Towards the Predictability of Drug-Lipid Membrane Interactions: The pH-Dependent Affinity of Propranolol to Phosphatidylinositol Containing Liposomes

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Purpose. Prediction of the pH-dependent affinity of (*RS*)-[^{3}H]propranolol to mixed phosphatidylcholine (PhC)/phosphatidylinositol(Phl) membranes from the partitioning in the single lipid liposome/buffer systems.

Methods. Partition studies in liposome/buffer systems were performed by means of equilibrium dialysis at 37°C between pH 2 and 11 at a molar propranolol to lipid ratio of 10^{-6} to 10^{-5} in the membrane.

Results. The Phl membrane more strongly attracts the protonated (*RS*)-[^{3}H]propranolol than the neutral solute, i.e. the partition coefficient of the protonated base (P_i) is 17'430 ± 1320, P of the neutral compound (P_n) is 3110 ± 1650. In the PhC-liposome system P_i is 580 ± 17, P_n 1860 ± 20. The partition coefficients show an exponential dependence on the molar Phl fraction in mixed liposomes. The partitioning in mixed PhC/Phl membranes is predictable from P_n and P_i in the single lipid liposome systems.

Conclusions. The negative charge of biological lipid membranes causes strong electrostatic interactions with positively charged solutes. This strong attraction is not predictable from the octanol/buffer partition system, but it is important regarding drug accumulation in the tissue and drug attraction by certain lipids in the vicinity of membrane proteins.

KEY WORDS: lipophilicity; partitioning; liposome; propranolol; drug-lipid membrane interactions; phosphatidylinositol.

INTRODUCTION

The lipophilicity of a solute, as expressed by its partition coefficient, i.e. the concentration ratio between a lipophilic and a hydrophilic phase, is the sum of electrostatic and hydrophobic interactions (1). In the octanol/buffer partition system, which is widely used for the characterisation of the lipophilicity, electrostatic interactions between the lipophilic phase and the solute play a minor role, since octanol carries no charge. With ionizable compounds P values of the neutral species are considerably higher than those of the ionized species, which prefer the polar water phase. In contrast, lipid membranes, the major lipophilic phase *in vivo*, contain various amounts of charged lipids providing a polar environment at the membrane surface with predominantly negative charges. A strong attraction of positively charged molecules, e.g. bases with pK_a values above the physiological pH or quaternary amines, can thus be observed (2–4).

We recently published that negatively charged lipid membranes containing deprotonated free fatty acids (FFA) beside phosphatidylcholine (PhC) attract the protonated base (*RS*)-[^{3}H]propranolol stronger than the neutral drug, as revealed from partition experiments in the liposome/buffer system. Below pH 7, where the FFA within the membrane are protonated and therefore neutral, the apparent partition coefficient D was similar to D determined with net neutral PhC membranes (5). Similar behaviour was found for other drugs in the PhC/phosphatidic acid liposome/buffer system (6).

In this work we investigate whether the pH-dependent affinity of the base (*RS*)-[^{3}H]propranolol to a lipid membrane containing the negatively charged phosphatidylinositol (Phl) beside PhC can be predicted from the partitioning in the single lipid membrane/buffer systems. Partition studies in liposome/buffer systems were carried out by means of equilibrium dialysis. Phl is negatively charged above pH 3 due to the deprotonated phosphate group. Below pH 3 the latter is protonated, i.e. Phl carries no charge. PhC on the other hand is a zwitterion above pH 2.5 due to the deprotonated phosphate group and the positively charged quaternary amine group of the choline. Extrusion of multilamellar vesicles of Phl and PhC/Phl mixtures results in unilamellar liposomes which are stable between pH 2 and 10 for several hours at 37°C. We show that the attraction between the negatively charged headgroup of Phl and the protonated (*RS*)-[^{3}H]propranolol is much stronger than the one between (*RS*)-[^{3}H]propranolol and net neutral PhC. The partition profiles follow the ionization curves of the solute and the lipids as we already described for other liposome/buffer systems (5,7). In a partition system consisting of mixed PhC/Phl-liposomes D is exponentially dependent on the Phl fraction in the membrane. The exponent is pH-independent between pH 2 and 11. These findings allow the prediction of the partition profiles of (*RS*)-[^{3}H]propranolol in mixed PhC/Phl liposome/buffer systems.

In recent studies (5,7) we used the zetapotential of the liposomes at various pH values in order to correct apparent pK_a values of solute and lipids, as determined in partition studies, for the pH difference between the stationary buffer layer above the lipid membrane and the bulk aqueous phase. The examination of the negatively charged Phl- and PhC/Phl-liposomes shows that this correction is necessary in order to get a reliable description of the partition profile, which is based on the P values of the solute and the pK_a values of solute and lipids.

MATERIAL AND METHODS

Chemicals

(*RS*)-Propranolol HCl #P-0884 was supplied by Sigma, (*RS*)-[4-^{3}H]propranolol hydrochloride, 533 GBq/mmol #TRK 495 by Amersham Int.. Solvents were purchased from Romil (Shepshed, UK), HPLC quality. Egg PhC and wheat germ PhI, grade I, were supplied by Lipid products (Nutfield, UK). As

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NOTATIONS: D, Apparent partition coefficient, distribution coefficient; FFA, Free fatty acids; LUV, Large unilamellar vesicles; MLV, Multilamellar vesicles; P_n, True partition coefficient of the neutral solute; P_i, True partition coefficient of the ionized solute; PhC, Phosphatidylcholine; Phl, Phosphatidylinositol; ζ, Zetapotential.

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liquid scintillation counting (LSC) cocktail Ultima Gold from Packard was used. All other chemicals were of analytical grade.

Liposomes

Liposomes were prepared by the extrusion method (8). 10 to 60 mg PhC, Phl or mixtures of PhC and Phl were dried from chloroform/methanol solutions to a thin layer in a round flask. The lipids were hydrated with 1 to 9 ml PBS pH 7.4 to form multilamellar vesicles (MLV). The MLV preparation was treated by 5 cycles of freeze and thaw in liquid nitrogen and at 37°C. Large unilamellar vesicles (LUV) were then prepared by extrusion of the MLV preparation through 0.1 μm polycarbonate filters by means of the extruder from Lipex (Canada). PhC-liposomes, as used to check the partition equilibrium between outer and inner layer, were extruded by means of a LiposoFast (Avestin, Canada) through 0.1 μm polycarbonate filters. The resulting size distributions of the liposomes were measured with a Malvern Zetasizer 3. The following diameters were found: LiposoFast PhC-liposomes, 130 nm; extruder Phl-Liposomes, 90 nm; extruder PhC/Phl-liposomes, 80 to 100 nm. The polydispersity factors of all preparations were lower than 0.1. The size distribution was stable during the partition experiments within the studied pH range 2 to 11.

Zetapotential Measurements

Zetapotentials were determined from interference effects on two cross laser beams in the liposome preparations, which were exposed to an electric field, using a Zetasizer 3 from Malvern.

Partition Experiments

Partition experiments were performed by means of equilibrium dialysis at 37°C during 5 h (>99.9% of equilibrium concentrations reached). As aqueous phase a standardised universal buffer solution, SUBS, was used, which contains phosphate, citrate, borate and sodium chloride adjusted to a physiological osmolality and with a constant ionic strength of 0.23 M (9). The lipid concentration in equilibrium dialysis was 2 mg/ml. If not stated otherwise (*RS*)-[^{3}H]propranolol was added to the liposome suspension in a concentration of 10^{-9} to 10^{-8} M at the start of the dialysis. Radioactivity of both chambers was determined by LSC (95–100% recoveries) using a Beckman LS6800. The exact PhC and Phl concentrations after dialysis were determined by either an enzymatic choline quantification (PhC) (10) or by HPLC (Varian #9012), using a Spherisorb S3W Si-60 column from PhaseSep (Norwalk, CT, USA) according to (11). The lipids were detected by an ACS 950/14 (UK) mass detector. The liposome preparation procedure did not change the PhC/Phl ratios of the lipid mixtures.

Calculations and Data Analysis

Calculations and data analysis are described in detail in (5 and 7). For the calculation of the various parameters we used the following equations:

Difference between the bulk pH and the pH of the stationary buffer layer above the membrane (ΔpH):

$$\Delta\ pH = pH(bulk) - pH(above\ membrane) = -\frac{F \cdot \zeta}{2.303 \cdot R \cdot T} \quad (1)$$

pH(bulk) is the operational bulk pH, as measured with an Ingold Ag/AgCl combination micro electrode. F, Faraday constant; R, gas constant; ζ, zetapotential.

Curve fitting of D as a function of pH was completed with the program proFit 4.1 (Quantum Soft, Switzerland) using the Levenberg-Marquardt algorithm. The parameters resulting in the lowest χ^2 were chosen at a confidence level of 90%.

For the PhI- and the PhC/Phl-liposome/SUBS systems the following equation was used:

$$\begin{aligned} D = {} & \frac{1}{1 + 10^{pK_a - pH + \Delta pH}} \cdot \alpha[>pH_{m2}] \cdot P_n[>pH_{m2}] \\ & + \frac{1}{1 + 10^{pH - pK_a - \Delta pH}} \cdot (\alpha[<pH_{m1}] \cdot P_i[<pH_{m1}] \\ & + \alpha[pH_{m1}/pH_{m2}] \cdot P_i[pH_{m1}/pH_{m2}] \\ & + \alpha[>pH_{m2}] \cdot P_i[>pH_{m2}]) \end{aligned} \quad (2)$$

where

$$\alpha[<pH_{m1}] = \frac{10^{-2pH}}{10^{-2pH} + 10^{-pH-pH_{m1}} + 10^{-pH_{m1}-pH_{m2}}} \quad (3)$$

$$\alpha[pH_{m1}/pH_{m2}] = \frac{10^{-pH-pH_{m1}}}{10^{-2pH} + 10^{-pH-pH_{m1}} + 10^{-pH_{m1}-pH_{m2}}} \quad (4)$$

$$\alpha[>pH_{m2}] = \frac{10^{-pH_{m1}-pH_{m2}}}{10^{-2pH} + 10^{-pH-pH_{m1}} + 10^{-pH_{m1}-pH_{m2}}} \quad (5)$$

pK_a in eq. 2 equals the thermodynamic pK_a of propranolol at 37° C, i.e. 9.24 (9). It is kept fixed for the curve fitting. P_n is the true partition coefficient of the neutral (*RS*)-[^{3}H]propranolol. The term in brackets behind P_n denotes the ionization state of the membrane, e.g. [$>pH_{m2}$], the ionization state which predominates above pH_{m2}, [pH_{m1}/pH_{m2}], between pH_{m1} and pH_{m2}. pH_{m1} and pH_{m2} correspond to the inflection points of the D-pH-profile. pH_{m1} results from the ionization of the phospholipids phosphate groups, pH_{m2} is assigned to the protonation/deprotonation of free fatty acids or other hydrolysis products of the phospholipids. pH_m values have to be corrected according to eq. 1 in order to get the pK_a of the membrane lipids. α gives the pH-dependent molar fraction of one particular membrane ionization state.

The function for the PhC-liposome/SUBS system (eq. 6) is used as described elsewhere (7).

$$D = \frac{1}{1 + 10^{pK_a - pH}} \cdot \frac{P_n[>pH_{m1}]}{1 + 10^{pH_{m1} - pH}} + \frac{1}{1 + 10^{pH - pK_a}} \cdot \left[\frac{P_i[>pH_{m1}]}{1 + 10^{pH_{m1} - pH}} + \frac{P_i[<pH_{m1}]}{1 + 10^{pH - pH_{m1}}}\right] \quad (6)$$

RESULTS

Zetapotential of Liposomes

In order to estimate the pH within the stationary buffer layer above the membrane, liposomes were characterised for their zetapotentials as described under Materials and Methods. Fig. 1 shows the pH-dependent zetapotentials of Phl-, PhC/Phl (7/3 mol/mol)- and PhC-liposomes. The Phl-liposomes have the most negative ζ, i.e. between −20 mV (pH 3.7) and −26 mV (pH 10.2). ζ of PhC/Phl (7/3 mol/mol)-liposomes is between

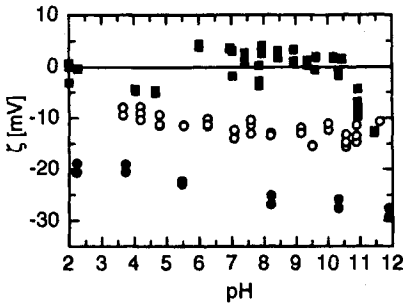

Fig. 1. Zetapotentials of (■)PhC-, (●)Phl- and (○)PhC/Phl (7/3 mol/mol)-liposomes in SUBS. Liposomes were prepared and characterised as described under Materials and Methods. The zetapotential ζ was analysed in SUBS (ionic strength 0.23 M) by micro electrophoresis and dynamic light scattering using a Zetasizer 3 (Malvern Instruments).

−8 mV (pH 3.7) and −15 mV (pH 10.6). PhC-liposomes under the examined conditions have ζ around 0 mV between pH 2 and 10.5 (5).

Partition Equilibrium of (*RS*)-[^{3}H]propranolol Between the Outer and Inner Membrane Layers of PhC-liposomes

PhC-liposomes (10 mg/ml) were prepared in PBS pH 7.4 by extrusion (see Materials and Methods). For partition experiments, the liposome suspension was diluted to 2 mg lipid per ml SUBS pH 7.4 and pH 10, respectively. At pH 7.4, most of the solute in the aqueous as well as in the lipid phase is protonated, since the pK_a of the solute in the membrane = aqueous $pK_a - \log P_n + \log P_i = 8.73$ (Table II). At pH 10 most of it is neutral in both phases. (*RS*)-[^{3}H]propranolol was either added to the chloroform/methanol solution of PhC before liposome preparation or alternatively to the liposomes at pH 7.4 or pH 10 at the start of the equilibrium dialysis. The resulting D values are listed in Table I. As can be seen, D values are similar whether the solute is added before or after liposome preparation, which means that the equilibria between the two lipid layers of the membrane and the outer and inner buffer phases are reached within the dialysis time (5 h at 37°C).

Table I. Equilibration of Propranolol in the Membrane

	D of (*RS*)-[^{3}H]propranolol	
	protonated[a]	neutral[b]
(*RS*)-[^{3}H]propranolol added before liposome preparation	754±13	1734±6
(*RS*)-[^{3}H]propranolol added to LUV of pH 7 and 10 at start of dialysis	714±10	1700±30

Note: Partition coefficients of mainly neutral and protonated (*RS*)-[^{3}H]propranolol in the PhC-LUV/SUBS system when the solute was added before or after liposome preparation.
[a] pH 7.26 and pH 7.36, respectively
[b] pH 10.06. n = 3.

pH-dependent Distribution of (*RS*)-[^{3}H]propranolol in the PhI-liposome/SUBS System and the PhC/Phl (Molar Ratio 7/3) Liposome/SUBS System

In order to investigate the influence of a negatively charged phospholipid on the distribution profile of a drug, which can be protonated, i.e. positively charged, the partitioning of the base (*RS*)-[^{3}H]propranolol in the Phl- and PhC/Phl (7/3 mol/mol)-liposome/SUBS systems was studied. Partition experiments were performed as described under Materials and Methods. Fig. 2 shows the distribution profiles of (*RS*)-[^{3}H]propranolol in the Phl-, PhC/Phl- (7/3 mol/mol) and PhC- liposome/SUBS systems. Data for the latter are from (9). The neutral (*RS*)-[^{3}H]propranolol is more strongly attracted by the PhC-membrane than the protonated drug. In contrast, the protonated (*RS*)-[^{3}H]propranolol—at pH values lower than 9—shows higher affinity to the membranes containing Phl than the neutral (*RS*)-[^{3}H]propranolol. The lower D values at pH < 4 result from the protonation of the phosphate group of Phl and PhC, as was described by (9) for the PhC-liposome/SUBS system.

Besides the changes of D around the pK_a of propranolol and of the lipid phosphate groups, a further change in the partition profile is found around neutral pH with Phl-containing systems. We postulate that it results from the protonation/deprotonation of hydrolysis products of Phl, as e.g. FFA. Under the

Table II. Fit Parameters of the pH-dependent Partitioning of (*RS*)-[^{3}H]propranolol in Phl-, PhC/Phl- and PhC-liposome/SUBS Systems

Liposomes	$pK_a+\Delta pH$ of propranolol[a]	Membrane derived inflection points		P at various membrane states				
				below pH_{m1}[c]	above pH_{m1}/ below pH_{m2}		above pH_{m2}	
		pH_{m1}	pH_{m2}	Pi	Pn	Pi	Pn	Pi
Phl	9.66	3.49 ± 0.31	6.85 ± 0.92	3470 ± 2300	[c]	17430 ± 1320	3110 ± 1650	20590 ± 1120
PhC/Phl (7/3 mol/mol)	9.43	3.13 ± 0.17	6.22 ± 0.53	720 ± 340	[c]	4510 ± 220	2040 ± 250	5410 ± 150
PhC[b]	9.24	2.46 ± 0.54	—	220 ± 180	1858 ± 24	580 ± 17	—	—

[a] ΔpH is calculated acc. to eq. 1. $pK_a+\Delta pH$ was kept fixed during the fit procedure (see Materials and Methods).
[b] Experimental data from (9).
[c] $P_n[<pH_{m1}]$ and missing $P_n[pH_{m1}/pH_{m2}]$ are not relevant for the curve description. The experimental data from partition studies were fit with a combination of Henderson-Hasselbalch equations (Materials and Methods). The fit curves are shown in Fig. 2.

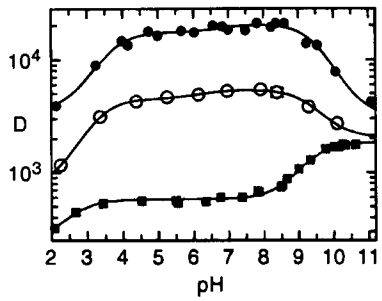

Fig. 2. Partition profiles of (*RS*)-[^{3}H]propranolol in (■)PhC-, (●)Phl- and (○)PhC/Phl (7/3 mol/mol)-liposome/SUBS systems. (——) Data were fit using eq. 2 and 6 (Materials and Methods), the curve with the lowest χ^2 is shown. Fit parameters see Table II.

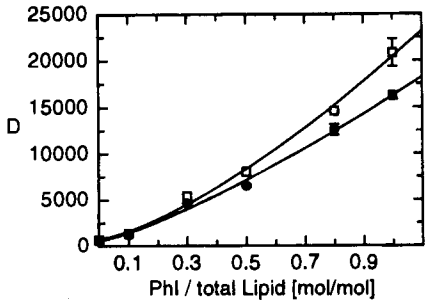

Fig. 3. Apparent partition coefficients D of (*RS*)-[^{3}H]propranolol between PhC/Phl-liposomes of various lipid ratios and SUBS at pH 6 (●) and 8 (□). (——) D values are fit using eq. 8 (see text).

same experimental conditions, the pK_a of membranous FFA was found between 7.5 and 7.8 (5).

Calculation of the Partition Parameters of (*RS*)-[^{3}H]propranolol Between Phl-containing Liposomes and SUBS

True partition coefficients of the neutral (P_n) and the ionized (P_i) solute and the pK_a of the membrane lipids were calculated by curve fitting of the experimental data as described in Materials and Methods (eqs. 2 and 6). The partition parameters are summarised in Table II. Table III gives the pK_a values of the membranous lipids. The protonation/deprotonation of the unknown lipid(s) which are probably identical with hydrolysis products of Phl (see above) was considered in the equations (pH_{m2}). They not only show up in the partition profiles but also in the HPL chromatograms (not shown) and in the zetapotential measurements, where they cause a slight change of ζ around neutral pH (Fig. 1). In the examined system, the presence of Phl in the lipid membranes causes higher P_i than P_n values, i.e. with pure Phl-liposomes $P_i[pH_{m1}/pH_{m2}]$ = 17'430 is 5.6 times higher than P_n. The P_n value, i.e. 3110 ± 1650, is of the same order as the one found with the standard PhC-liposome/SUBS system, i.e. 1858 ± 24. The deprotonation between pH 6 and 7 of the unknown membrane constituent in the Phl-containing liposomes causes an increase of P_i by a factor of about 1.2, i.e. from 17'430 to 20'590.

Influence of the Amount of Phl in PhC/Phl-liposomes on the Partitioning of (*RS*)-[^{3}H]propranolol

Liposomes consisting of various ratios PhC and Phl were prepared by the extrusion method and the partitioning of (*RS*)-[^{3}H]propranolol was determined at pH 6 and 8 as described in Materials and Methods. Fig. 3 shows D at pH 6 and 8 as a function of the molar Phl fraction in the liposomes. At all PhC/Phl ratios D is higher at pH 8 than at pH 6, as was already described for the PhC/Phl (7/3)-liposomes (see above). At pH 6 as well as at pH 8 D seems to be exponentially dependent on the Phl fraction. Therefore the data were fit using eq. 7. From these data it cannot be distinguished whether D is also exponentially dependent on the PhC-fraction or not. From the D values as shown in Fig. 3, exponent x is fit as 1.25±0.18 for pH 6 and 1.36±0.21 for pH 8.

$$D_{PhC/Phl} = f_{PhC} \cdot D_{PhC} + f_{Phl}^{x} \cdot D_{Phl} \qquad (7)$$

Prediction of the Partitioning Profile of (*RS*)-[^{3}H]propranolol in the PhC/Phl (Molar Ratio 7/3) Liposome/SUBS System Based on the P_n and P_i Values in the Single Lipid Liposome Systems

In order to check whether eq. 7 is valid over the whole pH range and can therefore be used to predict partitioning in a mixed PhC/Phl liposome system, D values of eq. 7 are replaced by the functions for the pH-dependent D in the single lipid liposome systems (eq. 8).

$$D_{PhC/Phl}(pH) = f_{PhC} \cdot D_{PhC}(pH) + f_{Phl}^{x} \cdot D_{Phl}(pH) \qquad (8)$$

In Fig. 4 the calculated curve according to eq. 8 is compared to the experimental partition data and to the fit curve as found with eq. 2 and as presented in Fig. 2. The exponent x in eq. 8

Table III. pK_a of Membranous PhC, Phl and Unknown Lipid(s)

Liposomes	pK_a of phosphate	pK_a of unknown lipid(s)
Phl	3.2±0.3	6.5±0.9
PhC/Phl (7/3 mol/mol)	3.0±0.2[a]	6.0±0.5
PhC	2.5±0.5	

Note: pK_a values of the membranous lipids were calculated from the fit inflection points (pH_m) of the partitioning curves (Tab. II) and from ζ (Fig. 1) (Materials and Methods).
[a] Apparent pK_a of the lipid mixture in the PhC/Phl-liposomes.

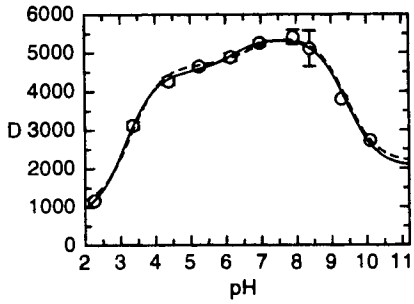

Fig. 4. The experimental partition coefficients of (*RS*)-[^{3}H]propranolol in the PhC/Phl (7/3 mol/mol)-liposome/SUBS system were fit using the predicted function based on the partitioning in single lipid membrane systems acc. to eq. 8 (----). (——), fit curve as shown in Fig. 2 using eq. 2.

was set to 1.18 resulting in the lowest χ^2 for the calculated curve. In the function $D_{Phl}(pH)$ as used in eq. 8, pH_m values were calculated according to eq. 1 from the lipid pK_a values in Table III and the zetapotentials of the PhC/Phl (7/3)-liposomes at the corresponding pH (Fig. 1). Fig. 4 shows good agreement of the resulting curve with the experimental data over the whole pH range. The calculated curve according to eq. 8 follows the curve as fit using eq. 2. The influence of the Phl fraction in the mixed lipid membrane on the partition coefficients as found at pH 6 and 8 is valid over the whole pH range. Prediction of the partition profile in the mixed lipid membrane system is possible.

DISCUSSION

Most biological lipid membranes carry a net negative charge which is the result of the presence of phosphatidylserine, Phl, phosphatidic acid, FFA or other negatively charged lipids. A strong attraction of positively charged solutes like protonated bases or quaternary amines can therefore be expected. Discussion is open whether this effect should be defined as being part of the lipophilicity of a molecule or not. Obviously this type of interaction is not predictable from the octanol/buffer partition system. It is not known how the strong electrostatic attraction of charged molecules contributes to their permeation through membranes *in vivo*. However, the electrostatic interactions certainly influence drug accumulation in membranes, membrane effects of drugs and the activity of membrane protein active drugs.

In this work, we examined the influence of negatively charged Phl within Phl- and PhC/Phl-membranes on D of the secondary amine (*RS*)-[^{3}H]propranolol. We could show that the partition profile of this compound in PhC-membranes containing increasing amounts of Phl can be predicted from the one in the pure PhC- and Phl-membranes, respectively. Parameters for this prediction were obtained by measuring D with various PhC/Phl ratios at pH 6 and 8. By curve fitting, it was found that D depends exponentially on the amount of Phl. Since the contribution of the PhC to D is relatively small, it was not possible to find out whether this is also true for PhC. D as a function of the PhC/Phl ratio was not determined for the neutral propranolol, i.e. above the bulk pH 9.7, as P_n in the PhC- and the Phl-liposome/SUBS systems are relatively close, i.e. 1858 ± 24 and 3110 ± 1650, respectively, and because D is strikingly pH-dependent in this pH range. The good agreement of the fit curve using eq. 8 with the experimental partition profile in the PhC/Phl (7/3)-liposome/SUBS system shows that eq. 7 is also valid at pH values higher than the pK_a of propranolol. From these results, P_n and P_i of (*RS*)-[^{3}H]propranolol can be calculated for every PhC/Phl ratio. This allows the prediction of the whole partition profile of the drug in mixed PhC/Phl-membrane systems. At a molar fraction of 0.14 Phl in a PhC/Phl membrane, P_n equals P_i, i.e., 1839, as calculated by eq. 7. An amount of 6 mol % Phl in a lipid membrane would double D at physiological pH when compared to a PhC-membrane. This has to be considered when predicting drug-membrane interactions from partition studies with PhC-membranes. We do not have a convincing explanation for the exponential dependence of D on the Phl fraction. It could be caused by an asymmetric distribution of the lipids between the two membrane layers (12). Further investigations are needed to shed light on this.

For the membranous Phl we found a pK_a of 3.2 ± 0.2. It is higher than the pK_a of PhC, which was fit previously (5), i.e. 2.46 ± 0.54. This is in agreement with other authors (13). The fit inflection point in the PhC/Phl (7/3 mol/mol)-liposomes was found at pH 3.0 ± 0.2. It corresponds to the value that would be expected in a 7/3 molar mixture of PhC and Phl, i.e. the weighted (mol/mol) mean of the pK_a values of the lipids.

Zetapotential measurements are used to correct the pK_a of the solute for the difference of the pH between the bulk aqueous phase and the stationary buffer layer above the membrane. It is assumed that the potential stays constant over the whole stationary layer and is equal to the surface potential (14). When the experimental data from the partition experiments using PhC/Phl-liposomes are fit using the sum of the distribution functions in the PhC-and Phl-liposome/SUBS systems, the pK_a values of (*RS*)-[^{3}H]propranolol and of the lipids have to be corrected for the actual zetapotential in order to get reliable fits. However, χ^2 is similar if the molar weighted mean of the propranolol apparent pK_a, i.e. $pK_a + \Delta pH$, resulting from the two pure lipid liposome systems is used, i.e. 9.37 instead of 9.43, which is calculated from ζ.

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