Biochimica et Biophysica Acta, 945 (1988) 361–366
Elsevier

BBA 70582

BBA Report

Interaction of electrically charged drug molecules with phospholipid membranes

Dieter Barthel, Olaf Zschoernig, Kay Lange, Royald Lenk and Klaus Arnold

Institute for Biophysics, School of Medicine, Karl-Marx-University Leipzig, Leipzig (G.D.R.)

(Received 26 July 1988)

Key words: Phospholipid membrane; Surface potential; Amphiphilic drug; Partition coefficient; Drug–membrane interaction

Model membranes (egg-yolk PC liposomes) were exposed to the cationic form of amphiphilic drugs. Microelectrophoresis was used to measure the change of the electrokinetic potential as a function of the drug concentration. By use of the Gouy-Chapman theory the surface potential and surface charge density were calculated. A theoretical model postulating a simple partition equilibrium of the charged drug molecules between the membrane and the aqueous phase in the vicinity of the membrane failed to describe the experimental results. Modification of the partition law by introducing a mechanism of saturation at high drug concentrations, however, resulted in concordance of model and experiment. Some parameters of the model can be used as a means of evaluating the efficiency of neuroactive drugs.

A number of drugs influencing the excitability of nerve cell membranes are of amphiphilic character. The molecules of such drugs coexist in a cationic and an electrically neutral form. The percentage of molecules of the cationic form depends on the pH value of the tissue where the drug is applied. For pH chose sufficiently low ($pH \ll pK_a$) most of the molecules carry one positive electrical charge.

The adsorption of the charged form of local anesthetics onto lipid membranes was to some extent studied by Ohki [1], who investigated the influence of charged local anesthetics on the electrophoretic mobility, electrokinetic potential, surface potential, and surface charge of multilamellar liposomes. In general, Ohki found that increasing the bulk concentration of the drug in the buffer solution provoked a shift towards positive values in all parameters mentioned above. In particular in uncharged membranes (egg-yolk PC) he found the ratio of the concentrations of drug molecules adsorbed onto the membrane and in the aqueous phase in the vicinity of the membrane to be rather constant, at least at bulk concentrations not too large. Furthermore, this ratio correlated with the ability of the drugs to inhibit the excitation of nerve membranes in the normal physiological environment. Similar observations were published by Westman et al. [2]. So this ratio turned out to be of pharmacological relevance. Consequently, it should be useful (1) to find out if similar results could be obtained from other amphiphilic drugs; (2) to develop a theoretical model based on the assumption of a constant ratio of drug concentrations on and before the membrane in order to extract a maximum amount of information from the measurements.

Multilamellar egg-yolk PC liposomes were prepared following the method of Bangham et al. [3]. In order to do so egg-yolk PC, purified according to Singleton et al. [4] was dried in a rotary

Correspondence: D. Barthel, Institute for Biophysics, School of Medicine, Karl-Marx-University Leipzig, Liebigstrasse 27, Leipzig 7010, G.D.R.

evaporator. Then the buffer solution was added and the suspension was shaken vigorously. The size of the liposomes produced in this way was widely distributed.

The following buffers were used:

Buffer I: 6.8 mM Na_2HPO_4/KH_2PO_4, 10.0 mM NaCl, 262.0 mM saccharose (pH 6.8);

Buffer II: 6.8 mM Na_2HPO_4/KH_2PO_4, 140.0 mM NaCl, 20.0 mM saccharose (pH 6.8).

The drugs investigated were amphiphilic psychotropic agents, β-blockers and local anesthetics. Because of the pH chosen the drug molecules were expected to exist predominantly in the cationic form. The electrophoretic mobilities were measured at a temperature of 25°C by means of a Parmoquant-2 cell electropherometer (VEB Carl Zeiss, Jena, G.D.R.), the mean value of 100 individual measurements being calculated. The viscosities were measured by use of a Hoeppler-type viscosimeter.

As primary results for each drug we obtained the dependence of the electrophoretic mobility, μ, on the bulk concentration of the drug, c. Beginning with a small negative value ($|\mu| < 0.1 \cdot 10^{-8}$ $m^2 \cdot V^{-1} \cdot s^{-1}$) for zero concentration the mobility shifted monotonically to positive values. The extent of the shift depended on the drug under investigation.

Using Smoluchovsky's formula

$$\zeta = \frac{\mu \cdot \eta}{\varepsilon \cdot \varepsilon_0} \tag{1}$$

(η, viscosity; $\varepsilon \cdot \varepsilon_0$, permittivity of the suspension medium) and assuming $\varepsilon = 81$ we calculated the electrokinetic potential ζ, which is identical with the electrostatic potential $\psi(X_S)$ in the hydrodynamic shear plane and shows the same tendency as the electrophoretic mobility. A representative example is given in Fig. 1. (chlorpromazine, buffer I).

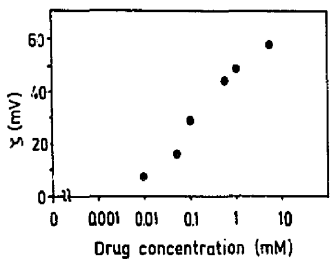

Fig. 1. Electrokinetic potential, ζ, of phosphatidylcholine multilamellar vesicles in 10 mM NaCl suspension solution (pH 6.8) as a function of the concentration of chlorpromazine.

Assuming the distance between the membrane and the shear plane to be $X_S = 0.2$ nm (Eisenberg et al. [5]), and applying the Gouy-Chapman theory [1,5,6–8] in its special form valid for 1–1 electrolytes we calculated the following parameters as a function of the bulk concentration of the drug, c.

(1) Surface potential, ψ_0, according to

$$\psi_0 = \frac{4RT}{F} \cdot \operatorname{artanh}\left[\tanh\left(\frac{F \cdot \psi(X_S)}{4RT}\right) \cdot \exp(\kappa X_S)\right] \tag{2}$$

(see Fig. 2.)

(2) Surface charge density, σ, by use of the Gouy-Chapman equation

$$\sigma = \kappa \varepsilon \varepsilon_0 \cdot \frac{2RT}{F} \cdot \sinh\left(\frac{F\psi_0}{2RT}\right) \tag{3}$$

see (Fig. 3.)

(3) Surface concentration of the drug, c_S (amount of drug adsorbed per area of membrane):

$$c_S = \frac{\sigma}{F} \quad (\text{mol/m}^2) \tag{4}$$

(4) Volume concentration of the drug in the immediate vicinity of the membrane, $c(0)$ (amount of drug per volume of suspension), according to Boltzmann's law:

$$c(0) = c \cdot \exp\left(-\frac{F\psi_0}{RT}\right) \quad (\text{mol/m}^3) \tag{5}$$

(5) Surface partition coefficient, K, defined by

$$K = \frac{c_S}{c(0)} \quad (\text{m}) \tag{6}$$

(R, universal gas constant; F, Faraday's constant; T, temperature; κ, Debye Huckel parameter).

K is a characteristic length. It should be noted that Ohki [1] preferred to define a surface partition coefficient by

$$K_{\text{Ohki}} = \frac{\sigma}{e_0 \cdot c(0)} = N \cdot K \quad (\text{m/mol}) \tag{7}$$

where N is Avogadro's constant.

We found, as did Ohki, K to be relatively independent of the bulk concentration, c, at least at low concentrations. So, in accordance with the approach of Westman et al. [2] we postulated a simple partition equilibrium of the cationic form of the drug, strictly speaking, a Henry-type relation between c_S and $c(0)$:

$$\frac{c_S}{c(0)} = K = \text{constant} \tag{8}$$

Then in addition to the Gouy-Chapman Eqn. 3 σ and ψ_0 are interconnected by

$$\sigma = KFc \cdot \exp\left(-\frac{F\psi_0}{RT}\right) \tag{9}$$

There exists a unique solution $[\psi_0, \sigma]$ of Eqns. 3 and 9, which can be written in terms of elementary transcendental functions. So, if K is known and c is given, ψ_0 and σ can be calculated by use of a set of formulas.

Unfortunately this transparent model proved to be unable to describe the relation between surface potential and drug concentration over the whole range of measurements, whatever drug was considered. The best thing one could do was to fit the parameter K to the first three or four points of a set of measurements. For higher concentrations the values of the surface potential predicted were systematically greater than those measured. Fig. 2 shows this deviation for chlorpromazine (broken line). So we had to conclude that at higher concentrations a mechanism of saturation had to be taken into account. Such a mechanism is provided by a Langmuir adsorption isotherm, as was shown by Lee [6], Rooney et al. [8], Seelig et al. [9] and other authors. Provided that n distinct binding sites (lipid molecules) bind one drug molecule the Langmuir adsorption isotherm connects c_s and $c(0)$ by

$$\frac{c_S}{(c_L - n \cdot c_S) \cdot c(0)} = K_L = \text{constant} \quad (\text{m}^3/\text{mol}) \tag{10}$$

(c_L, amount of lipid per area of membrane; K_L, binding constant).
The limited availability of binding sites restricts c_S to $c_S \leqslant 1/(n \cdot N \cdot A_L)$, where A_L is the area in the membrane occupied by one lipid molecule.

One can doubt whether distinct binding sites for drug molecules really exist. Seelig et al. [9] preferred the idea of drug molecules being intercalated between the lipid molecules and introduced a law of partition equilibrium which differs from that of Eqn. 8:

$$\frac{X_b}{c(0)} = K_P = \text{constant} \quad (\text{m}^3/\text{mol}) \tag{11}$$

(K_P, Seelig's partition coefficient).
X_b, the degree of binding (n_D drug molecules per n_L lipid molecules), is equal to the ratio $X_b = c_S/c_L$. The more drug molecules are intercalated the more the membrane expands. c_S depends on the actual area of the membrane, while X_b does not. Consequently, c_S and X_b are related, not by simple proportionality, but by

$$c_S = \frac{X_b}{N} \cdot \frac{1}{A_L + X_b \cdot A_D} \tag{12}$$

(A_D: area in the membrane occupied by one drug molecule).
So this type of partition equilibrium allows an unlimited growth of the degree of binding, X_b, but limits the surface concentration of the drug to $c_s \leqslant 1/(N \cdot A_D)$.

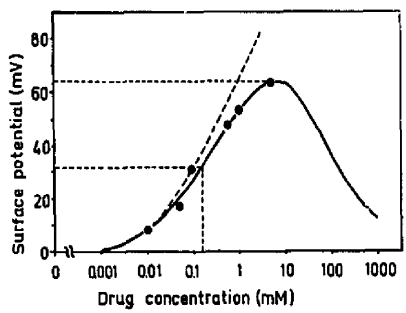

Fig. 2. Surface potential, ψ_0, as a function of the concentration of chlorpromazine. ●, calculated from Fig. 1; broken line: model based on simple partition equilibrium; solid line: model including saturation. $K = 2666$ nm, $a = 5.4$ nm^2. Maximum surface potential 64 mV. $c_\psi = 0.15$ mM corresponds to 50% of maximum surface potential.

The Langmuir adsorption isotherm as well as Seelig's law can be transformed to

$$\frac{c_S}{c(0)} = \frac{K}{1 + N \cdot a \cdot K \cdot c(0)} \quad K: (\mathrm{m}) \quad a: (\mathrm{m}^2) \tag{13}$$

with

$$K = \frac{K_L}{N \cdot A_L}, \quad a = n \cdot A_L \quad \text{Langmuir}$$
$$K = \frac{K_P}{N \cdot A_L}, \quad a = A_D \quad \text{Seelig} \tag{14}$$

The generalized surface partition coefficient K is a characteristic length. The saturation parameter a is a characteristic area, restricting c_S to $c_S \leqslant 1/(N \cdot a)$. For low concentrations the original Ohki postulate holds.

As shown above two incompatible concepts resulted in formally identical relations. Possibly there exist further mechanisms of saturation yielding a similar law. A decision in favour of one of several alternatives cannot be founded only on electrophoretic measurements. Therefore we used Eqn. 13 as a model with two parameters, K and a, but avoided to opt for one specific interpretation.

A combination of Eqns. 13, 5, and 4 gives a relation between σ and ψ_0 which replaces Eqn. 9 and is more complicated than this. Consequently, if K and a are known and c is given, ψ_0 and σ must be calculated by an iterative procedure.

It turned out that in every case by use of a least squares fitting procedure the parameters K and a of the modified model could be chosen so that the values of the surface potential as well as those of the surface charge density were predicted correctly over the whole range of concentrations investigated so far (Figs. 2. (solid line) and 3).

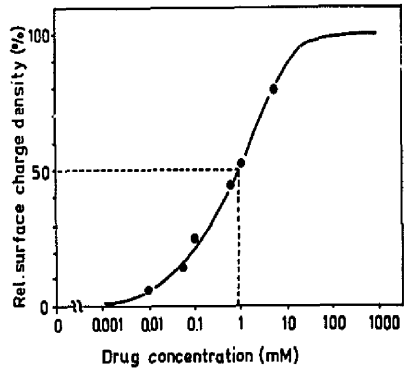

Fig. 3. Relative surface charge density, σ/σ_{max}, as a function of the concentration of chlorpromazine. ●, calculated from Fig. 1; solid line: model including saturation. $K = 2666$ nm, $a = 5.4$ nm^2. Maximum surface charge density: 0.19 $e \cdot$nm^{-2} ≙ 100%. $c_\sigma = 0.97$ mM corresponds to 50% of maximum surface charge density.

Schlieper et al. [10] interpreted measurements of the electrokinetic potential dependent on the drug concentration in terms of a dose-effect relation described by a logit function with two parameters. One of the parameters represented the mean effective dose (ED_{50}) of the drug. Following this reasoning one can calculate that concentration c_σ which leads to 50% of the maximum charge density σ_{max}, or that concentration c_ψ which leads to 50% of the maximum surface potential ψ_{max}. The procedure is indicated in Figs. 2 and 3. c_σ and c_ψ

TABLE I

CALCULATED PARAMETERS OF INVESTIGATED DRUGS

Buffer I (10 mM NaCl).

Drug	K (nm)	a (nm^2)	σ_{max} ($e \cdot$nm^{-2})	c_σ (mM)	ψ_{max} (mV)	c_ψ (mM)
Bunitrolol	37	1.9	0.53	149.35	44	4.45
Tetracaine	169	2.7	0.37	37.06	54	1.41
Alprenolol	293	5.5	0.19	6.19	48	0.67
L-Propranolol	689	6.2	0.16	2.44	52	0.34
DL-Propranolol	694	6.1	0.16	2.46	52	0.35
D-Propranolol	785	5.4	0.19	3.17	56	0.35
Chlorpromazine	2666	5.4	0.19	0.97	64	0.15

TABLE II

CALCULATED PARAMETERS OF INVESTIGATED DRUGS

Buffer II (140 mM NaCl).

Drug	K (nm)	a (nm^2)	σ_{max} ($e \cdot nm^{-2}$)	c_σ (mM)	ψ_{max} (mV)	c_ψ (mM)
Tetracaine	327	3.3	0.30	5.56	38	1.76
L-Propranolol	1192	2.4	0.41	2.43	51	0.86
DL-Propranolol	1112	2.0	0.49	3.88	56	1.08
Chlorpromazine	5148	2.4	0.42	0.61	56	0.25

can be used as measures of the efficiency of a drug.

In Tables I–III the primary model parameters K and a and the derived parameters σ_{max}, c_σ, ψ_{max}, c_ψ are given. For convenience we used appropriate non-SI units. Tables I and II represent series of measurements using buffer solutions I (10 mM NaCl) and II (140 mM NaCl), respectively. Additionally we proceeded in the same way with data published by Ohki [1], which represent the dependence of the elektrokinetic potential of PC liposomes on the concentration of local anesthetics. The results are given in Table III. For comparison the surface partition coefficients published by Ohki himself, transformed to K according to Eqn. 7, are given in brackets. Obviously the two-parameter model produces slightly increased surface partition coefficients. In the case of dibucaine this shift does not affect the good agreement between Ohki's partition coefficient and that of Seelig [9] derived by UV and ^{2}H-NMR spectroscopy, for $K_p = 640\ M^{-1}$ (Ohki) and $K_p = 670\ M^{-1}$ (two-parameter model) are both compatible with Seelig's measurements ($A_L = 0.68\ nm^2$ [9]).

The values of the parameter a seem to be generally greater than the area occupied by one intercalated drug molecule (dibucaine: $A_D \approx 0.5\ nm^2$, [9]). This could indicate that because of electrostatic repulsive forces between the drug molecules the effective area per drug molecule is greater than expected geometrically. On the basis of the Langmuir isotherm one would conclude that several binding sites per drug molecule are involved.

Zachowski and Durand [11] discussed their experiments on the binding of chlorpromazine to various types of membranes in terms of the Langmuir isotherm. For a PC membrane at a temperature of 37°C in a buffer containing 130 mM NaCl with pH 7.4 they found two bindings, with the dissociation constants $K_{d,1} = 17.3\ \mu M$, $K_{d,2} = 158.3\ \mu M$, and the numbers of drug molecules per lipid molecule $N_1 = 0.108$, $N_2 = 0.378$. With $n_i = 1/N_i$, $K_{L,i} = 1/(n_i \cdot K_{d,i})$ and Eqn. (14) these data can be transformed to

$K_1 = 15300$ nm, $a_1 = 6.3\ nm^2$ for the first binding,

$K_2 = 5853$ nm, $a_2 = 1.8\ nm^2$ for the second binding.

The latter seems to correspond to our findings (Table II) $K = 5148$ nm, $a = 2.4\ nm^2$. It must be noted, however, that the authors did not apply the Gouy-Chapman theory. Consequently they ob-

TABLE III

CALCULATED PARAMETERS OF DRUGS INVESTIGATED BY Ohki [1]

100 mM NaCl, pH 6. K, Ohki's results in brackets.

Drug	K (nm)	a (nm^2)	σ_{max} ($e \cdot nm^{-2}$)	c_σ (mM)	ψ_{max} (mV)	c_ψ (mM)
Procaine	103 (74)	6.8	0.15	3.88	23	2.12
Tetracaine	580 (498)	1.7	0.57	15.07	61	1.91
Dibucaine	1638 (1549)	1.7	0.61	6.09	69	0.92

tained apparent dissociation constants, and the comparison is somewhat questionable.

The difficulty in generalizing from the adsorption of the cationic form to the adsorption of the drug in both the cationic and the neutral form cannot be removed by a method detecting changes in electrostatic properties like electrophoresis. On the other hand some of the features of the model seem to reflect the relations in the efficiency of the drugs quite well. In particular the surface distribution coefficient and the concentrations corresponding to 50% of the maximum surface charge or surface potential bring the drugs into the same relative order. So perhaps these parameters could be used in a routine test procedure to evaluate the efficiency of a neuroactive drug.

We would like to thank Professor Dr. M. Mueller, Institute for Pharmacology, Karl-Marx-University Leipzig for placing the drugs investigated at our disposal.

References

1 Ohki, S. (1984) Biochim. Biophys. Acta 777, 56–66.
2 Westman, J., Eriksson, L.E.G. and Ehrenberg, A. (1984) Biophys. Chem. 19, 57–68.
3 Bangham, A.D., Hill, M.W. and Miller, N.G.A. (1974) Meth. Membr. Biol. 1, 1–68.
4 Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53–56.
5 Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5233.
6 Lee, A.G. (1978) Biochim. Biophys. Acta 514, 95–104.
7 Bentz, J. (1982) J. Coll. Interface Sci. 90, 164–182.
8 Rooney, E.K., East, J.M., Jones, O.T., McWhirter, J., Simmonds, A.C. and Lee, A.G. (1983) Biochim. Biophys. Acta 728, 159–170.
9 Seelig, A., Allegrini, P.R. and Seelig, J. (1988) Biochim. Biophys. Acta 939, 267–276.
10 Schlieper, P., Medda, P.K. and Kaufmann, R. (1981) Biochim. Biophys. Acta 644, 273–283.
11 Zachowski, A. and Durand, P. (1988) Biochim. Biophys. Acta 937, 411–416.