New Model of Charged Molecule Redistribution Induced in Spherical Vesicles by Direct Current Electric Field

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ABSTRACT A new electrophoresis model of charged components in a spherical phospholipid vesicle is proposed. In the new model the effective local tangential electric field is a result of the uniform external electric field modified by the electric field of redistributed charges. The modification is calculated on the basis of the Gouy-Chapman surface potential theory. Numerical calculations of steady-state distribution of charged molecules and the transmembrane potential are performed. The results show significant difference from the old, simplified model that neglects modification of the external electric field caused by redistributed charges.

INTRODUCTION

Free lateral diffusion of lipids and proteins in the membrane plane has been studied in a number of laboratories during the past decades. Many molecules that undergo long-range movements bear electric charges. Such membrane components, when subjected to electric field, are capable of moving within the plane of the membrane. This phenomenon, known as electrophoresis of membrane components, has been studied both theoretically and experimentally by a number of scientists (e.g., Jaffe, 1977; Poo and Robinson, 1977; Poo et al., 1979; Poo, 1981; Sowers and Hackenbrock, 1981). Electrophoresis and free lateral diffusion of charged molecules in the membrane lead to alterations in the transmembrane potential due to shifts of the membrane surface potentials.

The present knowledge of membrane component electrophoresis is based on equilibrium analysis of the phenomenon presented in 1977 by Jaffe, and on further analysis of the dynamics of the process carried out by Poo (Poo et al., 1979). The starting point for the model of equilibrium distribution of charged molecules is the equation for balance between electrophoretic and diffusional transport. Electrophoretic transport is attributable to the tangential field E_ϕ at the cell surface. In the model all interactions between the molecules are neglected, and E_ϕ is assumed to be attributable only to the external field E_0. No investigation of influence of the electric field associated with the induced surface charge redistribution has been carried out so far. It has been found as a result of calculations that this additional field, which always opposes the externally applied field, is not negligible in many situations. Therefore, in the present paper a new model of steady-state redistribution of charged membrane components caused by the external electric field is described. In this model local modification of the external electric field caused by the redistributed molecules is included. In the new model an effect of the ionic strength of the electrolyte on the redistribution is taken into account.

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THE MODEL

Let us assume a model of a cell that is a spherically shaped bilayer of radius a composed of molecules bearing zero net charge and $n\%$ population of molecules bearing a single electronic charge (negative in Fig. 1). The cell is also assumed to be immobilized by either adhesion to the solid surface of the substratum or by sufficiently high viscosity of the surrounding medium.

Additionally, we assume that the bilayer is immersed in electrolyte of orders of magnitude higher conductivity than conductivity of the membrane, which is always true under normal physiological conditions (Poo, 1981). If the external uniform electric field of magnitude E_0 is applied, the charged molecules on the outer surface of the bilayer encounter an electrophoretic force proportional to the tangential component E_ϕ of the electric field $\mathbf{E}_0$. Because the conductivity of the aqueous medium is orders of magnitude higher than that of the membrane, the electric field inside the cell is orders of magnitude smaller than that outside and its effects can be neglected. The system must satisfy the equation of continuity (Poo, 1981):

$$\frac{\partial\sigma(\phi, t)}{\partial t} + \nabla \cdot (m\mathbf{E}\sigma(\phi, t) - D\nabla\sigma(\phi, t)) = 0, \quad (1)$$

where $\sigma(\phi, t)$ is the time-dependent surface concentration of charged molecules, D is the diffusion constant, and m is the electrophoretic mobility. At equilibrium, back-diffusion and electrophoretic drag compensate, and because the surface concentration becomes time independent, the equation takes form

$$\frac{d\sigma(\phi)}{d\phi} - \frac{m}{D} \cdot a \cdot \sigma(\phi) \cdot E_\phi = 0. \quad (2)$$

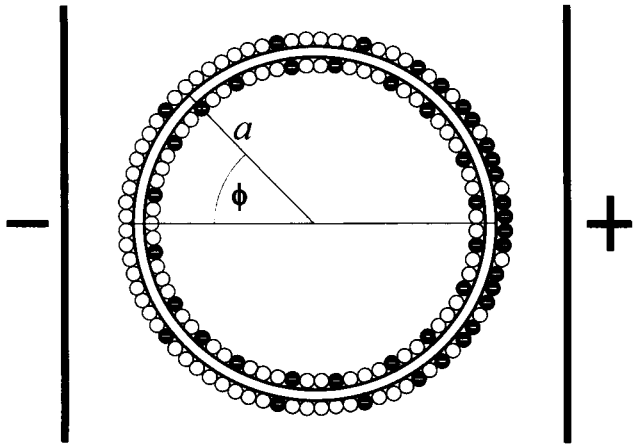

FIGURE 1 Schematic illustration of the equilibrium state distribution of negatively charged and neutral molecules on the inner and outer surfaces of the spherical vesicle membrane, induced by the external DC electric field.

The tangential component, E_ϕ, of the electric field is a sum of the tangential component of the external field $E_{0\phi}$ and the tangential component of the field generated by the redistributed charges $E_{\sigma\phi}$.

Introducing the additional field $E_{\sigma\phi}$ means taking into account the electrostatic interactions among charged molecules that was neglected in the previous model. For the cell as a nonconducting sphere (Cole, 1969),

$$E_{0\phi} = 1.5 \cdot E_0 \cdot \sin(\phi). \tag{3}$$

Neglecting $E_{\sigma\phi}$, we obtain the well-known solution to Eq. 2:

$$\sigma(\phi) = \sigma_0 \cdot \beta \cdot \operatorname{csch}(\beta) \cdot e^{-\beta \cdot \cos(\phi)}, \tag{4}$$

where $\beta = 1.5E_0am/D$, and σ_0 is the surface concentration with no external field present.

For such a solution the shape of the distribution function depends only on the external field intensity, E_0, the cell radius, a, and on the ratio of the electrophoretic mobility to the diffusion constant, m/D. According to that solution the shape of the function and the so-called asymmetry index, defined as

$$A = \frac{\sigma(0) - \sigma(180°)}{\sigma(0) + \sigma(180°)}, \tag{5}$$

depend neither on σ_0 nor on the ionic strength of the electrolyte.

Because $\mathbf{E}$ equals $-\nabla\Psi$, where Ψ is the electrostatic potential, the redistribution of charges on the surface of the membrane generates the electric field $\mathbf{E}_\sigma$. Because of an axial symmetry the tangential component of the field $E_{\sigma\phi}$ can be calculated as

$$E_{\sigma\phi} = -\frac{1}{a}\frac{d\Psi(\phi)}{d\phi}. \tag{6}$$

The electrostatic potential Ψ at the surface of the cell can be calculated on the basis of the Gouy-Chapman surface potential theory (McLaughlin, 1977). This theory assumes a uniform charge density at the membrane surface, which is not a case in our model. However, the use of the Gouy-Chapman theory can be justified, as the change in the charge distribution in the membrane plane at a distance on the order of the Debye length is negligible. This assumption of the theory will be discussed further (see Results and Discussion). For a symmetrical salt at 20°C, of ionic strength K in mol/liter and surface charge density σ expressed in the number of electronic charges per Å^2, the relation between surface potential and surface charge density takes form (Honig et al., 1986)

$$\Psi = 51 \text{ mV} \cdot \sinh^{-1}\left(\frac{136 \cdot \sigma}{K^{1/2}}\right). \tag{7}$$

Because $\sigma = \sigma(\phi)$, we can define a distribution function $C(\phi)$ of charged molecules as

$$C(\phi) = \frac{\sigma(\phi)}{\sigma_0}. \tag{8}$$

With the above definition the surface potential can be expressed as

$$\begin{aligned}\Psi(\phi) &= 51 \text{ mV} \cdot \sinh^{-1}\left(\frac{136 \cdot \sigma_0 \cdot C(\phi)}{K^{1/2}}\right) \\ &= k \cdot \sinh^{-1}(\Gamma \cdot C(\phi)),\end{aligned} \tag{9}$$

where $k = 51$ mV and $\Gamma = 136 \cdot \sigma_0/K^{1/2}$ are constants. Thus the $E_{\sigma\phi}$ is

$$E_{\sigma\phi} = -\frac{k \cdot \Gamma}{a((\Gamma \cdot C(\phi))^2 + 1)^{1/2}} \cdot \frac{dC(\phi)}{d\phi}. \tag{10}$$

Introducing the above expression and Eq. 3 into Eq. 2, we obtain

$$\begin{aligned}&\frac{dC(\phi)}{d\phi} - \beta \cdot \sin(\phi) \cdot C(\phi) \\ &\qquad + \frac{S}{(\Gamma^2 + 1/(C(\phi))^2)^{1/2}} \frac{dC(\phi)}{d\phi} = 0,\end{aligned} \tag{11}$$

where $S = k\Gamma m/D$.

When the ratio $136 \cdot \sigma_0/\sqrt{K}$ is relatively small, then $\Gamma < 1$ (for $K = 0.1$ mol/liter and $n = 5\%$ of charged molecules $\Gamma = 0.27$), and $\sinh^{-1}(\Gamma C(\phi))$ can be replaced by $\Gamma C(\phi)$, because $C(\phi)$ is on order of 1. With the above simplification Eq. 11 can be replaced by

$$\frac{dC(\phi)}{d\phi} - \beta \cdot \sin(\phi) \cdot C(\phi) + S \cdot \frac{dC(\phi)}{d\phi} \cdot C(\phi) = 0. \tag{12}$$

Transforming to the exponential form, the result of integration of the above equation gives

$$C(\phi) \cdot \exp(S \cdot C(\phi)) = \lambda \cdot \exp(-\beta \cdot \cos(\phi)). \tag{13}$$

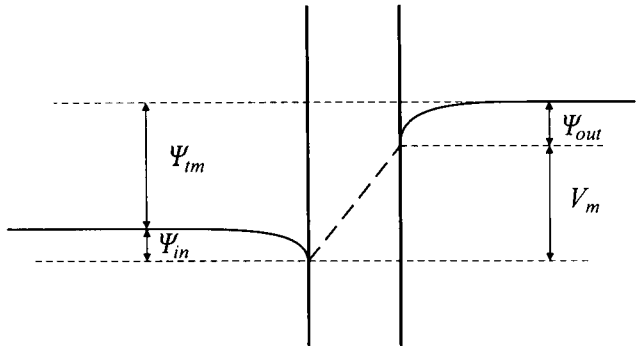

FIGURE 2 Schematic drawing of the electric potential across the membrane and its vicinity. Ψ_{tm}, transmembrane potential; Ψ_{in}, Ψ_{out}, inside and outside surface potentials; V_m, potential difference across the membrane.

The λ constant must be such that the total charge is preserved:

$$\int_0^{\pi} C(\phi) \cdot \sigma_0 \cdot \sin(\phi) d\phi = \int_0^{\pi} \sigma_0 \cdot \sin(\phi) d\phi = 2\sigma_0. \quad (14)$$

In the case of a weak electrolyte and high surface charge concentration, the $\Gamma < 1$ condition is no longer valid. However, for extremely low ionic strength of the electrolyte and a very high surface charge density, $\Gamma^2 \gg 1$ (for K = 0.01 mol/liter and n = 50% Γ^2 = 72). The term $(\Gamma^2 + 1/(C(\phi))^2)^{1/2}$ in Eq. 11 can be replaced by Γ, and the solution to the equation can be easily found as

$$\sigma(\phi) = \sigma_0 \cdot \beta_1 \cdot \mathrm{csch}(\beta_1) \cdot e^{-\beta_1 \cdot \cos(\phi)}, \quad (15)$$

where $\beta_1 = \beta \cdot (1 + k \cdot m/D)^{-1}$.

However, the case of extremely low ionic strength of the electrolyte and a very high surface charge density is of rather small physiological significance.

TRANSMEMBRANE POTENTIAL

The transmembrane potential Ψ_{tm} is the electric potential difference established between the bulk outside and inside aqueous phases of the cell (Fig. 2). One can write the simple relation between the transmembrane potential, the inner surface potential (Ψ_{in}), the outer surface potential (Ψ_{out}), and the trans bilayer potential (V_m):

$$\Psi_{tm} = \Psi_{out} + V_m - \Psi_{in}. \quad (16)$$

For the system at equilibrium with no external field present, the transmembrane potential can be calculated from the Nerst equation, as the concentrations and relative permeabilities of the various membrane-permeable ions are known. The value of the potential for biological systems is typically between 10 and 100 mV, with the inside polarized negatively relative to the outside (Honig et al., 1986).

Immediately after application of the external electric field, both the inside and the outside potentials change, which causes ions inside the cell to move along the field. When the accumulation of ions on the surfaces of the membranes establishes with a typical time constant of order of 0.3 μs for an erythrocyte (Kinoshita and Tsong, 1977), the inside potential becomes virtually constant. The degree of change in the transmembrane potential, however, depends on the surface charge redistribution, as shown by Gross (1988), because surface charge redistribution alters the surface potential of the outer layer of the membrane. Immediately after application of the field, before the redistribution occurs, the transmembrane potential is directly modified by the external field. The direct effect is described by (Kinoshita and Tsong, 1977)

$$\Delta\Psi_{tm0} = \Delta V_m = 1.5E_0 \cdot a \cdot \cos(\phi) \cdot (1 - e^{-1/\tau}), \quad (17)$$

where the time constant τ, as already mentioned, is on the order of a microsecond for an erythrocyte. Thus the process

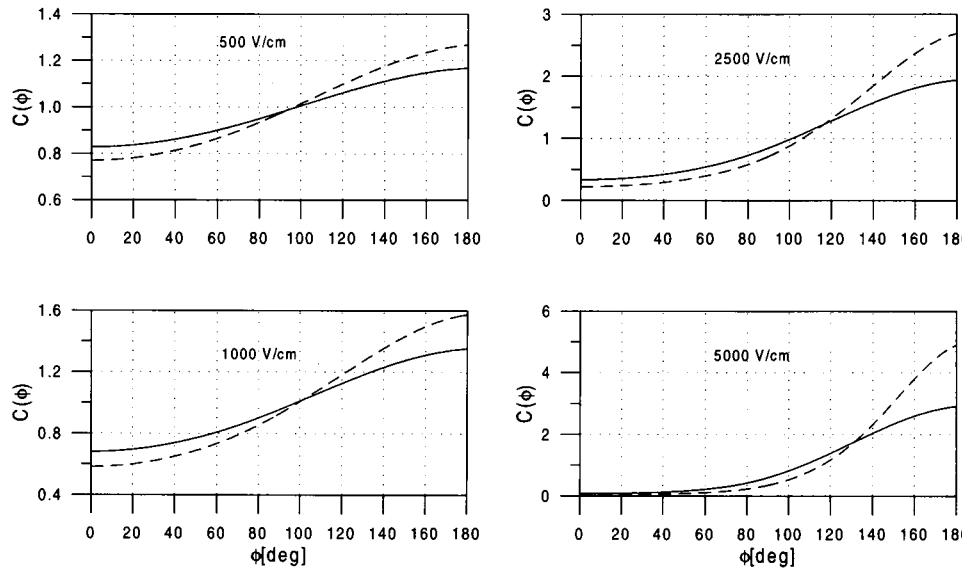

FIGURE 3 Equilibrium distributions $C(\phi)$ of charged molecules on the surface of the cell for various external electric field strengths. - - -, Distribution according to the model I, neglecting the electric field associated with the redistributed charges; ———, distribution according to model II. All calculations are for a spherical vesicle of 0.2 μm diameter, a charged lipid concentration of 5%, and an ionic strength of electrolyte of 100 mM.

TABLE 1 Asymmetry indexes for various electric field strengths

	500 V/cm	1000 V/cm	2500 V/cm	5000 V/cm
A1 (model I)	0.24	0.45	0.85	0.99
A2 (model II)	0.17	0.33	0.71	0.95

Ionic strength: 100 mM; charged lipid concentration: 5%.

TABLE 2 Asymmetry indexes for various ionic strengths

	5 mM	10 mM	100 mM	1 M
A2 (model II)	0.27	0.31	0.40	0.44
A1 (model I)	0.45	0.45	0.45	0.45

Field strength: 1000 V/cm; charged lipid concentration: 2%.

is very fast compared with the surface charge redistribution, which needs minutes or even hours to establish (e.g., Poo et al., 1979), and for analysis of the surface charge redistribution process the time-dependent exponential term in Eq. 17 can be dropped. When the surface redistribution is established, the surface potential difference opposes the direct effect, because the negative charges on the outer surface move to the side of the cell exposed to the positive electrode, and thus,

$$\Delta\Psi_{tm} = \Delta V_m + \Delta\Psi_{out}, \quad (18)$$

because $\Delta\Psi_{in} \approx 0$.

Thus the degree of change in the transmembrane potential depends on the surface charge distribution function. The analysis of the phenomenon presented by Gross is based on the simple redistribution model described at the beginning. Because the new model presented here gives significantly different distribution of surface charges induced by the external DC electric field, the transmembrane potential change was also recalculated.

RESULTS AND DISCUSSION

The surface charge density function $C(\phi)$ resulting from our new model (model II) was found numerically with the use of MathCad5+ software, for various charge densities, ionic strengths, and external electric field intensities. The results were compared with the surface charge density obtained for the previous, simplified model (model I), according to Eq. 4. The calculated $C(\phi)$ function for various electric field strengths is presented in Fig. 3. For the calculations it was assumed that the cell was a large unilamellar liposome of 0.2 μm diameter, composed of 95% lipids bearing zero net charge and 5% single-charged lipids. The electrolyte was assumed to be a 100 mM solution of NaCl. The ratio of the electrophoretic mobility and the diffusion constant (m/D) was 33, which is a typical value reported in the literature (Poo, 1981; Zimmermann, 1982). Asymmetry indexes for distributions a, b, c, and d obtained for electric fields 500 V/cm, 1000 V/cm, 2500 V/cm, and 5000 V/cm, respectively, are summarized in Table 1.

Because $\beta = 1.5E_0am/D$, the same results for $C(\phi)$ can be obtained for a 20-μm-diameter cell and field strengths of 5 V/cm, 10 V/cm, and 50 V/cm, respectively, which are typical field intensities used experimentally for studying electric field influence on cells (e.g., Poo, 1981; Poo et al., 1979; Ryan et al., 1989; Stollberg and Fraser, 1989; Rajnicek et al., 1994).

The influence of the ionic strength of electrolyte is presented in Fig. 4. For the calculations it was assumed that 2% of lipids bear a single charge and that E_0 = 1000 V/m. Asymmetry indexes for various ionic strengths of electrolyte are presented in Table 2.

It is apparent from both the plots and the above table that for electrolytes of the ionic strength smaller than 100 mM the difference between the two analyzed models is signifi-

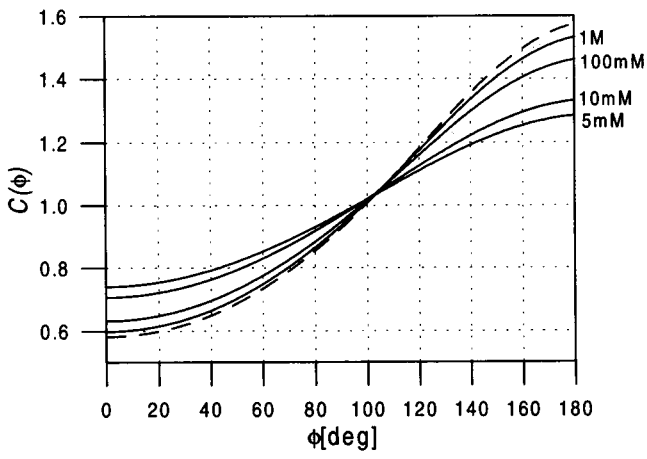

FIGURE 4 Equilibrium distribution $C(\phi)$ of charged molecules on the surface of the spherical vesicle for various ionic strengths of electrolyte. External field strength (1000 V/cm) and lipid concentration (2%) were kept constant. - - -, Distribution according to model I.

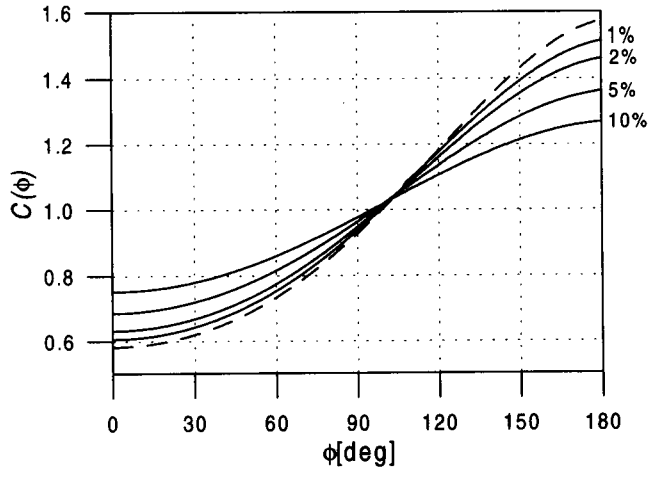

FIGURE 5 Equilibrium distribution $C(\phi)$ of charged molecules on the surface of the spherical vesicle for various concentrations of charged lipids. External field strength (1000 V/cm) and ionic strength (100 mM) were kept constant. - - -, Distribution according to model I.

TABLE 3 Asymmetry indexes for various charged lipid concentrations

	1%	2%	5%	10%
A2 (model 2)	0.43	0.40	0.33	0.26
A1 (model 1)	0.46	0.46	0.46	0.46

Field strength: 1000 V/cm; ionic strength: 100 mM.

cant, even for charged lipid concentration as low as 2%. This fact is a direct implication of the Gouy-Chapman electrostatic theory. As was mentioned before, the Gouy-Chapman model assumes a uniform charge density. In our model the charge density changes within the membrane plane. This change, however, is not significant at the distance of the Debye length, which is typically on the order of nanometers. Assuming the 3-nm Debye length and the 15-μm radius of the cell, and taking the highest calculated gradient of the charge density (see Fig. 3) one can calculate, the change in the charge density in the membrane plane over the Debye length is smaller than 0.025%. This means that the use of the Gouy-Chapman theory in our model seems to be justified. The electrostatic interactions between charged lipids on the surface of the cell and ions of the electrolyte cause an increase in the concentration of the counterions and a decrease in the concentration of coions near the surface of the cell. The charge density gradient in the membrane plane is orders of magnitude smaller than that in the direction perpendicular to the membrane surface. This means that the shape of the ion cloud surrounding the cell is determined mainly by the surface charge density and that the effect of the external electric field on the shape of the ion cloud can be neglected. The changes in the concentration of coions and counterions result in a decrease in the surface potential, according to Eq. 7. Because the surface charge density with no field present (σ_0) directly affects the surface potential, the difference between the two models increases with the increase in σ_0. The influence of the charged lipid concentration on the distribution $C(\phi)$ is shown in Fig. 5, and the asymmetry indexes of the distributions are presented in Table 3.

Even this simple analysis of the influence of the charged lipid concentration and ionic strength of the electrolyte on the redistribution of the charged membrane components induced by externally applied electric field shows that the effects discussed above cannot, in many cases, be neglected. Only in the case where surface charge density is very low or the ionic strength of the electrolyte is very high do the distributions of charged molecules become similar in the two models.

The dependence of the transmembrane potential change $\Delta\Psi_{tm}$ on the electric field strength was calculated according to Eq. 18. For the calculations it was assumed that the concentration of the charged lipids is 10%, and that the ionic strength of the electrolyte is 100 mM. The transmembrane potential change was calculated knowing the distribution function $C(\phi)$ and the relation between the surface charge density and the surface potential, as given by Eq. 7. The results were compared with the transmembrane potential change calculated with the use of the simple redistribution model, as was done by Gross. The results of the calculations are presented in Fig. 6. Furthermore, the direct effect described by Eq. 17 is plotted to indicate the compensating effect of surface charge redistribution. As one can see from the plots, the compensating effect is much smaller than that expected from the simple model of redistribution, because the degree of redistribution can be significantly smaller.

CONCLUSIONS

In the old model of the surface charge redistribution in the cell membrane induced by the external DC electric field, it was assumed that the local electric field seen by a charged molecule is time independent. In the new model the local electric field is a sum of the external electric field and the

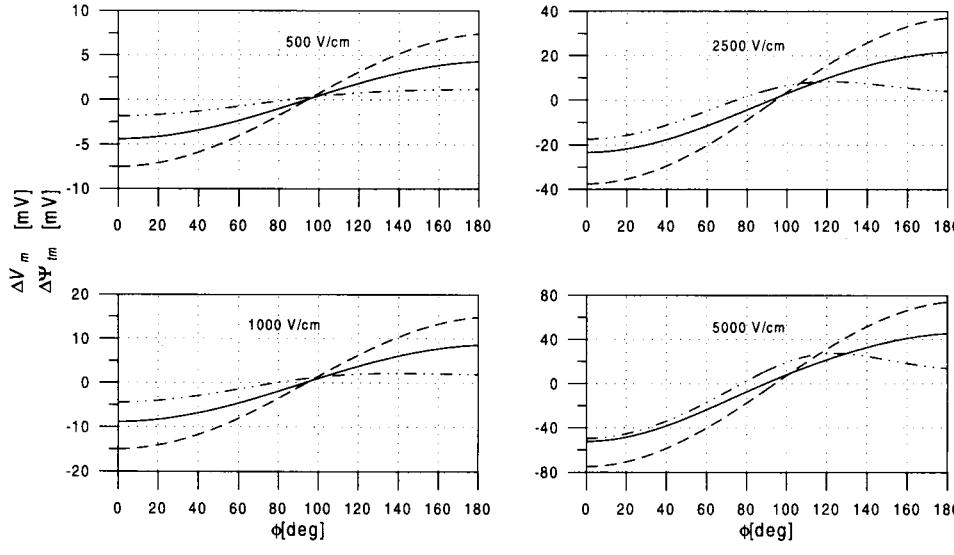

FIGURE 6 Change in the transmembrane potential ($\Delta\Psi_{tm}$) for the new model II (———) and for the model I (– · · –) compared with the direct effect of change in the potential ΔV_m across the membrane change (- - -). The calculations were performed for various external field strengths. Charged lipid concentration (10%) and ionic strength of electrolyte (100 mM) were kept constant.

field that originates from the charged molecule gradient. This field is time dependent and decreases during the process of establishing the distribution of charged membrane components. The local field reaches a minimum at the equilibrium state. The two models give the same local electric fields at the moment of turning on the external electric field. In the time course used, the local fields calculated according to the two models diverge. This leads to different steady-state distributions of charged molecules in the membrane. The difference depends on the charged molecule concentration, ionic strength of the electrolyte, and the external electric field intensity. In most cases that difference cannot be neglected. The difference is especially pronounced in the presence of a strong external electric field. In this case the old model gives an unrealistic angular dependence of the transmembrane potential change, which has a maximum at some intermediate angle (approximately 120°, Fig. 6, 5000 V/m electric field strength), rather than at the pole exposed to the positive electrode ($\phi = 180°$). Thus it can be concluded that the effect of charge distribution gradient on the local field that is seen by the charged molecules should always be taken into account when studying the effect of the external DC electric field on the distribution of charged molecules on a surface of a cell.

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