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Nanopore occlusion: A biophysical mechanism for bipolar cancellation in cell membranes

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ABSTRACT

Extraordinarily large but short electric field pulses are reported by many experiments to cause bipolar cancellation (BPC). This unusual cell response occurs if a first pulse is followed by a second pulse with opposite polarity. Possibly universal, BPC presently lacks a mechanistic explanation. Multiple versions of the "standard model" of cell electroporation (EP) fail to account for BPC. Here we show, for the first time, how an extension of the standard model can account for a key experimental observation that essentially defines BPC: the amount of a tracer that enters a cell, and how tracer influx can be decreased by the second part of a bipolar pulse. The extended model can also account for the recovery of BPC wherein the extent of BPC is diminished if the spacing between the first and second pulses is increased. Our approach is reverse engineering, meaning that we identify and introduce an additional biophysical mechanism that allows pore transport to change. We hypothesize that occluding molecules from outside the membrane enter or relocate within a pore. Significantly, the additional mechanism is fundamental and general, involving a combination of partitioning and hindrance. Molecules near the membrane can enter pores to block transport of tracer molecules while still passing small ions (charge number ±1) that govern electrical behavior. Our extension of the standard model accounts for key BPC behavior.

1. Introduction

Over the past few years several publications have reported and partially characterized the phenomenon of "bipolar cancellation" (BPC), using a variety of in vitro experiments with isolated cells [1–8]. BPC manifests as reduction or cancellation of bioeffects, specifically the uptake of tracers such as YO-PRO-1, propidium or calcium. BPC occurs when two pulses of opposite polarity (not necessarily of same amplitude) are applied in rapid succession [1–8]. The extent of cancellation decreases with increased separation of the two opposite polarity pulses.

One striking feature is that BPC requires short, very large fields (nsPEF or nanosecond pulsed electric fields). These are not the longer, smaller field pulses used in conventional cell electroporation (EP) since the 1970s [9–11], but are nsPEF pulses used in supra-EP studies [12–16]. While not yet understood mechanistically, BPC is reported to mainly occur for widely separated mammalian cells in vitro, for applied electric field pulse strengths of 4–100 kV/cm and durations of 10–600 ns.

In some experiments pulse trains predominate, which greatly complicates interpretation because of memory effects due to pore lifetimes. Other studies employ single pulses, which is more relevant to basic understanding, and therefore the focus of the present work [17,18].

An unusual BPC feature is that the second part (reversed polarity) of the pulse should move tracer molecules "uphill", against the concentration gradient. The potential implications of BPC are tantalizing, but initial explanatory hypotheses have failed. To our knowledge, the present paper is the first report of a biophysical model that can account for functional features of BPC.

Significantly, attempts to use the standard cell EP model to account for BPC all failed. The standard model always predicts a large number of pores such that the diffusive influx always leads to an increase in intracellular tracer molecule. Essentially all EP delivery/extraction protocols accelerate transport down a solute concentration gradient. For BPC the second pulse should do the opposite. This apparently simple change greatly increases the problem difficulty: how can tracer molecule entry be slowed?

The standard EP model is based on lipidic transient pores (TPs) that form in lipid bilayer membranes in contact with aqueous

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electrolytes on both sides, and is consistent with many experimental observations [17,19–21]. The standard model is essentially an extension of the Schwann model for either spherical or cylindrical cells [17,20]. By adding TP creation for supra-physiologic transmembrane voltages the resulting model exhibits non-linear TP creation that begins at one (anodic) pole, followed by poration at the other (cathodic) pole, and then with time during a porating pulse, additional pore creation further away from the cell's poles [17,22].

Here we propose a mechanism for BPC that is based on increased pore occlusion and a corresponding decrease in tracer transport due to the presence of charged molecules within pores. The increased occlusion is represented in the model by a decrease in the occlusion factor magnitude. We also account for the possibility of a weak interaction of the inserted molecule with the membrane pore by allowing the recovery of the occlusion factor.

2. Methods

2.1. Cell electroporation EP model

Cell EP inescapably involves spatially distributed, highly nonlinear and hysteretic interactions throughout a cell system model. We use a cylindrical cell membrane contacting electrically conducting extracellular and intracellular media [18,20,21,23,24]. These complex interactions are solved computationally with an isolated cylindrical cell model (Fig. 1). We describe the system using the meshed transport network model (MTNM) elsewhere (above publications). The cylindrical plasma membrane (PM) has 5 μm radius, 6.7 μm height, and 4 nm thickness (Fig. 1A). The extracellular region is represented by 2077 nodes (or Voronoi cells which are the local regions), and the intracellular region is represented by 891 nodes. Of these, 150 node pairs (one extracellular and one intracellular) span the PM (Fig. 1B). The two electrolytes are represented by passive models that describe conductive and capacitive properties of the electrolyte [25]. The PM node pairs (Fig. 1C) contain a complete dynamic EP model that provides the local kinetics of membrane pore creation, evolution, and destruction, and include associated changes in transmembrane voltage and membrane conductance.

We use $D_p = 2 \times 10^{-13}$ m^2/s for the diffusion coefficient in pore radius space and a maximum pore radius, $r_{p,max}$ of 12 nm with a pore lifetime of 100 s. The details of the local membrane EP model are described elsewhere [18,20,23,24]. The local membrane models also include a −50 mV resting transmembrane voltage source. Other parameters for describing membrane EP within local membrane areas (regions associated with a transmembrane node pair) and adjacent aqueous media are given elsewhere [26].

2.2. Occluded transport

We assume that once a lipidic transient pore (TP) is created in the membrane, one or more charged molecules enter the pore. The presence of a charged molecule in the pore causes occlusion that hinders the movement of ions and tracer molecules. Some of the molecules are weakly bound to the pore wall and with time leave the pore. However, in the case of a bipolar pulse, the second pulse draws more molecules into the pores, increasing occlusion.

Accordingly we modify the standard model of electroporation by introducing an occlusion factor, O(t), that accounts for a decrease in pore-mediated transport of both small ions and tracer molecules. O(t) represents the total occlusion due to external molecule hindrance and partitioning. In addition, O(t) kinetics can account for the partial recovery of the membrane by the release of weakly bound molecules from the pore walls. In this way O(t) accounts for the decrease in tracer transport through a pore in the presence of external molecules in the pore.

2.3. Applied field

Our model can readily accommodate experimental waveforms with complex characteristics, including a decaying sinusoid. We model the response of two different but related electric field pulses: bipolar (BP; + and - 24 kV/cm, 200 + 200 ns; Fig. 2A) and unipolar (UP; 24 kV/cm, 200 ns; Fig. 2B). These pulses are digitized version of the experimental pulses of Gianulis et al. [3].

2.4. Electrolytes

The extracellular and intracellular media have electrical conductivities of 1.2 S/m and 0.3 S/m, respectively. The extracellular medium also contains 1 μM YO-PRO-1 (YP), a fluorescent dye with molecular properties: charge number: +2, molecular length: 1.7 nm, molecular radius: 0.53 nm, extracellular diffusion coefficient: 5.39×10^{-10} m^2/s, and intracellular diffusion coefficient: 1.35×10^{-10} m^2/s [20].

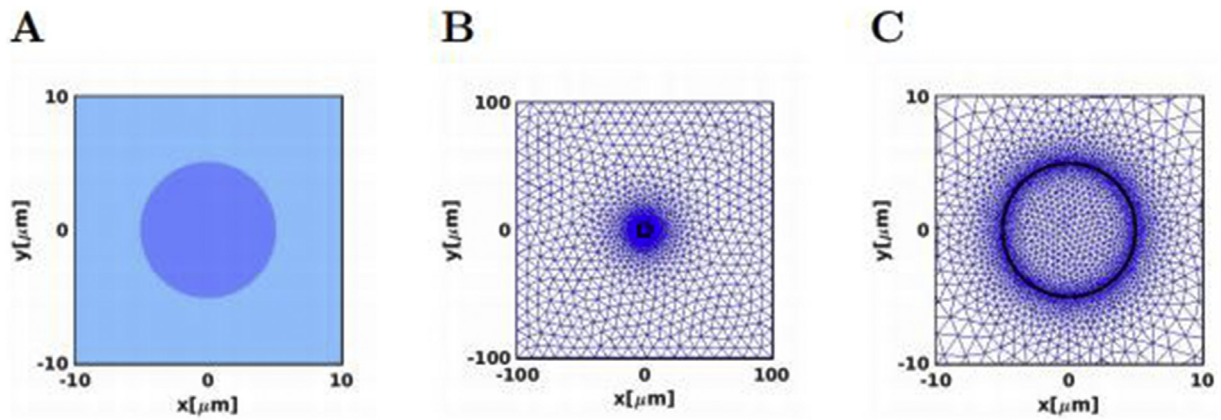

Fig. 1. Isolated cell model. The 5 μm radius cylindrical cell (A) is contained in a 200 μm × 200 μm system model (B). The cell meshed transport network model (MTNM) [20] is represented by 150 transmembrane node-pairs (C) that describe local transmembrane voltages, pore distributions, hindrance, partitioning of molecules and ions into the pores, and molecular transport. The 4-nm thick membrane has a resting potential of −50 mV due to a fixed current source [20]. The field is created by applying external pulse generator voltages to the top and bottom rows of nodes of the simulation box. Each of the local areas associated with a transmembrane node-pair is regarded as a very small planar membrane patch (a Voronoi cell) endowed with a resting potential source and a complete dynamic EP model [20].

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