Interaction of [2-(Alkyloxy)-phenyl]-2-(1-piperidinyl)ethyl Esters of Carbamic Acid with Dipalmitoylphosphatidylglycerol Model Membranes: A Calorimetric Study*

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Abstract. As detected by adiabatic differential scanning microcalorimetry, [2-(alkyloxy)-phenyl]-2-(1-piperidinyl)ethyl esters of carbamic acid (C_nPPEECA, n is the number of carbon atoms in the alkyloxy substituent) with local anesthetic and antiarrhytmic activities interact with 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] model membranes (DPPG). C_nPPEECAs form solid-like solutions with DPPG at low C_nPPEECA concentrations and with short alkyloxy chain lengths ($n < 4$), while at higher concentrations and with longer alkyloxy chains ($10 \geq n \geq 5$) demixing and separation of C_nPPEECA+DPPG clusters of unknown stoichiometry occurs in the gel phase. The temperature of the gel – liquid crystal phase transition T_m is depressed in the presence of C_n PPEECA; the depression of T_m scaled for unity C_nPPEECA concentration in the lipid phase indicates higher intrinsic perturbation activity of the charged form of C_nPPEECA than that of the basic form of C_nPPEECA. It is suggested that this might be caused by a deeper location of the basic form of C_nPPEECA in the lipid bilayer.

Key words: Local anesthetics — Scanning microcalorimetry — Drug-lipid interaction — Dipalmitoylphosphatidylglycerol

Introduction

Amphiphilic drugs such as tertiary amine local anesthetics are known to interact with membrane lipids and to change the physico-chemical properties of the lipid bilayer. In local anesthesia, the anesthetic effect seems to be a result of these

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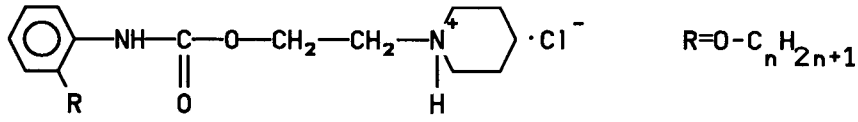
changes (Seeman 1972; Jain et al. 1975; Lee 1976a). This has prompted intensive studies of the physico-chemical properties of the lipid bilayer in biological as well as in model membranes in the presence of local anesthetics.

One of the important parameters characterizing the properties of model membranes prepared from synthetic lipids is temperature T_m of the main phase transition from the gel to the fluid liquid crystalline state. Several groups of authors have reported tertiary amine local anesthetics to decrease the value of T_m and this decrease to correlate well with the local anesthetic activity (Lee 1976b; Ueda et al. 1977; Lee 1978; Račanský et al. 1984). Račanská et al. (1990) and Gallová et al. (1992) reported recently that the T_m depression in 1,2-di-palmitoylglycero-*sn*-3-phosphorylcholine (DPPC) model membranes in the presence of a homologous series of monohydrochlorides of [2-(alkyloxy)-phenyl]-2-(1-piperidinyl)ethyl esters of carbamic acid



(C_nPPEECA, n is the number of carbon atoms in the alkyloxy substituent) displays a similar quasi-parabolic dependency on the number of carbon atoms n in the alkyloxy substituent as found for the local anesthetic activities. In this paper we present the results of a study into the effects of C_nPPEECA on phase transitions in 1,2-di-palmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DPPG) model membranes using adiabatic differential scanning microcalorimetry (DSC).

The value of pK = 8.86 – 8.88 has been measured for C_nPPEECAs in aqueous solutions (Pešák et al. 1980). Consequently, in physiological conditions, C_nPPEECA is present as a mixture of the neutral base and the positively charged protonated form. To reveal the difference in the effects between these forms, we studied the effects of C_nPPEECA on DPPG phase transitions at pH values well below and above the pK value for C_nPPEECA.

Materials and Methods

DPPG (Sigma, St. Louis, USA) and C_nPPEECA with n = 1, 3, 5, 6–10 (prepared as described by Čižmárik and Borovanský (1975)) were mixed in organic solvents. The solvents were then evaporated in a stream of nitrogen gas followed by evacuation using a diffusion pump. The dry samples were suspended in aqueous solvent A (103 mmol/l Na_2HPO_4, 48.5 mmol citric acid, pH 6.1) or in aqueous solvent B (25 mmol/l Na_2HPO_4, pH 9.7), and pH was adjusted to the given values; the final DPPG concentration was 680.2 μmol/l. Before the measurements, the samples were vigorously agitated and incubated at 50 °C for

1 h, and allowed to equilibrate for at least 2 h at room temperature. The curves of excess apparent specific heat capacity C_p vs. temperature (scan rate 1 K/min) were recorded with a Privalov DASM-4 microcalorimeter (Academy of Sciences, Moscow, Russia) connected, via an A/D converter, to a microcomputer. The first derivative dC_p/dT vs. T curve was obtained by numerical derivation at a T step of 0.1 K or less.

Results and Discussion

The curves of excess apparent specific heat capacity C_p vs. temperature T for DPPG in the presence of C_nPPEECA at concentrations c are shown in Fig. 1.

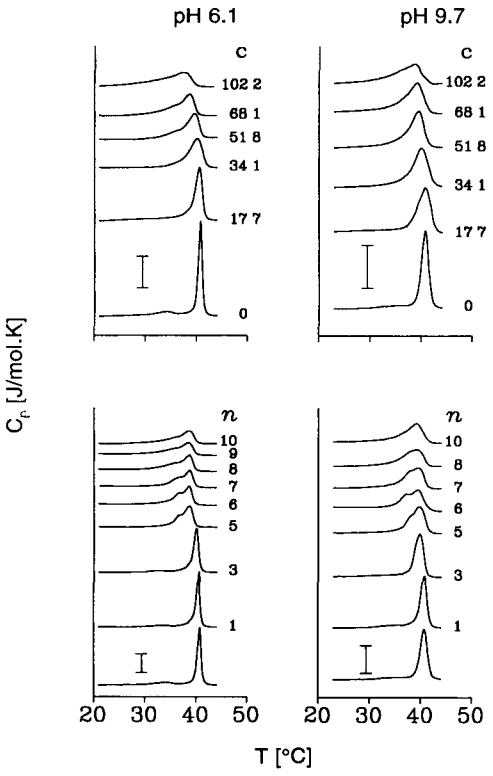


Figure 1. Variation of excess apparent specific heat capacity C_p with temperature T for DPPG in the presence of different C_{10}PPEECA concentrations c (in μmol/l) and in the presence of C_nPPEECA with different alkyloxy chain lengths n at the concentration 68.1 μmol/l. The sample pH is indicated. The vertical capped bars indicate the value of C_p = 10 kJ/mol·K for each series of curves.

The two maxima of C_p in the absence of C_nPPEECA and at pH 6.1 observed at $T_p^0 = 34.1 \pm 0.2$ °C and at $T_m^0 = 40.8 \pm 0.1$ °C correspond well with the pre-transition temperature between the gel phase $L_{\beta'}$, and the rippled gel phase P_β and with the main $P_\beta \longrightarrow L_\alpha$ liquid crystal phase transition temperature, respectively, observed in DPPG by other authors at pH values 7.0–7.4 (Boggs et al. 1986; Wilkinson and McIntosh 1986; Cevc and Marsh 1987) and at pH 6.0–6.7 (Hanpft and Mohr

1985). Lipid bilayers in $L_{\beta'}$, and P_β phases are solid-like while those in the L_α phase are fluid. At pH 9.7, the pre-transition C_p peak was broad and, due to experimental noise, it was impossible to obtain the value of T_p^0. The broadening of the pre-transition C_p peak at pH 9.7 might indicate a non-identified impurity in the preparation of DPPG used (Sigma); this however, is highly improbable because of the absence of effects at pH 6.1. Another reason might be a not-yet-found impurity in chemicals used for preparation of pH 9.7 samples. The second possibility is also improbable as the same batches of chemicals were used in both preparations. Moreover, there was no effect of these chemicals on the pre-transition in DPPC model membranes (Gallová et al. 1992). As suggested by a referee of the present paper, the differences in the calorimetric traces at pH 6.1 and 9.7 could be explained by a formation of different types of ultramolecular structure of DPPG at neutral and alkaline pH. In comparison to pH 6.1, the main phase transition C_p peak was broadened too, but the value of the main phase transition temperature at this pH was $T_m^0 = 40.7 \pm 0.1\,°C$. Taken the experimental error, this is equal to that at pH 6.1. This is in agreement with Cevc and Marsh (1987) who found the value of T_m^0 in dimyristoylphosphatidylglycerol membranes to be constant within a pH range of 4.5–14.

With the increasing C_{10}PPEECA concentration c and with the increasing alkyloxy chain length n at a constant C_nPPEECA concentration c, the main peak of C_p broadens and shifts to lower temperatures and becomes increasingly asymmetric at temperatures below the maximum temperature T_m at both pH values studied. According to the theoretical models of Sturtevant (1982), Kaminoh et al. (1988) and Jørgensen et al. (1991), broadening and asymmetry of the main transition peak are indications of the formation of C_{10}PPEECA-DPPG solid-like solution in the gel phase.

A close inspection of the C_p vs. T curves as well as of their first derivatives dC_p/dT vs. T (not shown) for pH 6.1 and for C_{10}PPEECA concentrations $c \geq 51.8$ μmol/l has shown that the main peak splits into two components; this indicates demixing of the solid-like drug-lipid solution. Similar splitting of the main peak has been observed by several groups of authors in model membranes in the presence of amphiphilic drugs (Cater et al. 1974; Frenzel et al. 1978; Kursch et al. 1983; Hanpft and Mohr 1985; Mohr and Struve 1991; Gallová et al. 1992). Dörfler et al. (1990) have clearly demonstrated that this demixing in a solid-like C_7PPEECA-DPPC system results in the formation of separated drug-lipid clusters differing in their composition. At a constant C_nPPEECA concentration $c = 68.1$ μmol/l and pH 6.1, splitting of the main C_p peak has been observed for C_nPPEECA derivatives with alkyloxy chain lengths $n \geq 5$ but not $n \leq 3$. Since most probably the demixing occurs at a defined C_nPPEECA:lipid molar ratio in the membrane, this indicates low solubility of the long-chain C_nPPEECA homologs in the solid-like bilayers. Similar effects have been observed also at pH 9.7. Splitting of the main peak has

been observed for long-chain ($n \geq 5$) homologs at concentration $c = 61.8$ μmol/l, but not for short-chain homologs ($n \leq 3$).

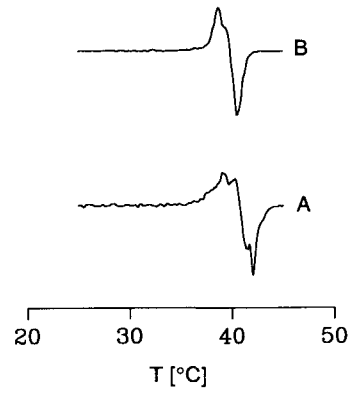


Figure 2. First derivative dC_p/dT of the curve of excess apparent specific heat capacity C_p vs. temperature T for DPPG in the presence of C_{10}PPEECA ($c = 17.7$ μmol/l) and pH 9.7 (curve A), and in the presence of C_3PPEECA ($c = 68.1$ μmol/l) and pH 9.7 (curve B). The vertical amplitude is the value of dC_p/dT in relative units.

The main C_p peaks were broader at pH 9.7 than at pH 6.1 at corresponding both concentrations and alkyloxy chain lengths. It is noteworthy that at the lowest C_{10}PPEECA concentration studied ($c = 17.7$ μmol/l) and at pH 9.7, the main peak seemed to be a superposition of two C_p vs temperature curves; both curves had maxima at temperatures coinciding within experimental error, but their widths differred significantly. An indication of a superposition of two curves with different widths is the first derivative dC_p/dT curve shown in Fig. 2 (curve A). Superposition of two curves is seen also for the C_3PPEECA homolog at $c = 68.1$ μmol/l (Fig. 2, curve B). The superpositions observed at a low C_{10}PPEECA concentration and that for C_3PPEECA at a higher concentration could be caused a) by a non-identified impurity in the lipid which influences the main phase transition at pH 9.7 but not at pH 6.1, b) by a non-identified impurity in C_nPPEECA which influences the main phase transition at pH 9.7 but not at pH 6.1, or c) by two types of solid-like C_nPPEECA-DPPG solutions coexisting in a sample. A support for alternative a) could be the broadening of both the pre-transition and the main transition C_p peaks in the absence of C_nPPEECA seen in Fig. 1 and discussed above. An underlying reason for alternative b) could be alkaline hydrolysis of C_nPPEECA during the preparation and incubation of samples at pH 9.7 and 50°C. However, Stankovičová et al. (1990) have found a second-order rate constant for alkaline hydrolysis of the order of $k \simeq 10^{-4}$ l/mol.s at 50°C, so that this process cannot be expected to influence our results significantly. Moreover, the partition of tertiary amines in the lipid phase is known to inhibit the alkaline hydrolysis (Bianconi et al. 1988). In addition, using the same chemicals and procedures in experiments with the same drugs and DPPC model membrane we have found no effect of sample

preparation and incubation at pH 10 (Gallová et al. 1992). Alternative c) will be discussed later.

The van't Hoff and true (calorimetric) enthalpies and the ratio of partition coefficients in the solid-like and fluid lipid phases can be obtained from an analysis of the shapes of C_p vs. temperature curves (Sturtevant 1982; Kaminoh et al. 1988). Because of the unsolved problems with the broadening and superpositions of the main C_p peak (see above) these thermodynamic parameters have not been obtained. However, since the described effects do not influence (within experimental error) the positions of the C_p maxima, the C_p curves were used to obtain T_m and, where possible, T_p values. The dependence of the depression of the main transition C_p peak temperature $\Delta T_m = T_m^0 - T_m$ on the C_{10}PPEECA concentration is presented in Fig. 3. At both pH values studied, the main phase transition temperature decreased with the increasing concentration up to a maximum concentration used in our work (c = 102.2 μmol/l). From theoretical models of Sturtevant (1982), Kaminoh et al. (1988) and Jørgensen et al. (1991) it follows that the decrease of T_m with the increasing c is an evidence for the C_nPPEECA partition coefficient between aqueous solution and DPPG model membrane in the solid-like state being lower than that in the fluid state.

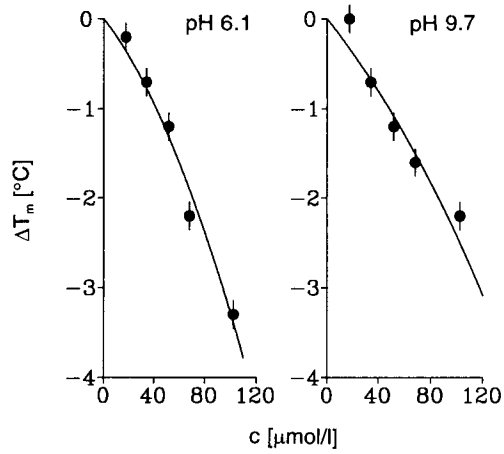


Figure 3. Depression ΔT_m of the main phase transition temperature for DPPG in the presence of different C_{10}PPEECA concentrations c (in μmol/l). The vertical bars indicate the experimental error.

The pre-transition peak was observable only for short alkyloxy chain C_nPPEECA homologs ($n \leq 5$) at pH 6 (C_nPPEECA concentration c = 68.1 μmol/l). In the presence of C_nPPEECA the peak was broader and asymmetric; for n = 5 it was nearly masked by background noise. As is clearly seen in Fig. 1, the demixing in the solid-like solution (indicated by the main peak splitting) starts at the alkyloxy chain length n = 5. The disappearence of the pre-transition peak and the splitting of the main transition peak might thus be interconnected. With the increasing n

the value of T_p decreases (Fig. 4). These experimental findings indicate that in the experimental conditions used, C_nPPEECAs form solid solutions with both $L_{\beta'}$ and P_β gel phases of DPPG, and that at a constant C_nPPEECA sample concentration the concentration of C_nPPEECA in the phase P_β is higher than that in the phase $L_{\beta'}$.

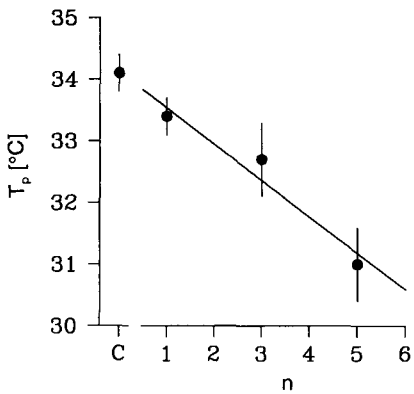


Figure 4. The dependence of the pre-transition temperature T_p for DPPG in the presence of C_nPPEECA at concentration $c = 68.1$ in μmol/l. The vertical bars indicate the experimental error. C, control sample without C_nPPEECA.

The mechanism responsible for the increased C_nPPEECA binding in phase P_β is not clear. Most probably, the increase is not caused by changes in the bilayer hydrophobic region at the $L_{\beta'} \longrightarrow P_\beta$ phase transition: in both phases, the DPPG acyl chains are in the all-*trans* conformation and are tightly packed, and the only difference is a change in the angle of their symmetry axes to the bilayer midplane from tilted to perpendicular and rippling of the bilayer surface in phase P_β (Cevc and Marsh 1987). There is another possibility which is differences in lateral heterogeneity of the bilayers of these phases. Any solid-like structure comprises structural defects and, as suggested by Ivkov and Berestovskij (1982) and Sackmann (1983), a lipid bilayer consists of domains of densely packed lipids and of defects between these domains. These defects can be considered as lateral vacant points in the surface of a bilayer. Evidence for the existence of defects and domains in lipid bilayers has been provided by the small angle diffusion scattering of neutrons (Bezzabotnov et al. 1987). The phase transitions in lipid bilayers are associated with the formation of domains of one phase in the matrix of the second phase and vice versa. The domain formation is a dynamic process, and the domains fluctuate in size and position near the phase transition temperature, this resulting in a fluctuation-induced lateral heterogeneity of bilayers (Mouritsen and Zuckermann 1985; Jørgensen et al. 1991). In the solid-like phase this domain structure is frozen due to slow lateral diffusion of lipids. In our experimental conditions, the $L_{\beta'}$ phase was equilibrated for a long time (minimum 2 h) at room temperature, while

the P_β phase was scanned in the calorimeter in less than 10 min., i.e. in the $L_{\beta'}$ phase the bilayers are annealed and in the P_β phase they are non-annealed. The P_β phase could thus contain more lateral defects than the $L_{\beta'}$ phase. Since the defect interfacial region between the domains is the site where drugs accumulate in the bilayer (Jørgensen et al. 1991), this lateral heterogeneity model could explain the observed increase in C_nPPEECA binding. This assumption is further supported by the observed hysteresis in the T_p values during reversed temperature scan (preliminary observations). An independent indication for this mechanism seems to be the observed alteration of the surface potential of the uncharged DPPC bilayers at the pre-transition in the presence of calcium ions (Tatulian 1987).

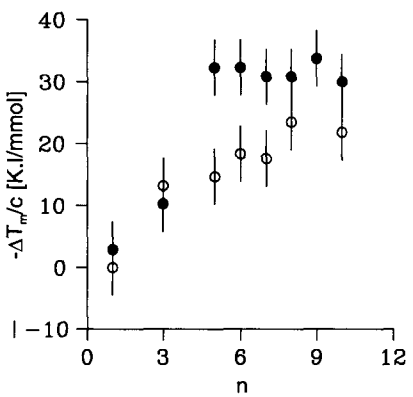


Figure 5. The dependence of the slope of depression ΔT_m of the main phase transition temperature vs. C_nPPEECA concentration c on the C_nPPEECA alkyloxy chain length n at pH 6.1 (filled circles) and at pH 9.7 (open circles). The vertical bars indicate the experimental error.

Fig. 5 shows the dependence of the depression of the main phase transition temperature ΔT_m normalized for the 1 mmol/l C_nPPEECA sample concentration evaluated from data obtained at $c = 68.1$ μmol/l. At pH 6.1, the efficiency of C_nPPEECA to decrease the main phase transition temperature increases with the increasing alkyloxy chain length n up to $n = 5$ and then levels off (within experimental error). At pH 9.7, this levelling off probably starts at $n = 6$. At equal sample concentration, the C_nPPEECAs are more efficient membrane perturbers at pH 6.1 than at pH 9.7. The observed saturation or levelling-off cannot be explained by the limited solubility of the long-chain C_nPPEECAs in the aqueous phase as suggested by Račanská et al. (1990) in their work with C_nPPEECAs and DPPC model membranes, because the most lipophilic C_{10}PPEECA does not show saturation or levelling- off in its effect on the ΔT_m value up to concentrations (see Fig. 3) significantly higher than those used in experiments the results of which are shown in Fig. 5. The dependencies in Fig. 5 thus must be a combined function of two effects, the intrinsic perturbing activity of the compounds studied on the lipid bilayer and the partition equilibria in the sample. Therefore, to evaluate the

experimental data in more detail the concentration of C_nPPEECA in the bilayer must be known. As a first rough approximation, this can be obtained by using the equilibria scheme of the protonated form of C_nPPEECA HA^+ and its base A between the aqueous phase (subscript *a*) and the bilayer (subscript *b*)

$$\begin{array}{ccccc} & & K_a & & \\ & HA_a^+ & \rightleftharpoons & H^+ + A_a & \\ K_p^+ & \downarrow\uparrow & & \uparrow\downarrow & K_p \\ & & K_b & & \\ & HA_b^+ & \rightleftharpoons & H^+ + A_b & \end{array}$$

where K_a and K_b are the dissociation constants of C_nPPEECA located in the aqueous phase and in the bilayer, respectively, and K_p^+ and K_p are the respective volume partition coefficients. In this scheme we have not included the effects of electrical double layer of the negatively charged DPPG bilayer surface and the change in the electrical surface potential due to insertion of both charged and basic forms of C_nPPEECA between the lipids in the bilayer. The second approximation is a consequence of the fact that the partition coefficients of C_nPPEECA between the DPPG bilayers and the aqueous phase are not known. Nevertheless, we decided to use all the available experimental parameters to study the trends in the data because this might be instructive and inspirative for further studies of other authors.

The values of partition coefficient K_p^+ have been measured for C_nPPEECA with $n = 3$, 5, and 7 and the fluid bilayer of egg yolk phosphatidylcholine (Hanus 1990; Balgavý et al. 1992). Since $\log K_p^+$ is a linear function of n within homologous series of amphiphilic long-chain compounds (see Devínsky et al. (1990) for Discussion and References), the experimental values of K_p^+ can be used to calculate K_p^+ for the whole homologous series of C_nPPEECA. The pK values of tertiary amine local anesthetics located in phosphatidylcholine bilayers are shifted from their normal values in aqueous solutions. For example, for tetracaine $\Delta pK = pK_a - pK_b = 1.5 \pm 0.1$ (Rooney and Lee 1983; Schreier et al. 1984; Kelusky et al. 1986; Švajdlenka et al. 1987). From the equilibria scheme it follows that $\Delta pK = \log(K_p : K_p^+)$, so that the values of K_p can be calculated from experimental values of ΔpK, pK_a and K_p^+. The values of $\log K_p$ are not appreciably influenced by the surface charge of the bilayer. Kelusky et al. (1986) have found $\log K_p = 2.78$ for the tertiary amine local anesthetic tetracaine in uncharged phosphatidylcholine bilayers and $\log K_p = 2.63$ in negatively charged phosphatidylserine bilayers. However, the K_p^+ values depend strongly on the lipid type. The value of $\log K_p^+ = 2.84$ found for the phosphatidylserine bilayers is considerably greater than $\log K_p^+ = 1.34$ found for phosphatidylcholine bilayers (Kelusky et al. 1986). Using the above data, we used the following relationships:

$$\log K_p(\mathrm{PG}) = \log K_p^+(\mathrm{PC}) + \Delta pK(\mathrm{PC}) \qquad (1)$$

$$\log K_{\mathrm{p}}^{+}(\mathrm{PG}) = \log K_{\mathrm{p}}(\mathrm{PG}) + 0.21 \tag{2}$$

where PG and PC denote the particular partition coefficients in the phosphatidylglycerol and phosphatidylcholine membranes, respectively, $\log K_{\mathrm{p}}^{+}(\mathrm{PC})$ is the experimentally found dependence on the C_nPPEECA alkyloxy chain length n

$$\log K_{\mathrm{p}}^{+}(PC) = 0.52 + 0.37 \cdot n \tag{3}$$

in the fluid phosphatidylcholine bilayers (Balgavý et al. 1992), $\Delta \mathrm{p}K(\mathrm{PC}) = 1.5$ is the value obtained from potentiometric experiments (Schreier et al. 1984; Švajdlenka et al. 1987), and 0.21 is the experimental correction for the electrical double layer in charged bilayers (Kelusky et al. 1986). Using the density of 1 g/ml for DPPG, the experimental $\mathrm{p}K_{\mathrm{a}}$ value of 8.88 for C_nPPEECA (Pešák et al. 1980), and the volume ratio of the lipid and aqueous phase as used in our experiments, we calculated molar ratios of C_nPPEECA:DPPG for both the charged HA^+ and the basic A forms of C_nPPEECA at a concentration $c = 68.1$ μmol/l and alkyloxy chain length $n = 5 \div 10$ (Table 1). It is seen that the charged HA^+ form of C_nPPEECA

Table 1. Molar ratios of charged HA^+ and basic A forms of C_nPPEECA (at concentration $c = 68.1$ μmol/l) and DPPG at pH 6.1 and 9.7 and the intrinsic activity α to decrease the main phase transition temperature in DPPG as a function of the alkyloxy chain length n.

	pH 6.1			pH 9.7		
n	A:DPPG	HA^+:DPPG	α [K]	A:DPPG	HA^+:DPPG	α [K]
5	$6.99 \cdot 10^{-5}$	$8.60 \cdot 10^{-2}$	25.6 ± 2.3	$7.11 \cdot 10^{-2}$	$8.74 \cdot 10^{-4}$	12.5 ± 2.5
6	$7.60 \cdot 10^{-5}$	$9.35 \cdot 10^{-2}$	23.5 ± 2.1	$8.04 \cdot 10^{-2}$	$9.90 \cdot 10^{-4}$	13.8 ± 2.2
7	$7.80 \cdot 10^{-5}$	$9.72 \cdot 10^{-2}$	21.6 ± 2.1	$8.52 \cdot 10^{-2}$	$1.05 \cdot 10^{-3}$	12.5 ± 2.1
8	$8.03 \cdot 10^{-5}$	$9.88 \cdot 10^{-2}$	21.2 ± 2.0	$8.75 \cdot 10^{-2}$	$1.08 \cdot 10^{-3}$	16.3 ± 2.0
9	$8.09 \cdot 10^{-5}$	$9.96 \cdot 10^{-2}$	23.1 ± 2.0	$8.85 \cdot 10^{-2}$	$1.09 \cdot 10^{-3}$	9.1 ± 2.0
10	$8.12 \cdot 10^{-5}$	$9.99 \cdot 10^{-2}$	21.5 ± 2.0	$8.89 \cdot 10^{-2}$	$1.09 \cdot 10^{-3}$	15.0 ± 2.0

is the dominant one in the bilayer at pH 6.1 (more than 99.9%) while at pH 9.7, the charged HA^+ form of C_nPPEECA molecules in the bilayer represents about 1.2% of the total amount and dominant is the basic A form (98.8%) at all the alkyloxy chain lengths n studied. As expected, the molar ratios A:DPPG and HA^+:DPPG increase with the increasing alkyloxy chain length n at both pH values studied and the total molar ratio (A+HA^+):DPPG is higher at pH 6.1 than at pH 9.7. This higher value arises from an electrostatic interaction between the positively charged HA^+ form and the negatively charged DPPG ($\mathrm{p}K = 2.9$, see Watts et al. (1978)).

Using the values of molar ratios from Table 1 we further calculated the intrinsic activity of C_nPPEECA to change the main transition temperature as

$$\alpha = |\Delta T_m/(C_n\text{PPEECA/DPPG})| \quad (4)$$

where C_nPPEECA/DPPG is the molar ratio of C_nPPEECA and lipid in the fluid lipid phase. The values of α are shown in Table 1. We did not see any systematic dependence of α on the alkyloxy chain length n outside the experimental error, which is rather high. However, the values of α at pH 6.1 are significantly higher than that at pH 9.7, i.e. the C_nPPEECA molecules exert a stronger perturbing activity on the DPPG bilayers at pH 6.1 than at pH 9.7 at the same concentration in the fluid lipid phase. Similarly to other surface-active drugs, the molecules of C_nPPEECA intercalate between the DPPG molecules in the bilayer, their polar parts interact with the polar DPPG head-groups and their non-polar fragment penetrate between the DPPG fatty acyl chains. *Ab initio* molecular orbital method study of the interactions between models of tertiary amine local anesthetics and phospholipids has shown that the protonated amine forms a very strong hydrogen bond with the phosphate anion (Remko and Scheiner 1988a). Using the crystal structure data of dimyristoylphosphatidylglycerol (Pascher et al. 1987) and that of C_7PPEECA (Pavelčík et al. 1986), and the $P\text{–}O^- \ldots H\text{–}N^+$ intermolecular separation of 0.263 nm as observed for the crystal structure of dilauroylphosphatidyldimethylethanolamine (Pascher et al. 1987), we studied possible conformations of the charged form HA^+ of C_nPPEECA in the DPPG bilayer (Balgavý et al., in preparation). We found that the first carbon atom of the alkyloxy chain is located on the level of the first carbon atom of the DPPG acyl chain. This location causes lateral expansion of the lipid bilayer and affects the packing of the fatty acyl chains. Due to the mismatch between the lengths of the DPPG acyl chains and the C_nPPEECA alkyloxy chain, this intercalation will create free volume in the bilayer hydrophobic region below the terminal alkyloxy methyl groups. On C_nPPEECA deprotonation the hydrogen bond between the C_nPPEECA amino nitrogen and the DPPG phosphate oxygen is interrupted and the C_nPPEECA carbamate nitrogen can form hydrogen bonds with the DPPG acyl chain carbonyl oxygens (Remko and Scheiner 1988b). Furthemore, the C_nPPEECA aromatic ring can interact through dispersion forces with the acyl chains of DPPG. As a result of these interactions the C_nPPEECA molecules will penetrate more deeply into the DPPG bilayer hydrophobic core. On deprotonation the free volume below the terminal alkyloxy methyl group of C_nPPEECA will thus decrease. This model is in agreement with the experimental data of Boulanger et al. (1981) and Kelusky et al. (1986) who have observed that on deprotonation, the tertiary amine local anesthetic tetracaine changes location within the phosphatidylcholine and phosphatidylserine bilayers, respectively, and penetrates deeper into the hydrophobic core of bilayers. The free

volume could thus be a defect responsible for the different intrinsic perturbation activities of C_nPPEECA at pH 6.1 and 9.7.

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