Biochemical Pharmacology, Vol. 42, Suppl., pp. S61–S65, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

THE ABILITY OF CATIONIC AMPHIPHILIC COMPOUNDS TO DEPRESS THE TRANSITION TEMPERATURE OF DIPALMITOYLPHOSPHATIDIC ACID LIPOSOMES DEPENDS ON THE SPATIAL ARRANGEMENT OF THE LIPOPHILIC MOIETY*

KARIN BORCHARDT,† DIETER HEBER,‡ MARTIN KLINGMÜLLER,‡ KLAUS MOHR†§ and BERND MÜLLER†

†Department of Pharmacology, University of Kiel, Hospitalstrasse 4 and ‡Department of Pharmacy, University of Kiel, Gutenbergstrasse 76–78 D-2300 Kiel 1, Federal Republic of Germany

(*Received* 17 *July* 1991; *accepted* 2 *September* 1991)

Abstract—The hypothesis was tested with the help of model compounds that the ability of cationic amphiphilic drugs to depress the phase-transition temperature T_t of dipalmitoylphosphatidic acid (DPPA) liposomes depends on the spatial arrangement of the lipophilic moiety. The main structure of the compounds with identical cationic side chain was 1-dimethylamino-3-phenylpropane (compound I). A further phenyl ring was introduced either at C3 of the propane chain (compound II) to broaden the lipophilic moiety, or in para-position of the phenyl ring (compound III) to elongate it. As shown by differential scanning calorimetry, the reduction of T_t (control 64°) amounted for compound I to 29°, for compound II to 28° and for compound III to 53°. In order to assess the binding affinity of the compounds to DPPA, their inhibitory effect on $^{45}Ca^{2+}$-binding to DPPA films was measured. The IC_{50} values were 2100 μM for compound I, 40 μM for compound II, and 9 μM for compound III. Thus, binding affinity corresponded with the hydrophobicity of the compounds. In contrast, the depressing effect on the transition temperature was only augmented by the additional phenyl ring when substituted in the elongating position.

Various drugs are cationic amphiphilic, such as local anesthetics, antiarrhythmics, β-blockers, antimalarials, and antidepressants. The amphiphilic nature causes the drugs to bind to phospholipid membranes, where the hydrophilic protonized nitrogen is located near the negatively charged phosphate groups of the phospholipid and the lipophilic aromatic ring system is directed into the fatty acid chain region (e.g. Ref. 1). The binding to phospholipid membranes has been related to the biological effects of the drugs, for instance sodium channel blockade (e.g. Ref. 2) and drug-induced lipidosis (e.g. Refs 3 and 4). One aspect of the drug–phospholipid interaction is the perturbation of phospholipid bilayers, and cationic amphiphilic drugs have been shown to lower the phase-transition temperature (T_t) of the liposomes of various phospholipids (e.g. Refs 5–8). With dipalmitoylphosphatidic acid (DPPA‖) it was found that the drugs induced the formation of domains within the phospholipid, the transition temperature of which was depressed to different extents depending on the added catamphiphilic drug [7, 9]. It has been suggested that the "intrinsic activity" of a catamphiphilic drug in depressing T_t is governed by the depth of penetration into the bilayer [7, 9, 10], i.e. the activity should not simply depend on the hydrophobicity but on the spatial arrangement of the lipophilic moiety. However, the drugs investigated so far were picked from various pharmacological groups and varied with respect to the structure of both the hydrophilic and the lipophilic moiety.

In order to circumvent this problem, cationic amphiphilic compounds were synthesized in which only the lipophilic moiety was modified [11]. The structural formulae of the model compounds and their pK_a, and log P′ values are compiled in Table 1. The hypothesis to be tested was that the depressing action on the phase-transition temperature T_t of DPPA liposomes does not correspond with the hydrophobicity of the compounds but depends on the spatial arrangement of the lipophilic moiety. The transition temperature was measured by differential scanning calorimetry.

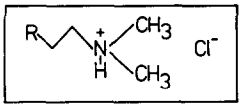
Hydrophobicity is known to be a major determinant of the binding of cationic amphiphilic drugs to phospholipids [12]. It was checked whether the affinity of the drugs to bind to DPPA layers paralleled the hydrophobicity. For this purpose, the drug action on ^{45}Ca binding to DPPA films was studied. Intercalation of cationic molecules reduces the ability of phospholipid films to bind $^{45}Ca^{2+}$; inhibition of

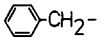
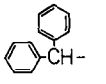
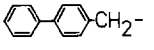
* The manuscript has been dedicated to Prof. Dr Dres. h.c. H. Oelschläger, Frankfurt a.M., F.R.G., on the occasion of his 70th birthday.

§ Corresponding author.

‖ Abbreviation: DPPA, dipalmitoylphosphatidic acid.

Table 1. Structure and activity of the applied cationic amphiphilic compounds.



R—	pK_a	log P'	ΔT_t [°C]	IC_{50} [μM]
	11.0	1.5	29	2100
	10.0	2.8	28	40
	9.9	3.0	53	9

pK_a, dissociation constant; log P', log (partition coefficient octanol/buffer pH 7.4); ΔT_t, reduction of the transition temperature of DPPA; IC_{50}, concentration inhibiting ^{45}Ca-binding to DPPA films by 50%.

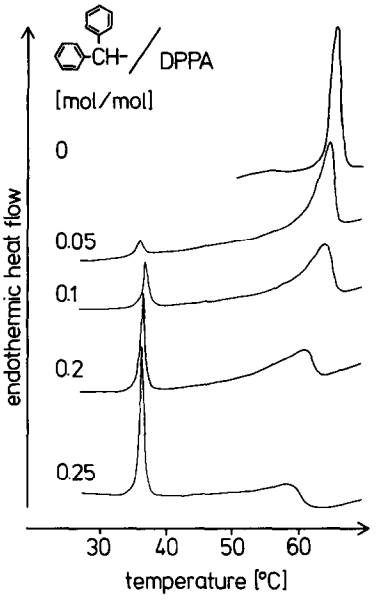


Fig. 1. Phase-transition signals of DPPA liposomes obtained by differential scanning calorimetry under control conditions and in the presence of increasing amounts of compound II (mol/mol of DPPA). Ordinate: peaks indicate extra heat flow into the sample containing the liposome suspension. Abscissa: temperature of the sample.

^{45}Ca binding thus reflects binding of the cationic compound [13].

Part of the results was presented previously in abstract form [14].

MATERIALS AND METHODS

Phase-transition temperature of DPPA liposomes. The preparation of the drug–liposome suspensions and measurements by differential scanning calorimetry were performed as described in detail previously [9]. In short, 5 mg of DPPA (1,2-dipalmitoyl-*sn*-glycero-3-phosphate monosodium salt, purity >99%; Sigma Chemical Co., München, F.R.G.) were suspended together with an appropriate amount of the test compound in 100 μL of a 14 mM TES [*N*-tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid]/histidine buffer, pH 6, at 70° for 2 hr. Ten microlitres of the suspension were encapsulated in aluminium pans (Perkin–Elmer, Überlingen, F.R.G.) for differential scanning calorimetry (DSC-2C/intracooler II, Perkin–Elmer). A capsule filled with 10 μL of distilled water was the reference. The pans were heated at a rate of 5° per min from 12° to 72°; the difference between the heat flows into sample and reference was recorded at a sensitivity range setting of 0.5 mcal/sec. The onset of the transition signal was taken as the transition temperature: the baseline of the thermogram was extrapolated, a straight line was fitted to the upward deflection of the signal and from the intersection of these lines a perpendicular was drawn to the temperature axis of the thermogram. Reproducibility was checked by repeated (up to N = 6) measurements for some molar ratios of substance/DPPA; results differed by less than 1°. In case of repeated recordings, the mean value of the transition temperature was used for further analysis (see Results).

^{45}Ca binding to DPPA films. The method for measurement with phosphatidylserine at pH 7.5 has been described before [9, 15]. In the present study, DPPA was used at pH 6 in order to yield the conditions for the calorimetric measurement. In short, 10 nmol of DPPA dissolved in 3 μL chloroform were applied at room temperature (about 20°) on the surface of 5 mL buffer in a Teflon planchette with a diameter of 4.5 cm. The buffer contained 0.01 mM $CaCl_2$, 5 mM NaOH, 2 mM TES, 2 mM histidine, pH 6, and trace amounts of $^{45}CaCl_2$ (New England Nuclear, Dreieich, F.R.G.). The planchette was placed under a Geiger–Müller counting tube (Frieseke & Hoepfner, Erlangen, F.R.G.) to detect ^{45}Ca radiation (equipment by Berthold, Wildbad, F.R.G.). The radioactivity amounted to about 1200 cpm in the absence of the phospholipid and doubled after DPPA was applied. The additional radiation indicated ^{45}Ca adsorption to the DPPA film. With a test compound added to the buffer, ^{45}Ca adsorption was reduced. For evaluation, ^{45}Ca adsorption in the presence of the compound was expressed as a percentage of the ^{45}Ca adsorption under control conditions. Curve fitting was performed with the Inplot software (GraphPad, San Diego, U.S.A.).

RESULTS

The compounds tested reduced the phase-transition temperature of DPPA liposomes. This is illustrated with differential scanning calorimetry tracings obtained for various doses of compound II (Fig. 1). The transition temperature under control

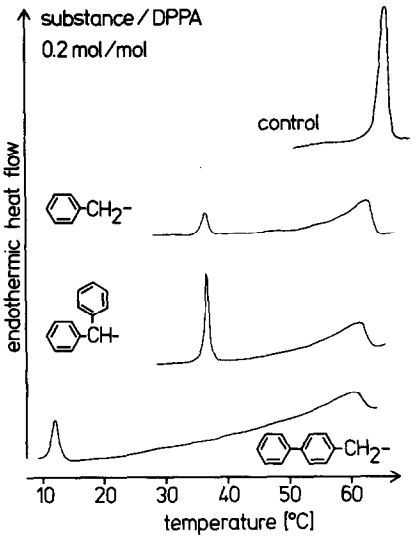


Fig. 2. Phase-transition signals of DPPA liposomes recorded under control conditions and in the presence of the compounds I, II, and III, each added at a dose of 0.2 mol/mol of DPPA.

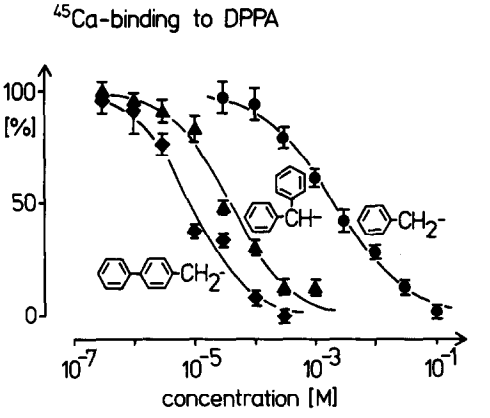


Fig. 3. Inhibition of $^{45}Ca^{2+}$ binding to DPPA layers by the compounds III, II, and I (from left to right). Ordinate: $^{45}Ca^{2+}$ binding as percentage of the control value recorded in the absence of a test compound. Abscissa: concentration of the test compounds. Indicated are means ± SE of N = 9 measurements for compound III and N = 6 for II and I. Sigmoid curves were fitted by nonlinear regression analysis; Hill coefficients were −0.94, −0.90 and −0.73 for III, II, and I, respectively.

conditions was T_t = 63.7 ± 0.1° (mean ± SE, N = 21), thus matching the values reported in the literature (e.g. Refs 16 and 17). In the presence of 0.05 moles of compound II per mole of DPPA an additional transition signal occurred at a temperature of 35°. With the addition of increasing amounts of compound II, this signal grew whereas the control signal became smaller and was shifted to lower temperatures. The temperature of the additional signal, however, was independent of the dose. The compound was applied in a total of 12 molar ratios between 0.04 and 0.5 mol/mol DPPA. The transition temperatures of the signals induced by the various doses of compound II were averaged: T_t amounted to 36.0 ± 0.1° (mean ± SE, N = 12). The effect of compound II on the transition of DPPA is qualitatively identical to the effects observed previously with cationic amphiphilic drugs [7, 9, 18]. Accordingly, the transition signal induced by compound II is interpreted as indicating the formation of substance-containing domains, the size of which dose-dependently increases at the expense of the rest of the phospholipid. The constant temperature of this signal suggests that the composition of the compound II-containing domains does not change with increasing doses of the compound. In any case, the difference ΔT_t between the onset temperature of the control signal and the signal induced by compound II was independent of the dose. For compound II the depression of the transition temperature amounted to ΔT_t = 28°.

Figure 2 shows thermograms obtained with the applied compounds at a dose of 0.2 mol/mol of DPPA. The transition signal for compound I occurred at the same temperature as the compound II-induced signal. A total of seven molar ratios was investigated; the onset temperature of the signal induced by compound I was also independent of the molar ratio and the transition temperature amounted to T_t = 35.0 ± 0.2° (mean ± SE, N = 7). Thus, the transition temperature was depressed by ΔT_t = 29°. The transition signal induced by compound III was found at a considerably lower temperature (T_t = 11.1 ± 0.1°, mean ± SE, for N = 9 molar ratios between 0.07 and 0.6 moles of compound III per mole DPPA). ΔT_t amounted to 53°. It should be mentioned that this, to our knowledge, is the greatest depression of the transition temperature of DPPA encountered so far with a catamphiphilic drug. The reported ΔT_ts (Table 1) were checked in two further sets of experiments; each set was performed by a different investigator and included the three compounds.

The binding of $^{45}Ca^{2+}$ to DPPA monolayers was inhibited by the compounds concentration-dependently (Fig. 3). The concentration–response curves had similar slopes, and IC_{50} values were read from the curves as a measure of potency (Table 1). Since the displacement of $^{45}Ca^{2+}$ from phospholipid layers by cationic amphiphilic compounds probably corresponds to drug-binding [13, 15], the IC_{50} can be taken to reflect the binding affinity of the compounds.

DISCUSSION

Compound II and compound III, which are structural isomers with similar hydrophobicity, differed widely in their ability to reduce the transition temperature of DPPA liposomes. Vice versa, compound II and compound I, although differing widely in hydrophobicity, lowered T_t to the same extent. Obviously, the hydrophobicity of cationic amphiphilic compounds does not govern their perturbing action on DPPA bilayers. In contrast, the binding affinity to DPPA layers increased in parallel with the hydrophobicity. The inverse

correlation between the IC_{50} values in inhibiting ^{45}Ca binding to DPPA layers and the log P′ values is in keeping with findings obtained with phosphatidylserine [9, 15, 18]. In general, hydrophobicity is a main determinant of the binding of catamphiphilic drugs to phospholipid membranes [12]. Since differences in binding are unlikely to account for the differing actions of the structural isomers II and III on the transition temperature, the way binding causes the effect should differ depending on the spatial arrangement of the lipophilic moiety.

As described previously [7], the depression of the transition temperature in DPPA presumably results from two actions of the intercalated drug molecules on the bilayer. Firstly, the drugs interrupt specific interactions between the polar head-groups of DPPA which are responsible for the more than 20° higher transition temperature of DPPA compared with dipalmitoylphosphatidylcholine. Secondly, the drug molecules disturb interactions between the fatty acid chains of the phospholipid. The extent of the latter effect has been proposed to depend on the depth of penetration into the interior of the bilayer [7, 9].

As found with other cationic amphiphilic drugs [1, 19–21], the cationic amino-group of the compounds will interact electrostatically with the negatively charged phosphate of DPPA (pK_{a1} 3.5, pK_{a2} 9 [22]) and anchors this part of the molecule in the polar head-group region of the lipid. The depth of penetration into the bilayer then depends on the length of the compound. Hence, compound III should penetrate considerably deeper into the bilayer interior than compound II which, in turn, should penetrate as deeply as compound I. Accordingly, the depth of penetration of the compounds would closely correspond with their effect on the transition temperature. It has, however, to be recognized that the depth of penetration was not measured directly.

In any case, some more general conclusions can be drawn from the results obtained with the model cationic amphiphilic drugs: hydrophobicity alone does not govern all of the aspects of drug–phospholipid interaction, also, the spatial arrangement of the lipophilic moiety may be a determinant [23, 24]. If a biological action of drugs does not correlate with a simple measure of hydrophobicity such as the octanol/water partition coefficient, a drug–phospholipid interaction might nevertheless be involved in the drug effect.

REFERENCES

1. Cerbon J, NMR evidence for the hydrophobic interaction of local anaesthetics. Possible relation to their potency. *Biochim Biophys Acta* **290**: 51–57, 1972.
2. Lee AG, Model for action of local anaesthetics. *Nature (Lond)* **262**: 545–548, 1976.
3. Lüllmann H, Lüllmann-Rauch R and Wassermann O, Drug-induced phospholipidosis. *CRC Crit Rev Toxicol* **4**: 185–218, 1975.
4. Hein L, Lüllmann-Rauch R and Mohr K, Human accumulation potential of xenobiotics: potential of catamphiphilic drugs to promote their accumulation via inducing lipidosis or mucopolysaccharidosis. *Xenobiotica* **20**: 1259–1267, 1990.
5. Cater BR, Chapman D, Hawes SM and Saville J, Lipid phase transitions and drug interactions. *Biochim Biophys Acta* **363**: 54–69, 1974.
6. Kursch B, Lüllmann H and Mohr K, Influence of various cationic amphiphilic drugs on the phase-transition temperature of phosphatidylcholine liposomes. *Biochem Pharmacol* **32**: 2589–2594, 1983.
7. Hanpft R and Mohr K, Influence of cationic amphiphilic drugs on the phase-transition temperature of phospholipids with different polar headgroups. *Biochim Biophys Acta* **814**: 156–162, 1985.
8. Thomas PG and Verkleij AJ, The dissimilar interactions of the calcium antagonist flunarizine with different phospholipid classes and molecular species: a differential scanning calorimetry study. *Biochim Biophys Acta* **1030**: 211–222, 1990.
9. Girke S, Mohr K and Schrape S, Comparison between the activities of cationic amphiphilic drugs to affect phospholipid-membranes and to depress cardiac function. *Biochem Pharmacol* **38**: 2487–2496, 1989.
10. Mohr K and Struve M, Differential influence of anionic and cationic charge on the ability of amphiphilic drugs to interact with DPPC-liposomes. *Biochem Pharmacol* **41**: 961–965, 1991.
11. Klingmüller M, Synthese und cardiodepressive Wirkung substituierter *N,N*-Dimethyl-3-phenylpropylamine. Ph.D. Thesis, University of Kiel, 1990.
12. Lüllmann H and Wehling M, The binding of drugs to different polar lipids *in vitro*. *Biochem Pharmacol* **28**: 3409–3415, 1979.
13. Vogelgesang R, Wood G, Peters T and Scheufler E, pH-Dependent influence of membrane-incorporated flunarizine on Ca-binding to phosphatidylserine monolayer membranes. *Biochem Pharmacol* **37**: 1597–1600, 1988.
14. Heber D, Klingmüller M, Margraf S, Mohr K and Müller B, Comparison between the antiarrhythmic potency and the perturbing action on phospholipid-membranes of structural isomers of cationic amphiphilic drugs. *Naunyn Schmiedebergs Arch Pharmacol* **339**(Suppl): R56, 1989.
15. Lüllmann H, Plösch H and Ziegler A, Ca^{2+}-replacement by cationic amphiphilic drugs from lipid monolayers. *Biochem Pharmacol* **29**: 2969–2974, 1980.
16. Jacobson K and Papahadjopoulos D, Phase transitions and phase separations in phospholipid membranes induced by changes in temperature, pH, and concentration of bivalent cations. *Biochemistry* **14**: 152–161, 1975.
17. Blume A and Eibl H, A calorimetric study of the thermotropic behaviour of 1,2-dipentadecylmethylidene phospholipids. *Biochim Biophys Acta* **640**: 609–618, 1981.
18. Seydel JK, Cordes HP, Wiese M, Chi H, Croes N, Hanpft R, Lüllmann H, Mohr K, Patten M, Padberg Y, Lüllmann-Rauch R, Vellguth S, Meindl WR and Schönenberger H, QSAR and multivariate data analysis of amphiphilic benzylamines and their interaction with various phospholipids determined by different methods. *Quant Struct Act Relat* **8**: 266–278, 1989.
19. Yeagle PL, Hutton WC and Martin RB, Molecular dynamics of the local anesthetic tetracaine in phospholipid vesicles. *Biochim Biophys Acta* **465**: 173–178, 1977.
20. Herbette L, Katz AM and Sturtevant JM, Comparisons of the interaction of propranolol and timolol with model and biological membrane systems. *Mol Pharmacol* **24**: 259–269, 1983.
21. Brasseur R, Ruysschaert J-M and Chatelain P, Semi-empirical conformational analysis of propranolol interacting with dipalmitoylphosphatidylcholine. *Biochim Biophys Acta* **815**: 341–350, 1985.
22. Träuble H and Eibl H, Electrostatic effect on lipid

phase transitions: membrane structure and ionic environment. *Proc Natl Acad Sci USA* **71**: 214–219, 1974.

23. Ondriaš K, Staško A, Jančinová V and Balgavý P, Comparison of the effect of eleven β-adrenoceptor blocking drugs in perturbing lipid membrane: an ESR spectroscopy study. *Mol Pharmacol* **31**: 97–102, 1987.
24. Ondriaš K, Staško A, and Balgavý P, Spin label study of the perturbation effect of the local anaesthetics tetracaine and dibucaine on synaptosomes at pharmacological concentrations. *Biochem Pharmacol* **36**: 3999–4005, 1987.