

MEMBRANE DESTABILIZATIONS SUPPORTING ELECTROPERMEABILIZATION

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Abstract: The cell membrane can be permeabilized when subjected to calibrated short electric pulses. This membrane alteration can be reversible, leaving cell viability unaffected. This set of events is called Electroporation. This is now used in clinical applications to introduce hydrophilic drugs into the cytoplasm. While an empirical control of the electrical parameters is obtained, our knowledge of the molecular mechanisms supporting the membrane alteration is very limited. This paper gives a critical review of the limits of the models which are proposed by taking into account the different experimental approaches.

Key Words: Lipid Bilayer, Cell Membrane, Electroporation, Electroporation

INTRODUCTION

Since the 60's, it has been known that after being submitted to short high intensity electric pulses, cells can be lysed. Their plasma membranes are permeabilized during this process. Using more calibrated electrical conditions, it was shown that this permeabilization can be a transient effect (i.e. reversible) [1, 2]. This set of events is called Electroporation. This is now used in clinical applications (Electrochemotherapy) [3, 4]. While this approach has been used with great success for almost 10 years, there is a general agreement that the molecular processes behind these membrane alterations are not well known. Some biophysical points are clearly described. But only rather primitive molecular descriptions are proposed, which may be misleading for the safe development of an *in vivo* approach.

TEMPORAL ASPECTS

The cell can be considered as a spherical leaky capacitor which is charged when a field is present. This gives rise to an increase in the membrane potential difference which is position dependent on the cell surface [5]. When this potential alteration is locally brought to a critical value, the membrane organization is altered. Modern digitized videomicroscopy observes these

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processes at the single cell level with the aid of fluorescent probes [6, 7]. Several steps are sequentially present in this transition: the charging time of the membrane to reach the threshold value in potential (microseconds); the induction of minute defects (nanoseconds); local expansion of the alteration (micro to milliseconds) [8, 9]. As soon as the field is weakened, a stabilization step occurs (millisecond), nevertheless leaving the membrane in a non-equilibrium state (it remains leaky) [6]. A strongly temperature dependent resealing phenomena follows (second to minutes) [10]. This step is a first order process, the time constant of which is under the control of the pulse duration. Permeabilization is therefore annihilated. A key parameter in the kinetics of electroporation is that the induction step is very fast, as observed via conductimetry or spectrophotometry [1, 11]. This is not observed in experiments where a planar bilayer membrane (BLM) is used as the model for a cell membrane [12]. A lag is observed between the onset of the voltage and the first conductance fluctuations which are indicative of the permeabilization [13] ("poration").

Electroporation is controlled by the field intensity. The conclusion based on work with cells is that it is the geometry of the cell surface brought to the permeabilized state which is dependent on the intensity. The local level of permeabilization is not controlled by the intensity (when larger than a threshold) but by the pulse duration [7-9, 14].

STRUCTURAL INVESTIGATIONS

Dramatic changes should affect the membrane structure to support the free transmembrane exchange of polar compounds. Short lived cracks are transiently present on erythrocyte membranes but disappear as soon as the field is switched off. [15]. EM studies show that some wide pores are present on erythrocytes but only under hypoosmolar conditions [16]. Furthermore they are detected only a long time after the pulses, while the high conducting state of the membrane is observed during the pulses. They appear to be due to the induced osmotic swelling which is always present but gives hemolysis only in the case of red blood cells [1, 17]. P^{31} NMR studies show that the polar head regions of lipids are affected while no change in the fatty acid chain region is detected [18]. This appears to be a global effect suggesting that most lipids in the permeabilized part of the cell membrane are altered. This change is temporally associated with electroporation. The polar head region returns to normal after membrane resealing. All other structural conclusions are in fact indirect [11, 19].

STRUCTURAL MODELS

Taking into account the thermodynamics of lipid bilayers, results from work on BLMs are used to explain the physics of Electroporation. It was first considered that, as in the case of a capacitor due to the electrocompression of the film, an irreversible breakdown was taking place. But electrocompression of the film and viscoelastic waves cannot explain the reversible character of electroporation. A transition of hydrophobic fluctuations to hydrophilic pores is proposed to create cylindrical pores where a rotation of the polar heads brings a hydrophilic surface to the pore [12]. These pores can be present only as long as the field is present, while experimental observations show that permeabilization is present long after the field is switched off [20, 21]. As emphasized in a critical analysis, some of the key hypothesis in the "poration" model are crude approximations, and cannot give an accurate description of the molecular events affecting the lipid assembly [22]. A very limited number of hydrophilic pores can be present, as predicted by the conductance experiments [12, 13, 23], while all experimental observations indicate that a large number of defects are present on the cell surface [6-9,14, 24, 25] with the exception of the hemolytic pores detected in erythrocytes [16, 26]. A more recent model proposes that it is linked to mismatches in the membrane assembly as in lipid phase transition [27, 28]. A more complex description takes into account the internal dynamic of membranes. It can then be proposed that permeabilization is associated with an increased level of density fluctuations in the membrane. The movement of polar compounds can be linked to a reptation process as described in the case of polymer chains [29]. Most of these descriptions treat the cell membrane as a lipid bilayer, while membrane proteins and the cytoskeleton are clearly involved [1, 11].

CONCLUSION

At the present state of our knowledge, it is impossible to obtain a reliable description of the molecular events supporting electroporation. There is a need for more investigations to obtain information on the structure of the modified membrane using spectroscopic methods, computer simulations, quick freezing EM and atomic force microscopy.

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