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The conductance of cellular membranes at supra-physiological voltages

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ABSTRACT

Membrane permeabilization by pulsed electric fields (electroporation), that is supposed to be caused by the formation of aqueous pores, is widely used in biomedicine and biotechnology. It is detected most precisely by measuring membrane conductance. When whole-cell patch-clamp experiments are used to screen a wide voltage range, poration becomes manifest by large currents elicited at extreme hyper-/depolarization. The slope conductance, G_{slope}, can be obtained from non-linear current–voltage relations by differentiation of the current–voltage curve. Alternatively, the chord conductance, G_{chord}, is defined as the slope of straight lines connecting each point on the current–voltage curve with the zero-current (reversal) potential on the voltage axis. Here, Boltzmann functions were fitted to plots of G_{chord} versus voltage recorded on protoplasts from bright-yellow-2 tobacco cells. These plots are supposed to reflect transition from a non-porated to a porated membrane state. Consistently, G_{chord} saturated at extremely negative and positive voltages at values well below those expected for a complete demolition of the membrane (half-maximum voltages: ~−332 mV and +294 mV, respectively). The slope factor allowed inferring the change in dipole moment associated with water intrusion into the bilayer. It was $-6.19\ 10^{-4}$ and $3.35\ 10^{-4}$ C∗m∗mol^{-1}, respectively. Outside-out patches rendered similar results, but half-maximum voltages were shifted to more extreme voltages with respect to whole-cell experiments.

1. Introduction

Exposure of cells to strong pulsed electric fields is known to induce a transient or permanent increase in the conductance of the cellular membrane. This effect is usually explained by the formation of aqueous pores in the membrane, known as electroporation. Electroporation is a versatile tool in biotechnology and biomedicine, which is employed, among other things, for introduction of DNA into cells, for triggering apoptosis in tumour cells or for decontamination of wastewater. The first application requires the cells to recover from the treatment and integrate DNA into their genome (reversible electroporation), whereas the remaining ones aim at a permanent destruction of the cells (irreversible electroporation).

The membrane conductance increase elicited by a pair of external electrodes has been monitored in detail by means of impedance spectroscopy. Impedance is a very useful parameter to monitor e.g. the kinetics of pore formation and subsequent pore closure upon a treatment with pulsed electric fields ([1–4]), but the technique is not suitable for a more detailed analysis of membrane processes at the single-cell level. This is because the impact of an electric field on a cell is highly inhomogeneous and depends on the local orientation of the membrane with respect to the field (for review see e.g. [5]). An alternative for studying the effects of field exposure on the membrane conductance is provided by the whole-cell configuration of the patch clamp technique [6–9]; by establishing an electrical access to the cell interior via a fine-tipped glass electrode, the membrane can be clamped homogenously to voltages that exceed by far the physiological voltage range. By recording the current response, single-cell current–voltage curves can be obtained that reflect, among other things, the voltage dependence of pore formation.

The focus of this communication will be on the voltage dependence of the membrane conductance that can be extracted from current–voltage curves in two ways: The slope conductance, G_{slope}, is effectively the first derivative of the current–voltage relation. Alternatively, the conductance can be calculated using an ohmic approximation: For every point on the current–voltage curve, the current level is divided by the respective voltage (termed chord conductance, G_{chord}). Zero voltage is set to the potential at which no current passes through the pores (reversal potential, E_{rev}); this allows a mechanistic interpretation of G_{chord}-values in terms of currents passing through a variable, field-dependent number of individual electropores [10]. The reversal potential has to be determined separately by applying voltage ramp protocols [7–9]. Plots of chord

Abbreviations: ATP, adenosine triphosphate; BTP, bis-tris-propane; BY-2, bright yellow-2; d, membrane thickness; EGTA, ethylene glycol tetraacetic acid; E_M, electric field across the membrane; E_{rev}, reversal potential; Mes, 2-(N-Morpholino)ethanesulfonic acid; SEM, standard error of the mean; M, dipole moment difference between non-porated and porated membrane; G_{slope}, slope conductance; G_{chord}, chord conductance; U_M, membrane potential.

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conductance versus voltage will be analysed here in terms of a thermodynamic approach introduced by Neumann et al. [11], that is based on a successive, electric field-induced transition from a non-porated to a porated state of the membrane. Note that this approach bears some similarity with the formalism used to analyse the voltage-dependent open probability of ion channels (compare e.g. [12]).

2. Materials and methods

2.1. Experimental

Experiments were performed on protoplasts prepared from the tobacco cell line bright yellow 2 (BY-2). Cell culture, protoplast isolation, electrophysiological equipment and the patch clamp procedure were described in more detail previously [7]. Patch clamp media were composed as follows (concentrations in mM): bath, 30 K-gluconate, 5 $CaCl_2$, 2 $MgCl_2$, 10 Mes/BTP with pH adjusted to 5,8; pipette, 120 K-gluconate, 10 EGTA, 2.68 $MgCl_2$, 3.91 $CaCl_2$, 2.13 Mg-ATP, 2 Mes, pH adjusted to 7.2 with BTP (free Ca^{2+}: 0.1 μM, free Mg^{2+}: 2 mM, Mg-ATP: 2 mM). The organic buffers Mes and BTP are used for pH adjustment in different pH ranges (around 5–6 and 6–9, respectively). Osmolality of both solutions was adjusted to 472 mosmol/kg by adding mannitol. Adjustment of the osmotic pressure is required to avoid osmotic swelling of protoplasts with electrolyte concentrations being asymmetric between pipette and bath media. Asymmetric ionic composition of pipette and bath solution was chosen to mimic conditions in intact cells when treated with pulsed electric fields. Voltage was controlled by means of an EPC-10 patch clamp amplifier, and data were recorded and partly evaluated using Patchmaster software (HEKA electronics, Lambrecht, Germany). A sequence of 10-ms voltage pulses usually ranging from −1000 mV to +1000 mV was applied with alternate polarity, starting at the extreme values. Between successive pulses, the membrane was clamped to 0 mV or −80 mV for 20 s (holding potential). The whole voltage range was scanned at increments of 40 mV. The current level at the end of each voltage pulse was averaged over a short segment (about 500 μs). Currents were plotted against the actual membrane potential that was calculated from the clamped voltage as described and verified experimentally in previous publications [7,8]. Briefly, when the voltage imposed by the amplifier was stepped by a certain value ΔU_{clamp} away from the holding potential, the fraction that actually dropped at the membrane (U_M (t)) could be calculated as a function of time using the following equation (based on the assumption that $U_M \approx U_{clamp}$ at the holding potential):

$$U_M(t) = U_{clamp} - (I(t)/I_0)\Delta U_{clamp}. \quad (1)$$

I(t) is the recorded current response of the cell and I_0 is the initial amplitude of the capacitive current spike, that is obtained by fitting an exponential relation to the time course of current relaxation after stepping U_{clamp} to a new value:

$$I_{cap}(t) = I_0 * \exp(-t/\tau) \quad (2)$$

with I_{cap} being the capacitive current and τ being the time constant of current relaxation. The validity of the approach was tested experimentally by measuring the voltage drop across the membrane with a voltage-sensitive fluorescent probe, ANNINE-6. Membrane potentials calculated according to Eq. (1) perfectly matched fluorescent intensities recorded on cells stained with this dye when both parameters were plotted as a function of U_{clamp} [8].

Further processing of the data after correction for the voltage drop at the access resistance, including the fitting of equations (see below) with the least square method, was performed with the graphic programme 'Origin' (Originlab, Northampton, MA, USA).

2.2. Theory

Following Neumann et al. [11], electroporation can be described by a transition of the membrane from a non-porated to a porated state induced by the electric field:

$$C \longleftrightarrow P.$$

Both symbols also denote the fractions of the membrane surface attributed to these states. Furthermore, it is supposed that the membrane conductance $G_{chord}(U)$ is a measure of P ($G_{chord}(U)$ α P). $G_{chord}(U)$ is calculated in the following way:

$$G_{chord}(U) = \frac{I(U)}{U_M - E_{rev}}. \quad (3)$$

With U_M being the membrane potential and E_{rev} the reversal potential of open pores. Note that for I(U) only currents passing through electropores are considered, i.e. background currents due to ion channel activity and an imperfect seal are linearly extrapolated and subtracted from the overall current values before calculating G_{chord}. When the whole membrane surface is in the porated state, a maximum chord conductance, ($G_{chord,max}$) is attained. Hence, *C* is proportional to ($G_{chord,max} - G_{chord}(U)$). The ratio of the two states is then:

$$f = \frac{G_{chord}(U)}{G_{chord,\,max} - G_{chord}(U)}. \quad (4)$$

Eq. (4) is based on the assumption that the background conductance of the membrane in the absence of pores is negligible. Note that *f* can be expressed by the following Boltzmann distribution [11]:

$$f = \exp\left(-\frac{\Delta\hat{G}}{RT}\right) \quad (5)$$

with $\Delta\hat{G}$ being the difference in Gibbs energy between the non-porated and the porated state R and T are the gas constant and the absolute temperature, respectively. $\Delta\hat{G}$ can be split into an electric field-dependent and an independent part, $\Delta\hat{G}_0$ [11]:

$$\Delta\hat{G} = \Delta\hat{G}_0 + M^*E_M = \Delta\hat{G}_0 - \frac{M}{d}{}^*U_M \quad (6)$$

assuming that the field drops linearly across the membrane. Membrane thickness, d, was supposed to be 5 nm. M is the difference in dipole moment between non-porated and porated state. Combining Eqs. (5) and (6) renders:

$$f = \exp\left(-\left(\frac{\Delta\hat{G}_0 d}{M} - U_M\right)\frac{M}{dRT}\right) \quad (7)$$

The expression $\frac{\Delta\hat{G}_0 d}{M}$ is equivalent to the voltage, at which *f* attains unity, i.e. at which half of the membrane is in the P state. It will be denoted as the half-maximum voltage, $U_{0.5}$. For the fraction of the membrane that is in the electroporated state as a function of voltage, we obtain the following equation:

$$\frac{P}{P+C} = \frac{1}{1+\exp\left((U_{0.5} - U_M)\frac{M}{RTd}\right)} \quad (8)$$

or, in terms of the membrane chord conductance (compare Eq. (3)):

$$G_{chord}(U) = \frac{G_{chord,\,max}}{1+\exp((U_{0.5} - U_M)S)} \quad (9)$$

With the slope factor $S = \frac{M}{RTd}$ (in mV^{-1}).