form at pH 8.5 and 7.4, respectively, and practically no drug is present in a double-ionized state. It seems that both the non-linearity and pH-dependence of concn plots in Fig. 2 can be explained from the general model developed for membrane binding of cationic drugs [16, 17, 30]. According to this model both charged and uncharged forms of the drug bind to phosphatidylcholine liposomes. The initial binding of the protonated form results in a positive charge of the originally neutral phosphatidylcholine bilayer. This hinders further binding of the drug leading thus to negative deviations from linearity in concn plots. At higher pH less drug is present in an ionized form. The electrostatic effects resisting the binding of quinidine to the bilayer are therefore of smaller significance; hence the influence of the drug on the thermotropic behaviour of phosphatidylcholine is stronger.

The depression of the phase transition temp, caused by quinidine, is much more pronounced for negatively charged dipalmitoyl phosphatidic acid than for neutral dipalmitoyl phosphatidylcholine. Two factors may contribute to the stronger interaction of quinidine with acidic phospholipids compared to neutral ones. Firstly, the presence of negatively charged lipids in the bilayer should neutralize the effects attributed previously to the build-up of positive charge on liposomes and thus enhance perturbations produced by the drug. Secondly, it is possible that specific electrostatic complexes between anionic head groups of acidic phospholipids and charged drug molecules are formed. The importance of charge–charge interaction and the formation of electrostatic drug–lipid complexes have been previously suggested for the membrane action of some other cationic drugs [14, 15, 18, 24].

The membrane-perturbing action of quinidine was confirmed by permeability measurements. The ability of the drug to depress the lipid phase transition temp might suggest that the increased liposome permeability to calcein in the presence of quinidine is due to the increase in membrane fluidity [31]. Such an interpretation would be, however, premature. Permeability measurements were performed under conditions where the lipids used are in the liquid–crystalline state, far above their transition temp. The results of very recent spin-labeling experiments indicate that the quinidine-induced decrease in the phase transition temp does not imply further fluidization of phospholipid membranes when they are already in the liquid–crystalline state. On the contrary, the drug tends to decrease the fluidity of liquid–crystalline bilayers [32]. This should rather depress the solute transport through the membrane. The quinidine-induced increase in calcein permeability of liquid–crystalline liposomes should be attributed, therefore, to other mechanisms than changes in membrane fluidity. Some local defects or instabilities in the bilayer, induced by quinidine, and/or alterations in the membrane surface charge may be suggested as possible and likely factors responsible for the increased calcein efflux from liposomes.

The work reported here was designed as an approach to the understanding of quinidine-triggered events in biological membranes. The use of simple well-defined membrane models, liposomes, enabled us to detect concn-dependent effects of quinidine on the physical state of bilayer lipids. These effects are especially strong with acidic phospholipids. Similar disturbances in lipid structure are very likely to occur also in biological membranes. Such dusturbances might trigger diverse functional perturbations, depending on drug concn and membrane type. Although great caution should be exercised in attempting to extrapolate from drug-induced effects in model membranes to those in biological systems, it may be suggested that quinidine–lipid interaction contributes to the mechanisms by which the drug exerts its physiological and pharmacological effects. This is particularly relevant to the local anesthetic action of quinidine [1]. The similarity between the effects produced in model membranes by quinidine and local anesthetics [14–17] is consistent with the results of voltage clamp experiments showing that some of the effects of quinidine on excitable tissues resemble those of local anesthetics [1]. Further studies are, however, required to elucidate to what extent the lipid-perturbing and local anesthetic effects of quinidine are related to its specific antiarrhythmic action.

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REFERENCES

1. J. Z. Yeh and T. Narahashi, *J. Pharmac. exp. Ther.* **196**, 62 (1976).
2. H. Nawrath, *J. Pharmac. exp. Ther.* **216**, 176 (1981).
3. P. A. van Zwieten, *Br. J. Pharmac.* **35**, 103 (1969).
4. M. C. Fishman and J. Spector, *Proc. natn. Acad. Sci. U.S.A.* **78**, 5245 (1981).
5. J. A. L. Arruda and S. Sabatini, *J. membrane Biol.* **55**, 141 (1980).
6. V. L. Lew and H. G. Ferreira, *Nature, Lond.* **263**, 336 (1976).
7. J. Atwer, C. M. Dawson, B. Ribalet and E. Rojas, *J. Physiol., Lond.* **288**, 575 (1979).
8. J. A. C. Harrow and N. S. Dhalla, *Biochem. Pharmac.* **25**, 897 (1976).
9. W. Thorpe, *Can. J. Physiol. Pharmac.* **51**, 499 (1973).
10. J. A. C. Harrow and N. S. Dhalla, *Can. J. Physiol. Pharmac.* **53**, 1058 (1975).

11. F. Fuchs, E. W. Gertz and F. N. Briggs, *J. gen. Physiol.* **52**, 955 (1968).
12. D. V. Godin, T. Au and M. E. Garnett, *Biochim. biophys. Acta* **512**, 388 (1978).
13. H. Komai and H. A. Berkoff, *Biochem. Pharmac.* **28**, 1501 (1979).
14. D. Papahadjopoulos, *Biochim. biophys. Acta* **265**, 169 (1972).
15. D. Papahadjopoulos, K. Jacobson, G. Poste and G. Shepherd, *Biochim. biophys. Acta* **394**, 504 (1975).
16. A. G. Lee, *Molec. Pharmac.* **13**, 474 (1977).
17. A. G. Lee, *Biochim. biophys. Acta* **514**, 95 (1978).
18. W. K. Surewicz and W. Leyko, *Biochim. biophys. Acta* **643**, 387 (1981).
19. W. K. Surewicz, I. Fijakowska and W. Leyko, *Biochem. Pharmac.* **30**, 839 (1981).
20. W. K. Surewicz, *Biochem. Pharmac.* **31**, 691 (1982).
21. D. V. Godin, T. W. Ng and J. M. Tuchek, *Biochim. biophys. Acta* **436**, 757 (1976).
22. N. S. Dhalla, J. A. C. Harrow and M. B. Anand, *Biochem. Pharmac.* **27**, 1281 (1978).
23. M. W. Hill, *Biochim. biophys. Acta* **356**, 117 (1974).
24. E. Goormaghtigh, P. Chatelain, J. Caspers and J. M. Ruysschaert, *Biochim. biophys. Acta* **597**, 1 (1980).
25. A. G. Lee, *Biochim. biophys. Acta* **472**, 237 (1977).
26. P. N. Yi and R. C. MacDonald, *Chem. Phys. Lipids* **11**, 114 (1973).
27. N. O. Peterson and S. I. Chan, *Biochim. biophys. Acta* **509**, 111 (1978).
28. J. N. Weinstein, S. Yoshikami, P. Henkart, R. Blumenthal and W. A. Hagins, *Science* **195**, 489 (1977).
29. T. M. Allen and L. G. Cleland, *Biochim. biophys. Acta* **597**, 418 (1980).
30. W. K. Surewicz and W. Leyko, *J. Pharm. Pharmac.* **34**, 359 (1982).
31. K.-Y. Pang, T. L. Chang and K. W. Miller, *Molec. Pharmac.* **15**, 729 (1979).
32. W. K. Surewicz, *Biochim. biophys. Acta* **692**, 315 (1982).