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Brief Communication

Electroporation of a Lipid Bilayer as a Chemical Reaction

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When a cell's transmembrane potential is increased from a physiological one to more than 370 mV, the transmembrane current increases more than hundredfold within a millisecond. This is due to the formation of conductive pores in the membrane. We construct a model in which we conceive of pore formation as a voltage sensitive chemical reaction. The model predicts the logarithm of the pore formation rate to increase proportionally to the square of the voltage. We measure currents through frog muscle cell membranes under 8 ms pulses of up to 440 mV. The experimental data appear consistent with the model. Bioelectromagnetics 25:634–637, 2004. © 2004 Wiley-Liss, Inc.

Key words: electroporation; pore formation; pore kinetics

INTRODUCTION

A cell membrane is about 5 nm thick and consists for the most part of a bilayer of phospholipid molecules. The polar phosphate groups of these phospolipids are directed toward the aqueous intracellullar and extracellular solution. In the middle of the bilayer, the apolar tails, consisting of 16–18 carbons, are directed toward each other. The membrane is held together only by hydrophobic and hydrophylic forces, but it is nevertheless a remarkably robust structure. Cells generally use the available energy to generate a transmembrane potential of about 100 mV, that is, a field of several tens of megavolts per meter. But even under this strong field, the membrane remains essentially impermeable to water and ions.

Intracellular and extracellular fluid are very good conductors, so any externally imposed electrical potential across living tissue will be distributed over cell membranes. When a human being touches a kilovolt source with the hand, muscle cell membranes in the arm can be subjected to potentials of more than half a volt. Under such a voltage, the membrane integrity can be lost with the onset of formation of small pores. When electroporation is too widespread or when electropores become too large or too stable, intracellular and extracellular fluid start mixing and the cell will die. In a laboratory setting, single cells are routinely placed in electric fields if they are to be opened up temporarily so certain foreign material, like DNA, can be inserted. In such procedures, the main challenge lies in finding the right compromise between minimizing the damage and creating sufficiently durable and large openings. Finally, electroporation is supposed to play a major role in the workings of electro shock therapy.

To study electroporation experimentally, one needs a way to control the voltage across a patch of cell membrane and measure the resulting current. The challenge lies in constructing a setup in which the seals (where the membrane adheres to the device) are sufficiently robust to withstand voltages of over 400 mV. A long cylindrical muscle cell in a double vaseline gap leads to good results [Chen and Lee, 1994; Bier et al., 2002]. A muscle cell of about 0.5 mm in length is clamped all

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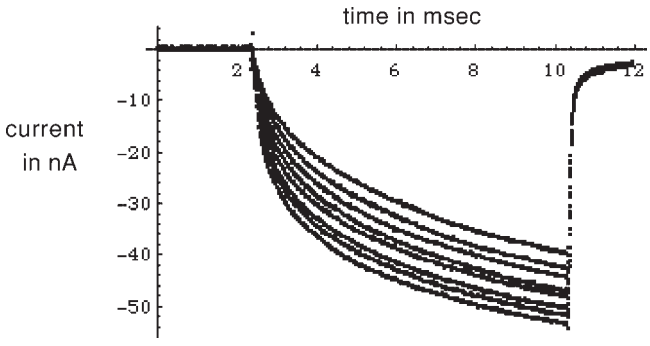

Fig. 1. A series of 8 ms pulses of amplitude 370 – 440 mV (in steps of 10 mV) is applied across the membrane of a frog muscle cell in a double vaseline gap. The electroporation currents that result from these pulses are shown.

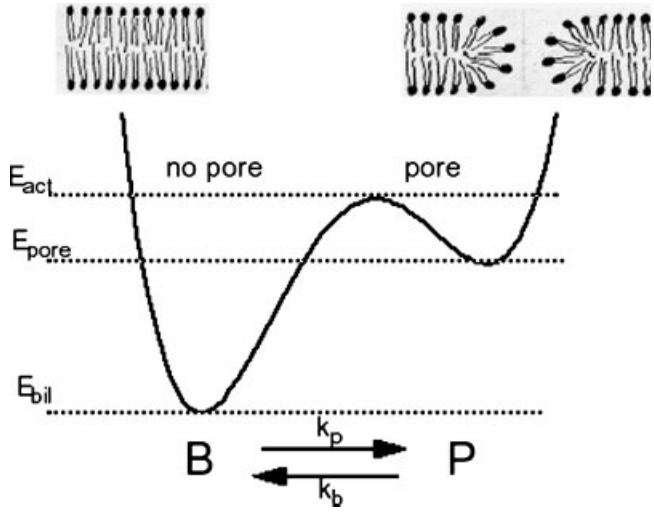

Fig. 2. The top shows how pore formation is a rearrangement of a number of phospholipids in a bilayer. We view the process as a chemical reaction with an activation barrier at E_{act}. B stands for the bilayer state and E_{bil} is the energy of this state. P represents the pore state and E_{pore} is the energy of the pore state. The rates for pore creation (k_p) and pore destruction (k_b) follow Equation 2. The energy difference $E_{pore} - E_{bil}$ involves a term proportional to V^2.

around on both ends. Both end tips are next permeabilized and the cell is then essentially a tube with two open ends in which the electric potential between inside and outside can be controlled. Figure 1 shows the result of a typical experiment. For 8 ms, the transmembrane voltage is stepped up from a rest voltage of 90 mV to a shock voltage. The picture shows currents through one and the same patch for shock voltages from 370 to 440 mV (in 10 mV steps). Ion channels were blocked in this experiment and capacitive currents were subtracted. The higher the voltage, the larger the electroporation current is relative to the capacitive current. For the high voltage (>370 mV) that we consider, shock to shock variations in the capacitance are no longer a significant source of noise. What the graph shows is purely the result of electroporation. During the first millisecond of the shock, the transmembrane conductance increases by a factor of more than 100. For a complete description of the materials and methods, we refer to Chen and Lee [1994].

MODEL

Figure 2 shows how pore formation can be thought of as a chemical reaction. A number of lipids rearrange themselves and the process is analogous to a conformational change of a protein. During the first millisecond of the stepped up electric potential, the increase appears linear for all of the shock voltages in Figure 2. In a recent study [Bier et al., 2002], we present evidence that the vast majority of pores in this type of experiment is just above the necessary minimum radius to permit single-file passage of water and small ions (Na^+, K^+, Cl^-). This minimum radius is generally assumed to be about 0.3 nm [Hille, 1992]. Growth of the pore radius in the course of the shock is, in principle, possible. Such growth would be a result of just the Brownian motion of the lipid molecules in the pore lining. However, growth like that is unlikely, as it would face a lot of frictional resistance and would, furthermore, have to go against a steep energy gradient.

The energy profile of a chemical reaction can be depicted with a so-called reaction coordinate or reaction parameter (Fig. 2). For a segment of membrane, the minimum on the left represents a bilayer arrangement and the minimum on the right represents a pore arrangement. The maximum in the middle represents the activation barrier for the transition.

In modeling the shock in our double vaseline gap setup, we take the entire membrane as being initially, at $t = 0$, in the bilayer configuration. When the transmembrane potential is stepped up, the energy levels of Figure 2 change and significant pore formation, that is, $B \rightarrow P$, starts. The linear increase of the current at the beginning of the shock indicates that pore formation is effectively a one step process. If it is in fact a multistep process, then the observed linearity indicates that one step is rate limiting. If pore formation were a process involving n steps of similar duration, then the current would have increased as $I(t) \propto t^n$. From the mere fact that the slope of the initial current increase depends on the shock voltage, we infer that the rate limiting step of pore formation is voltage sensitive.

Eventually the "pore well" P will fill up to a level where pore creation ($N_B k_p$) equals pore destruction ($N_P k_b$). Approaching this equilibrium corresponds to the electroporation current asymptotically approaching

a constant level. It is technically impossible to impose a shock voltage long enough to see an accurate approximation to this level. The seals degrade, stable leaks are formed and permanent damage accumulates long before a constant electroporation current is reached. The results presented in this study are based on two of many experiments in which the seals and the membrane held up under the repeated 4 or 8 ms shocks.

In a recent publication [Bier et al., 2002], it was, furthermore, derived that, even at the highest electroporation currents, the pores occupy a negligible fraction (10^{-8}) of the entire membrane surface area. Let N be the number of pores that a membrane can maximally sustain. We then have $N_B + N_P = N$, where N_P is the number of pores. N_B can be thought of as the number of potential pores. With the 10^{-8} fraction, it is obvious that $N_P \ll N$, and hence $N_B \approx N$ at all times during the shock.

It is hard to come to an estimate of the barrier height $E_{\text{act}} - E_{\text{bil}}$ for pore formation. However, we can describe how $E_{\text{bil}} - E_{\text{pore}}$ depends on the shock voltage V. When there is no pore, electric field lines are directed perpendicularly to the membrane surface. Water has a much higher dielectric permittivity ($\varepsilon_w = 80$) than lipid ($\varepsilon_t \approx 2$), so when a pore is filled with water the entire system can lose energy if field lines "bend" to go through the pore (Fig. 2). At first order in the pore radius r the total pore energy equals

$$E(r) = (2\pi\gamma - \varepsilon_0\varepsilon_w V^2)r. \quad (1)$$

Here γ represents the line tension, that is, the energy necessary to create one unit length of highly curved pore lining [Litster, 1975]. The second term describes the energy that is released when the electric field lines reconfigure themselves. This term was derived in 1987 by Winterhalter and Helfrich [1987]. The derivative $-dE(r)/dr$ represents the force towards increasing pore radius. As V is changed, it is the second term in Equation 1 that is being varied. The transition rates obey the relation:

$$\frac{k_p}{k_b} = \exp\left[-\frac{(E_{\text{pore}} - E_{\text{bil}})}{kT}\right] = \exp\left[-\frac{E(r)}{kT}\right], \quad (2)$$

where k represents the Boltzmann constant and T represents the absolute temperature. This equation guarantees that at the Boltzmann equilibrium, that is, $N_P/N_B \approx \exp[-(E_{\text{pore}} - E_{\text{bil}})/kT]$, the pore creation $N_B k_p$ is equal to the pore destruction $N_P k_b$. From Equation 2, we derive $\ln k_p - \ln k_b = C + \varepsilon_0\varepsilon_w V^2 r/kT$, where C is a constant that involves, among other things, the line tension γ and the pore radius r. The voltage dependence on the right hand side is apportioned over the two transition rates, that is,

$$\begin{aligned} \ln k_p &= \alpha(C + \varepsilon_0\varepsilon_w V^2 r/kT) \text{ and} \\ \ln k_b &= -\beta(C + \varepsilon_0\varepsilon_w V^2 r/kT), \end{aligned} \quad (3)$$

where, obviously, $\alpha + \beta = 1$. An α and β emerge whenever there is a variation of the energy difference between reactant(s) and product(s) that is to be distributed over forward and backward transition rates. The α and β are often called symmetry factors [Bockris and Reddy, 1977]. Mathematically, α and β can take on any value within their degree of freedom. However, in practice, it turns out that if the energy level of the reactant state or the product state goes up, then the level of the activation barrier in between goes up with it part of way. The α's and β's are generally found to fall in a range between 0.2 and 0.8 [Bockris and Reddy, 1977]. We, furthermore, assume that α is independent of the voltage V.

RESULTS AND DISCUSSION

Given our model, we can determine the value of α from the experimentally found dependence of $(dI/dt)(t \to 0) = \dot{I}_{t\to 0}$ on V^2. From $I(t \to 0) \propto N_P(t \to 0) \propto k_p t$, we infer $\dot{I}_{t\to 0} = A k_p$, where A is a constant. We thus have $\ln \dot{I}_{t\to 0} - \ln B = \alpha\varepsilon_0\varepsilon_w V^2 r/kT$, where the constant B has absorbed both the constants A and C. We can use the value of $\dot{I}_{t\to 0}$ at 370 mV to fix the value for B:

$$\ln\left\{\frac{\dot{I}_{t\to 0}(V)}{\dot{I}_{t\to 0}(V = 370\,\text{mV})}\right\} \propto \alpha\frac{\varepsilon_0\varepsilon_w r}{kT}V^2. \quad (4)$$

Figure 3 shows how the left hand side of Equation 4 varies with V^2 for the experiment depicted in Figure 1. The data points appear to well fit the predicted straight line. A least squares fit leads to a slope of 18.0 V^{-2}. For $r = 0.3$ nm, $\varepsilon_0 = 8.8\ 10^{-12}$ C^2/Nm^2, and $\varepsilon_w = 80$

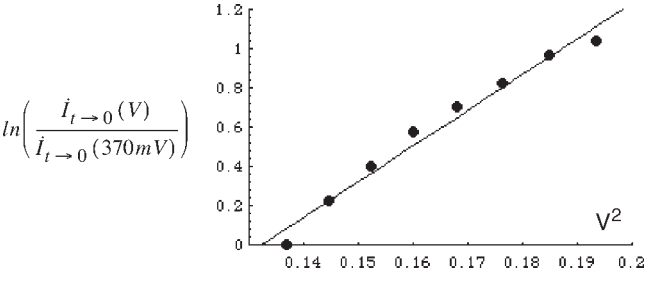

Fig. 3. The logarithm of the slope *dI/dt* right after the transmembrane potential's elevation at $t = 0$ versus the square of the elevated potential. Data points were taken from the experiment depicted in Figure 1 and appear to largely follow the straight line that the model predicts.

this leads to an estimate of $\alpha = 0.36$. The other successful experiment returned a value for the slope of 11.1 V^{-2}, which leads to $\alpha = 0.22$.

The model is successful in that the parameter α is indeed found to be close to 1/2. We thus obtain an indication that transient electroporation indeed involves for the most part pores with a radius of about 0.3 nm, that is, just large enough to let water and small ions through. Pore formation appears to be well described as a chemical reaction with a genuine activation barrier. Moreover, the first linear term in the pore radius r is sufficient to account for the observed quadratic voltage dependence of the energy of transient electropores.

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