Biochimica et Biophysica Acta, 778 (1984) 276–280
Elsevier

BBA 72303

INTERACTION OF INFLUENZA VIRUS PROTEINS WITH PLANAR BILAYER LIPID MEMBRANES

II. EFFECTS OF RIMANTADINE AND AMANTADINE

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(Received December 6th, 1983)

Key words: Influenza virus protein; Membrane protein interaction; Amantadine, Rimantadine; Antiviral agents; Surface potential; (Lipid bilayer)

The dependence of the surface potential difference (ΔU), transversal elasticity module (E_1) and membrane conductivity (G_0) on the concentrations of the antiviral drugs, rimantadine and amantadine was studied in the planar bilayer lipid membrane system. The method used was based on independent measurements of the second and third harmonics of the membrane capacitance current. The binding constants of bilayer lipid membranes obtained from the drug adsorption isotherms were $2.1 \cdot 10^5$ M^{-1} and $1.3 \cdot 10^4$ M^{-1} for rimantadine and amantadine, respectively. The changes in G_0 took place only after drug adsorption saturation had been achieved. The influence of rimantadine and amantadine on the interaction of bilayer lipid membranes with matrix protein from influenza virus was also investigated. The presence of 70 μg/ml rimantadine in the bathing solution resulted in an increase in the concentration of M-protein at which the adsorption and conductance changes were observed. The effects of amantadine were similar to those of rimantadine but required a higher critical concentration of amantadine. The results obtained suggest that the antiviral properties of rimantadine and amantadine may be related to the interaction of these drugs with the cell membrane, which can affect virus penetration into the cell as well as maturation of the viral particle at the cell membrane.

Introduction

Adamantane derivatives amantadine (1-amino-adamantane hydrochloride) and rimantadine (α-methyl-1-adamantyl-methylamine hydrochloride) are used clinically for the prevention of influenza virus infection. At the present time, there is a variety of hypotheses on the nature of the antiviral effect of adamantane derivatives. It has been shown that the drugs affect either the early stages of the adsorption of viruses on the membrane and their penetration into the cell [1], or the later stages of virus macromolecular component synthesis and M-protein synthesis, in particular, by the infected cell [2]. The M-protein gene product of influenza virus has been associated with amantadine sensitivity in studies utilizing temperature sensitive mutants [3,4]. It has also been suggested that rimantadine acts on the stage of viral ribonucleoprotein deproteinization [5], i.e. M-protein separation from viral ribonucleoprotein on the nuclear membrane.

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In an earlier study, the influence of rimantadine on the packing and mobility of fatty acid chains of the phospholipids of the influenza virus membrane and artificial vesicular membranes made from the total lipids of influenza virus was studied [6]. It was shown that the drug decreases the temperature of phase transition in the virus membrane as observed with the aid of spin-labelled derivatives of stearic acid as probes [6]. This is probably a consequence of the penetration of rimantadine molecules into the hydrophobic regions of the lipid bilayer. The absence of this effect in artificial lipid membranes has led the authors to conclude that the drug affects lipid-protein interactions. In the presence of rimantadine, the spin label concentration in the membrane increased which suggests adsorption of the positively charged drug molecules on the membrane [6].

In our previous papers we studied the interactions of M-protein, total surface glycoproteins and neuraminidase of influenza virus with bilayer lipid membranes [7,8]. It was shown that bilayer lipid membranes may be used for modelling the main features of the viral protein interactions with the membrane of the infected cell, i.e., adsorption and incorporation.

In this paper, we report results of the interaction of rimantadine and amantadine with planar bilayer lipid membranes and the effects of rimantadine and amantadine with planar bilayer lipid membranes and the effects of rimantadine and amantadine on the interactions of influenza virus and M-protein with bilayer lipid membranes.

Materials and Methods

The techniques for preparation of influenza viruses and M-protein and the methodology employed for measurement of the surface potential difference on the membrane, the bilayer lipid membrane conductivity and its mechanical module are described earlier [8]. Amantadine was obtained from Sigma Chemical Co., and rimantadine was the kind gift of Dr. M.K. Indulen from Kirchenstein Institute of Microbiology, Latvian SSR Ministry of Health.

Results

The kinetics of surface potential difference, ΔU (the second harmonic of capacitance current), changes after two consecutive additions of rimantadine to one side of an asolectin bilayer lipid membrane as shown in Fig. 1. A saturation level is observed for ΔU (lower curve) after each addition of rimantadine; the current through the membrane being unchanged. The adsorption of amantadine on successive addition produces similar results.

Fig. 2 demonstrates the dependence of surface potential difference, i.e., the quantity of molecules adsorbed on the concentration of rimantadine (a) or amantadine (b). The adsorption of the drugs induces a positive potential on that side of the membrane to which they are applied. The membrane affinity for rimantadine is higher than that for amantadine and isothermal adsorption of rimantadine reaches a saturation at a concentration approximately 10-times lower than in the case of amantadine. In both cases, adsorption isotherms are not of the Langmuir type. After the surface of the asolectin bilayer lipid membrane has been saturated with the adsorbed molecules of either rimantadine or amantadine, the surface

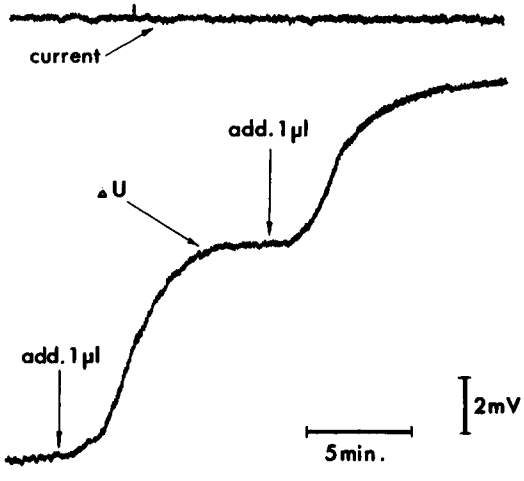

Fig. 1. The kinetics of surface potential difference, ΔU, changes after two consecutive additions of rimantadine to one and the same side of an asolectin bilayer lipid membrane. The arrows designate the time at which rimantadine was added. The rimantadine concentration is 12 µg/ml (1) and 15 µg/ml (2) The bilayer lipid membrane was formed in solution containing KCl (0.2 M), Tris buffer (5 mM, pH 7.4 at 20°C).

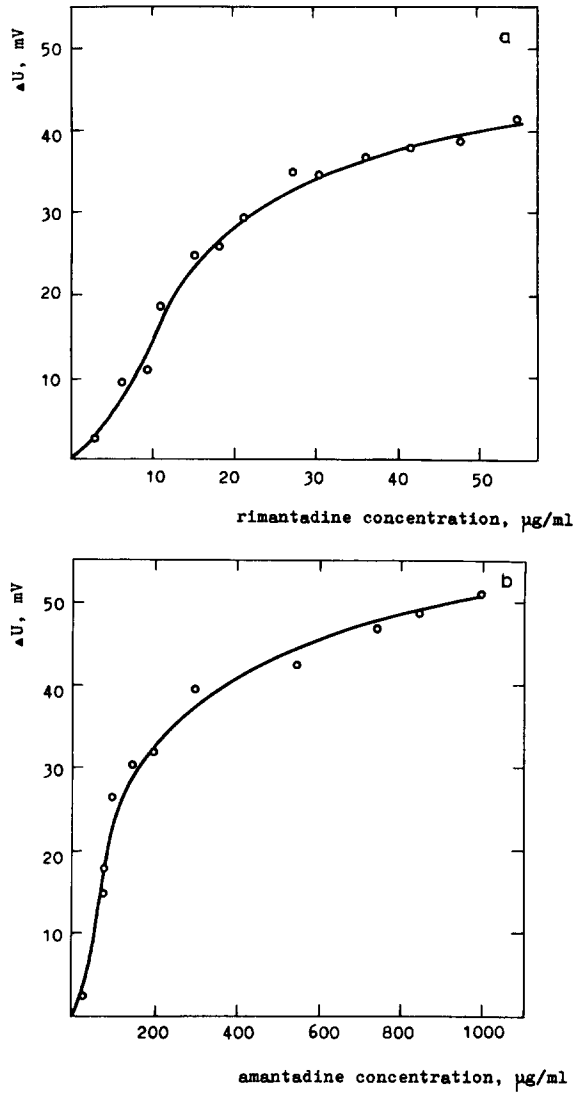

Fig. 2. The surface potential difference dependence on rimantadine (a) and amantadine (b) concentrations for an asolectin bilayer lipid membrane. See also Fig. 1.

potential difference equals approx. 50 mV in both cases.

The asolectin membrane conductance begins to increase at a rimantadine concentration of approx. 100 μg/ml and an amantadine concentration of approx. 800 μg/ml, i.e., after adsorption has reached saturation. The membrane conductance increased with concentrations of rimantadine up to 450 μg/ml and amantadine up to 1000 μg/ml. At higher concentrations, the membrane became unstable and was destroyed.

TABLE I

MODIFYING CONCENTRATION OF RIMANTADINE FOR BILAYER LIPID MEMBRANE WITH DIFFERENT COMPOSITIONS

Membrane composition	Rimantadine concentration (μg/ml)
Oxidized cholesterol	40
Cephalin + cholesterol	100
Asolectin	110

The influence of the membrane lipid composition on the minimal concentrations of the drugs modifying the membrane conductance was minor (Table I).

The elasticity module of an asolectin membrane was calculated from the third harmonic of the capacitance current increasing from the initial value of $7.2 \cdot 10^6$ Pa to the maximal value of $1.1 \cdot 10^7$ Pa at 100 μg/ml of rimantadine. Further increase of the rimantadine concentration did not produce any changes of the elasticity in spite of the increasing membrane conductance. At the amantadine concentration of 800 μg/ml, the elasticity module of the asolectin bilayer lipid membrane reached $8.6 \cdot 10^6$ Pa.

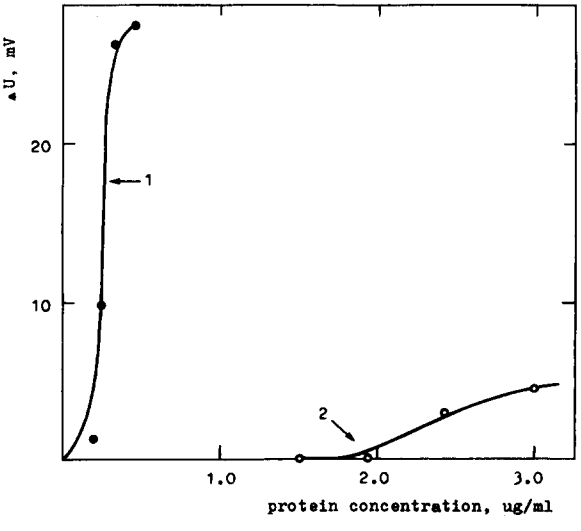

Fig. 3. The surface potential difference dependence on the concentration of M-protein in the absence (1) and presence of 70 μg/ml rimantadine (2). See also Fig. 1.

Rimantadine and amantadine affected the interaction of M-protein with bilayer lipid membrane. M-protein adsorption in the presence of rimantadine (curve 2 in Fig. 3) is decreased significantly as compared with adsorption in the absence of rimantadine (curve 1 in Fig. 3). Rimantadine also inhibited M-protein incorporation into the membranes so that modification of the membrane began at 5 μg/ml of protein as compared with 0.6 μg protein/ml in the absence of rimantadine (Fig. 4). All of the effects of rimantadine could be observed only if the rimantadine was added 10–15 min before the M-protein.

The effects of amantadine were found to be similar to those of rimantadine. At a concentration of 100 μg/ml of amantadine, the modifying concentration of M-protein was increased to 3.6 μg/ml.

Addition of native influenza viruses to the solution bathing the bilayer lipid membrane also resulted in an increase of membrane conductivity. Fig. 5a shows the kinetics of such changes for an oxidized cholesterol membrane. The membrane conductivity increases discretely with considerable fluctuations of the transmembrane current. Rimantadine inhibited the membrane modifying effect of influenza viruses (Fig. 5b). The critical modifying concentration of viruses in the absence of rimantadine was approx. 0.3 μg/ml (virus protein concentration) and 0.66 μg/ml in the presence of rimantadine at a concentration of 30 μg/ml.

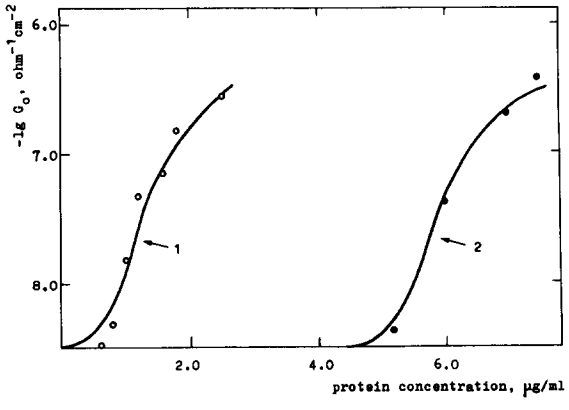

Fig. 4. The dependence of the asolectin bilayer lipid membrane conductance on the concentration of M-protein in the absence (1) and in the presence of 70 μg/ml of rimantadine (2). See also Fig. 1.

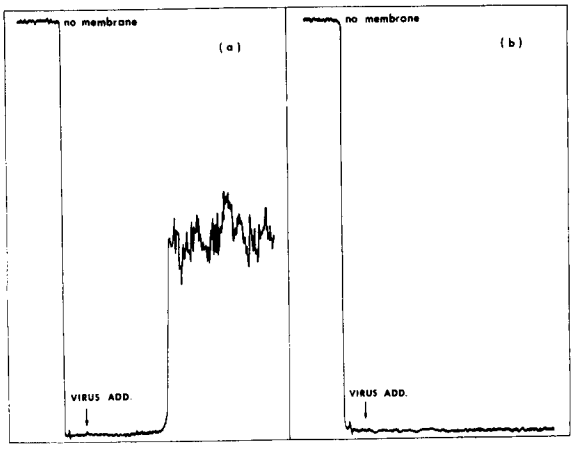

Fig. 5. The kinetics of the conductance changes of oxidized cholesterol bilayer lipid membrane in the presence of influenza viruses. Conductivity of the membrane: (a) in the absence and (b) in the presence of 30 μg/ml rimantadine.

Discussion

Rimantadine and amantadine molecules have a positive charge equal to 1 at neutral pH values. Therefore, on adsorption to the negatively charged surface of an asolectin bilayer lipid membrane, the drugs induce a positive electrical potential difference on the membrane. The hydrophobic radical of adamantyl is probably penetrating into the hydrophobic phase of the lipid bilayer. Rimantadine shows a higher affinity for the bilayer lipid membrane than does amantadine. This is probably a result of the higher energy of hydrophobic interactions in the case of rimantadine. This would result in deeper penetration of the rimantadine molecules into the bilayer. The incorporation of hydrophobic radicals into the bilayer may be suggested on the basis of our data on the membrane elasticity and conductance changes as well as earlier published data on structural changes in viral membranes caused by rimantadine [6].

To the best of our knowledge concerning the charge of the rimantadine and amantadine molecules, we can estimate the surface density of the adsorbed molecules. It was shown that after the

external compensation of the intramembranous electric field (experimentally, this can be observed as the disappearance of the second harmonic), the potential difference may be determined by the Hui-Chapman approach [9]. Using the experimental value of $\Delta U = 50$ mV which corresponds approximately to saturation of one side of the membrane with adsorbed molecules of rimantadine or amantadine and 0.1 C/m^2 for the initial surface charge density of asolectin bilayer lipid membrane [11], we obtain a value of $6 \cdot 10^{-2}$ C/m^2 for the surface charge. This density corresponds to approx. 300 $Å^2$ of the surface area per unit charge. This means that the average distance between adsorbed molecules each carrying a unit of positive charge is approx. 20 Å. The observation that the membrane surface saturation with adsorbed rimantadine and amantadine molecules is achieved at different drug concentration may be related to the different binding constants for these drugs.

The adsorption process may be considered as a reversible reaction

$$R + L \rightleftarrows RL$$

The saturation function for binding centers in this reaction is:

$$Y = \frac{K(L)^{n_H}}{1 + K(L)^{n_H}}$$

where K is the binding constant, L is the reactant concentration, and n_H is Hill's coefficient. The value of K calculated from this formula is $2.1 \cdot 10^5$ M^{-1} for rimantadine and $1.3 \cdot 10^4$ M^{-1} for amantadine. The cooperativity coefficient, n_H, for both compounds is the same and equals 1.33.

Rimantadine and amantadine antiviral activity are related to a number of factors as discussed in the Introduction. The change of the bilayer lipid membrane surface potential caused by adsorption of rimantadine or amantadine molecules hinders the M-protein binding on the bilayer lipid membrane surface and correspondingly its penetration into the bilayer (Figs. 3 and 4).

The change of the membrane surface charge density causes subsequent change of the electrostatic free energy of the bilayer and influences the phase behaviour of the lipid molecules [10]. Consequently, the possible influence of these drugs on the structure of the membrane should also be considered. Perhaps the incorporation of influenza viral proteins into the membrane may be influenced by the same factors.

It seems likely that penetration of the adamantyl radical into the lipid bilayer prevents the formation of a specific structure in the host-cell membrane which is necessary for formation and 'maturation' of the viruses. It is also possible that rimantadine and amantadine on being bound to M-protein complexes, change the structure of the complex to hinder penetration of the bilayer. It was shown in particular, that the affinity of M-protein from rimantadine-insensitive influenza viruses is lower than that for rimantadine-sensitive strains [5].

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