The Effect of Rimantadine on the Structure of Model and Biological Membranes

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Abstract. The action of the antiviral drug rimantadine on the structure of bilayer lipid membranes (BLM) and RBC membranes was investigated. Structural changes in BLM were recorded by ionophore conductivity changes and by changes in the third harmonic of capacity current signal due to lateral compression of BLM in an electric field. It was shown that the adsorption of rimantadine on BLM results in an increase in ionophore mobility in bilayer membranes of dioleolyllecithin (DOL) and common lipids of bovine brain (CL) and in a decrease in those of azolectin (A). Relative changes in the third harmonic signal also depend on the membrane composition and have different signs. The results may be explained by the rimantadine action on the lipid bilayer structure: "rigidification" of A-membranes and "fluidization" of BLM from DOL and CL. Structural reorganization of RBC membranes as investigated by the ability of the cells to enter a micropipette (inner diameter $\geq 3\,\mu$m) thereby undergoing deformation. It was shown that rimantadine influences RBC deformability due to drug induced inhomogenous mechanical membrane properties. Also, rimantadine accelerated the process of artificially induced aggregation of erythrocytes. The relation of the effects on artificial and biological membranes, and the structural changes in the lipid phase of membrane are discussed.

Key words: Drug — BLM — Human erythrocytes — Deformability

Introduction.

Rimantadine and other aminoderivatives of adamantane are widely used as antiviral agents in medicine: however the detailed mechanism of their pharmacological activity is not known as yet. The results obtained by Kato and Eggers (1969) have revealed that these drugs act on biomembranes and this has been

shown to underly their effects on viruses (Kharitonenkov et al. 1979). Rimantadine has been shown to induce shape changes in erythrocytes under certain condition (Tverdislov et al. 1985; Paulitschke et al. 1986a) and to alter RBC membrane properties (Herrmann et al. 1985). On the other hand, rimantadine changes the properties of artificial bilayer lipid membranes (BLM). This has been suggested by electric field distribution after the adsorption of the positively charged form of rimantadine (Simonova et al. 1984 and 1986a), by results of transport studies of the neutral form of rimantadine (Simonova et al. 1986b) and by investigations of BLM-elasticity (Kharitonenkov et al. 1979).

The idea seems attractive that the interactions of rimantadine with the lipid part of biomembranes are responsible for its biological effects. This prompted us to carry out experiments with membrane interaction of rimantadine with both natural and artificial membranes to show whether the drug induces changes in the lipid bilayer structure.

Materials and Methods

1. Measurements on BLM

Bilayer membranes were formed on the hole ($A = 1\,mm^2$) in a teflon vessel using the MULLER-RUDIN technique. The membranes were formed from decane solutions of the following lipids: common lipids of bovine brain (CL) in a concentration of 40 mg/ml, 50 mg/ml of L-α-phosphatidylcholine (azolectin, A) (Sigma), 20 mg/ml egg lecithin (Koch-Light) mixed with 10 mg/ml cholesterol (BDH), 40 mg/ml dioleolyllecithin (DOL) (Sigma) and a mixture of DOL and cholesterol (2 : 1). All measurements were carried out in 0.1 mol/l KCl solutions. The buffer solutions contained 10^{-3} mol/l of $C_4H_5O_6K$, MES (Serva), Tris (Calbiochem), HEPES (Serva) and sodium acetate. Ag/AgCl electrodes were used to measure the capacity current of BLM.

The method of inner membrane electric field compensation (IFC) (Sokolov and Kuzmin 1980) was used to measure the boundary potential difference $\Delta\varphi_B^c$ on both sides of BLM and its changes in the presence of rimantadine at one side of the membrane. Changes in boundary potentials were also determined by measuring BLM conductivity G/G_o (McLaughlin et al. 1971) in the presence of different ionophores (nonactin and pentachlorphenol). Changes in BLM conductivity were recorded after the addition of rimantadine to the solutions at both sides of membrane and upon the application of a constant voltage of 20 mV. $\Delta\varphi_B^c$ was calculated by Eq. (1):

$$\Delta\varphi_B^c = -\,RT/zF * \ln(G/G_o) \tag{1}$$

where $RT/F = 25.3$ mV at 20 °C, and z is the charge number of the ionophore complex, carrying electric current across the membrane.

The third harmonic of the capacity current was measured in an arrangement based on a modified device for the analysis of the highest harmonics in a range from 20 Hz to 20 kHz (BKF-10, Radiometer, Denmark), or on a selective amplifier (U 2—8, USSR) at a fixed frequency of 385 Hz. An AC voltage of 10—50 mV was applied to the BLM, and the capacity current was recorded with an amplifier (Keithley-427, UK). The amplitude of the third harmonic may be related to the modulus of transversal compressibility of BLM (Passechnik and Hianik 1979) or to modulus of BLM bending and surface tension (Lejkin 1985). For our considerations it is essential that the third

harmonic signal characterizes mechanical properties of the lipid bilayer membrane.

2. Measurement of erythrocyte deformability

Deformability investigations of unwashed erythrocytes from one-day-old donor blood (ACD-AG, blood group ARh, male; Bezirksinstitut für Transfusionswesen, Berlin) were performed in isotonic phosphate-buffered salt solution (PBS) (Paulitschke et al. 1986b), which contained different rimantadine concentrations. The rested-state biconcave shape of untreated RBC in PBS was stabilized by 0.5 % human serum albumin.

The cells were suspended in the respective rimantadine solution in a ratio of about 1 : 6000 and incubated at 37 °C for 30 min. After cooling the samples to room temperature (25 ± 1)°C, RBC deformability was quantified by a capillary passage time analyzer, a modified micropipette technique described in detail elsewhere (Paulitschke et al. 1986b; Paulitschke 1987). In brief, single cells were aspirated under a defined pressure difference ($\Delta P = 200$ Pa) into a micropipette with an inner diameter of (3 ÷ 3.5)μm. The impulse-like changes in conductivity induced by the cell entering the glass capillary were recorded, digitized and mean values of the time and amplitude parameters of conductivity changes of about 100 erythrocytes for each blood sample were evaluated by a microcomputer.

3. Erythrocyte aggregation

Erythrocytes from one-day-old donor blood (see above) were centrifugated and washed twice in isotonic PBS (pH 7.4). The washed RBC were resuspended at a volume concentration of $p = 45$ % in glucose-containing PBS (5 mmol/l glucose) and incubated at 37 °C for 1 h. Then the sample was divided, 10^{-3} mol/l rimantadine was added to one part and incubated again at 37 °C for 30 min. The other part served as control. The aggregation process was carried out in the gap of a horizontally arranged cylinder system as described in detail by Lerche et al. (1979). In brief, RBC were made to collide due to a laminar shear flow. If they aggregate, a decrease in particle number (single cells and aggregates) occurs. From temporal changes in particle numbers N_t (determined microscopically in a cell counting chamber) the collision efficiency α was determined as a quantitative measure for the probability that the collision of two cells result in a stable aggregate. In isoosmotic solutions of decreased ionic strength (5.6 mmol/l) and reduced pH (6.1) washed erythrocytes aggregate spontaneously (Lerche 1982; Hessel and Lerche 1985). This aggregation behaviour was compared with and without rimantadine.

Results

1) Effect of rimantadine on the induced ion transport across BLM

BLM conductivity was induced by ionophores of both signs: pentachlorphenol as an anion carrier, nonactin and valinomycin as cation carriers. The addition of rimantadine to both sides of BLM decreases membrane conductivity for cations and increases that for anions (Fig. 1). Relative changes in BLM conductivity for hydrophobic cations and anions were found to differ in their absolute values. The presence of rimantadine induced larger changes in anion-induced

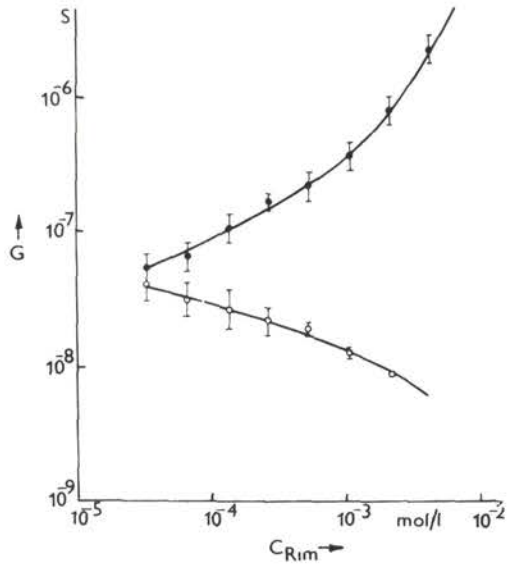

Fig. 1. The dependence of DOL-BLM conductivity (40 mg/ml in decane) on rimantadine concentration. The changes in BLM conductivity were induced by ionophores of opposite sign: filled circles: pentachlorphenol (6×10^{-5} mol/l), empty circles: nonactin (7.2×10^{-6} mol/l).

conductivity of BLM consisting of DOL and CL than in that induced by cations. The opposite was observed with asolectin membranes.

To explain this asymmetry of effects it should be suggested that the changes in conductivity are connected not only with membrane boundary potential alterations caused by rimantadine adsorption. Boundary potential changes at different rimantadine concentrations were calculated from conductivity changes by Eq. (1) (Figs. 2, 3). If only the bounary potentials changed due to the adsorption of rimantadine, the computed potential values should be identical for ionophores of both signs. In other words, BLM conductivity for hydrophobic cations should decrease to the same extent as it increases for hydrophobic anions. However, experimental data show that another rimantadine-associated factor has to be assumed. This factor which we propose to be associated with membrane structural changes, leads to an increase in DOL- and CL-BLM conductivity equally for cations and anions, and to a decrease in asolectin- BLM conductivity.

This assumption was verified by measuring boundary potential changes using the IFC method: This method was chosen as its results do not depend on structural changes in BLM (curve 3, Figs. 2, 3). A comparison of the potentials

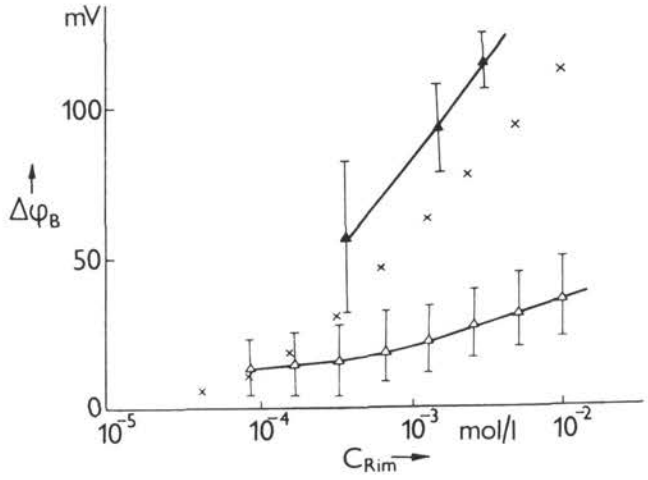

Fig. 2. The dependence of $\Delta\varphi_B$ potentials calculated from values of BLM conductivity by Eq. (1) (filled triangles: induced by PCP (3.3×10^{-5} mol/l); open triangles: induced by nonactin (3.3×10^{-7} mol/l)) on rimantadine concentration at both sides of CL-BLM (40 mg/ml in decane). Crosses illustrate the influence of rimantadine added to one side of the BLM on $\Delta\varphi_B$ measured by the IFC method.

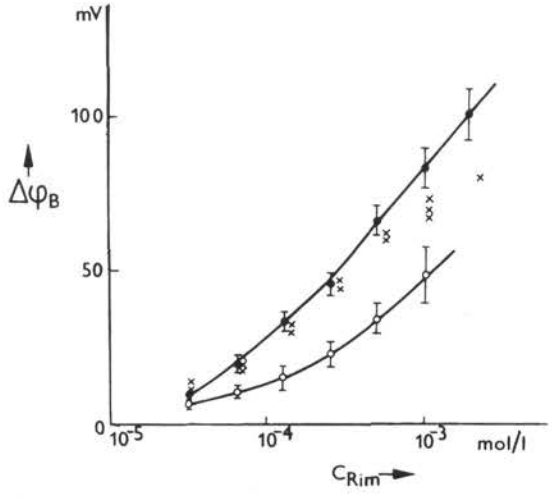

Fig. 3. Values of $\Delta\varphi_B$ calculated from measurements of BLM conductivity (filled and empty circles) and obtained by the IFC method (crosses), respectively, for different rimantadine concentrations. The membranes were prepared of azolectin (50 mg/ml). Filled circles: 5×10^{-6} mol/l of nonactin; empty circles: 3.3×10^{-6} mol/l of pentachlorphenol; 2.5×10^{-3} mol/l of mixed buffer solution (pH 6.7) were added to the solutions.

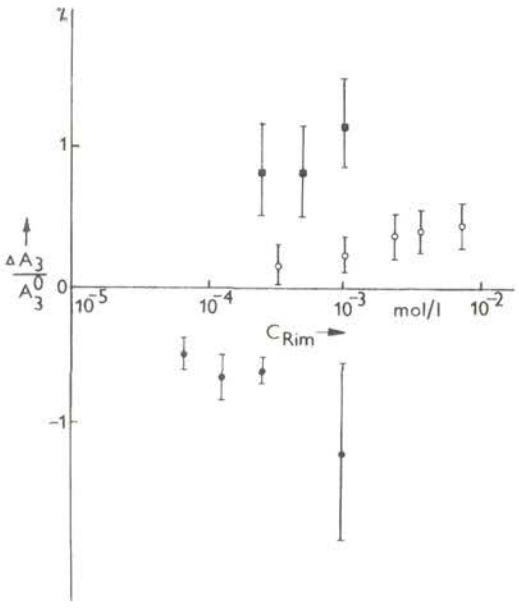

Fig. 4. The dependence of relative changes in the third harmonic amplitude of capacity current on rimantadine concentration for BLM of different composition. Squares: BLM of DOL (40 mg/ml); filled circles: BLM of azolectin (50 mg/ml), and empty circles: BLM of CL (40 mg/ml).

calculated from conductivity changes as measured by the IFC method is instructive. The measurement of BLM conductivity yields the "error" of potential calculation connected with ion movements in the hydrophobic part of the membrane; this is changed due to rimantadine effects on the lipid bilayer structure.

In addition, changes in BLM structure were detected by measuring the amplitude of the third harmonic of the capacity current. After the addition of rimantadine to both membrane sides, the signal changes differed for membranes of A and those of CL (Fig. 4). For CL-BLM these changes depended on the frequency of the alternate voltage applied to the membrane (Fig. 5). The simplest explanation is that these differences in the third harmonic amplitudes result from changes in the modulus of transversal compressibility of the BLM (Passechnik and Hianik 1979). Another possibility proposed by Lejkin (1985) is that the changes have to do with the bending modulus and the surface membrane tension, respectively. These changes in amplitude of the third harmonic suggest an alteration of membrane mechanical properties. The results shown in Fig. 4 thus reflect effects of rimantadine on bilayer structures, resulting in a

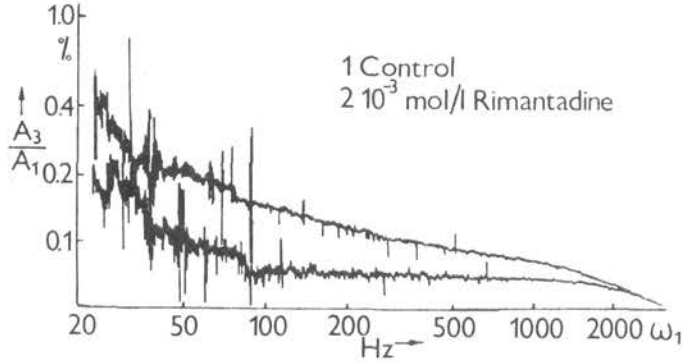

Fig. 5. The dependence of amplitude changes of the third harmonic of capacity current A_3, relative to the first harmonic amplitude A_1, respectively, on the frequency of applied voltage. Measurements were carried out on BLM of CL (40 mg/ml); curve 1 relates to the control sample and curve 2 to a sample containing 10^{-3} mol/l of rimantadine.

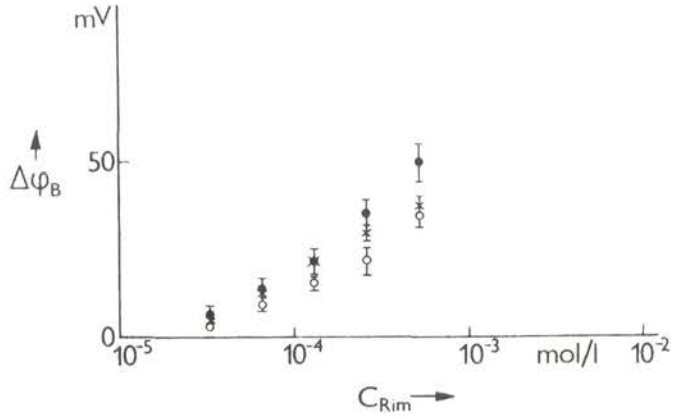

Fig. 6. Values of $\Delta\varphi_B$ calculated from measurements of BLM conductivity (filled and empty circles) and obtained by the IFC method (crosses) for different rimantadine concentrations. The membranes were prepared of azolectin (50 mg/ml). Filled circles: 3.3×10^{-5} mol/l pentachlorphenol; empty circles: $(1.8 - 4.3) \times 10^{-6}$ mol/l of nonactin. The solutions contained 10^{-1} mol/l KCl and 10^{-3} mol/l $C_4H_5O_6K$ (pH 4.2).

higher "rigidity" of an azolectin- BLM and a higher "fluidity" of DOL- membrane. A more rigid membrane structure impairs the transmembraneous ion transport. The frequency dependence of rimantadine effects on DOL-BLM is due to kinetic characteristics. The interpretation of these effects is based on the theoretical model for the description of mechanical properties of BLM (cf. Lejkin 1985; Passechnik and Hianik 1979).

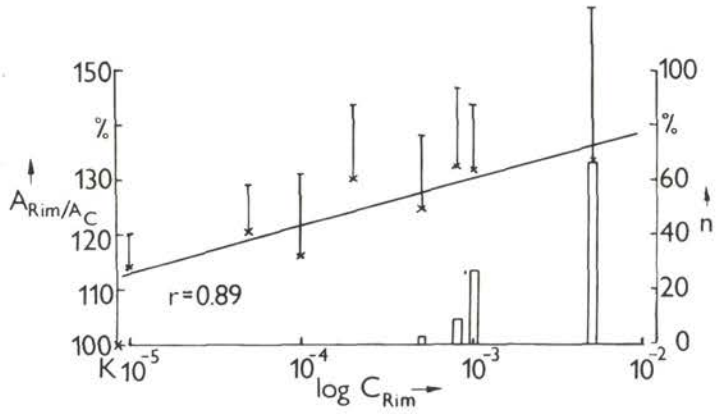

Fig. 7. Relative changes in the amplitude of the passage signal of RBC A_{Rim}/A_C incubated in PBS with different rimantadine concentrations (mean values of 6 experiments) and relative number n of RBC unable to enter the capillary.

Rimantadine molecules adsorbed to BLM in a neutral form were shown to be able to cross the membrane and to reach the opposite monolayer (Simonova et al. 1986b). Variation of pH can change the concentration of neutral rimantadine molecules in the membrane. In our experiments, pH was varied to distinguish between the effects of charged and neutral form of rimantadine on membrane structure. The dependence of structural alterations on pH is clear from Figs. 3 and 6. A pH decrease strongly reduced rimantadine induced conductivity changes measured using the IFC method and the corresponding boundary potentials; thus, the effect on membrane structure was less pronounced. At very low pH rimantadine-produced changes in the third harmonic become negligible.

2) Effect of rimantadine on RBC deformability

Fig. 7 shows the effect of rimantadine on amplitude A, a parameter sensitive to changes in cell surface excess and membrane mechanical properties (Paulitschke 1987). The relative increase was linear with the logarithmic increase in rimantadine concentration, and corresponded to the decrease in RBC deformability:

$$A_{Rim}/A_c = 8.6 * \log C_{Rim} + 156 \qquad (r = 0.89) \qquad (2)$$

At rimantadine concentrations as low as 10^{-5} mol/l the mean value of A_{Rim} is significantly higher than the corresponding control value. Entry time and passage time do not correlate with the rimantadine concentration in the suspension

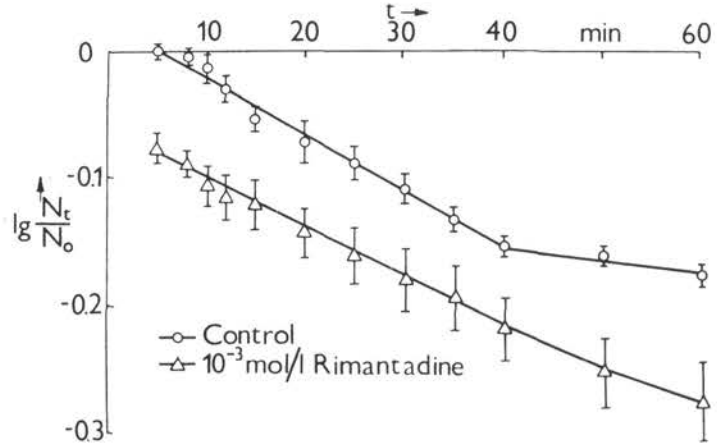

Fig. 8. The kinetics of artificially induced erythrocyte aggregation (C_{NaCl} = 5.6 mmol/l; pH = 6.1) of normal (circles) and rimantadine-incubated (triangles) RBC (10^{-3} mol/l rimantadine).

media. Averaging all blood samples and rimantadine concentrations a moderate increase in entry time (+ 13 %) and passage time (+ 7 %), respectively, was observed. Generally speaking, these results clearly indicate a decreased whole cell deformability. In addition, a subpopulation of RBC seems to lose the ability to pass through capillaries. At rimantadine concentrations exceeding 5×10^{-4} mol/l the number of erythrocytes not passing the glass capillary increased rapidly (percentages of non-deformable RBC are shown as columns in Fig. 7) and correlated with the rimantadine concentration. It is also interesting to note that the surface of the erythrocytes seemed to turn inhomogenous. The dimple region of stomatocytes folds with increasing rimantadine concentrations (Paulitschke et al. 1986a). The frequently folded dimple regions of stomatocyte RBC are less fluid than the other parts of the cells. The cells thus rotate so that they can be deformed leaving the rigid cell part almost not deformed.

With increasing rimantadine concentrations the surface excess of increasing numbers of RBC is folded, and the cells cannot be deformed and do not enter the micropipette (cf. Fig. 7). At 5×10^{-3} mol/l of rimantadine this was observed for more than 60 % of about 600 RBC investigated. It should be stressed, therefore, that the values of A_{Rim}/A_c shown in Fig. 7 characterize only „deformable" RBC.

3) Artificial aggregation of human erythrocytes

The effect of rimantadine on the kinetics of artificial aggregation is shown in Fig.

their suspension stability they aggregate.

Erythrocytes incubated with 10^{-3} mol/l of rimantadine aggregate without any latency. This should be discussed in terms of local protein redistribution in the membrane rather than of a direct rimantadine effect on the properties of membrane proteins. Electrophoretic results were interpreted in a similar way (Tverdislov et al. 1985).

Structural alterations in the RBC membrane seem to be the likely reason for rimantadine-induced discocyte-spherostomatocyte shape changes (Tverdislov et al. 1985; Paulitschke et al. 1986a), and for the impaired RBC deformability observed in our investigations. It seems interesting that over the whole range of rimantadine concentrations used a logarithmic dependence on rimantadine concentration was observed for cells, which were able to pass through the micropipette. Starting with a concentration of 5×10^{-4} mol/l rimantadine a subpopulation of RBC could be detected, which at the hydrodynamic conditions employed ($\Delta P = 200$ Pa, radius and effective length of capillaries 3.2 μm and 150 μm, respectively) were completely unable to pass. The reason may have been an extremely increased negative spontaneous curvature of the membrane (Deuling and Helfrich 1976; Markin and Kozlov 1984; Paulitschke et al. 1986a, Lerche et al. 1987b).

Our assumption is based on the insertion of asymmetrically formed rimantadine molecules predominantly in the inner monolayer. Such an insertion of a progressive number of rimantadine molecules in the lipid phase should lead to structural changes in the lipid phase of the cell membrane, and secondarily, to higher lateral mobility of membrane proteins. Due to the positively charged rimantadine the electrostatic energy of the glycocalyx will be changed and therefore also some alternation of the macromolecules has to be expected (Lerche 1987a). Facilitated lateral diffusion allows interactions between different membrane proteins to occur, resulting in changes in the cell membrane structure, and thereby, in a decreased deformability of the erythrocytes.

Experimental investigation of rimantadine action on BLM and RBC membranes showed the existence of similar effects, which can be associated with structural changes in the membrane matrix. Still, detailed knowledge is lacking about the nature of the structural changes and their dependence on membrane composition. However, there is no doubt about the importance of such effects for many processes taking place in biological membranes.

Appendix I

We suppose that the original conductivity of a membrane for some lipophilic ions i is G_{0i}, and G_i after rimantadine addition. The change in boundary

potential is described by $\Delta\varphi_B$ or in dimensionless form by $\Delta\psi = F/RT^*\Delta\varphi_B$ where F, R and T have their usual meanings. Due to changes in the boundary potential the conductivity for ions i with a charge number z_i should change exp $(-z_i^*\Delta\psi_B)$ times. The parameter λ was introduced as the relative change in ion mobility in the membrane under the condition that the membrane conductivity is changed e times. I.e., positive values of λ correspond to increasing mobility of lipophilic ions in the membrane, and negative values to decreasing mobility. If structural characteristics of the membrane are not changed, $\lambda = 0$. The conductivity of a modified membrane (by rimantadine molecules) is given as:

$$G_i = G_{oi}\exp(\lambda - z_i\Delta\psi_B) \tag{3}$$

The values of λ and $\Delta\psi_B$ do not depend on the type of ions passing the membrane. For the common case this suggestion requires further experimental support.

The parameters λ and $\Delta\psi_B$ are determined under the following assumption: The conductivities of the original and the modified membrane are defined for two different lipophilic ions ($i = 1; 2$), and can be described by

$$\begin{aligned} G_1 &= G_{o1}\exp(\lambda - z_1\Delta\psi_B) \\ G_2 &= G_{o2}\exp(\lambda - z_2\Delta\psi_B) \end{aligned} \tag{4}$$

If $z_1 = z_2$ it must hold that

$$G_1/G_{o1} = G_2/G_{o2} \tag{5}$$

i.e., relative changes in BLM conductivity are identical for similarly charged lipophilic ions (membranes modified by rimantadine). If z_1 and z_2 are different, the system (4) can be solved relative to λ and $\Delta\psi_B$:

$$\lambda = \frac{z_1}{z_1 - z_2}\ln\frac{G_2}{G_{o2}} - \frac{z_2}{z_1 - z_2}\ln\frac{G_1}{G_{o1}} \tag{6}$$

$$\Delta\psi = \frac{1}{z_1 - z_2}\ln\frac{G_2}{G_{o2}} - \frac{1}{z_1 - z_2}\ln\frac{G_1}{G_{o1}} \tag{7}$$

or, by taking into account values of boundary potentials, calculated by Eq. (3) for each of the two ions $\Delta\varphi_1$ and $\Delta\varphi_2$ ($z_1 = 1; z_2 = -1$):

$$\lambda = -F/2RT^*(\Delta\varphi_1 - \Delta\varphi_2) \tag{8}$$

$$\Delta\psi = F/2RT^*(\Delta\varphi_1 + \Delta\varphi_2) \tag{9}$$

Expression (9) yields the mean value of the boundary potential, measured by the conductivity method with the help of different lipophilic ions. As can be seen from Figs. 2 and 3, these values do not differ greatly from those measured by the IFC method. This means that after rimantadine addition to the system

anions and cations change their mobility in BLM effectively in the same manner.

Using Eq. (8) and the results shown in Figs. 1, 2 and 3 we can determine the values of λ under the effect of rimantadine for BLM composed of different lipids. The results are shown in Fig. 9, and they reflect changes in membrane structure revealed by the mobility of lipophilic ions. A comparison of Figs. 4 and 9 shows a good agreement of the parameter with the data obtained by the method of measurement of the third harmonic of the capacity current. Parameter λ introduced by us thus determines quantitative structural membrane changes that may be useful for analyzing data obtained by different methods.

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References

Deuling H. J., Helfrich W. (1976): Red blood cell shapes as explained on the basis of curvature elasticity. Biophys. J. **16,** 861–868

Helenius A., Kartenbeck I., Simons K., Fries E. (1980): On the entry of Semliki Forest virus into BHK-21 cells. J. Cell. Biol. **84,** 404–420

Herrmann A., Lentzsch P., Lassmann G., Landhoff A. M., Donath E. (1985): Spectroscopic characterization of vesicle formation on heated human erythrocytes and the influence of the antiviral agent amantadine. Biochim. Biophys. Acta **812,** 277–285

Hessel E. (1985): Experimentelle Quantifizierung der Wechselwirkung der roten Blutzelle in ionalen Medien unter besonderer Berücksichtigung der elektrostatischen und sterischen Oberflächenstruktur. Dissertation A. Humboldt-Universität Berlin

Hessel E., Lerche D. (1985): Cell surface alterations during blood-storage characterized by artificial aggregation of washed red blood cells. Vox. Sang. **49,** 86–91

Kato N., Eggers H. T. (1969): Inhibition of uncoating of fowl plaque virus by L- adamantanamine hydrochloride. Virology **37,** 632–641

Kharitonenkov I. G., Poltorak V. I., Ruuge E. K. (1979): Investigation of the myxoviruses structure by the spin probes method: II. Influence of rimantadine on the structure of viral and artificial lipid membranes. Molekulyarnaya Biofizika **13,** 1035–1043 (in Russian)

Lejkin S. L. (1985): Thermal fluctuations and capacitance of bilayer lipid membranes in a electric field. Biol. Membrany **2,** 820–831 (in Russian)

Lerche D., Hessel E., Donath E. (1979): Investigation of the La^{3+}-induced aggregation of human red blood cells. Stud. Biophys. **75,** 95–106

Lerche D. (1982): Spontaneous aggregation of washed human erythrocytes in isotonic media of reduced ionic strength. Conclusions about the spatial arrangement of the N-terminal part of glycophorins. Biorheology **19,** 587–598

Lerche D. (1983): The importance of the electrostatic and steric properties of the red cell membrane for RBC–RBC and RBC-surface interaction. Ann. N. Y. Acad. Sci **416,** 66–81

Lerche D. (1987a): Electrostatic fields and their influence on surface structure, shape, and deformation of red blood cells. In: Mechanistic Approaches to Interactions of Electromagnetic Fields with Living Systems. (Eds. M. Blank and E. Findl), pp. 349—364. New York, Pergamon Publish. Corp.

Lerche D., Kozlov M. M., Markin V. S. (1987b): Electrostatic free energy and spontaneous curvature of spherical charged layered membrane. Biorheology **24,** 23—34

Markin V. S., Kozlov M. M. (1984): Progress in Science and Technology. VINITI Biofizika Membran **3,** 49—53 (in Russian)

McLaughlin S., Szabo G., Eisenman G. (1971): Divalent ions and the surface potential of charged phospholipid membranes. J. Gen. Physiol. **58,** 667—687

Passechnik V. I., Hianik T. (1979): The measurement of elastic properties of lipid bilayers under different influences. Biofizika **24,** 460—464 (in Russian)

Paulitschke M., Cherny V. V., Lerche D., Kozlov M. M., Markin V. S. (1986a): Influence of rimantadine on erythrocyte shape. Stud. Biophys. **113,** 65—73

Paulitschke M., Lerche D., Meier W., Kucera W. (1986b): A method for determining parameters of dynamic deformation on single erythrocytes. Biol. Membrany **3,** 167—172 (in Russian)

Paulitschke M. (1987): Aufbau und Testung eines mikrorechnergestützten Verfahrens zur Bestimmung der Deformierbarkeit von Erythrozyten (Kapillar-Rigidometer). Dissertation A, Humboldt-Universität Berlin

Simonova M. V., Cherny V. V., Tulkes S. G., Markin V. S. (1984): The influence of adsorbed adamantane derivatives on the electric field distribution. Biol. Membrany **1,** 516—523 (in Russian)

Simonova M. V., Cherny V. V., Donath E., Sokolov V. S., Markin V. S. (1986a): Boundary potentials on bilayer membrane in the presence of rimantadine: Analysis of three methods of measurement. Biol. Membrany **3,** 846—858 (in Russian)

Simonova M. V., Cherny V. V., Sokolov V. S., Markin V. S. (1986b): The transport of neutral form of rimantadine through the planar bilayer lipid membrane. The role of pH gradient. Biol. Membrany **3,** 397—403 (in Russian)

Sokolov V. S., Kuzmin V. G. (1980): The measurements of the difference of the surface potentials of bilayer membranes with help of the second harmonic of capacity current. Biofizika **25,** 170—172 (in Russian)

Tverdislov V A., El-Karadagi S., Kharitonenkov I. G., Glaser R., Donath E., Hermann A., Lentzsch P., Donath J. (1985): Interaction of the antivirus agents rimantadine and amantadine with lipid membranes and the influence on the curvature of human red cells. Gen. Physiol. Biophys. **4,** 61—75

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