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CONCENTRATION-DEPENDENT BINDING OF THE CHIRAL β-BLOCKER OXPRENOLOL TO ISOELECTRIC OR NEGATIVELY CHARGED UNILAMELLAR VESICLES*

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Abstract—Large unilamellar vesicles (LUVs) of different lipid compositions were used to study the type of binding of the β-blocking cationic agent oxprenolol to the lipid matrix of biological membranes at a physiologic pH value of 7.4. When isoelectric membranes of pure egg lecithin or egg lecithin/cholesterol (7:3 mol/mol) were used, a linear relationship between membrane-bound and free oxprenolol indicated a constant molar partition coefficient of 54 or 44 between the liposomal and the aqueous phase over a wide concentration range of the drug up to 25 mM. This pointed to deep insertion of the drug molecules into the hydrophobic membrane interior. Drug binding to membranes of negatively charged phosphatidylserine from bovine brain was cooperative with a Hill coefficient h of 3.4 at concentrations below 0.5 mM and a molar ratio R_e of bound drug per lipid of 1:10. Above drug concentrations of 2.5 mM and $R_e = 1:5$, a constant molar partition coefficient of 33 could be estimated. *R*-oxprenolol or *S*-oxprenolol, as well as the racemic drug, showed no differences in membrane binding, even with egg lecithin LUVs containing 20 mol% of the negatively charged (2*S*, 4*R*)-*N*-(hexadecanoyl)-4-hydroxyproline, which has a pronounced chiral headgroup. Our results suggest that enantioselective interactions of the chiral oxprenolol with the chiral lipids of biological membranes can be excluded. Furthermore, surface adsorption of the drug is probable only on the negatively charged cytosolic side of biological plasma membranes, whereas on the isoelectric exterior the cationic drug is inserted deeply into the membrane.

Key words: oxprenolol; membranes; liposomes; binding; partition; chirality

β-Blockers as cationic amphiphiles interact with biological membranes not only by high-affinity binding to β-adrenoreceptors, but also by unspecific binding to the lipid matrix of the membranes. Numerous studies have shown that hydrophobic propranolol induces alterations in the lipid arrangement after binding to model membranes [1–6], and even the position of the bound drug in model membranes has been elucidated by using neutron diffraction [4]. Drug partition in liposome/buffer systems therefore provides better information than octanol/buffer about the lipophilicity of the drugs, their membrane permeation rate, and the relationship between their structures and biological activity [6].

Nevertheless, several problems have often been disregarded when liposomes were used as models for studying the interaction of β-blockers with biological membranes:

(1) bound drug molecules influence membrane structure and possibly the binding strength of additionally added molecules. Partition between membrane and buffer should therefore be evaluated over a wide drug concentration range;

(2) adsorption of charged amphiphiles preferentially or exclusively to the outer membrane leaflet should be envisaged, as shown for bile salts [7], when membrane penetration of the amphiphile is slow [8]. In this case, LUVs‖ are better suited as membrane models than MLVs. Furthermore, the amount of non-bulky water, which complicates the estimation of the size of the aqueous phase [9], is negligible in highly diluted LUV suspensions;

(3) phospholipids in biological membrane bilayers are asymmetrically distributed, with negatively charged lipids being exclusively in the inner (cytosolic) membrane monolayer [10]. Possible differences in binding to each membrane surface should be investigated by using membranes with isoelectric (e.g. phosphatidylcholine) as well as negatively charged lipid head groups (e.g. phosphatidylserine or phosphatidylglycerol);

(4) phospholipids in membranes of higher organisms are exclusively of the L-configuration type. Selective interaction of membrane lipids with drug enantiomers may, in addition to different receptor

* Dedicated to Professor Josef Hermann Roth on the occasion of his 65th birthday.

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‖ Abbreviations: BPS, bovine brain phosphatidylserine; Cho, cholesterol; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; EPC, egg phosphatidylcholine; HDP, (2*S*, 4*R*)-*N*-(hexadecanoyl)-4-hydroxyproline; LUV, large unilamellar vesicle; MLV, multilamellar large vesicle; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; OG, *n*-octyl-β-D-glucopyranoside; Op, oxprenolol.

binding, therefore be responsible for side-effects of racemic mixtures.

In initial experiments, we found that the extensively studied and highly lipophilic β-blocker propranolol [5] dissolves liposomal membranes at higher concentrations. Op does not exert such membrane-damaging properties. Therefore, we used this less lipophilic drug for a first study on the binding properties of β-blockers to various liposomal membranes. LUVs served as model membranes with different charges and chiral headgroups to determine binding profiles of the drug enantiomers and racemic mixtures over a wide concentration range.

MATERIALS AND METHODS

Materials. EPC, BPS and Cho were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Cho was recrystallized from methanol before use. Sodium cholate was from Serva (Heidelberg, F.R.G.). OG from Calbiochem GmbH (Frankfurt, F.R.G.). HDP was prepared according to the method of Kochetkov *et al.* [11]. *R,S*-oxprenolol was a gift from Ciba-Geigy (Basel, Switzerland). [^{3}H]-inulin and [^{14}C]-DPPC were purchased from NEN Chemicals (Dreieich, F.R.G.).

Chiral separation and detection of oxprenolol. Racemate separation of Op was achieved by preparing the diastereomeric salts of glutamic acid (Ciba-Geigy, personal communication): for the isolation of *R*-oxprenolol, 20 g of the racemate were mixed with 11 g of *S*-glutamic acid, dissolved in 35 mL water at 70° and filtered. After addition of 170 mL ethanol, the mixture was incubated at 5° for 24 hr. The formed precipitate was washed with a small amount of ethanol and dried, then 13.6 g were recrystallized twice by dissolving in 7.5 mL water, adding 60 mL methanol, and incubating for 15 hr at 5°. The diastereomeric salt was vacuum-dried at 60° for 8 hr (yield: 6.7 g; $[\alpha]_D^{20}$: 7.2, 1% in water; m.p. 179°) and was then dissolved in aqueous ammonia. The free base was extracted with methylene chloride, redissolved in methanol and mixed with concentrated hydrochloric acid. After removal of methanol under reduced pressure, 2.4 g of the hydrochloride of *R*-Op crystallized at 5°. For the isolation of *S*-Op, the procedure followed was identical, this time using *R*-glutamic acid. The melting point of each final product was 74° and the specific rotation of *R*-Op or *S*-Op hydrochloride was $[\alpha]_D^{20} = +19°$ or $-19°$ (1%, EtOH).

The concentration of racemic or enantiomeric Op was detected by UV-absorbance at 225 nm.

Liposome preparation. LUVs were prepared using the method of fast and controlled dialysis of mixed detergent/lipid micelles [12]. In round-bottom flasks, EPC/Cho (7:3 mol/mol) or EPC/HPD (8:2 mol/mol) were dissolved together with sodium cholate in methanol to yield a molar lipid/detergent ratio of 0.55. Pure BPS or EPC were dissolved together with OG to yield a molar lipid/detergent ratio of 0.2. Solvent was then completely removed under reduced pressure and the dry lipid/detergent mixtures dissolved in MOPS buffer (10 mM; NaCl, 150 mM; pH 7.4). The clear mixed micelle solutions were adjusted to lipid concentrations of 17 mM, and 7.5 mL was dialysed for 15 hr at room temperature against a continuous buffer flow using a commercially available apparatus (Lipoprep, Dianorm Munich, F.R.G.) and a highly permeable dialysis membrane with a cut-off of 10,000 Da (Dianorm). Liposomes were essentially free of detergents, as found in parallel experiments using radioactively labelled cholate or OG. Lipid concentrations of the final liposome stock solutions were measured by their phosphorus content [13] and adjusted to 15 mM.

Incorporation of HPD into EPC membranes was investigated by ultracentrifugation (see below) and differential canning calorimetry. In the supernatants of vesicle preparations, no HPD could be detected, whereas the endothermal peak of fully hydrated HPD at 40° completely disappeared in the EPC/HPD lipid mixture. In addition, ^{31}P-NMR studies showed that membranes were formed up to an HPD content of 50%. Increasing the HPD resulted in the formation of hexagonal phases of the phospholipids.

For preparing radioactively labelled liposomes, 5 kBq [^{14}C]-DPPC was added to the lipids in the organic solution and 500 kBq [^{3}H]-inulin to the mixed micelle solution. After dialysis, non-entrapped inulin was removed from encapsulated inulin by gel chromatography on Sepharose 4B-CL (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Liposome size and homogeneity were measured by photon-correlation spectroscopy (Nanosizer, Coulter, Harpenden, U.K.). For the binding studies, liposomes were used within 3 days after their preparation.

Equilibrium binding studies. Binding equilibria to liposomal membranes were measured with concentrations of Op hydrochloride (racemate or enantiomers) varying between 0.06 and 30 mM. The final lipid concentration in all liposome/Op mixtures was 1.5 mM. Twenty-two mixtures of 1 mL each were incubated for 10 min at room temperature in thick-wall polycarbonate tubes and then ultracentrifuged (140,000 *g*, 3.5 hr; 25°, Beckman Ti 50.4 rotor) to pellet the liposomes. Free Op concentrations were then determined in 0.5 mL of the supernatants, which were free of membrane lipids.

A possible drug adsorption to the tube walls, which may result in misinterpretations of binding data especially at low drug concentrations, was tested by comparing the UV-absorbance of the solutions before filling into the tubes and after incubation for 3 hr in the tubes. Identical drug concentrations before and after incubation suggested no adsorption to the tube material.

The stability of EPC/Cho (7:3 mol/mol) liposomes upon increasing Op concentrations was determined in a parallel experiment also using the ultracentrifugation method by measuring the amounts of [^{3}H]-inulin or [^{14}C]-DPPC in the supernatants as probes for membrane leakiness or solubilization.

RESULTS

Liposomes

Liposomes of egg lecithin/cholesterol (7:3 mol/mol), pure egg lecithin, bovine phosphatidyl-

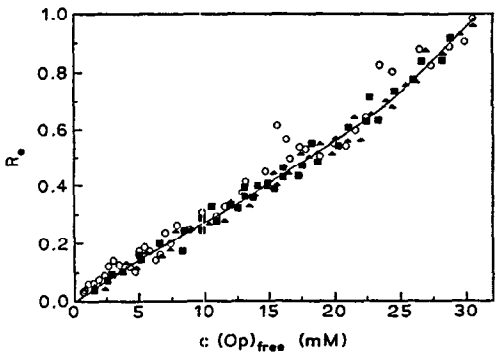

Fig. 1. Binding plot of oxprenolol enantiomers and racemic mixture to unilamellar vesicles of EPC/Cho 7:3 (mol/mol). $c(Op)_{free}$, Molar concentration of oxprenolol in the buffer phase; R_e, molar ratio of oxprenolol concentration $c(Op)_{bound}$ in the membrane and the lipid concentration; ○, racemic drug mixture; △, *S*-enantiomer; □, *R*-enantiomer.

serine, or egg lecithin/HPD (8:2 mol/mol) had a narrow size distribution around diameters of 90, 160, 80 or 120 nm as measured by photon-correlation spectroscopy, and were essentially unilamellar as determined by freeze-fracture and negative-staining electron micrographs.

Liposome stability upon oxprenolol titration

EPC/Cho liposomes radioactively labelled with [^{14}C]-DPPC in the membranes and with [^{3}H]-inulin entrapped in the vesicle interior were incubated with racemic oxprenolol (Op) for 3 hr. No release of hydrophilic inulin (M_r = 5000) or membrane lipid was detected up to an Op concentration of 50 mM in the supernatants after the liposomes were pelleted by ultracentrifugation. Inulin release increased to approximately 7% of the initially entrapped amount at 100 mM Op, whereas no released lecithin was detected at this high drug concentration. This clearly indicates that Op leads to increased permeability only at very high doses, whereas membrane solubilization by the amphiphilic drug is negligible.

Oxprenolol binding to liposomes.

Figure 1 shows a binding plot of membrane-bound vs free oxprenolol upon increasing concentration, using LUVs composed of EPC/Cho (7:3 mol/mol). R_e is the effective Op binding ratio, i.e. mol of bound Op per mol lipid, and represents the drug concentration in the lipophilic phase, whereas $c(Op)_{free}$ represents Op concentration in the aqueous phase. The ratio of the drug concentrations in the lipophilic and aqueous phases is constant up to a total oxprenolol concentration of approximately 25 mM. The slope of the curve corresponds to an apparent lipid-standardized constant partition coefficient P_l of 29 M^{-1}, which may be converted (see Appendix) into other partition coefficients often used (Table 1).

A slight increase in membrane partition above 25 mM points to an enhanced insertion of Op molecules in a disturbed membrane. Differences in membrane binding of the *R*- and *S*-enantiomers or the racemic mixtures are not detectable over the whole concentration range.

Using LUVs from pure EPC, the binding plot also has an essentially linear shape (data not shown). The slopes of the curves of the racemate as well as of both enantiomers correspond to an apparent P_l of 41 M^{-1} (see Table 1). This increased value, compared to cholesterol-containing EPC LUVs, tends to suggest that insertion of Op is favoured in more fluid membranes.

The interaction of Op with negatively charged membranes clearly differs from that with isoelectric

Table 1. Partition coefficients, association constants, and Hill coefficients of oxprenolol in various unilamellar liposomal systems

Lipid composition	Partition coefficients: lipid-stand. P_l*	lipid-stand. P_{li}*	molar or molal P_c,P_b	molar or molal P_{ci},P_{bi}	mole fractional P_x	mole fractional P_{xi}	Binding parameters K_a†	h‡
EPC	41	5200	54	6860	2280	290,000	—	—
EPC/Cho (7.3 mol/mol)	29	3680	44	5560	1600	204,000	—	—
BPS	25	3170	33	4190	1390	176,000	$4.0 \cdot 10^{10}$	3.4
EPC/HDP (8:2 mol/mol)	42	5330	60	7620	2340	297,000	$4.8 \cdot 10^{4}$	1.8

Lipid-standardized partition coefficients P_l were determined in MOPS-buffered saline, pH 7.4, estimated from the linear parts in the binding diagrams and converted into molar (P_c), molal (P_b), mole fractional (P_x) and the corresponding ion-corrected partition coefficients P_i, according to the formulae given in the Appendix. Apparent association constants K_a and Hill coefficients h of cooperative Op binding to negatively charged membranes were calculated from Hill plots.

* Dimension of lipid-standardized partition coefficients: M^{-1}, the other partition coefficients are dimensionless.

† Association constant (M^{-1}).

‡ Hill coefficient, assuming that maximum drug binding number to one lipid is $n = 1$.

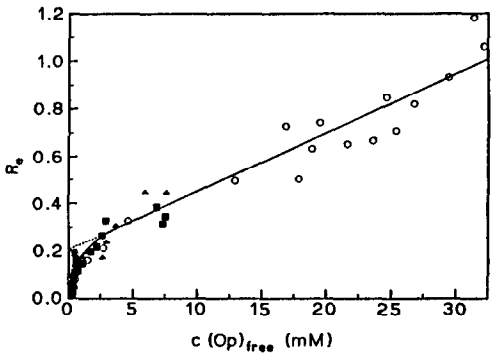

Fig. 2. Binding plot of oxprenolol enantiomers and racemic mixture to unilamellar vesicles of BPS. $c(Op)_{free}$, Molar concentration of oxprenolol in the buffer phase; R_e, molar ratio of oxprenolol concentration $c(Op)_{bound}$ in the membrane and the lipid concentration; ○, racemic drug mixture; △, *S*-enantiomer; □, *R*-enantiomer.

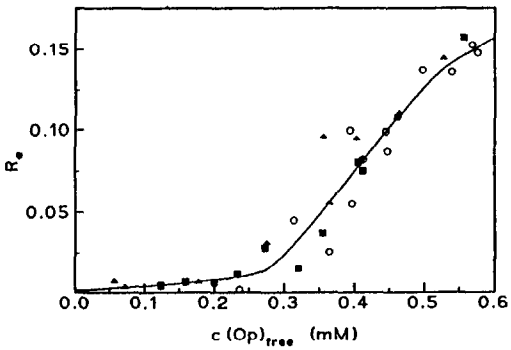

Fig. 3. Enlarged version of Fig. 2 at low oxprenolol concentrations.

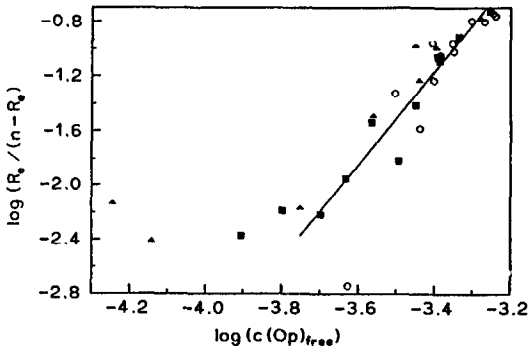

Fig. 4. Hill plot of oxprenolol binding to BPS membranes at low drug concentration. log ($c(Op)_{free}$), Logarithm of molar concentration of oxprenolol in the buffer phase; R_e, molar ratio of oxprenolol concentration $c(Op)_{bound}$ in the membrane and the lipid concentration; n, maximum binding of drug per lipid molecule (in this case, $n = 1$); ○, racemic drug mixture; △, *S*-enantiomer; □, *R*-enantiomer. Data were estimated using the Hill equation: $\log (R_e/(n - R_e)) = -\log K_d + h \cdot \log (c(Op)_{free})$. From the value of 10.6 of the interception with the *y*-axis, a dissociation constant of $2.5 \cdot 10^{-11}$ M was calculated. The slope of the linear part corresponds to a Hill coefficient $h = 3.4$.

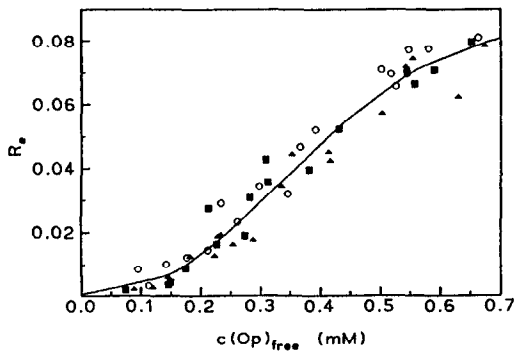

Fig. 5. Binding plot of oxprenolol enantiomers and racemic mixture at low concentrations to unilamellar vesicles of EPC/HDP 8:2 (mol/mol). $c(Op)_{free}$, Molar concentration of oxprenolol in the buffer phase; R_e, molar ratio of oxprenolol concentration $c(Op)_{bound}$ in the membrane lipid concentration; ○, racemic drug mixture; △, *S*-enantiomer; □, *R*-enantiomer.

membranes. In Fig. 2, the binding curve to BPS-containing LUVs turns to a linear partition only above 5 mM Op, with an apparent coefficient P_1 of 25 M^{-1}. The initial non-linear binding curve below 5 mM is obviously due to the negative charges of the phosphatidylserine headgroups and suggests a saturable anionic lipid/cationic drug interaction at the membrane surface. The enlarged version of the binding curve of Op up to 0.6 mM (Fig. 3) shows cooperative membrane binding (sigmoid curve) at a very low drug concentration range.

Cooperativity is also reflected by the Hill plot (Fig. 4). Data were calculated assuming that the maximum number of Op per lipid, bound by charge interaction, is $n = 1$. Above free Op concentrations of 0.2 mM [i.e. $\log (c(Op)_{free} = -3.7)$] up to 0.6 mM, the plot is essentially linear.

From the interception with the *y*-axis ($-\log K_d = 10.6$; dissociation constant $K_d = 2.5 \cdot 10^{-11}$ M), an apparent mean association constant K_a of $4.0 \cdot 10^{10}\ M^{-1}$ may be calculated. The slope of the linear part corresponds to a Hill coefficient $h = 3.4$.

In spite of additional charge interactions between both chiral drug and membrane lipid, enantioselective differences in binding are not detectable. This is also true for EPC membranes containing 20 M% HPD, which has a pronounced chirality centre in its headgroup. Binding characteristics of Op to HPD membranes are comparable to those shown for BPS membranes in Figs 2–4. At Op concentrations up to 0.7 mM, drug binding is cooperative as found by a sigmoid binding curve (Fig. 5). The Hill plot (not shown) is essentially linear between free Op concentrations of 0.08 mM and 0.7 mM, with an

apparent association constant $K_a = 4.8 \cdot 10^4\ M^{-1}$ and a Hill coefficient of 1.8, when also assuming a maximum binding of one Op molecule per lipid. Surface binding is saturated approximately at an effective ratio R_e of 0.2 bound Op per lipid. Upon a further increase of concentration, drug molecules show a nearly linear lipid standardized partition coefficient of $P_1 = 42\ M^{-1}$ up to 20 mM Op (see Fig. 5). Above this concentration, a second binding compartment, probably the hydrophobic interior of the membrane also seems to be saturated.

DISCUSSION

The major results of our study of oxprenolol/liposome interactions are that adsorption of the drug by negatively-charged membranes differs dramatically from that of isoelectric membranes, and that stereoselective drug/lipid interactions are in no case significant.

At a physiologic pH value of 7.4, the number of uncharged Op molecules being negligible (pK_a = 9.5), we therefore quantified the adsorption of the drug cation to the membrane. Binding to lipid membranes may indeed change the pK_a values of drugs. However, in contrast to oil/water systems, where the partition coefficient of a drug correlates with the ratio of the number of uncharged to charged molecules, in membrane/water systems the charged drug portion is also able to bind to the membrane due to interactions at the membrane–water interface. This has been shown for membrane adsorption of anaesthetics and other amphiphiles. For example, Boulanger *et al.* [14] investigated the position of both uncharged and charged tetracaine in the lecithin bilayer in a detailed NMR study. Owing to less pronounced hydrophilic interactions, the deprotonated base inserts with its hydrophobic domain deeply into the membrane, while the cationic form inserts in a less pronounced but similar way. This is in line with our finding of a constant lecithin membrane/buffer partition coefficient of Op over a wide concentration range. It may be concluded that predominant hydrophobic interactions, similar to those of an uncharged drug with organic solvent molecules in an oily phase, result in uniform partition behaviour.

Many different partition coefficients have been used in the literature, including the ratios of the mole fractions [15] or molar [16] and molal concentrations [17] of the drug in the organic and aqueous phases. In addition, a lipid-standardized distribution coefficient has been introduced [18] for membrane/buffer systems to standardize the amount of drug in the membranous compartment by taking the lipid concentration into consideration. Detailed instructions for converting these partition coefficients are given in the Appendix, making an improvement over the procedure of Kajama *et al.* [19].

From each of these estimated apparent partition coefficients, an ion-corrected value may be calculated from the pK_a and the actual pH [6], as shown in Table 1. These true partition coefficients are useful in oil/water systems (e.g. in standard use of octanol/buffer), because they reflect a situation in which all drug molecules are uncharged. In membrane systems, where charged molecules are also adsorbed, a "true" partition coefficient clearly overestimates the drug portion in the membrane and is therefore not useful for a quantitative calculation of structure–activity relationship. This was demonstrated by Betageri and Rogers [6], who found that the apparent rather than the ion-corrected partition coefficients of β-blockers in membrane/buffer systems correlated with the biological effects of the drug. The apparent molar or molal partition coefficients (P_c or P_b) of 33 to 60 for different membranes used in this study (see Table 1) compare to those of around 30 for membranes in the fluid crystalline phase used by these authors, whereas they markedly differ from a P_b value of 5.2 for octanol/buffer pH 7.4 [6].

When membranes with negative charges through phosphatidylserine or a hydroxyproline derivative (with a pronounced chiral headgroup) are used, strong charge interactions necessarily lead to Op/lipid contact on the membrane surface. This kind of interaction was found to deviate from concentration-independent partition and showed a sigmoid binding curve. Linearity in the Hill plot points to identical binding sites on the surface in a pharmacologically relevant Op concentration range below 0.6 mM. Hill coefficients (h) of 3.4 for pure phosphatidyserine membranes and of 1.8 for a mixture of 20% of a negatively charged hydroxyproline derivative in egg lecithin show that cooperativity of drug binding correlates with the density of negative surface charges on the membrane surface. The cooperative effect may partially be explained by a perturbation of the membrane structural order upon binding of a low drug amount, which makes additional membrane charges more accessible. In the case of cooperative ligand binding to polymers such as proteins, the Hill coefficient h can adopt only values of $h < n$ (n = maximum binding sites of the ligand on the polymer). In our case of drug binding to negatively charged membrane surfaces, a maximum amount n of one drug molecule can be bound to one membrane lipid by charge interactions. However, we found that $h > n$, when using phosphatidyserine or HPD-containing vesicles. The reason for the deviation from the theoretically expected values may be an additional interaction of the drug binding molecules (i.e. the membrane lipids).

At approximately 2 mM drug concentration, i.e. at a ratio $R_e = 0.2$ of bound Op per lipid in BPS membranes (or $R_e = 0.15$ in HPD-containing membranes), surface binding is saturated. An increasing amount of evenly charged drugs may then be forced to insert deeper into the membrane, resulting in a constant partition coefficient similar to that in isoelectric membranes.

Enantioselective interaction between the oxprenolol isomers and the chiral membrane lipids was not found. When pure egg lecithin or lecithin–cholesterol membranes are used, this finding is explained by a deep drug insertion, which results in reduced interaction between the chiral hydrophilic groups of drug and lipid molecules. A lack of enantioselective interaction is also true for the binding of Op enantiomers to L-DMPC vesicles at 10° below the phase transition (unpublished data) and is therefore

in principle independent of membrane fluidity. Using lipids with negative charges additional to the chiral molecular parts, however, one could expect a different binding strength of drug stereo-isomers. According to Dalgliesh [20], at least three fixing points are necessary for stereoselective recognition. This prerequisite is fulfilled by the cooperative binding of Op molecules to charged lipids and an additional orientation of the hydrophobic part of the drug into the membrane interior. The lack of chiral discrimination of the membranes may be due to the high rotational motility of the membrane lipids, which results in a reduced fixing of the drug molecule.

The biological consequences of our study may be summarized as follows. Stereoselective interactions of cationic drugs such as β-blockers with the lipid matrix of biological membranes, resulting in pharmacologic side effects of racemic drug mixtures, are negligible. However, even the lipid matrices of biological membranes without any receptor proteins are in fact able to bind considerable amounts of such drugs. Owing to lipid asymmetry in the plasma membrane, in which the negatively charged lipids are orientated exclusively towards the cytosolic side, cationic drugs are adsorbed mainly by this membrane surface; they also partially insert into the hydrophobic membrane interior. Through insertion of even low amounts of drug molecules, the fluidity of the membrane is perhaps altered, which in turn can influence the conformation of membrane proteins and their activity or binding strength.

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APPENDIX

Definitions for calculating partition coefficients P *of a drug d*

X_l, X_w:	molar fractions of a drug d in lipophilic and aqueous phases; $X_l = n_{dl}/(n_{dl} + n_l)$; $X_w = n_{dw}/(n_{dw} + n_w)$,
n_{dl}, n_{dw}	molar quantity (mol) of d in lipophilic and aqueous phase;
n_d	total quantity of d; $n_d = n_{dl} + n_{dw}$
n_l, n_w	quantity (mol) of lipid, water
m_{dl}, m_{dw}	mass (g) of drug d in lipophilic, aqueous phase
m_d	total mass (g) of d; $m_d = m_{dl} + m_{dw}$
m_l, m_w	mass (g) of lipid, water
M_l	mean molar mass (g/mol) of membrane lipids
M_w	molar mass of water; $M_w = 18{,}015$ g/mol
V_l, V_w	volume (L) of lipophilic, aqueous phase
V_t	total volume (L); $V_t = V_l + V_w$
V_l°, V_w°	partial molar volume (mL/mol) of lipid, water
ρ_l, ρ_w	volumic mass (g/mL) of lipidic, aqueous phase
c_{dl}	molar concentration of d (mol/L phase volume) in the lipophilic phase; $c_{dl} = n_{dl}/V_l$
c_{dw}	molar concentration of d (mol/L phase volume) in the aqueous phase; $c_{dw} = n_{dw}/V_w$
c_b	molar concentration (mol/L total volume) of the lipid-bound portion of d; $c_b = n_{dl}/V_t$
c_f	molar concentration (mol/L total volume) of the free (aqueous) portion of d; $c_f = n_{dw}/V_t$
c_l	molar concentration of lipophilic solvent in the lipophilic phase; $c_l = n_l/V_l$
c_w	molar concentration of water in the aqueous phase; $c_w = n_w/V_w$
cL	molar concentration of lipid in the liposome suspension; $cL = n_l/V_t$
cW	molar concentration of water in the liposome suspension; $cW = n_w/V_t$

Definitions of various partition coefficients at low drug concentrations (activity coefficients are equal to 1)

Partition coefficient of molar fractions:

$$P_x = X_l/X_w = \frac{n_{dl}}{n_l + n_{dl}} \Big/ \frac{n_{dw}}{n_w + n_{dw}}. \quad \text{(A1)}$$

For $n_{dl} \ll n_l$ and $n_{dw} \ll n_w$, P_x can be approximated as

$$P_x = \frac{n_{dl} \cdot n_w}{n_l \cdot n_{dw}}. \quad \text{(A2)}$$

Partition coefficient of molal concentrations:

$$P_b = \frac{m_{dl}}{m_l} \Big/ \frac{m_{dl}}{m_w} = \frac{n_{dl}}{n_{dw}} \cdot \frac{n_w}{n_l} \cdot \frac{M_w}{M_l}. \quad \text{(A3)}$$

Partition coefficient of molar concentrations:

$$P_c = c_{dl}/c_{dw} = \frac{n_{dl}}{V_l} \Big/ \frac{n_{dw}}{V_w}. \quad \text{(A4)}$$

Lipid-standardized molar partition coefficient (for liposomes used as membrane models):

$$P_l = \frac{c_b}{c_f \cdot cL}\,(M^{-1}) = \frac{n_{dl}/V_t}{(n_{dw}/V_t) \cdot (n_l/V_t)} = \frac{n_{dl}}{n_{dw}} \cdot \frac{V_t}{n_l}. \quad \text{(A5)}$$

All "apparent" partition coefficients shown above can be converted to "true", i.e. ion-corrected partition coefficients P_i:

(a) for monobasic acidic drugs:

$$P_{ia} = P \cdot (1 + 10^{pH - pKa}) \quad \text{(A6)}$$

(b) for monoacidic basic drugs:

$$P_{ib} = P \cdot (1 + 10^{pKa - pH}) \quad \text{(A7)}$$

(e.g. oxprenolol; $pK_a = 9.5$)

Conversions of various partition coefficients, using the approximation for P_x

Dividing Eqn (A3) by Eqn (A4), P_c and P_b can be converted by

$$\frac{P_b}{P_c} = \frac{M_w \cdot V \cdot n_w}{M_l \cdot V_w \cdot n_l} = \frac{M_w}{M_l} \cdot \frac{\bar{V}_l^o}{\bar{V}_w^o} = \frac{\rho_w}{\rho_l}. \quad \text{(A8)}$$

The volumic mass of lipid membranes depends on lipid composition and temperature, but in most cases is close to the volumic mass of water. Therefore, P_b/P_c is approximately 1.

Using Eqns (A2) and (A3) P_x and P_b can easily be converted by

$$\frac{P_x}{P_b} = \frac{M_l}{M_w}. \quad \text{(A9)}$$

With mol. wts of EPC or BPS: 760; cholesterol: 387; HPD: 369; the ratios P_x/P_b can be calculated for EPC or BPS as 42.2; for EPC/Cho (7:3 mol/mol) as 36.8 and for EPC/HPD (8:2 mol/mol) as 38.8.

In Eqn (A10), derived from Eqns (A1) and (A3),

$$\frac{P_x}{P_c} = \frac{n_w \cdot V_l}{n_{dl} \cdot V_w} = \frac{V_l/n_l}{V_w/n_w} = \frac{\bar{V}_l^o}{\bar{V}_w^o}. \quad \text{(A10)}$$

the partial molar volume of lipid has to be estimated for the various membranes. Using an approximated value of $P_b/P_c = 1$ [see Eqn (A8)], the values for P_x/P_b can be used for the ratio P_x/P_c.

Dividing Eqn (A2) by Eqn (A5) results in

$$\frac{P_x}{P_l} = \frac{n_w}{V_t} = cW\,(\text{mol/L}) \quad \text{(A11)}$$

which is approx. 55.6 for diluted liposomal suspensions.

The ratio P_b/P_l can be calculated by using Eqns (A3) and (A5):

$$\frac{P_b}{P_l} = \frac{n_w}{V_t} \cdot \frac{M_w}{M_l} = cW \cdot \frac{M_w}{M_l}\,(\text{mol/L}) \quad \text{(A12)}$$

which is equal to the ratio of Eqn (A11) to Eqn (A9). According to the assumptions made for Eqn (A9), P_b can be calculated from P_l using the factors of 1.32 for EPC or BPS, 1.51 for EPC/Cho (7:3 mol/mol) or 1.43 for EPC/HPD (8:2 mol/mol).

Eqn (A13), derived from Eqns (A4) and (A5),

$$\frac{P_c}{P_l} = \frac{V_w \cdot n_l}{V_l \cdot V_t} = cL \cdot \frac{V_w}{V_l} = cW \cdot \frac{V_w^o}{V_l^o}\,(\text{mol/L}) \quad \text{(A13)}$$

is equal to Eqn (A11)divided by Eqn (A10). The P_c values can then be calculated from P_l by approximately the same factors to be used for the calculation of P_b.