Biochimica et Biophysica Acta, 506 (1978) 265—280

BBA 77899

INFLUENCE OF STEROLS ON ION TRANSPORT THROUGH LIPID BILAYER MEMBRANES

ROLAND BENZ and DANIELE CROS *

Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz (G.F.R.)

(Received May 2nd, 1977)

Summary

Charge-pulse relaxation experiments with the negatively charged lipophilic ions, dipicrylamine and tetraphenylborate, (as well as with the positively charged carrier system Rb^+-valinomycin) have been carried out in order to study the influence of sterols on the ion transport through the lipid bilayer membrane. The mol fraction of the sterols (cholesterol, epicholesterol, ergosterol, stigmasterol, dihydrocholsterol, epicoprostanol and cholesterololeate) as referred to total lipid was varied in a wide range (mol fractions 0—0.8).

The monoolein/sterol or dioleoylphosphatidylcholine/sterol mixtures were dissolved in *n*-hexadecane in order to minimize effects of the sterol on the membrane thickness.

Cholesterol had a strong influence on the transport of the lipophilic ions. Its incorporation into monoolein membranes increased the rate constant k_i of translocation up to 8-fold, but incorporation into phosphatidylcholine membranes had virtually no influence on k_i. The other sterols with one hydroxy group and cholesterololeate had no influence on the rate constant or the partition coefficient β. The results are discussed on the basis of a possible change of dipole potential of the membrane caused by cholesterol and its derivatives.

In the case of valinomycin-mediated Rb^+ transport only cholesterol had a strong influence on transport properties. The rate constants of association (k_R) as well as the rate constants of translocation of the complex (k_{MS}) and of the free carrier (k_S) were reduced by incorporation of cholesterol up to eightfold. The decrease of k_S and k_{MS} are possibly caused by a decrease of membrane fluidity, whereas the decrease of k_R may be due to an increase of surface potential. The different action of cholesterol on the two transport systems is discussed under the assumption that the adsorption plane of the lipophilic ion is located more towards the aqueous side and that of the ion-carrier complexes more towards the hydrocarbon side of the dipole layer.

* Permanent address: Laboratoire de Chimie Physique, Université des Sciences et Techniques du Languedoc, Place E. Bataillon, 34060 Montpellier, France.

Introduction

Sterols are common constituents of biological membranes. Some mammalian membranes such as erythrocyte or myelin membranes contain high amounts of cholesterol (the major mammalian sterol), up to mol fractions between 0.5 and 0.67 (referred to total lipid) [1]. Besides this major sterol component small amounts of other sterols like 7-dehydrocholesterol, cholestanol or desmosterol are present in mammalian membranes [2—4]. Ergosterol and stigmasterol have been found in plants [5].

Sterols were found to have a large influence on the structure of monolayers and model membranes [6,37]. It has been shown that the addition of cholesterol to monolayers of phosphatidylcholine with unsaturated fatty acid residues leads to a condensation of the monolayer [7]. A similar condensation effect has been observed with lipid vesicles. In the presence of cholesterol the osmotically induced swelling rate of liposomes from phospholipids in the liquid crystalline state decreased considerably [8], indicating a decreased water permeability. A similar influence on the permeability of other uncharged molecules has been found. In contrast to these findings the permeability of liposomes from lecithin in the crystalline state increases, if cholesterol is present [8,9].

Calorimetric measurements showed that the phase transition of phospholipids from the crystalline to the liquid crystalline state gradually disappears with increasing cholesterol content [10]. Nuclear magnetic resonance studies with mixed phospholipid/cholsterol systems in water indicated a strong interaction between the fatty acid chains of the phospholipids and cholesterol [11]. The mobility of the chains has been found to decrease above the phase transition temperature and to increase below the phase transition point.

Experiments with different sterols, in particular with the 3 α-hydroxy isomers like epicholesterol showed that for the interaction of sterols with the membrane phospholipids certain structural properties are necessary. Besides a planar configuration of the sterol molecule, the presence of the 3-β-hydroxy-group and of the intact side chain is critical. Epicholesterol, for instance, does not influence the structure of model membranes to an appreciable extent [5].

The action of cholesterol on planar lipid bilayer membranes has also been studied in the past. It has been found that the permeability of membranes is altered in the presence of cholesterol [12—17]. Whereas for positively charged molecules a decrease of conductivity has been observed [12,15,16]; the opposite effect has been found for negatively charged "probes" [14,15,17]. From these results it has been concluded that not so much the above-mentioned condensation effect is responsible for the permeability changes but rather the high dipole moment of the cholesterol molecule (it should be noted that under the conditions of these experiments the lipid bilayer membranes were always in the liquid bilayer membranes were always in the liquid crystalline state). Cholesterol is assumed to change the dipole potential in the surface of the membrane, thus altering the transport kinetics of the charged probes [13—15,18]. In addition, the concentration of the charged molecules adsorbed to the membrane-water interface may be influenced by the change of the dipolar potential.

Most of these investigations have been performed on a more qualitative basis, i.e. the change of membrane conductivity has been measured, which does not

allow the separation of the above-mentioned effects [12—14]. From relaxation studies it is possible to separate the influence of cholesterol on the translocation rate constant as well as on the partition coefficient. Such studies have been described in three recent publications [15—17]. In addition, it has been found that increasing the content of cholesterol in membranes made from monoolein or dioleoyllecithin dissolved in *n*-decane leads to an increase in the specific capacity of the membranes [16,17,19]. Experiments with lipophilic ions and membranes made from one and the same lipid dissolved in different n-alkanes have shown that thinning of the membrane leads to a dramatic increase of the translocation rate constant of the lipophilic ion [17]. In this study we have attempted to separate the thickness effect from the other effects of cholesterol on the transport properties. Therefore the lipids were dissolved in *n*-hexadecane where no further thinning of the membranes in the presence of cholesterol was observed. In addition, the influence of other sterols on the transport of charged probes was investigated. We used the negatively charged lipophilic ions tetraphenylborate and dipicrylamine as well as the positively-charged carrier system valinomycin/Rb^+ as probes. These three different transport systems are well established and have been used as model systems in a number of investigations [14—17,20—25].

Kinetic studies with lipophilic ions and carriers have been performed in the past using the voltage-jump relaxation method [15,20—22,24] and recently also by the charge-pulse relaxation technique [16,17,23,25]. The latter technique was used throughout this study. Its main advantage, besides a minimal pertubation of the membrane ($V_m \leq 10$ mv), lies in the increased time resolution which is given by the measuring circuit and not limited by the membrane conductance.

Description of the transport models

The models for the transport of lipophilic ions as well as for carrier mediated ion transport have been presented in full detail in previous publications [20—27]. The application of the charge pulse method to both transport systems has also been described extensively [23,25]. Therefore we give here only the main equations which relate the experimental data to the kinetic parameters of the model.

The transport of lipophilic ions across lipid bilayer membranes occurs in different steps, namely the adsorption-desorption reaction between the aqueous phase and the membrane interface (rate constants k_{am} and k_{ma}), and the exchange of the lipophilic ion across the central potential barrier in the membrane (rate constant k_i) [20]. For charge-pulse experiments in the limit of small voltages ($V_m << 25$ mV) the decay of the voltage across the membrane is governed by two exponentials with $\tau_1 << \tau_2$ [23]:

$$V_m(t) = V_m^0[a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)] \quad (1)$$

with

$$a_1 + a_2 = 1 \quad (2)$$

$$\tau_1 = \frac{1}{2k_i(1 + bN_t)} \quad (3)$$

$$a_1 = \frac{bN_t}{1 + bN_t} \tag{4}$$

$$b = \frac{F^2}{4RTC_m} \tag{5}$$

N_t is the total concentration of the lipophilic ions per cm^2, C_m the specific capacity of the membrane, R the gas constant, F the Faraday constant and T is the absolute temperature. It has been shown that in the presence of lipophilic ions the slow process is governed by diffusion polarisation and that it is not possible to derive from the experimental data the values for the rate constants k_{ma} and k_{am} [23]. Only the ratio of both rate constants, the partition coefficient β may be obtained according to the following equation:

$$\beta = \frac{N_t}{2c} = \frac{k_{am}}{k_{ma}} \tag{6}$$

The model for carrier mediated ion transport is based on the assumption that the association between carrier S and ion M^+ (aqueous concentration C_M) takes place at the membrane-solution interface with the rate constants of association and dissociation of the complex being k_R and k_D, respectively. Complex MS^+ and free carrier S cross the membrane with rate constants k_{MS} and k_S [26]. For charge-pulse experiments in the limit of small voltages ($[V_m] << 35$ mV) the decay of the voltage across the membrane with time is given by [25]:

$$V_m(t) = V_m^0[a_1 \exp(-\lambda_1 t) + a_2 \exp(-\lambda_2 t) + a_3 \exp(-\lambda_3 t)] \tag{7}$$

with:

$$a_1 + a_2 + a_3 = 1 \tag{8}$$

The four rate constants and the total carrier concentration N_0 in the membrane may be calculated according to the following equations [25]:

$$k_{MS} = \frac{1}{2}\left(\frac{P_5}{P_4} - P_4\right) \tag{9}$$

$$k_D = \frac{1}{2k_{MS}}\left[\frac{P_1 P_5}{P_4} - P_2 + \frac{P_3}{P_4} - \left(\frac{P_5}{P_4}\right)^2\right] \tag{10}$$

$$k_S = \frac{1}{2k_D}\frac{P_3}{P_4} \tag{11}$$

$$k_R = \frac{1}{c_M}(P_1 - P_4 - 2k_S - 2k_{MS} - k_D) \tag{12}$$

$$N_0 = \frac{2RTC_m}{F^2}\frac{P_4}{k_{MS}}\left(1 + \frac{k_D}{k_R C_M}\right) \tag{13}$$

The quantities P_i may be calculated from the relaxation times $\tau_i = 1/\lambda_i$ and the relative relaxation amplitudes a_i in the following way:

$$P_1 = \lambda_1 + \lambda_2 + \lambda_3 \tag{14}$$

$$P_2 = \lambda_1\lambda_2 + \lambda_1\lambda_3 + \lambda_2\lambda_3 \tag{15}$$

$$P_3 = \lambda_1\lambda_2\lambda_3 \tag{16}$$

$$P_4 = a_1\lambda_1 + a_2\lambda_2 + a_3\lambda_3 \tag{17}$$

$$P_5 = a_1\lambda_1^2 + a_2\lambda_2^2 + a_3\lambda_3^2 \tag{18}$$

Materials and Methods

Lipid bilayer membranes were obtained in the usual way [28] from a 1—3% (w/v) lipid solution in *n*-hexadecane (Merck, Darmstadt, G.F.R., standard for gas chromatography). The cell used for bilayer formation was made from Teflon. The circular hole in the wall between the two compartments had a diameter of either 2 mm or 1 mm. The smaller membrane area was used in certain critical cases in order to increase the lifetime of the membranes; otherwise the membrane area had no influence on the experimental results. The temperature was kept at 25°C throughout.

Dioleoylphosphatidylcholine was synthesized in our own laboratory according to ref. [29]. Monoolein was obtained from Nu. Check Prep. Elysian, Minn..

Various mixtures of the lipids with the following sterols were used for the membrane forming solutions (Fig. 1). Cholesterol (cholest-5-en-3β-ol, Eastman reagent grade), epicholesterol (cholest-5-en-3α-ol, Merck, analytical grade), stigmasterol (cholest-5,22-dien-24-ethyl-3β-ol, Sigma), ergosterol (cholest-5,7,22-trien-24-methyl-3β-ol, Sigma), dihydrocholesterol (5α-cholestan-3β-ol, Sigma) epicoprostanol (5β-cholestan-3α-ol, Sigma) and cholesterolloleate (Merck, analytical grade). Dipicrylamide (Fluka, Buchs, Switzerland, puriss), tetraphenylborate (Merck, analytical grade) and valinomycin (Calbiochem) were used as concentrated stock solutions in ethanol. Small amounts of the stock solutions were added to the aqueous solutions to get a final concentration between 10^{-7} M and 10^{-8} M. These concentrations were chosen in order to obtain a linear relationship between the concentrations of the probes in the aqueous phase and in the membrane [30], and in order to produce no "boundary potential" by

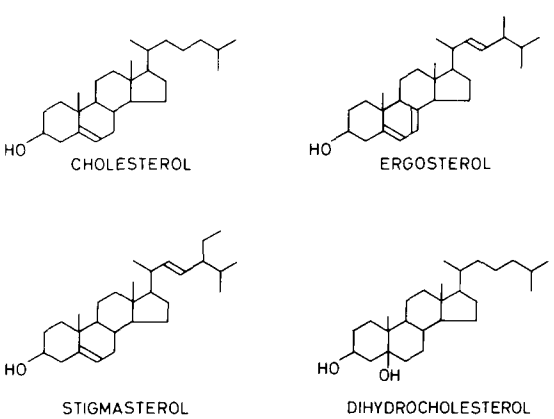

Fig. 1. Structure of some 3β-hydroxysterols, and one 3β,5α-dihydroxysterol (dihydrocholesterol). Epicholesterol and epicoprostanol (not included) are the α,β isomers of cholesterol and dihydrocholesterol, respectively.

the absorption of the lipophilic ions [39]. The ethanol content in the aqueous phase did not exceed 0.1% (v/v). The unbuffered aqueous solutions contained either 0.1 M NaCl (experiments with lipophilic ions) or 1 M RbCl (experiments with valinomycin). The experiments were performed 5—10 min after blackening of the membranes.

The charge pulse experiments were carried out as described in previous publications [23,25]. The membrane capacitance was charged up to a voltage of about 10 mV by a brief current pulse (20 ns to 50 ns duration) through silver/silverchloride or platinized platinum electrodes. The voltage transient across the membrane was recorded in different ways in the case of experiments with lipophilic ions or in the case of the carrier system. Whereas in the latter case the charge-pulse was applied repetitively, a single sweep was stored in the case of the experiments with the lipophilic ions. (Tektronix 7633/7A13/7A22 storage oscilloscope). The evaluation of the data from the oscillographic records was performed as described earlier [23,35].

For the calculation of the total concentration N_t of lipophilic ions and of the total carrier concentration N_o in the membrane the value of the specific membrane capacity C_m is needed. For some systems studied here the specific capacity has been determined previously [29,31]. For the others C_m was measured by applying rectangular voltage pulses of 10 mV to the membranes. The capacitive current was measured as a voltage drop across an external resistance with a storage oscilloscope (Tektronix 5115/5A22). The specific capacity of the membrane was calculated from the data as described earlier [29].

Results

Experiments with lipophilic ions

In all experiments with the negatively charged lipophilic ions dipicrylamine and tetraphenylborate two relaxations could be resolved. Whereas the first purely exponential relaxation process is coupled with the distribution of lipophilic ions within the membrane, the second, slow relaxation process reflects only the conductivity of undoped membranes because of slow aqueous diffusion [23]. From the observed relaxation time τ_1 and the relative relaxation amplitude a_1 of the fast process the rate constant of translocation k_i as well the total concentration N_t of lipophilic ions in the membrane were calculated according to Eqns. 3 and 4. For each set of experimental conditions at least 6 membranes were used. The standard deviations for k_i were usually less than 15% for k_i and less than 30% for N_t. The larger variations for N_t are presumably caused by the difficulty to obtain partition equilibrium because of the short lifetime of the membranes under certain conditions.

It is seen from Eqn. 5 that for the calculation of N_t from the experimental data the specific capacity C_m is needed. In the case of pure monoolein and pure dioleoylphosphatidylcholine dissolved in *n*-hexadecane the value for C_m was taken from the literature. For the other systems it was measured in the course of this study. In all cases the specific capacity was 600 ± 30 nF $\cdot$ cm^{-2} for membranes containing monoolein and 630 ± 30 nF $\cdot$ cm^{-2} for membranes containing dioleoylphosphatidylcholine, irrespective of the amount of sterol in the membrane.

Membranes were formed from mixtures of monoolein with different sterols and cholesterololeat dissolved in *n*-hexadecane. The results describing the kinetic behaviour of dipicrylamine and tetraphenylborate for the different systems are summarized in Tables I and II. It is seen that the time constant τ_1 of the fast relaxation process is strongly dependent on the molar ratio cholesterol/monoolein. For increasing mol fraction of cholesterol from 0 to 0.8 (as referred to total lipid) τ_1 decreases about eight-fold in the case of dipicrylamide and of tetraphenylborate. This decrease is paralleled with an increase of k_i for both lipophilic ions, whereas N_t remains virtually constant.

The influence of the other sterols like epicholesterol, ergosterol and stigmasterol on the transport kinetics of the lipophilic ions is comparably small. The same applies for cholesterololeate. In the case of the sterols with two hydroxy groups the influence is somewhat larger. But also in this case only the rate constant of translocation is changed whereas N_t remains unaffected.

It is seen from Table III that cholesterol, epicholesterol, ergosterol, stigmasterol and cholesterololeat have no influence on the kinetics of dipicrylamine

TABLE I

KINETIC PARAMETERS OF DIPICRYLAMINE TRANSPORT THROUGH MEMBRANES MADE FROM MONOOLEIN/STEROL MIXTURES DISSOLVED IN *n*-HEXADECANE

The mol fraction x of the sterols in the membrane-forming solution (referred to total lipid) is given in the first column. The aqueous phase contained 0.1 M NaCl and $3 \cdot 10^{-8}$ M dipicrylamine, 25°C. The results for pure monoolein were taken from ref. 17. For the calculation of N_t a specific capacity $C_m = 600$ nF · cm^{-2} was used.

x	τ_1 /μs	a_1	$k_i/10^3$ s^{-1}	N_t/pmol · cm^{-2}	$\beta/10^{-2}$ cm
Cholesterol					
0	87	0.18	4.7	0.14	0.23
0.17	76	0.18	5.4	0.14	0.23
0.33	54	0.17	7.7	0.13	0.22
0.50	26	0.21	15	0.17	0.28
0.67	16	0.19	26	0.15	0.25
0.80	11	0.19	38	0.15	0.25
Epicholesterol					
0.17	78	0.17	5.3	0.13	0.22
0.33	130	0.18	3.2	0.14	0.23
0.50	110	0.19	3.7	0.15	0.25
0.67	110	0.20	3.8	0.16	0.27
0.80	94	0.23	4.1	0.19	0.32
Ergosterol					
0.50	100	0.17	4.3	0.15	0.25
0.80	110	0.25	3.6	0.21	0.35
Cholesterololeate					
0.50	110	0.19	3.8	0.15	0.25
0.80	110	0.21	3.7	0.17	0.28
Stigmasterol					
0.80	75	0.21	5.3	0.17	0.28
Dihydrocholesterol					
0.50	36	0.20	11	0.16	0.27
0.80	22	0.24	17	0.20	0.33
Epicoprostanol					
0.50	51	0.17	8.2	0.13	0.22

TABLE II

KINETIC PARAMETERS OF TETRAPHENYLBORATE TRANSPORT THROUGH MEMBRANES MADE FROM MONOOLEIN/STEROL MIXTURES DISSOLVED IN *n*-HEXADECANE

The mol fraction x of the sterols in the membrane-forming solution (referred to total lipid) is given in the first column. The aqueous phase contained 0.1 M NaCl and 10^{-7} M tetraphenylborate; 25°C. For the calculation of N_t a specific capacity C_m = 600 nF · cm^{-2} was used.

x	$\tau_1/\mu s$	a_1	k_i/s^{-1}	N_t/pmol · cm^{-2}	$\beta/10^{-3}$ cm
Cholesterol					
0	3.0	0.22	130	0.18	0.90
0.17	3.3	0.21	120	0.17	0.85
0.33	1.8	0.19	230	0.15	0.75
0.50	0.74	0.20	540	0.16	0.80
0.67	0.59	0.19	690	0.15	0.75
0.80	0.37	0.19	1100	0.15	0.75
Epicholesterol					
0.80	3.2	0.24	120	0.20	1.0
Ergosterol					
0.50	2.8	0.23	140	0.19	0.95
0.80	3.0	0.21	130	0.17	0.85
Cholesterololeate					
0.80	3.3	0.21	120	0.17	0.85
Stigmasterol					
0.80	2.5	0.24	150	0.20	1.0
Dihydrocholesterol					
0.67	1.4	0.21	280	0.17	0.85
0.80	1.1	0.23	350	0.19	0.95
Epicoprostanol					
0.50	1.8	0.19	220	0.15	0.75

TABLE III

KINETIC PARAMETERS OF DIPICRYLAMINE TRANSPORT THROUGH MEMBRANES MADE FROM DIOLEOYLPHOSPHATIDYLCHOLINE/STEROL MIXTURES DISSOLVED IN *n*-HEXADECANE

The mol fraction x of sterol in the membrane-forming solution (referred to total lipid) is given in the first column. The aqueous phase contained 0.1 M NaCl and 10^{-8} M dipicrylamine; 25°C. The results for pure dioleoylphosphatidylcholine were taken from ref. 17. For the calculation of N_t a specific capacity C_m = 630 nF · cm^{-2} was used.

x	$\tau_1/\mu s$	a_1	$k_i/10^3$ s^{-1}	N_t/pmol · cm^{-2}	$\beta/10^{-2}$ cm
Cholesterol					
0	42	0.49	6.0	0.65	3.3
0.17	41	0.52	5.8	0.74	3.7
0.33	38	0.58	5.6	0.92	4.6
0.50	40	0.50	6.2	0.68	3.4
0.67	37	0.52	6.5	0.72	3.6
0.80	39	0.51	6.3	0.69	3.5
Epicholesterol					
0.80	37	0.50	6.7	0.67	3.4
Ergosterol					
0.80	43	0.43	6.2	0.59	3.0
Cholesterololeate					
0.80	45	0.48	5.8	0.62	3.1

TABLE IV

RELAXATION TIMES τ_i AND RELATIVE RELAXATION AMPLITUDES a_i OBTAINED FROM CHARGE-PULSE EXPERIMENTS WITH MEMBRANES MADE FROM MONOOLEIN/STEROL MIXTURES DISSOLVED IN *n*-HEXADECANE

The mol fraction x of sterol in the membrane-forming solution (referred to total lipid) is given in the first column. The aqueous phase contained 1 M RbC and 10^{-7} M valinomycin; T = 25°C. The experimental values for pure monoolein were taken from ref. 16.

x	$\tau_1/\mu s$	$\tau_2/\mu s$	$\tau_3/\mu s$	a_1	a_2
Cholesterol					
0	0.76	4.1	63	0.56	0.11
0.17	1.4	5.1	92	0.30	0.18
0.33	2.0	8.6	130	0.16	0.23
0.50	3.2	12	160	0.15	0.34
0.67	4.3	33	260	0.029	0.28
0.80	5.3	50	310	0.024	0.50
Epicholesterol					
0.17	1.1	3.8	130	0.34	0.11
0.33	1.0	3.7	110	0.30	0.12
0.50	1.1	2.9	120	0.30	0.20
0.67	0.90	3.1	140	0.44	0.16
0.80	1.2	3.4	180	0.21	0.18
Ergosterol					
0.67	1.3	3.3	120	0.23	0.18
Cholesterololeate					
0.67	0.95	3.1	98	0.35	0.12
Stigmasterol					
0.80	1.3	3.4	150	0.20	0.15

transport through membranes from dioleoylphosphatidylcholine. In a previous publication [17] we showed that the addition of cholesterol caused an increase of k_i in the case of membranes from the same lipid dissolved in *n*-decane (instead of *n*-hexadecane). According to the results obtained in this study it is very likely that the earlier observed increase is mainly caused by a thinning of the membranes. Similar considerations apply to the finding of Szabo [15] that cholesterol leads to increase of k_i for tetraphenylborate in monoolein/*n*-decane membranes of about 40-fold if the mole fraction of cholesterol is increased from 0 to 0.91. As cholesterol in monoolein in *n*-decane membranes causes a membrane thinning [16] this strong increase of k_i may also partly be caused by the thickness effect.

Experiments with valinomycin

The experimental results obtained with membranes from monoolein/sterol mixtures dissolved in *n*-hexadecane are given in Table IV. The mol fraction of cholesterol and epicholesterol was varied between 0 and 0.8.

The three relaxations predicted by the model could be resolved. As shown in Table IV, all three relaxation times strongly increase, while a_1 the amplitude of the fastest relaxation, shows an opposite behaviour. In contrast to this finding, epicholesterol, ergosterol and stigmasterol as well as cholesterololeat show only a small influence on the kinetics of valinomycin mediated Rb^+-transport.

TABLE V

INFLUENCE OF STEROL ON THE RATE CONSTANTS OF VALINOMYCIN-MEDIATED Rb^+-TRANSPORT, AS CALCULATED FROM THE EXPERIMENTAL DATA OF TABLE IV

For the calculation of N_t a value for the specific capacity C_m = 600 nF · cm^{-2} was used.

x	$k_R/10^4$ $M^{-1} \cdot s^{-1}$	$k_D/10^4$ s^{-1}	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s^{-1}	N_0/pmol · cm^{-2}
Cholesterol					
0	15	9	23	3.0	1.7
0.17	13	11	19	2.7	0.81
0.33	8.3	12	14	1.7	0.62
0.50	6.2	10	7.1	1.0	0.92
0.67	3.0	12	4.2	0.62	0.68
0.80	2.0	11	2.8	0.41	0.95
Epicholesterol					
0.17	19	8	25	3.4	0.62
0.33	19	10	28	3.7	0.58
0.50	29	14	23	2.7	0.75
0.67	25	12	26	2.1	0.91
0.80	26	13	24	2.2	0.48
Ergosterol					
0.67	25	12	21	3.5	0.54
Cholesterololeate					
0.67	23	10	28	4.2	0.68
Stigmasterol					
0.80	25	11	23	3.4	0.41

With dihydrocholesterol or epicoprostanol the experimental data are much more effected. In these two cases only two relaxations could be resolved. Possibly the first and the second relaxation process have approximately the same time constant or the amplitude of the fastest process is too small.

Table V contains the values of the rate constants and of N_0, which were calculated from the experimental results given in Table IV and from C_m according to Eqns. 9—13.

It is seen from Table V that cholesterol has a strong influence on the rate constants k_R, k_{MS} and k_S. As x_{chol} is varied between 0 and 0.8, the three rate constants k_R, k_{MS} and k_S decrease about 7 to 8-fold, whereas k_D remains virtually constant.

Tabel V contains also the results for the sterols with one hydroxy group and for cholesterinoleate. According to the finding that the relaxation date are not very much affected by these compounds, the rate constants and N_0 remain also virtually constant.

Discussion

From the results given in the previous section it is seen that there is a strong influence of cholesterol on the transport kinetics of lipophilic ions and carrier molecules. For an increasing mol fraction of cholesterol between 0 and 0.8 the translocation rate constant k_i for dipicrylamine and tetraphenylborate increases by a factor of about 8, whereas the partition coefficient β is almost insensitive

to the cholesterol content of the membrane. These findings are in contrast to the observed decrease of nonelectrolyte permeability of lipid vesicles in the presences of cholesterol [5,8]. These results have been explained in terms of a condensation effect of cholesterol [5] and a concomitant decrease of membrane fluidity.

In experiments with lipid bilayer membranes containing increasing amounts of cholesterol the permeability of positively charged probes decreases, whereas the opposite is valid for negatively charged molecules [14,15]. These findings can be explained by the assumption that the influence of cholesterol on the kinetics of charged probes is given mainly by the change of the dipole potential of lipid bilayer membranes and to a much lesser extent by the membrane fluidity [13,14]. No direct information is available on the magnitude of the dipole potential between aqueous phase and membrane interior. Measurements of surface potentials of lipid monolayers which may be considered as one halve of a membrane indicate that the interior of a membrane is more positive by several 100 mV with respect to the aqueous phase [32]. As indicated by monolayer experiments the dipole potential of membranes seems to be largely dependent on the lipid composition of the membrane [32]. Monolayers of monolein and lecithin, the lipids used in this study, have a surface potential of about 320 mV and 440 mV, respectively [32]. As the dipole potential V_D (Fig. 2) of a membrane affects in general, both the partition coefficient β and the translocation rate constant k_i, the product $k_i\beta$ for a given hydrophobic ion should be different by a factor of about 100 for membranes composed of either monoolein or lecithin. This is not the case, as seen from Tables I and III. From the deviation one may conclude that not only the variation of the dipole potential is responsible for the differences between the single lipids. The adsorption of hydrophobic ions to the zwitterionic polar head group or structural properties of the membranes may also play a role.

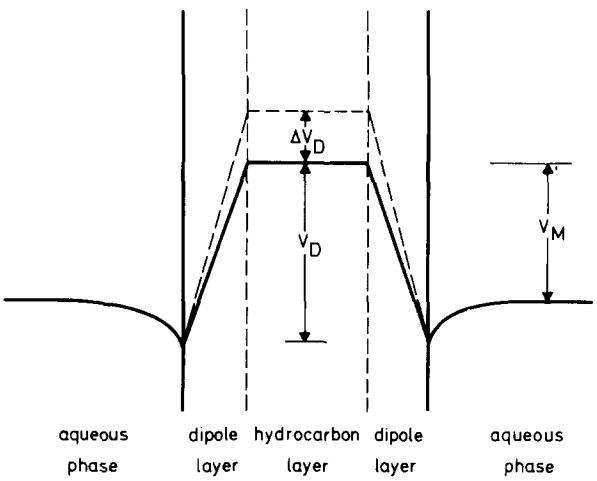

Fig. 2. Potential course in a membrane. V_D and V_M are the potentials of the membrane interior with respect to the membrane surface and the aqueous phase, respectively. ΔV_D is the change of the dipole potential V_D.

The change of the dipole potential ΔV_D (Fig. 2) of a membrane may affect in general the partition coefficient β as well as the translocation rate constant k_i. The relative magnitude of the effects on β and k_i depends on the location of the adsorption plane of the lipophilic ion. Under the assumption that the introduction of sterol molecules in the membrane affects only the surface potential by the amount ΔV_D but leaves other membrane parameters unchanged, the influence on βk_i is given by the following equation:

$$\beta k_i = \beta^* \exp(-z\delta F \Delta V_D/RT) \cdot k_i^* \exp(-z(1-\delta) F \Delta V_D/RT) \quad (19)$$

where z is the valency of the lipophilic ion, $\delta\Delta V_D$ and $(1-\delta)\Delta V_D$ the change of the dipole potential with respect to the adsorption plane of the lipophilic ions and to the translocation rate constant, respectively, and k_i and β are the constants for $\Delta V_D = 0$. If the adsorption plane is located outside (on the aqueous side) of the dipole layer then ΔV_D acts only on k_i leaving β unaffected ($\delta = 0$).

The results given in Tables I, II and III show that the partition coefficient is almost insensitive to the composition of the membrane, whereas the translocation rate constant k_i is found to increase in the case of monoolein membranes with increasing cholesterol content. In the case of lecithin membranes k_i seems to be nearly unaffected (Table III). These results are consistent with the finding that a monolayer of cholesterol has a surface potential of 390 mV [33], which is much larger than that of a monoolein monolayer (about 320 mV) [32]. A lecithin monolayer has a surface potential of 440 mV [32], k_i should therefore decrease in membranes from cholesterol lecithin mixtures with increasing cholesterol content. It is seen from Table III that no decrease was observed for k_i. The reason for this finding is not yet clear.

Using the assumption that cholesterol and monoolein are ideally mixed in the membrane and that the membrane has the same composition as the bulk phase, (an assumption, which is not completely fullfilled in cases where the composition of membranes containing cholesterol has been measured [34]) Eqn. 19 reduces in the case $\delta = 0$ to:

$$k_i = k_i^* \exp(F x \Delta V_D/RT) \quad (20)$$

when the valency $z = -1$ of dipicrylamine and tetraphenylborate has been introduced. x is the mol fraction of cholesterol. Fig. 3 shows the results for the two lipophilic ions. The theoretical curve was drawn with $\Delta V_D = 70$ mV. It is seen that the dependence of the ratio k_i/k_i^* on the mol fraction of cholesterol is very well described by Eqn. 20. This finding is not obvious, as some of the assumptions which are implicit in equation 20 seem doubtful. Especially in the case of epicholesterol, where a surface potential of monolayers in the order of −100 mV has been observed [33], it is not possible to describe the results for k_i/k_i^* in a similar way as for cholesterol. An explanation for this unexpected behaviour is also not yet clear, but monolayer studies may help to decide this question.

A possibility is that the sterols with one hydroxy group, besides cholesterol, are completely excluded from a bilayer. However, a complete exclusion of sterols from membranes seems not very likely. Similar considerations apply to cholesterololeat, where also no influence on the kinetics of ions transport was observed. Cholesterol, for instance is only excluded to a small extent at higher

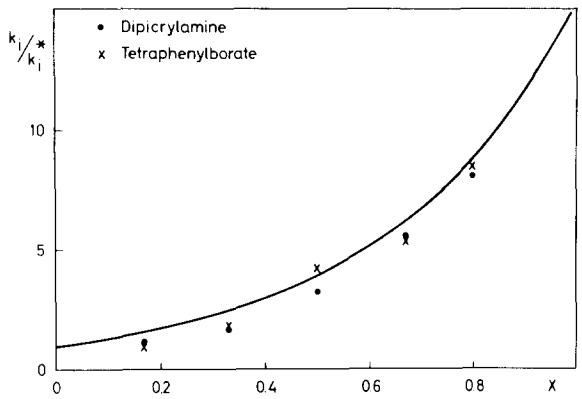

Fig. 3. Dependence of k_i/k_i^* on the mol fraction of cholesterol in monoolein/cholesterol mixtures. The theoretical curve was calculated according to Eqn. 20 using ΔV_D = 70 mV. For further explanation see text.

concentrations in the membrane forming solutions [34]. Monolayer studies with lipid/sterol mixtures, especially the measurement of the surface potential may help to decide this question.

The action of cholesterol on valinomycin mediated by Rb^+ transport is much more complex as compared with the action on lipophilic ions. It is seen from Table V that not only k_{MS} decreases with increasing cholesterol content in the membrane, but also k_R and k_S show a similar behaviour as k_{MS} (Fig. 4). The decrease of the three constants is of similar magnitude as the increase of k_i in the case of the lipophilic ions under identical conditions. A change in the dipolar potential of the membranes is able to influence the stability of the ion-carrier complex as well as the translocation rate constant k_{MS}. But its influence

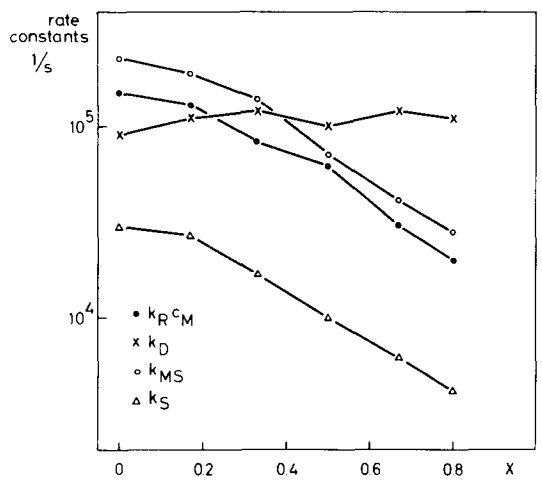

Fig. 4. Dependence of the rate constants of valinomycin mediated Rb^+ transport on the mol fraction of cholesterol in monoolein/cholesterol mixtures. The lipid was dissolved in *n*-hexadecane. The aqueous phase contained 1 M RbCl and 10^{-7} M valinomycin, T = 25°C.

on uncharged molecules should be negligible. The decrease of the translocation rate constant k_S of the free carrier may therefore be caused by a decrease of the fluidity of the membrane in the presence of cholesterol. A very similar condensing effect, which lowers the permeability of uncharged molecules, has been observed with model membranes in the fluid state [5]. The finding that the influence of cholesterol on k_S and k_{MS} is approximately the same seems to indicate that the change of the translocation rate constant k_{MS} is presumably caused by a fluidity change of the membrane and not by the change of the dipolar potential.

Depending on the location of the adsorbed carrier-ion with respect to the dipolar layer a change of the dipolar potential may affect the heterogeneous stability constant of the complex $K_h = k_R/k_D$, as well as the translocation rate constant k_{MS}. As k_{MS} and k_S show the same dependence and K_h decreases about 8-fold if the mol fraction x of cholesterol is increased from 0 to 0.8 the dipole potential change ΔV_D seems to act only on the stability of the complex. The decrease of K_h is caused by a decrease of the association rate constant k_R whereas k_D remains virtually constant. The finding that only k_R is affected by cholesterol may be explained by the assumption that the charged complex is located on the hydrocarbon side of the dipole layer. In this case, Rb^+ from the aqueous phase has to surmount the additional potential difference ΔV_D, thus apparently decreasing k_R. The decrease of k_R for increasing mol fraction x of cholesterol can be described in a similar way as given by Eqn. 20 for k_i. The experimental data for k_R fits very well for $\Delta V_D = 70$ mV. The hypothesis that the carrier-ion complex is located towards the membrane interior is supported by the fact that only part of an applied voltage seems to act on the carrier-ion complex [24,35,36].

The results obtained with the valinomycin/Rb^+ system in membranes containing the other sterols reflect in principle the results found with the lipophilic ions. Also in this case the transport parameters are not influenced by epicholesterol, ergosterol and stigmasterol. The same applies to cholesterololeate.

It is interesting to note that the 3β-hydrosterols ergosterol and stigmasterol show also in model systems like monolayers and liposomes a somewhat different behaviour than cholesterol [6,37]. For instance, the high collapse pressure for cholesterol monolayers is reduced in the case of these sterols [6] and the decrease of the permeability of lecithin liposomes for glucose, glycerol and Rb^+ is smaller [37]. In a recent publication [38] an opposite action of cholesterol on the permeability of lecithin vesicles has been reported. Whereas in the earlier publications [5,6,8,37] a reduced permeability of the liposomes obtained by stirring for charged and uncharged molecules has been found, the opposite is valid for vesicles obtained by sonication [38]. The contradictory results may caused by the different preparations of the model systems in the two cases [37,38].

From the results obtained with membranes containing cholesterol and related studies with membranes of different composition and thickness [16,17] it is possible to deduce which membrane properties act on the two transport systems, the negatively charged lipophilic ion and the positively charged ion-carrier complex. The transport kinetics of lipophilic ions seems to be mostly influenced by membrane thickness and magnitude of the dipole potential and

to a neglectible extent by the membrane fluidity. The translocation rate constants k_{MS} and k_S of carrier mediated ion transport are limited to large extend by the membrane fluidity, whereas the stability constant K_h seems to be partly influenced by the dipole potential. The different behaviour of the two transport systems may be caused by the different location of the adsorption planes. Whereas the lipophilic ions are possibly adsorbed towards the aqueous side of the dipole layer, the charged carrier-ion complex seems to be located on its hydrocarbon side.

Acknowledgements

The authors wish to thank Dr. P. Läuger for many helpful discussions, Mr. G. Ehmann and Mr. R. Schindler for excellent technical assistance and Mr. K. Janko for the synthesis of dioleoylphosphatidylcholine. One of us (D.C.) gratefully acknowledges the award of a fellowship of the Deutsche Forschungsgemeinschaft. This work has been financially supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 138).

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